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Gouvernance de la santé des plantes et gestion de la biodiversité cultivée - Le cas de la santé du haricot gérée par les membres de l'association « Croqueurs de Carottes »

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"Mais si on éprouve le besoin de se rassurer c'est qu'une angoisse hante constamment la pensée, si on délègue à la technique, magique ou positive, le soin de restaurer dans la norme souhaitée l'organisme affecté de maladie, c'est qu'on attend rien de bon de la nature par elle-même."

Georges Canguilhem, 1966

"Am I a part of the cure? Or am I part of the disease?"

Coldplay, 'Clocks', 2002

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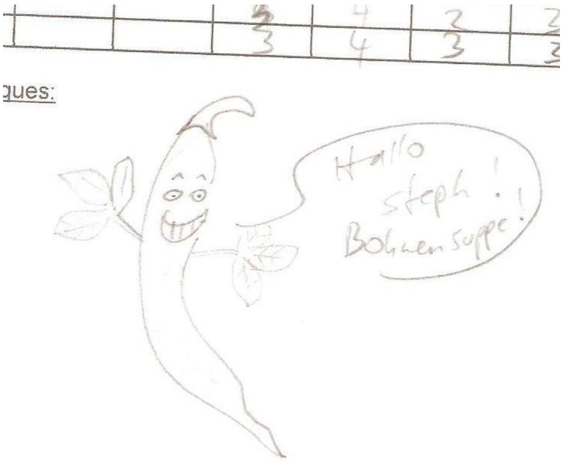

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Governance of plant health and management of crop diversity -

the case of bean health management among members of the association *Croqueurs de Carottes*

Abstract

All over the globe, networks of seed growers are cultivating crop diversity in fields and gardens. Their contribution to the maintenance of this diversity has been studied, but research has widely left aside their management of plant health. The governance of bean health practiced by an association of artisanal seed companies, *Croqueurs de Carottes*, is approached as a case study in the objective of specifying how management of crop diversity and governance of plant health are articulated. Their concern for the governance of bean health is elucidated from an agroecological perspective, taking an interdisciplinary and transformative approach. Actor-network theory constitutes the backbone of the thesis, situated between agronomy and sociology and drawing upon a threefold research device: on-farm experiments, semi-directive interviews and participant observation.

The *Croqueurs'* approach to bean health is described as *in situ* approach, in which plant populations are considered healthy if they are able to *live with* potential plant pathogens and adapt to their growing environments. Relying on ecological interactions, competences of plant health management are distributed throughout the production system. Both for plant health and crop diversity management, a seed lot is determined by a complex system of interactions. A clear boundary distinguishing plant populations from their growing environment cannot be drawn. This implies (i) that plant health must be judged upon *in situ* in the plants' growing environment and (ii) that the governance of plant health must be considered at the collective scale.

Key words: plant health, small-scale organic seed production, common bean, participatory research, agroecology

Gouvernance de la santé des plantes et gestion de la biodiversité cultivée -

le cas de la santé du haricot gérée par les membres de l'association *Croqueurs de Carottes*

Résumé

De multiples réseaux d'agriculteurs et de jardiniers maintiennent la biodiversité cultivée dans le monde. Leurs pratiques de gestion de la santé des plantes demeurent peu étudiées. La thèse a pour objectif de caractériser l'articulation entre gestion de la biodiversité cultivée et gouvernance de la santé des plantes, se saisissant du cas d'une association d'artisans semenciers, les *Croqueurs de Carottes*. Elle développe une approche interdisciplinaire et transformatrice pour décrire et comprendre la gouvernance de la santé du haricot par ces acteurs, dans une perspective agroécologique. La théorie de l'acteur-réseau est mobilisée pour situer l'analyse à l’intersection entre approches agronomique et sociologique, reposant sur les données produites par un triple dispositif : expérimentations à la ferme, entretiens semi-directifs et observation participante.

Nous qualifions d’*in situ* l'approche de la santé des plantes des *Croqueurs* dont l’objectif est de *vivre avec* les agents pathogènes potentiels. Fondées sur des interactions écologiques entre plantes et terroir, les compétences contribuant à la gestion de la santé des plantes sont distribuées à travers le système de production. Que ce soit en termes de santé ou de biodiversité, un lot de semence est l'expression d'un jeu complexe d'interactions. Il est alors difficile de délimiter des populations de plantes de leur terroir de manière précise. Par conséquent, (i) la santé des plantes ne peut être jugée qu'*in situ*, dans l'environnement dans lequel elles évoluent et (ii) la gouvernance de la santé des plantes doit être prise en compte à l'échelle du collectif.

Mots-clés: santé des plantes, artisans semenciers, haricot, recherche participative, agroécologie

Abbreviations

ANOVA	Analysis of Variance
AM	Arbuscular mycorrhiza
AQU	On-farm experimental site in Aquitaine (France)
ANT	Actor network theory
arbusc	arbuscules
BCMV	Bean common mosaic virus
BCMNV	Bean common necrotic mosaic virus
blight.leaf	Blight symptoms on leaves
blist	Leaf blistering symptom
brown.vein	Dark lesions on stems and leaf veins
BZH	On-farm experimental site in Brittany (France)
cal	Common bean cultivar 'Calima'
CBB	Common bacterial blight
COFRAC	*Comité français d'accréditation* - French accreditation body for certification bodies
Croqueurs	*Croqueurs de Carottes*
das	days after sowing
EPP	European plant passport
EPPO	European and Mediterranean Plant Protection Organization
EFSA	European Food Safety Authority
EU	European Union
feeding	Symptom of white-silvery spots left on leaves by leaf-sucking and pests
flc	Common bean cultivar 'Flageolet Chevrier'
FNR	Fonds National de la Recherche, Luxembourg
FSO	Farm Seed Opportunities, a EU-funded research project
GNIS	*Groupement national interprofessionnel des semences et plants* - French inter-branch union concerned with seeds and seedlings
HBB	Halo bacterial blight
HSD	Tukey's Honestly Significant Difference test
hyph	hyphae
INRA	*Institut National de Recherche Agronomique* - French national institute for research in agronomy.
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
ISTA	International Seed Testing Association
LUX	On-farm experimental site in Luxembourg

MCA	Multiple Correspondence Analysis
mosaic	Leaf mosaic symptom
%ndfa	percentage of N derived from the atmosphere
NGO	Non-governmental organisation
OPP	Obligatory passage point
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PLH	EFSA Panel on Plant Health
PRA	Pest Risk Assessment
Psp	Bacterial agent causing Halo Bean Blight: *Pseudomonas syringae* pv. *phaseolicola*
rdb	Common bean cultivar 'Roi des Belges'
rdc	Common bean cultivar 'Rognon de Coq'
RSP	*Réseau Semences Paysannes*, French umbrella organisation for peasant seed
ses	Common bean cultivar 'St. Esprit à œil rouge'
SNF	Symbiotic nitrogen fixation
SOC	*Service officiel de contrôle et certification* - Official service for inspection and certification of the GNIS
SRAL	*Service régional de l'alimentation* - French regional services for food and agriculture
TSW	Thousand-seed weight
vesic	vesicles
Xap/Xff	Bacterial agents causing Common Bean Blight: *Xanthomonas axonopodis* pv. *phaseoli* and/or *Xanthomonas fuscans* pv. *fuscans*

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Chapter 1: **General Introduction**

In 1972 Jack Harlan warned of the genetic erosion of humanity's crop plants in his article *Genetics of Disaster*. Based on his worldwide explorations of crop diversity as a plant breeder, he concluded that the success of modern breeding was threatening its own base, the world's crop genetic resources. There is now consensus about the threat that genetic erosion of crop diversity represents for global agriculture (Commission on Genetic Resources for Food and Agriculture, 2010). Considerable effort has been made in the second half of the last century to collect, characterize, conserve and store crop genetic diversity *ex situ* in national and international gene banks, but there is increasing evidence that *ex situ* conservation, although complementary, cannot substitute for maintaining crop diversity *in situ* in farmers' fields (Soleri and Smith, 1995; Tin et al., 2001; Fowler and Hodgkin, 2004). Only when regularly cultivated under practical conditions may local cultivars evolve in their natural and human environments. It has been recognised that, besides their "genetic raw material" conserved *ex situ* in gene banks, "crop genetic resources also comprise related species, agroecological interrelationships, and human factors" and that "ecological relationships such as gene flow between different populations and species, adaptation and selection to predation and disease, and human selection and management of diverse crop resources are components of a common crop evolutionary system that generate crop genetic resources" (Brush, 2000). Concerning common bean in particular, studies on Italian (Negri and Tiranti, 2010) and Nicaraguan (Gómez et al., 2005) landraces have shown that *ex situ* conservation did not maintain the full genetic diversity maintained by farmers. On one hand, local cultivars have been shown to continuously adapt to environmental conditions and crop management strategies. For example, local adaptation has been observed at the phenotypic level for wheat (Dawson et al., 2013), lentil (Horneburg and Becker, 2008), maize (Serpolay-Besson et al., 2014) and spinach (Serpolay et al., 2011) to name both autogamous and allogamous crop species. Genetic adaptation to local conditions has also been observed for common bean (Tiranti and Negri, 2007) and wheat (Thomas et al., 2012). On the other hand, crop and seed producers maintain the knowledge of crop selection and management associated to the development of local cultivars (Brush, 1995, 2000; Bretting and Duvick, 1997), as well as the knowledge about their use. One can speak of coevolution between crops, environment and Man, which is why the *in situ* maintenance and use of crop diversity on farms is also termed *dynamic conservation* by scholars of plant genetic resources. In this thesis, I will not speak of crop diversity *conservation*, but rather of *management, maintenance* and *safeguard* of crop diversity on farms. Thereby, the ambiguity of the term *conservation* - which appears as something static and isolated - is avoided. These terms also rejoin the terminology employed by the actors engaged in the maintenance of crop diversity on farms, including farmers and non-governmental organisations (NGO) (Demeulenaere, 2014).

1 Collaborative Research on crop diversity in France

In France, collaborations between seed-saving farmers, NGOs concerned with crop biodiversity and researchers from the public research institution INRA (*Institut National de Recherche Agronomique*) began at the turn of the millennium (Bocci and Chable, 2009). For example, research with cereal growers was driven by a common concern of farmers and researchers for bread wheat diversity maintained on farms at the national scale (Thomas, 2011; Enjalbert et al., 2011). In the region of Brittany, a lack of cabbage varieties suited for organic production led researchers and market gardeners to explore cabbage diversity stored in gene banks and to start participatory plant breeding (Conseil and Chable, 2009). Today, such participatory researches cover numerous crop species in France. It s conducted in contexts of organic and low-input farming (Chable and Serpolay, 2016). Many of the actors - farmers and consumers, but also bakers, cooks and other artisanal food workers - form part of the national umbrella organisation *Réseau Semences Paysannes* (RSP), literally "peasant seed network". RSP was founded in 2003 and now includes more than 80 associations "promoting and defending crop biodiversity and related know-how" (translated from RSP, 2007).

Among the member associations of RSP, the *Croqueurs de Carottes* are a group of small-scale organic seed companies. They qualify their seed production practices as *artisanal*, or handcraft, and regard themselves as *artisanal seed companies* (*artisans semenciers* in French). They pursue the aim of contributing to the safeguard of vegetable diversity in danger of genetic erosion (Semailles, 2016). This includes landraces[1], as well as *old varieties* that have undergone formal crop improvement and may even have been registered on a European variety catalogue. When plant protection for a formal variety expires, the variety "falls" into the public domain. The plant breeder can no longer claim royalties for the variety and is no longer legally responsible for its maintenance. The maintenance of public varieties is thus open to all. By making seed of vegetable varieties from the public domain available to home and market gardeners, the *Croqueurs de Carottes* - *Croqueurs* for short - take part in the safeguard of crop diversity. The *Croqueurs* operate both as partner and case study of the PhD research presented in this thesis.

From the start, the rediscovery and reappropriation of crop diversity was at the heart of co-constructed research projects, as well as building legitimacy for its cultivation and use on farms (Bocci and Chable, 2009; Demeulenaere, 2014). This went along with a focus on participatory plant breeding (Desclaux, 2005; Chable et al., 2008; Dawson et al., 2011; Rivière et al., 2015). In European research projects such as "Farm Seed Opportunities" (Chable, 2010), "SOLIBAM" (Chable, 2015) and, currently, "Diversifood" (Chable and Dibari, 2016), French collaborative research on crop diversity linked with scientific and farmer communities all over Europe. With time, the focus has also widened to the practices associated with the cultivation and use of crop diversity (Chable and Serpolay, 2016). For example, ongoing projects address the composition of variety mixtures (van Frank and Forst, 2016)

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[1] Landraces have been defined by Camacho Villa *et al.* (2005) as *'dynamic populations of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems'*.

and sowing densities of diverse wheat populations (Baltazar, 2016), as well as milling and cooking with diverse maize populations (Agrobio Périgord, 2016). This thesis forms part of this category of researches, as it addresses the governance of plant health practiced by small-scale seed companies engaged in the safeguard of crop diversity.

2 Crop diversity, agroecosystems and plant health

As for any crop (Anderson et al., 2004), seed-saving farmers engaged in the maintenance of crop diversity on their farms have to cope with plant pests and diseases in their fields. *Seed-borne diseases* represent a particular challenge for seed growers, because they are transmitted from one plant generation to the next *via* the seed (Wood and Lené, 1997; Lo Cantore et al., 2010). Farmers who maintain crop diversity and save seed need to manage plant health if they want to make a living from their work.

Crop diversity as such has been studied as a means to improve plant health. Crop genetic diversity may ensure plant health by enhancing agroecosystems, both directly and indirectly (Hajjar et al., 2008). Indirect effects may consist of sustaining diverse populations of pest predators or soil life, for example. Increased genetic diversity within crops - through intra-varietal genetic diversity, variety mixes or intercropping practices - has been shown to reduce pest incidence in various crop species including common bean (Fadda et al., 2010; Mulumba et al., 2012). To cite another example, mixtures of rice varieties grown in the traditional agro-system of the Yuanyang terraces in China combine different components of basal immunity and effector-triggered immunity against the rice blast fungus *Magnaporthe oryzaeh* and may limit the latter's spread in the landscape (Liao et al., 2016).

The coevolution of local cultivars with crop pests and diseases is cited regularly as one basic element of on-farm management of crop diversity (Maxted et al., 1997; Brown, 2000). Genetically uniform crops, with uniform disease resistance as it has been introduced by modern breeding, is met by the continuing evolution of new races of pests and pathogens able to overcome resistance genes, creating the phenomenon of boom and bust cycles (Mulumba et al., 2012). In contrast, more diverse crops - be it through intra-varietal genetic diversity, variety mixes or intercropping practices - may slow down this "arms race". In the framework of this co-evolution between local crop populations and plant pathogens, the potential of on-farm maintenance of crop diversity to give rise to novel resistance genes in crop plants has been questioned. It is uncertain whether the selection pressure exerted by pathogens in farmers' fields is sufficient for the emergence of resistance genes (Holden et al., 1993). The effect of crop distribution in time and space and of dispersal dynamics and survival structures of each pathogenic species is also unclear (Qualset et al., 1997). This has led Brown (2000) to the conclusion that *'the nature and pace of change of resistance structures in landrace populations conserved on farm are key topics about which there is much speculation and some dogma, but very little hard evidence'*.

In addition of crop diversity alone, diversity of whole agroecosystems play a role for plant health by providing for ecological interactions, also called ecological services (Matson et al., 1997; Altieri, 1999; see also references therein). In cropping systems, this includes the diversity of crops and weeds, pest-predator interactions (Losey and Denno, 1998; Wilby and Thomas, 2002; Tylianakis and Romo, 2010), as well as soil life (Mäder et al., 2002). Ecological interactions within agroecosystems, especially the maintenance of biological diversity and soil health, constitute a main pillar of plant health management in organic farming systems (van Bruggen et al., 2016). It is within such organic systems that the artisanal seed companies forming the *Croqueurs* operate.

3 Crop diversity and plant health regulations in the EU

Beyond plant diseases themselves, it has been argued for Europe that an additional risk for crop biodiversity may come from the very plant health regulations intended to protect crops from plant diseases. Non-profit organisations, small-scale seed companies and researchers have identified seed laws in general as the single most important barrier to the use and maintenance of crop diversity on farms (Cherfas et al., 1993; Anonymous, 2013). By reducing their potential market profitability, regulations may impede the use and maintenance of crop diversity on farms (Bretting and Duvick, 1997). Much public debate on EU seed legislation was sparked by the revision of the legislation bodies relevant for the European agricultural and food sectors. The revision process led to the adoption of a package of measures termed "Smarter Rules for Safer Food" by the European Commission in May 2013 (European Commission, 2013). The package consisted of 5 pieces of legislation, of which 3 concerned the seed market of the European Union (EU), namely the legislation bodies on “plant reproductive material” (including seeds), on plant health and on official controls. Critique of the legislation proposals by civil society focused on the first piece of legislation, explicitly addressing plant variety legislation and the seed market (Anonymous, 2013). While some actors in civil society warned about the potentially negative impacts of control mechanisms and plant health regulations for crop diversity, these pieces of legislation were rarely more than mentioned in debates and position papers of civil society organizations. As the legislative package entered technical negotiations between the European Parliament and the Council, the European Commission withdrew the proposal on plant reproductive organisms. Among the remaining pieces of legislation in the package, the one on plant health has just been voted[2] on in second reading in the European Parliament as I am writing this introduction. The new plant health legislation is to enter into force in 2017 and become applicable within three years. European plant health legislation is thus currently undergoing change.

The current plant health legislation of the EU consists of Council Directive 2000/29/EC of 8 May 2000 *on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community*. It lists *organisms harmful to plants or plant products* which are regulated by *protective measures* (so called "quarantine pests"). Protective

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[2] voted on November 25th, 2016

measures aim at preventing the introduction and spread of concerned pests in the EU: seeds and other plant propagation organs (tubers, plants) are tested for the presence of concerned pests and infected lots are banned from the EU market. The risk that such non-tariff measures for plant protection may constitute unjustified barriers to international trade is widely recognised and addressed by the Agreement on the Application of Sanitary and Phytosanitary measures (SPS) of the World Trade Organization (WTO) (MacLeod et al., 2010). However, the risk that such measures may represent market barriers for small-scale seed producers, seed savers' associations and crop biodiversity is seldom discussed within scientific spheres and among governing bodies.

4 Common bean diversity and bean diseases in Europe

This PhD research was triggered by increasing awareness that artisanal seed companies among the *Croqueurs* felt caught in the crossfire between the management of vegetable crop diversity in their fields on one hand and the legal requirements of European plant health regulations on the other. In particular, tensions became apparent regarding the plant health management of common bean (*Phaseolus vulgaris* L.).

Common bean is an important item in the seed catalogues of the *Croqueurs* members in terms of turnover and number of varieties. For example, it is the crop species that represented the second largest proportion of turnover for the member company *Germinance* in 2013, after the cabbages (*Brassica oleracea*) (Delmond, personal communication). Up to 31 varieties are proposed in the 2016 seed catalogues for *Croqueurs* members. This reflects the large number of common bean types and varieties developed on the Old Continent since the crop was introduced from the Americas by Columbus. Southern Europe is regarded as secondary centre of diversity for common bean (De Ron et al., 2010). The recombination between the Mesoamerican and Andean gene pools upon their arrival in Europe is likely to have created novel genetic variation (Gioia et al., 2013) before the crop spread across the continent (Gepts et al., 1988; Zewen, 1997; Maras et al., 2013). In general, common bean is a highly variable species and uses are diverse. Growth types range from determinate bush beans to indeterminate climbing ones. According to consumer preferences and bean varieties, either the pods are harvested as green beans or the mature seeds are harvested as dry beans. In Western Europe, dry beans were historically considered as "meat for the poor" and their consumption has dropped over the last decades (1.3 kg per capita in 2013)[3]. In this geographic region green beans, i.e. the fresh pods, represent the main mode of consumption. To meet the demand for labour-intensive fine beans all year around, the EU imported[4] 196.700 t of fresh green bean in 2015, mainly from Morocco (64% of imports), Kenya (16%), Egypt (10%) and Senegal (6%). Nevertheless, green beans remain an important crop in the EU. France alone cultivated 26 830 ha in 2014, i.e. 28% of the surface harvested in the EU (97 280 ha), mainly for the canning and freezing industry (Unilet, 2015). Given a decreasing

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[3] Consumption per capita was approximated by the produce available for consumption, calculated from FAOSTAT data (http://faostat3.fao.org/home/E) as follows: (Quantity produced - Quantity exported + Quantity imported) / Population

[4] Data on the imports of green beans into the EU retrieved from EuroStat: http://ec.europa.eu/eurostat/web/international-trade/data/database

demand for European dry beans on one hand and the development of green bean varieties adapted to growing conditions in Africa (Observatoire des marchés du Cirad, 2009) and for large-scale mechanized agriculture in Europe (Gry, 1995) on the other, traditional European bean diversity is at stake. For example, 64% of green bean surfaces in France were sown with varieties owned by three seed companies in 2014 (Unilet, 2015). Nevertheless, the proportion of home-grown produce in European plates remains considerable for fresh green beans[5]. The demand of organic market gardeners engaged in local food systems for crop diversity is also increasing (Kastler, 2006; Bocci and Chable, 2009; Brouwer et al., 2015). Home gardens and local organic food systems thus harbour potential for the use and maintenance of common bean diversity.

In the aim of meeting this demand for common bean diversity, *artisanal seed companies* (along with numerous non-profit seed-saver organisations) multiply seed of varieties from the public domain and provide it to home and market gardeners. Plant diseases sometimes intervene in their endeavour to cultivate and provide healthy bean plants. Several diseases affect bean production worldwide (Hall, 2005; Singh and Schwartz, 2010). Among the most important are the fungal diseases anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*), as well as bean common mosaic virus/bean common necrotic mosaic virus (BCMV/BCMNV), a *potyvirus*. The most important bacterial bean diseases cause bacterial blights (Rodiño et al., 2009). *Common bacterial blight* (CBB) is caused by the two species *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* (Xap/Xff). Halo bacterial blight (HBB) caused by *Pseudomonas syringae* pv. *phaseolicola*. Except for rust, all of these diseases are seed-borne; they are passed on from one plant generation to the next *via* the seed. This implies that that pathogens carried by seeds affect commercial seed quality, as they can represent a major source of inoculum in bean fields. This phytosanitary aspect of seed quality is also called <u>*seed health*</u> (ISTA Online, 2016). A common recommendation for the control of seed-borne diseases is to maintain disease-free seed stocks (Organic Seed Alliance, 2007). For common bean, the EU regulates CBB as a *regulated pest* in the entire EU zone. Specific *protective measures* ban common bean seed carrying the bacteria causing CBB from the EU seed market. These protective measures are contested by artisanal seed companies among the *Croqueurs de Carottes*.

5 Case study, hypothesis, and research questions

Croqueurs members argue that the protective measures against CBB are not compatible with their management of bean diversity and governance of bean health. The <u>underlying hypothesis</u> of this PhD research is that tensions between the protective measures and the *Croqueurs'* practices reveal the *Croqueurs'* concept of plant health management. Hence, the <u>*Croqueurs'* governance of bean health is approached as a case study</u> with the scientific aim of elucidating how they articulate management of

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[5] As is noted in the "Methods and Standards" section of the FaoStat homepage (http://faostat3.fao.org/mes/methodology_list/E): "...Production from family and other small gardens not included in current statistical surveys constitutes quite an important part of the estimated total production in certain countries: for example, Austria, France, Germany, Italy and the United States."

crop diversity and governance of plant health. By pushing this on the research agenda, the *Croqueurs* pursue the aim of showing the incompatibility between the current EU plant health regulation and their practices. These aims point to the dual scope of this research, for science and for society beyond the scientific arena. For science, this PhD research implies taking a peek outside the paradigm of plant disease eradication and taking into account an approach which consists of "living with" plant diseases. Beyond science, the PhD research questions current EU plant health regulations and their role for crop diversity.

In the framework of the case study, common bean is considered an appropriate model crop to study the *Croqueurs'* management of plant health for three reasons:

(i) Common bean is an emblematic crop among European crop diversity. Thus, it is representative of the stakes of maintaining this diversity on farms.
(ii) Common bean is prone to several seed-borne diseases which can challenge seed production and consequently the maintenance of bean diversity on farms.
(iii) One of its seed-borne diseases, CBB, figures among the EU list of regulated pests. Common bean seed is therefore subject to EU plant health regulations, making tensions between official plant health regulations and the *Croqueurs'* plant health management practices tangible.

In addition, the association *Croqueurs de Carottes* is considered as a spokesperson for a much wider network of actors concerned with crop diversity and farmers' seed autonomy, as it figures among the 80 collectives composing RSP. By contesting protective measures on CBB, the *Croqueurs de Carottes* emerge as a spokesperson for the wider network RSP facing plant health regulations.

The following research questions are addressed through the case study.

1. **Which are the specificities of bean health management practiced by artisanal seed companies among the association *Croqueurs de Carottes*? On which interactions between bean plants and their growing environments is this plant health management based?**

Founded on these two questions, ecological interactions between bean plants and their growing environments are further elucidated.

2. **What do analyses of some plant-environment interactions reveal of the ecological base of the *Croqueurs'* bean health management?**

The questions are approached from an interdisciplinary, agroecological perspective. The research combines methods taken from the disciplinary fields of sociology and crop ecology. It takes an agroecological stance as described by the Belgian interdisciplinary group of researchers in agroecology GIRAF (Stassart et al., 2012). These authors base agroecology as a scientific practice on a shift from the techno-economical (*productivity*) to the socio-technical domain. Agroecological research seeks to support the organisation of food systems in order "to face the diverse and multiple stakes and objectives concerning food supply, environment and equity". It is by definition an interdisciplinary practice and implies the redefinition of scientific and social boundaries.

6 Chapter set-up

A brief introduction to the subject has been given in this first chapter, including hints at relevant scientific and legislative references. It is followed by a chapter on the establishment and development of the research device during the research process. By specifying the socio-technical framing of the research, Chapter II renders the conditions under which knowledge was created explicit. The resulting research device combines methods from the disciplinary fields of sociology and agronomy. Chapter III then discusses results of the social science approach in the objective of specifying the *Croqueurs'* approach to bean health and how this approach is linked to interactions between bean plants and their growing environments. The following chapters report and discuss results of the agronomical approach in the objective of revealing some aspects of the ecological base of the *Croqueurs'* bean health management. Chapter IV explicates the general set-up of field experiments. In the four following chapters, Chapters V to VIII, the question of ecological interactions between bean plants and their growing environment is broken down into sub-questions: Chapter V addresses the plant health of bean plants observed in the field trials. Chapter VI focuses on interactions of bean plants with beneficial organisms in the soil, mycorrhiza and Rhizobia in particular. Chapter VII regards the microbial communities associated with common bean seeds. Chapter VIII addresses the phenotypic and genetic adaptation of bean populations to local growing environments. Finally, Chapter IX proposes a general discussion of the results presented in the thesis. Figure 1.1 gives an overview of the thesis set-up and may serve for orientation as the reader navigates between chapters.

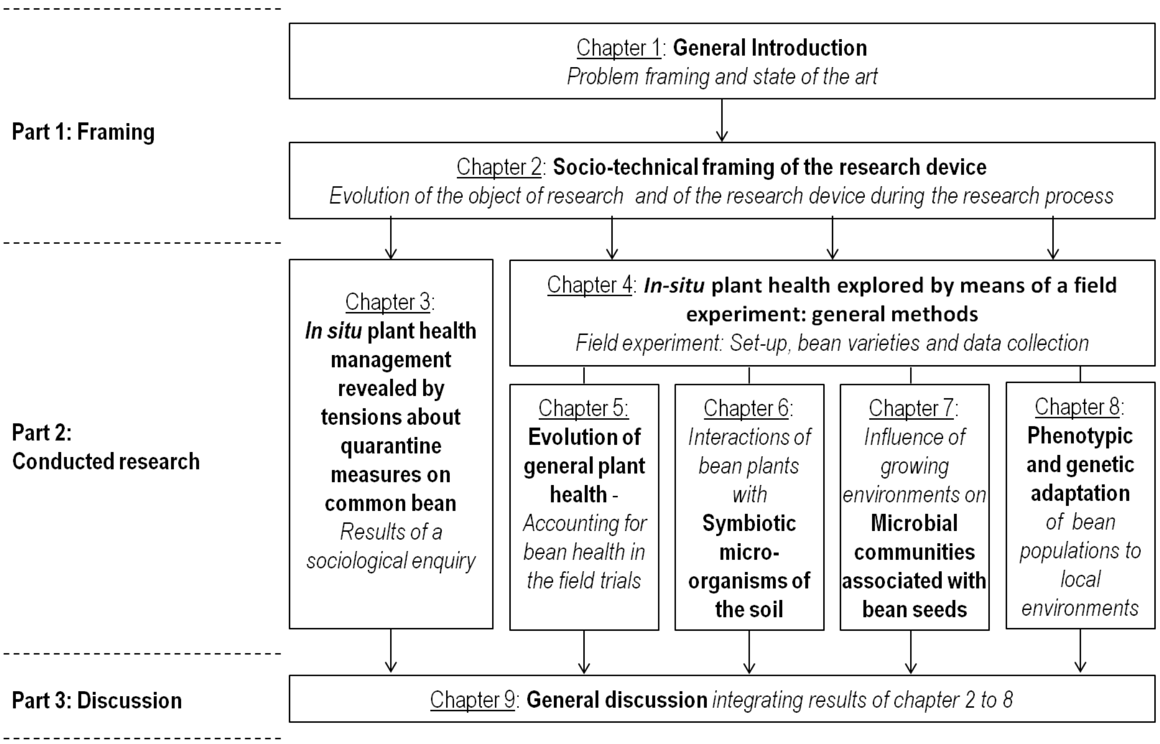

Figure 1.1: Set-up of the chapters of the thesis.

Chapter 2: **Socio-technical framing of the research device**

The doctoral research project was triggered by a debate on common bean seed health involving an association of small organic seed companies, *Croqueurs de Carottes.* Common bacterial blight (CBB), a bacterial disease of common bean, plays an important role in the debate. CBB is a regulated "quarantine pest" in the European Union. As a consequence of the debate, this thesis takes an interdisciplinary approach to elucidate plant health management in artisanal seed companies. Before going into the research results in the following chapters, this chapter questions the device and process which produced the data: Starting from the aforesaid debate, how did the research project and its research device emerge? I will trace its evolution. Although it takes place in the overall framework of a collective experience of participatory research about on-farm breeding and seed production, the project starts out with a focus on the ecological interactions between bean plants and their growing environments. During the research process, it evolves to question the rationale behind the plant health management of the *Croqueurs de Carottes*.

Any research directed at humans and animals can be considered "intervention" research in that it intervenes in the living conditions of these beings and transforms them. To this idea put forward by Thierry and Cerf (2009), I may add that the same is true for research directed at plants, as well as plant breeding - as also remarked, in other terms, by a former INRA chairman (Hervieu, 2004). Because these living beings are not indifferent to the research device "applied" to them as objects of research, the researcher is in the obligation of recognising that the research device is an artifice. The link between the research device and the "fact" that is to be accounted for is artificial. The knowledge created is not independent of the conditions in which it was created. Creating universal knowledge thus becomes impossible. By making the link between the research device and the construed "fact" explicit, the researcher's method is defined (Thiery and Cerf, 2009). Recognising the research device as an artifice is also the first of four principles of method carved out of an intervention research experience by Stassart *et al*. (2011). In this chapter, I propose a socio-technical framing of the research device employed. Thereby, links between the research device, its effects and the object of research are made explicit. By the same move, I will discuss how the four principles of method (Stassart et al., 2011) underlie the research process.

To address the evolution of the research device during the research process, I will discuss key moments which caused the researcher to hesitate and the research device to shift. These are moments where the device was put to the test and translated in the sense of actor-network theory (ANT), also called sociology of translation (Callon, 1986a). I will study the research device of the PhD research as an actor-network. Rather than taking the research device and its results as a stabilised given, ANT proposes to unfold the associations that compose the research device. One particularity of

ANT is to account for human and non-human entities alike. I shall trace how human and non-human *actants* associate to constitute the research device as a network. In the network, each actant may intervene as a *mediator*. As opposed to mere intermediaries, mediators "transform, translate, distort, and modify the meaning or the elements they are supposed to carry" (Latour, 2005). In other words, they *translate* meanings or elements according to their own understanding of what is relevant or problematic. The translations of an object by different actants can concur to form a common *problematisation*. They are then linked by this problematisation. For instance, a collaborative research project can be established only if partners with concurrent translations formulate a common problematisation (Audoux and Gillet, 2011). In the same way, non-human elements of the network must also concord with their translation. When translations of actants of a network do not concur, the network is put to the test by *competing translations* (Latour, 2007). Competing translations have also been called "anti-programs" to stress the obstacle they constitute for other translations of an object, i.e. for the "program" of other actants. We will see that keeping the research network together requires continuous effort from the researcher, not merely to maintain it, but to adjust it again and again. Every time the network is put to the test, the researcher hesitates and the research device is transformed. When the research project risks to be hampered by competing translations, the network is rearranged, either by externalising the competing translation or by integrating it in a renewed problematisation. In any case, the network is not left unchanged. When the network is put to the test, the object of research and the device established to study the object may shift.

In the first section, I will retrace how the object of research emerged and how the PhD research project was set-up. The actors contributing to the research will be described. In the second section, I will show how seed growers, bacteria and bean plants shape the research device by acting in unexpected ways. These happenings make space for a complementary social science approach in the research device. *Recalcitrance* as the basis of the second principle of method will be of some importance here. The third section will address the socio-technical framing of field experiments. I will show how getting a grasp on the object of research in the field experiments happens progressively. This is also where the third and fourth principles of method come into play, namely the absence of guarantee on results and the confidence that the device will produce meaningful scientific results. Finally, the fourth section, coming back on the first principle, acknowledges the artificial nature of the field experiment by questioning its link with the concern of the research partners.

1 A hot debate on the seed health of common bean

In October 2009, in a conference room in Marseille, in the South of France, a group of French farmers are hung to their headsets. They are trying not to miss the French translation of the presentation a Dutch scientist is giving in English. The Dutch scientist is a specialist in seed technology. He is presenting the final results on one aspect of Farm Seed Opportunities (FSO), a European research project in which they have all participated. The overall project focuses on seed regulations in the aim of investigating the status of heterogeneous crop varieties and the opportunities of on-farm breeding and seed production in Europe. The project encompasses several crop species and includes old varieties, landraces[6] and new crop populations bred on farm. Within one work package of the project, the Dutch seed technologist is in charge of seed quality analyses. He has performed seed tests on seed lots produced by participating seed growers over three years. Based on the seed tests, he concludes that "quality consciousness of farmers is limited" and "knowledge of processing is almost non-existent". The bacterial agent causing Common Bacterial Blight (CBB) on common bean - a "quarantine" pest - was among the pathogens detected on bean seeds. Indeed, CBB is a seed-borne disease that is carried from one plant generation to the next *via* the seed. The presence of CBB agents on bean seed thus affects what seed technologists call "seed health". It is one aspect of seed quality. Concerning common bean in particular, he concludes that "current EU germination standards can generally not [be met] for beans" and "seed health [...] is poor" (van der Burg, 2009). The seed growers and some of the researchers in the audience don't agree with the Dutch seed technologist's conclusions. A *hot* debate begins here and his not settled before the end of the FSO project.

The term *hot* situation is used according to the definition of Michel Callon (1999), who also termed such situations *hybrid fora*. In such hot situations, the disaccord concerns all aspects of a problem. Actors are unable to agree upon what constitutes causes or effects of the problem, nor on the knowledge necessary to solve it. Even a common definition of the problem cannot be agreed upon. In such *hot* debates, the involved actors propose visions for the future that are incompatible. Facts and values intertwine and become indistinguishable. The debate on bean seed quality appears as such a hot situation; the participants don't agree with the definition of bean seed health that the seed technologist considers a given. The debate extends beyond the conference and even beyond the FSO project, as we will see.

1.1 A seed growers' reply

One of the seed growers present at the conference forms part of a small-scale organic seed company named *BiauGerme*. It is located in the region of Aquitaine, in the South-West of France. *BiauGerme*, which he directs with ten other seed growers, is specialised in the production of seeds of old and

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[6] "Old varieties" are crop cultivars that have undergone formal plant breeding and that may have been registered on formal variety lists. "Landraces" are cultivars that have never undergone formal plant breeding (Villa et al., 2005).

heirloom vegetable varieties[7] (*variétés anciennes* in French). By doing so, the company pursues the stated aim of contributing to the safeguard of crop biodiversity (Biau Germe, 2011). After the conference, this seed grower addresses the coordinator of the FSO project with a letter. On behalf of his seed company, he describes seed growers' experiences with bean health. According to their experience, it is erroneous to try eradicating CBB agents. For some susceptible old bean varieties such as 'Rognon de Coq', blight symptoms are even perceived as part of the varieties' "typical traits". As a conclusion, he suggests to allow seed growers to live with the bacteria in a "dynamic equilibrium".

> “Our organization is followed by the French service of plant protection (“Protection des Végétaux”) and is submitted through an agreement, to the examination of parasites inside seed batches and to the visit of the fields in which the beans are multiplied. We usually have very few problems with the viruses. Exceptionally, on susceptible varieties and under some climatic conditions, we may observe some mosaic type symptoms.
>
> More generally, we are confronted to an endemic disease, the common bacterial blight caused by one kind of *Xanthomonas* bacteria which has become a quarantine parasite for some years, at the European level. It can be noted that some ancient varieties are very susceptible to the bacteria. And in some cases, this susceptibility is also part of their “typical” traits. For example, an expert of the GEVES (Groupe d’Etudes des Variétés et Semences), in charge in France of the registration test for the varieties, declared that the flageolet ‘Rognon du coq’, without the bacterial blight, is not a ‘Rognon du coq’!
>
> In 2008, our seed company, developed an area to produce beans without bacterial blight. In this area, no beans has been cultivated for 10 years and we will follow several kinds of cares and management practices such as those realised by other seed companies, according to an official protocol (established by the FNAMS in France).
>
> To be sure not to introduce the bacteria, we have bought new seed from seed companies which provide sanitary passport for these seed sample used as “base seed”. To check for the absence of bacteria, we asked for a control analysis by an official French laboratory: 3 of 8 samples were positive. From our own samples, the results were also inconsistent with regards to our observations.
>
> Based on our experience, we concluded that it is very difficult to get reliable tests of the disease presence and to produce healthy seed without bacteria, even for seed companies that have taken all the specific means to protect the crop. Hand-craft and industrial seed producers have to live with the bacteria.
>
> Nowadays, to my knowledge, we have no classic means to fight against the bacteria. With several organic seed producers, we are improving the thermotherapy, which allows a complete treatment and which respects the standards of organic agriculture, but may alter the germination rate. Nevertheless, by this way, we are able to diminish the presence of the bacteria even to suppress it when the charge is light.
>
> We wish to underline that, in the past, before the systematic controls, we were used to live with the bacteria. During many years, even under a hard bacteria incidence, we used to select the healthiest plants for seed yielding. Our customers did never complain about the sanitary quality of our seed. The common blight is endemic in France, and except very unfavourable climatic conditions, the presence of the bacteria did not prevent from very good yields. To my opinion, the eradication of the bacteria is not the good strategy general, the dynamic equilibrium of common parasites which, most often, are only slightly virulent, may be more profitable to the whole agro-ecosystem.”
>
> (Letter addressed to the coordinator of the FSO project in 2009 on behalf of *BiauGerme*, translated from French and published in FSO deliverable 3.1: seed quality recommendations)

The *hot* debate is not settled before the end of the FSO project. In the end, both opinions are represented in the final document of FSO dealing with seed quality. The Dutch seed technologist's opinion takes the lead in the "seed quality recommendations" produced by the research project. The

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[7] I translate "variété ancienne", which would literally mean "ancient varieties" as "old and heirloom varieties". Indeed the term "ancient varieties" is not common in English. The two terms "old" and "heirloom" are used to reflect their age and traditional aspect.

summary of the document states that bean seed of good quality can only be produced if seed growers get trained in disease detection and strengthen agronomic approaches.

> "It can be concluded that farmers in general can produce seed of reasonable to good quality fit for sowing, certainly for wheat, maize and spinach. Due to the nature of beans, which are notorious for their vulnerability to diseases, good seed can only be produced if farmers specialise on disease detection and use agronomic approaches to minimize the impact on quality." (van der Burg et al., 2010)

However, this conclusion is complemented by the *other opinion* on bean seed health at the end of the document. The letter addressed on behalf of *BiauGerme* is published in the "seed quality recommendations" as evidence of another approach, described as "living with" CBB.

The coordinator of the FSO project and future supervisor of this PhD thesis, Véronique Chable, had found it useful to argue for the inclusion of *BiauGerme*'s point of view in the final document. She is senior agronomist at the French National Institute for Agricultural Research (INRA). The FSO project is not the first research experience she makes with small-scale organic seed companies such as *BiauGerme*. From past experience, she perceives that the opinion expressed by the seed company is not an isolated one. Indeed, the seed grower who wrote the letter is also an active member of an association of small-scale organic seed companies, called *Croqueurs de Carottes*, literally "carrot munchers". From now on, they will be mentioned as "*Croqueurs"* (simply the "munchers"). Thus, the FSO coordinator, Véronique Chable, had argued that the point of view on bean seed health expressed by the seed grower must be accounted for in the final FSO document.

In the following, I will show that she pursues the topic beyond the FSO project by setting up a PhD research project in the framework of "SOLIBAM", the follow-up project of FSO. However, I shall first describe the *Croqueurs* in some more detail. As mentioned above, this association of seed companies counts the *BiauGerme* among its members and appears to share its view on bean seed health. The *Croqueurs* association will become the case studied in this PhD thesis. I thus propose to have a closer look at the *Croqueurs* association, which qualifies its members as "artisanal" seed companies.

1.2 The association *Croqueurs de Carottes*

The *Croqueurs* association was founded in 2005 (Semailles, 2016) and today comprises 8 small-scale seed companies, of which 6 are located in France and one each in Belgium and Spain. All produce and market exclusively organically produced seed of open-pollinated vegetable varieties from the public domain. Hence, the varieties they market are reproducible: open-pollination is the biological component to reproducible varieties, because they breed true to type, as opposed to F1 hybrid varieties. The public domain ensures the legal reproducibility of varieties, because they are not protected by any type of intellectual property right. The *Croqueurs'* stated aims are to contribute to the safeguard of old and heirloom varieties by identifying varieties in danger of genetic erosion, assessing and maintaining them and registering or re-registering the most promising varieties. A second stated aim is to favour the exchange of know-how and training on the maintenance of such varieties (Semailles, 2016). These aims also imply defending the right to produce and market non-registered varieties, as well as asserting and advocating for the practices of artisanal seed companies. A geographical overview of the seed companies forming the *Croqueurs* association is given in Figure 2.1.

By providing information on the legal framework concerning seeds and federating concerned associations, *Réseau Semences Paysannes* (RSP) supports the *Croqueurs* in their endeavour to position artisanal seed practices facing seed laws and seed inspection. As mentioned in the General Introduction (Chapter I) RSP is a national umbrella organisation of more than 80 associations active in the promotion and defence of crop biodiversity and related know-how, among which are the *Croqueurs*. RSP coins the term of *peasant seed*, thereby advocating the collective maintenance of crop diversity by farmers and reclaiming farmers' seed autonomy (Demeulenaere, 2014). Within RSP, the *Croqueurs* participate in a thematic group on vegetables ("groupe potagères"). Beyond seed production, this group comprises other actors concerned with vegetable seeds, in particular home and market gardeners. This collaboration of the *Croqueurs* with seed users in the RSP thematic group illustrates that they regard their customers as active players and partners in the maintenance of crop diversity. Beyond organic farming practices, the qualification of their activity as *artisanal* is of great importance, as is the qualification of their seed as *peasant* seed. Much more than the mere production of organic seed, these qualifications point to the *Croqueurs'* objective of contributing to the maintenance of crop diversity and reclaiming seed autonomy with their customers. In addition, the qualification of their activity as *artisanal* points to practices which can also be described as "handcraft". The term *artisanal* implies a particular form of know-how and a control of the entire process from seed production to marketing. It may also imply a limitation to company size and amounts of seed produced. As one seed artisan puts it: rather than having his company grow, he would prefer seeing other artisanal seed companies created. In any case, the seed companies among the *Croqueurs* are all micro-enterprises according to the criteria employed by the European Commission (European Union, 2016): none of the *Croqueurs* members has more than 5 employees.

The individual seed companies among the *Croqueurs* members differ somewhat in their translations of the qualifications "artisanal" and "peasant" into their individual practices and organisation schemes. For instance, some - like *BiauGerme* and *Graines del Pais* - are entirely led and managed by seed-growing farmers, whereas others - such as *Germinance* - are directed and managed by persons not directly involved in seed growing. Intermediary organisational schemes also exist - like *Semailles*- in which a few seed growers are in charge of processing and marketing the seed produced by a wider network of seed growers. In the following, actors will be named according to the practice of interest. The term "seed grower" will designate a person cultivating the seed crop and harvesting the seed. The term "seed artisan" will refer to a person in charge of processing and marketing seed, as well as managing the seed company. One and the same individual may be designated by both terms according to the role or practice of interest.

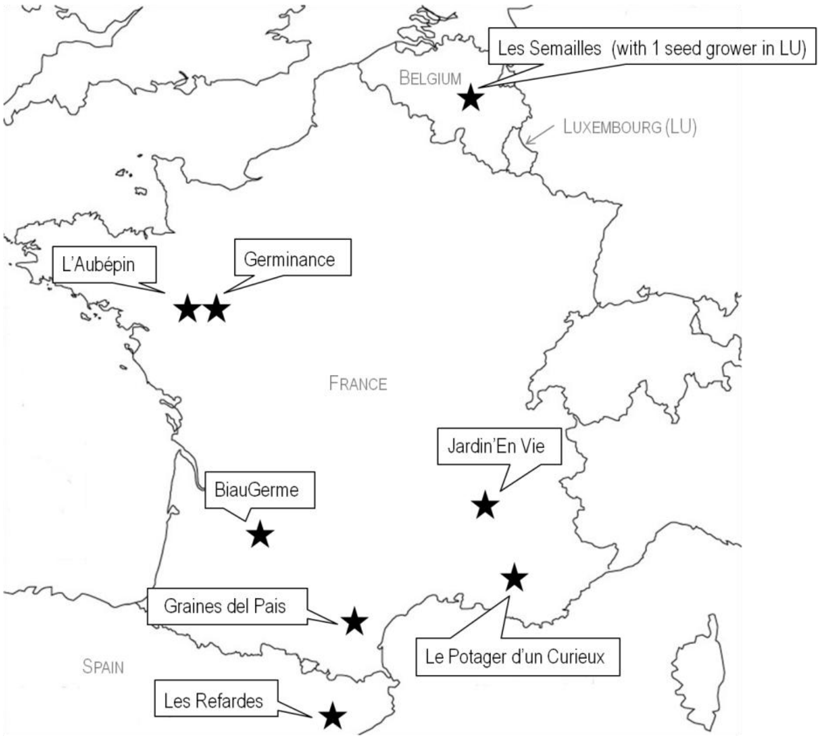

Figure 2.1: Geographical overview of the artisanal seed companies forming the association *Croqueurs de Carottes*

Not all *Croqueurs* members are confronted with CBB on common bean, as CBB is only able to develop in the warm climates of Southern Europe. Apart from the *BiauGerme* in Aquitaine, none of the members have (knowingly) faced CBB; nor have seed inspection authorities prevented them from marketing bean seed lots for phytosanitary reasons. Nevertheless, they are all apprehensive of the effects that ever more restrictive plant health regulations and seed inspections may have on their practices as artisanal seed companies, as well as on crop biodiversity. Even beyond seed production, some of the members perceive the debate on CBB as a showcase for increasing pressure of inappropriate hygiene regulations on small-scale, artisanal practices in agriculture. Comparisons with artisan cheese-makers, butchers and producers of seed sprouts are evoked. Thus, the seed grower from Aquitaine takes the lead in communicating his understanding of bean seed health in the *hot* debate sparked by the FSO conference. As he does so, he knows he can speak on behalf of his fellow *Croqueurs* members, who are ready to relay his message. Thereby, he becomes a spokesperson for the *Croqueurs* in the debate about bean seed health, and in the PhD project that follows. The artisanal seed companies consider that beyond mere bean seed production, this seed grower and the case of protective measures on CBB may also speak for many artisanal practitioners in the domain of food and agriculture. The case thus becomes a spokesperson for these practitioners facing hygiene regulations judged inappropriate for small scale, organic and handcraft activities.

1.3 Establishing the research device

Above, we have seen how Véronique Chable, coordinator of the FSO project, actively participates in the hot debate on bean seed health. She contributes to making a place for the seed growers' point of view in the "seed quality recommendations" produced by the project (van der Burg et al., 2010). Little later, she links with the *Croqueurs* to study their understanding of bean seed health in the framework of a follow-up project, SOLIBAM[8]. Véronique considers that the debates on bean seed health at the final FSO conference in Marseille arise from opposing concepts of plant health. Based on work of the founders of organic agriculture such as Albert Howard (1943), it appears to her that many measures set down in plant health regulations are incoherent with founding principles of organic agriculture. She considers recruiting a PhD student to work on the topic of bean health in the aim of embedding it in a broader reflection on plant health concepts.

With a little agronomic research experience on common bean[9], I enter the stage as potential PhD candidate the following year. We decide to set up a PhD project and apply for PhD funding by the Luxembourgish National Research Fund (FNR). Figure 2.2 gives an overview of the institutional framework of the resulting PhD project. It clearly shows how the stakes of the PhD project are built amidst the interplay between different actors: EU regulation - RSP/*Croqueurs* - EU research funding agency - EU research network - National regulation bodies.

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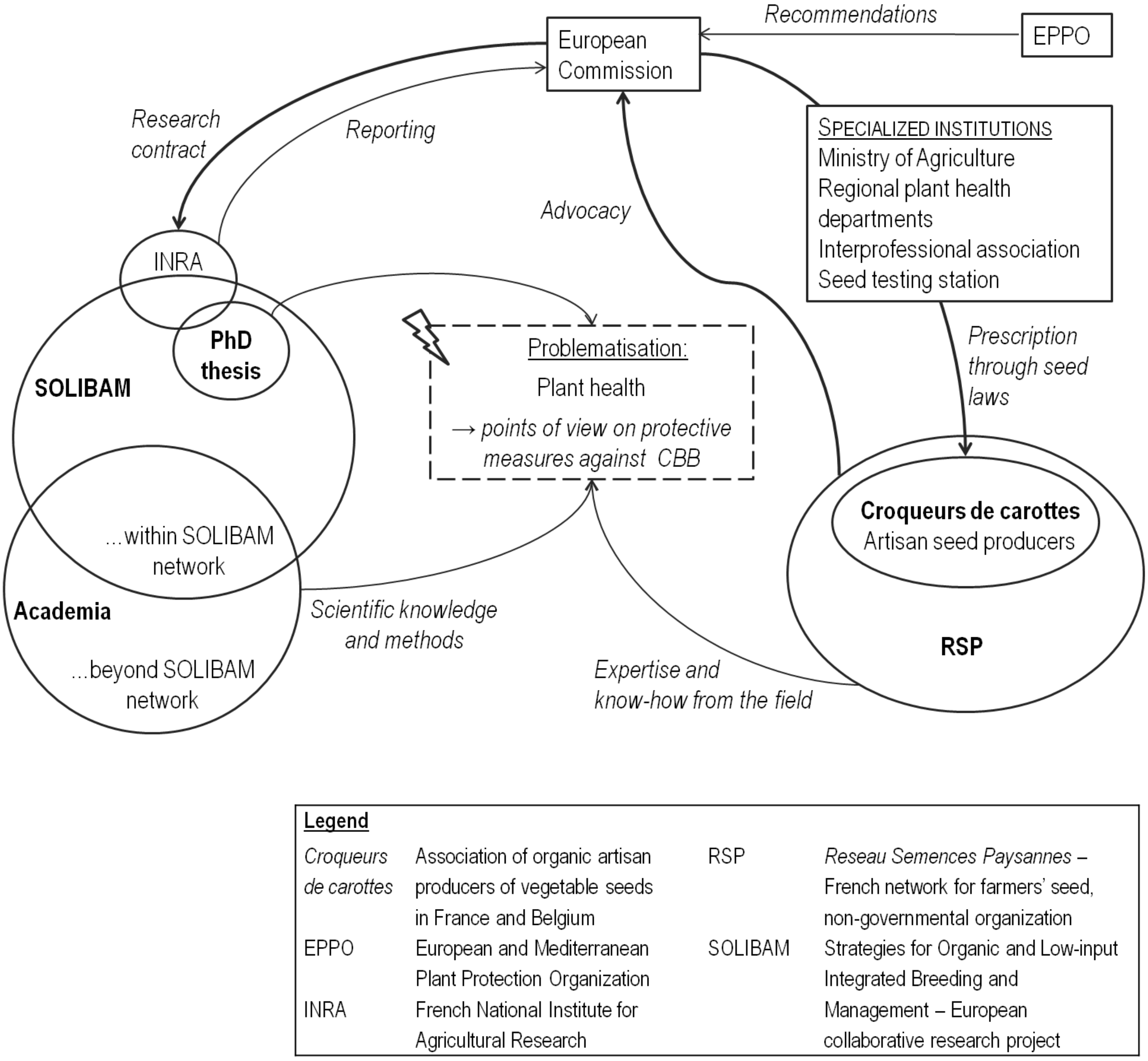
[8] Strategies for Organic and Low-Input Breeding And Management; http://www.solibam.eu

[9] and a strong ambition of working in the field of crop diversity and on-farm plant breeding



Croqueurs de carottes	Association of organic artisan producers of vegetable seeds in France and Belgium	RSP	*Reseau Semences Paysannes* – French network for farmers' seed, non-governmental organization
EPPO	European and Mediterranean Plant Protection Organization	SOLIBAM	Strategies for Organic and Low-input Integrated Breeding and Management – European collaborative research project
INRA	French National Institute for Agricultural Research		

Figure 2.2: Overview of the partners forming the PhD research project. Based on recommendations of expert groups within EFSA (European Food Safety Agency), the European Commission decides upon protective measures against plant diseases. These are transcribed to national law and implemented by specialised institutions in the Member States. Within RSP (*Réseau Semences Paysannes*), the *Croqueurs de Carottes* are subject to the prescribed protective measures. *Croqueurs* and RSP put the protective measures on common bacterial blight (CBB) into question. The RSP also relays the critique of protective measures through its advocacy work towards the European Commission. The PhD project links up with the problematisation of protective measures against CBB. The PhD project forms part of the European research project SOLIBAM, led by my PhD supervisor at INRA. SOLIBAM is funded by and reports back to the European Commission. As PhD student, I draw upon the expertise of researchers within and beyond SOLIBAM to contribute to the problematisation of protective measures on CBB. Thereby, the problematisation is fed both by the expertise and know-how of seed growers and scientific knowledge and methods. The figure was formerly published in an oral communication (Klaedtke et al., 2014).

Based on the final document of FSO and the narration of the problem by my future PhD supervisor Véronique, I set out to define the research problem and design a research device. I understand that the artisanal seed companies are less concerned about actual bean health than about tensions between phytosanitary regulations on one hand and their own experiences and practices on the other. Based on my training in plant breeding, I deduct that the *Croqueurs'* contestation of the "quarantine" measures can be explained by their way of selecting bean plants in their seed crops, rather than setting the focus on how they define plant health. This is reflected in the following extract from the PhD grant application.

> The overall aim of the PhD project is (i) to contribute to the understanding of the functional relationships between crop performance, plant health and the environment in crop production ecosystems and (ii) to determine the most relevant participatory selection procedure (including selection criteria) for on-farm breeding when taking the needs of stakeholders, seed regulations and the conservation of landraces into account. (Klaedtke, 2012: PhD grant application)

In the grant application, a participatory research project is devised, aimed at understanding, facilitating and informing on-farm selection for bean health. Several of the *Croqueurs'* bean varieties are to be cultivated in experimental plots on participating farms. Seed and vegetable growers are to be invited to observe and possibly select in the bean crop. The underlying hypothesis is that *Croqueurs* members and their bean plants are able to live with bean diseases by implicitly favouring genetic disease control and biocontrol mechanisms by their selection practices. This hypothesis focuses on ecological interactions in seed growers' fields. The objective is to link farmers' bean selection practices with genetic diversity within bean populations, their adaptation to local growing conditions and their health status. In this objective, diversity present in bean populations and their evolution in different growing environments shall be determined by genetic analyses. In addition, beneficial plant-associated microorganisms are to be assessed to demonstrate their ability to co-evolve with bean populations in different growing environments and reduce the impact of bean diseases.

In a given growing environment, the evolution and performance of bean populations are hypothesised to relate to seed growers' "decision rules". Seed growers are to participate in the experiment by observing and possibly selecting bean plants according to their "needs", while at the same time "expressing" and discussing needs and decision rules, as reflected from the following extract of the grant application.

> In a participatory and experiential process, the decision rules followed by the producers participating in the trials to meet constraints are explored in a qualitative manner. Their rational and breeding methods are observed, discussed and put in relation with the evolution and performance of the populations. [...] These participatory processes will allow exploring the coherence and dissonance between farmers' evaluations and the experimental approach that we develop. It could also give some indication about the coherence of current seed regulations with the needs expressed by producers. (Klaedtke, 2012: PhD grant application)

In the objective of accounting for "the needs of stakeholders", a co-supervision of the PhD research by senior social scientist Pierre M. Stassart is been arranged for. He is a rural sociologist at the University of Liège in Belgium. With him, I hope to learn how to analyse the seed growers' "decision rules" and "needs", which are expected to be directly and solely related to ecological interactions and agronomical results. In other words, I consider that the plant health management of the *Croqueurs'* emanates directly from the bean populations they cultivate. I expect the field trials to operate as platforms for a " participatory and experiential process", where farmers will join me in the observation of the experimental bean populations.

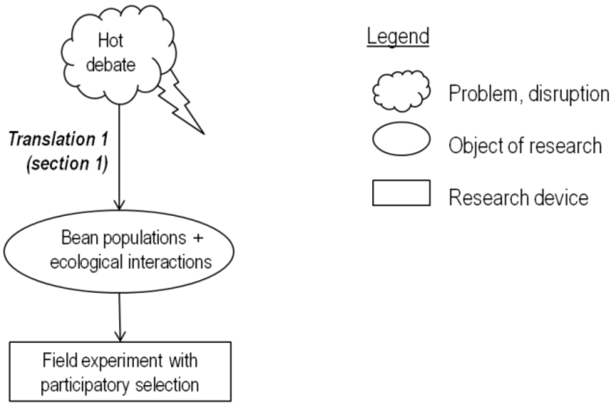


Figure 2.3: **Translation 1** of the research device. Departing from the *hot* debate in Marseille, the PhD research project sets its focus on bean populations and their ecological interactions with the growing environment. In the field experiment, participatory selection is to allow assess how bean growers select for bean health.

The hot debate at the origin of the PhD research project is thereby *translated* in the project. The *problematisation* is not just transported by the PhD student untouched, as by an *intermediary*. Indeed, the problematisation translates the *hot* debate in a field trial which is to operate as platform for a participatory process : The PhD student rather acts as an *mediator* and *translates* the problem (Latour, 2005). I do not only bring in my own problematisations - as agronomist and as PhD applicant - but also the possibilities of a three-year doctoral program, the requirements a doctoral school and the suggestions of the reviewers of the PhD grant application. In this translation of the problem, the bean populations constitute the object of research, which I plan to study *via* the analyses of plant-environment interactions and *via* farmer participation in the research project. Social science is thus attributed a minor role to complement the natural science approach, as reflected by the projected time plan: 75% of my time is attributed to the senior agronomist, 25% to the senior sociologist.

The field trial is established in 2012, although a PhD grant is obtained from the Luxembourgish National Research Fund (FNR) to begin PhD research only in spring 2013. Between the *hot* debate in Marseille and the establishment of the PhD research project, a first preliminary translation of the object of research has taken place. This first translation is summarised in Figure 2.3.

2 From ecological interactions to governance of bean health

In the first section, I have discussed how the PhD research project emerges from a *hot* debate and how it is set-up in the PhD project proposal. However, setting up the initial research device is only the beginning. The second principle of method put forward by Stassart *et al.* (2011) is based on *recalcitrance*. The first step is to recognise the research device as an artifice. The second is to construct the *right* artifice (Thiery and Cerf, 2009). The right artifice is able to "activate the recalcitrance of the people being investigated" (Stassart et al., 2011) - as well as that of other living beings being addressed by the research, I might add.

Recalcitrance is the ability of an object of research to object to research conducted in its regard. An object of research must be left the opportunity to reject a hypothesis formulated about its supposed behaviour (Latour, 1999; Stengers and Bensaude-Vincent, 2003). A particularity - a weakness - of the social sciences is that the objects of research - human beings - often lack recalcitrance, because they are not indifferent to research conducted in their respect. Instead of being recalcitrant, human beings can respond to any question posed by a scientist, even the most foolish one, out of politeness, submissiveness, curiosity or interest. In addition, social scientists regularly interpret these responses in one way or another without giving their object of research the opportunity to object to their interpretation (Stengers and Bensaude-Vincent, 2003). To a lesser extent, the same is true for animals (Thiery and Cerf, 2009) and any living being, as their behaviour is not independent of living conditions. Thus, to create the *right* artifice the social and the natural sciences must enable their objects of research to question and reject the research. Stengers and Bensaude-Vincent (2003) summarise this idea: "that those to whom a question is addressed shall have the ability to put the pertinence of that question at risk."[10]

By challenging the researchers' questions, the subjects make the research question evolve. *Recalcitrance* is not to be confused with defection or resistance. It is rather a form of involvement, or participation, in redefining the questions to ask (Stassart et al., 2011). The *defection* of actants, too, can cause translations of the research device. This happens when an actant does not take part in the problematisation and defects from a role in the network (Callon and Law, 1989). As a network, the research project then has to reorganise, either to realign its problematisation on that of the actant in question, or to make do without that actant. In this section, the research project is transformed both by events of recalcitrance and defection, as seed growers and bean plants do not act as expected in a research project that has been laid out for them.

2.1 Seed growers and bean plants put the research device to the test

In 2012, the field trials are sown on four farms and are ready for seed growers to participate in on-farm selection. Four bean varieties provided by *Croqueurs* members are grown in experimental sub-plots in threefold replication. However, within two years, two out of four experimental sites are challenged by what might be called *technical* difficulties, but what we will define as *defection* in the terms of ANT. Indeed, on-farm trials in Brittany and Luxembourg are located near the research institutions to which I am affiliated (INRA Rennes and ULg campus Arlon). Following up on crop management, field observations, seed storage and all activities which constitute a field trial is rendered easily feasible by my regular presence on site. However, the other two on-farm trials are located at a greater geographical distance further South, in Aquitaine and Umbria (Italy). Given that I wouldn't be present on these field sites on a regular basis, reduced protocols have been set up for field observations in these locations. This also implies a stronger reliance on the local seed growers hosting the trials to manage and observe the experimental plots. However, the plan doesn't work out. The seed grower in Aquitaine misplaces experimental seed lots he is storing over winter. They are lost. In Umbria, the trial is eaten by a neighbour's geese seeking something green to graze in the hot and dry summer months of the first year of the experiment. The second year, they are tasted by another neighbour's goats.

What happened? Once the autumn work peak of seed harvesting and cleaning had passed, the seed grower in Aquitaine couldn't remember what had become of the small experimental seed lots he had

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[10] "La première règle de la méthode scientifique serait alors : que ceux à qui une question s'adresse aient la capacité de mettre en risque la pertinence de cette question."

stored in addition to his regular ones. In Umbria, the craving of geese and goats for fresh greens outweighed the arrangements for the experiment. In the words of Latour (2007), *competing translations* overtook the field trials. The seed grower in Aquitaine did not operate as a mere *intermediary* between myself and the field trial. He is a *mediator*, acting upon the field trials according to the *translation* he makes of it. The field trial is regarded as secondary by the seed grower, something to do after his livelihood has been ensured and his regular work accomplished. Not so surprising, then, that five small experimental seed lots may get lost in the brisk activity of a work peak. In Umbria, even geese and goats imposed their own *translation* of fresh green bean plants upon the experimentation. Whereas strong links had been maintained with the field trials in Brittany and Luxembourg by my regular presence, I was not able to equip the Southern field trials with solid enough links. Other *competing translations* thus interfered, causing seed growers and bean plants to defect from enrolment in the research device.

Even on the farms located further North that I am able to visit regularly, the field trials designed by the researcher fail to awake the interest of the seed growers. The growers don't show up on the trial plots to share their breeding approach and express their needs. When encouraged to select for plant health in the experimental bean plots, they each have different reasons to decline the invitation: one grower replies that he doesn't care for diseases and prefers to select for maximum diversity in grain colours and forms. Another one explains that he never consciously selects common bean for health in the fields. He prefers to operate at the seed stage and shows me how he sorts, and thereby selects, bean seed after harvest. A third seed grower remarks that one cannot select bean plants on experimental subplots of only 5m^2. He needs a life-sized bean seed crop to select in. Although the seed growers have different reasons for "not participating", they all point to the same issue: "You are not asking the right question, the research device is not right!"

It is the seed grower from Aquitaine, who had acted as a spokesperson for the *Croqueurs* in the *hot* debate, who explains where the problem lies. Although he has misplaced the experimental seed lots from his farm, he is not willing to stop there. The following citation is derived from a later interview.

> "... that's why I reacted very quickly to your experimentations. It's because I had already gone through it once and in reality I aspire to doing another form of experimentation with you, as we are now starting with 'Roi des Belges'. [...] In fact, we will see it ['Roi des Belges'] in the fields, you see, already, I have an entire bed of 'Roi des Belges', I will keep it, I will harvest it, and from the second year on, I will have more of it. I will see how it behaves at my farm etcetera. And I will include it in my beans. That's when we will be able to start... comparing - and more in my own reality, you see." [CRO-280814c][11]

The seed grower explains that the field experiment comprising replicated subplots of only a few square meters does not correspond to his *reality*. He proposes "another type of experimentation", which would integrate better into his *reality* and into his management of the farm. Instead of observing five bean varieties on small experimental subplots, he suggests to introduce one of the varieties on his farm as a

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[11] "... c'est ça qui a fait que j'ai réagi très vite à tes expérimentations. C'est que je l'avais déjà vécu une fois et c'est vrai que j'aspire à faire avec vous une autre forme d'expérimentation, comme on est en train de la démarrer avec le 'Roi des Belges'. [...] D'ailleurs, c'est à dire, on le verra dans les champs, tu vois, là, déjà, j'ai toute une planche de 'Roi des Belges', je vais la garder, je vais le récolter, et à partir de la deuxième année, j'en aurai plus. Je vais voir comment il se comporte chez moi etc. Et je vais l'inclure dans mes haricots. Et c'est là où on va commencer à pouvoir le... le comparer - et plus dans ma réalité à moi, tu vois." [MP280814c]

variety to reproduce as a regular seed crop. Further, he proposes to multiply the seed until it can be cultivated on an entire bed before evaluating the variety on his farm. Not only does the bean variety have to adapt to the growing conditions on his farm, but he also needs to become acquainted with the new variety. The seed grower chooses the variety 'Roi des Belges' to begin with this *other type of experimentation*.

The *other type of experimentation* is pursued on the farm in Aquitaine only. As a consequence of the difficulties encountered with neighbouring livestock, the field trials in Umbria are given up. However, the requirements of a PhD thesis lead me to maintain the initial experimental set up in Brittany and Luxembourg. Indeed, the initial experimental set up is expected to yield sufficient data for a PhD thesis within three years, whereas the experimentation proposed by the seed grower in Aquitaine requires the development of completely different research methods and more time. In accordance with my PhD supervisors, I decide to pursue these field trials in order to ensure results in due time. The field trials are no longer considered as a participatory platform on which seed growers will explicate their decision rules and needs. They are researcher-led field trials aimed at studying the ecological interactions of the *Croqueur's* bean varieties and their evolution in various environments.

Nevertheless, the seed grower's proposition for *another type of research* extends the object of research from the mere bean plants to the entire environment the beans grow in, including himself: bean health cannot be accounted for by focusing solely on ecological interactions between beans and microorganisms. Seed growers' "needs" and "decision rules" are not independent of the bean variety, nor are they independent of the bean plot size. Whereas the ecological interactions of the bean plants with their growing environment may in part be captured by the experimental sub-plots, the seed growers reality of bean crop management and selection cannot. At this point, I realise that the seed growers' understanding of bean health is intimately linked with their general crop management. What starts out as an intuition based on experiences and exchanges with seed growers is confirmed by the encounter with a piece of scientific literature. The paper in question provides me with an example of how different approaches to plant health can be treated scientifically. Thereby, it encourages me to follow my impression that beyond ecological interactions of bean plants, the *Croqueurs'* understanding of plant health is at stake. Given the importance this scientific paper has had for the reorientation of the object of research, I shall briefly present it in the following subsection.

2.2 Intuition comforted by a bibliographic encounter

It is the lecture of a review article that comforts my impression that the *Croqueurs'* governance of bean health cannot be explained solely by ecological interactions, but that their approach to plant health must be accounted for. The review article is entitled "Concepts of plant health – reviewing and challenging the foundations of plant protection" and published in the journal 'Plant Pathology' in 2012. Based on philosophical debates in the field of human health, Thomas Döring and co-authors review concepts of plant health and position them within several philosophical controversies. By this means, contradictory conceptions are highlighted. In addition, the authors discuss how views on plant health may relate to mainstream and alternative approaches to plant health management (or plant protection). Philosophical controversies on health are combined to construct a framework in which to position any definition of plant health. Table 2.1, drawn from the paper, summarises that framework. The authors conclude that a univocal definition of plant health cannot be found among these numerous views. They recommend to take plant health as "an instigator of thought and debate rather than an objective entity". The construction of situated, *procedural* definitions of plant health is proposed in the form of organised debates on plant health issues. The conceptual framework for such a procedural definition consists of a set of questions to debate, as well as the rules for debating.

Table 2.1: Opposing views on plant health, discussed by Döring et al. (2012)

Criterion	Thesis	Antithesis
Values	Naturalist: be objective	Normativist: apply values
Discipline	Chemical: use molecules	Ecological: employ ecological interactions
Focus	Negative: kill the pathogen	Positive: strengthen the plant
Method	Reductionist: find rules	Holist: integrate
Interference	Functional: deliver	Resilient: be self-sufficient
Nature	Materialist: find the mechanism	Vitalist: feel the force
Ethics	Anthropocentric: fill the basket	Biocentric: support the plant
Definition	Definitive: be concise	Fuzzy: embrace complexity
Change	Conventional: maintain the status quo	Alternative: promote change
Mindset	Industrial: maximize production	Traditional: maintain multiple benefits

Each row can be viewed as an axis or dimension on which the thesis and antithesis positions correspond to the plus and minus side, respectively. Any use or definition of plant health can then be mapped in the resulting multidimensional space. Note that although within each column positions may have mutual affinity, there is no strict correlation between them.

In the context of my own research, this review of approaches to plant health operates like a key to understand the debate that had taken place in Marseille. The paper does not operate as a frame of analysis in the PhD research - the table above is not adopted as a theoretical framework - but rather as a precedent, an encouragement to treat approaches to plant health in the scientific arena. The lecture of the paper allows me to recognise the debate in Marseille as the confrontation of incompatible concepts of plant health, rather than a mere dispute on seed quality norms. The review also raises my awareness about preconceptions of the *Croqueurs'* plant health management that have been fostered in the PhD project. Up to now, I considered that understanding the *Croqueurs'* plant health management was about demonstrating how they managed to grow *healthy* bean plants despite pathogen-infected seed. The focus was thus on ecological interactions allowing for suppression of diseases: the role of intra-variety genetic diversity as a buffer against plant diseases, bio-control by beneficial soil microorganisms, etc. However, the existence of different, controversial approaches to plant health implies that plant health may be in the eye of the beholder. The paper thus triggers questions, such as: how is the *Croqueurs'* approach value-laden? What role do the bean plants play in the governance of their own health? Can this plant health management be reduced to a set of rules, or is a more holistic view necessary?

Hence, reading Döring *et al*. encourages me to elucidate the *Croqueurs'* understanding of bean health from a scientific point of view. An additional perspective is integrated into the research project. The object of research undergoes a second translation; it comprised only bean populations in field environments and now includes the *Croqueurs'* understanding of bean health. The research device is transformed accordingly: the social science approach of the research is strengthened, as described in the following subsection.

2.3 The *Croqueurs* as case study

To complement the agronomical approach, which focuses solely on the bean plants and their ecological interactions, the social science approach of the PhD project is strengthened (see Chapter III). Firstly, semi-directive interviews and a social theory, actor-network theory, are to account for approaches to bean health and health management practices. Secondly, an approach of participant observation is introduced - or rather, formalised - as part of the project. Indeed, I realise that informal exchanges with seed growers and actors alongside the field trials have been an important source of information all along. This unplanned form of participant observation is pursued throughout the research project - for example, when I ask seed growers for advice concerning the logistics of the field trials, when I meet them in their bean seed crops as I go to the field trial or when I participate in events organised by the *Croqueurs* or RSP[12].

It is within the social science approach that the governance of bean health practiced by the *Croqueurs* truly emerges as a singular case study. The *Croqueurs*, and the seed grower from Aquitaine in particular, have been understood as spokesperson for a larger range of small scale organic seed producers. For example, this becomes apparent in the initial layout of the field experiments. In the framework of the European project SOLIBAM, the experiments had been extended to a broader geographic scale with a field trial in Umbria (Italy). Thus, the sociological inquiry is initially set-up with the same idea: studying the bean health governance of small scale organic seed companies in Western Europe, beyond the *Croqueurs*. In this objective, interviews

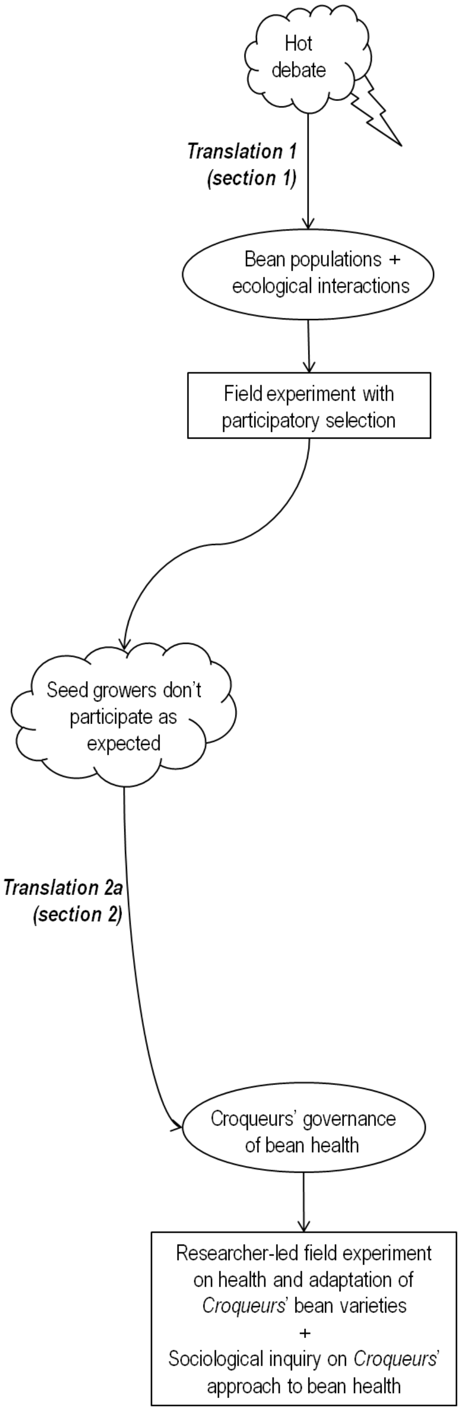

Figure 2.4: Translation 2a of the research device. Seed growers don't participate in the participatory selection as expected and cause the object of research to shift from focusing solely on bean plants and their ecological interactions to encompassing the *Croqueurs'* understanding of plant health. See legend in Figure 2.3.

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[12] Along with the research device, the PhD training plan is also transformed, as I have to get equipped with methods of social science.

were conducted with seed growers and managers of two small-scale organic seed companies in Germany. However, the institutional situations (e.g. company type, types of customers, legal framework of the countries) differ too much from those of the *Croqueurs*. In other words: the practices of the German seed companies do not respond to the same translation as the one of the *Croqueurs*'. Even beyond the geographic differences, the two actor-networks are dissociated networks, although there are some limited connections between the two.

The attempt to make a common analysis of their approaches to plant health leads to nothing more than vague generalisations. In order to proceed to an in-depth analysis of one approach to bean health, the research project settles on the *Croqueurs* as singular case study. Nevertheless, we will see how data obtained from one of the German seed companies contributes to the thesis. In Chapter III, division 1.2.2, it is brought forth as a contrast to the *Croqueurs'* approach. It thereby allows carving out the bean health approach of the *Croqueurs* more sharply, rather than *diluting* their approach in other approaches which were initially expected to be comparable. The recalcitrance of the German seed companies to behave as expected - i.e. as mere variants of the *Croqueurs'* approach - thus leads to a sharper focus on the *Croqueurs* as case study.

In summary, this section has traced how seed growers and bean plants forced the research project to transform by shifting its focus on a different object of research and redesigning the boundaries of its socio-technical object. Having focused on bean plants and their ecological interactions for some time, the project realigns on the *Croqueurs'* governance of plant health. After the initial establishment of the PhD project, this constitutes the second translation undergone by the project. This second translation is summarised in Figure 2.4. The field experiment comes out altered. The social science approach of the project is clarified, as the boundaries of the socio-technical network are redesigned around the *Croqueurs*. The governance of plant health practiced by the *Croqueurs* emerges as a singular case to be studied. However, aligning the field experiments on the renewed object of research - the governance of bean health practiced by the *Croqueurs* - takes some more hesitations. These hesitations are described in the next section.

3 Getting a grasp on bean health in the field experiments

Although the *hot* debate at the FSO conference is triggered by the presence of CBB on bean seeds, I have indicated in the previous section that the debate may result from opposing approaches to plant health in general, beyond CBB.

In this section, I will discuss the additional steps that are necessary to realign the field experiment on this renewed object of research. For the clarity of the analysis, I will come back on the translation discussed in the previous section from the perspective of the field trials, because linking with the governance of bean health as object of research was made possible by transformations of the field experiment. Opening this parenthesis is crucial to understand how the second translation, described in the previous section, was materially made possible.

The artisanal seed companies among the *Croqueurs* claim that they aim at "living with" bean diseases. However, *living with* bean diseases is more a matter of "concern" (*inquiétude* in French) for the *Croqueurs* and the researchers, rather than a clearly pre-defined question (Thiery and Cerf, 2009). At the onset of the project, the exact role played by CBB is not clear. There is concern over the governance of CBB on both sides in the debate, but the issue at stake is ill defined. This leads us to the third and fourth principles of method put forward by Stassart *et al*. (2011) for partnership-based intervention research. The researchers engage with partners without a guarantee of results, that is without a guarantee that the co-constructed project will produce the results initially expected - third principle. As the researchers and their partners co-evolve, the questions they pose link and the research question is carved out, narrowed down. For this to happen, however, "the researchers must trust that the research process, over the unpredictable course of its development, will create scientifically meaningful resources" (Thiery and Cerf, 2009; Stassart et al., 2011) - fourth principle. To enable this trust, the question posed at the start of the project must come in the form of an open matter of concern, rather than a predefined demand. Only then can researchers and their partners co-evolve in a reciprocal relation.

At the onset of the PhD project, *living with* bean diseases, with CBB in particular, appeared as such a matter of concern. In this section, I will trace how the object of research is progressively carved out in the field experiments.

3.1 CBB, a problematic plant disease

The field experiments set out to study the ecological base of the *Croqueurs'* approach of *living with* bean diseases. The co-habitation of seed growers with bean diseases constitutes the object of research. To study how seed growers live with plant diseases, the disease agents must be left uncontrolled[13]. Plant diseases are studied as they appear in a given farm environment. Whereas CBB is unlikely to occur in the cooler climates of Brittany and Luxembourg, *living with* bean diseases in the warmer climate of Aquitaine may well imply *living with* CBB.

However, *living with* CBB in an on-farm field experiment is not quite the same issue for a researcher as *living with* CBB in his seed crop is for a seed grower. A grower declaring a quarantine pest in one of his seed lots can expect to face the consequences for himself (prohibition to market the lot and perhaps obligation to start his selection from scratch on another seed lot; uncertainty on the possible infection of other bean crops). However, a researcher who detects and declares the disease can expect that other people, namely the growers involved in the project, will have to carry the negative consequences. Furthermore, the researcher may risk putting the reputation and identity of a group of actors at stake. This becomes clear when CBB is detected on bean leaves from Aquitaine in the

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[13] "Uncontrolled" in an experimental sense. The incidence of disease agents is not controlled to constitute pre-defined treatment levels. Nevertheless, some disease control takes place in an agronomical sense: the experimental plots are managed according to the practices of the hosting seed grower. These crop management practices include practices which may reduce disease incidence, e.g. avoiding an oversupply of plant nutrients. This is a very different situation from research projects studying specific plant diseases under controlled conditions, either in an experimental field or under laboratory conditions. In such research, plants are usually inoculated with known strains of the pathogen of interest at a pre-defined moment and under pre-defined conditions.

framework of the research project. Thanks to the collaboration with an INRA team specialised in bacterial plant diseases, I am enabled to test all the experimental seed lots from Brittany, Luxembourg and Aquitaine for disease agents of bacterial bean blights. The director of the unit, a senior bacteriologist, had accepted to host and train me in the detection and quantification of bacterial blight agents. No CBB agents are detected on any of the seed lots over three years of experimentation. However, as I test leaves sampled in the field trials, some turn out positive with CBB. Cautioned by the senior bacteriologist and with the approval of the local seed grower, I contact the responsible plant health inspection body. The official sends me a copy of the Plant Health legislation in which she has highlighted all the relevant paragraphs regarding common bean. She comes to the conclusion that only commercial bean seeds are concerned by the protective measures against CBB; leaves are not of interest for the inspection body. There is nothing to declare. Nevertheless, this experience raises my awareness of the risks implied with studying CBB in field experiments. CBB, as a regulated plant disease, is a hot potato. I am not ready to bear the responsibility of having to declare a "quarantine" pest in a seed grower's field and taking risks for which the seed growers will carry the consequences. Nor do I want to be accused for not doing so.

Doing research on CBB in seed growers' fields is problematic. Confronted with the reality of plant health regulations on CBB, I ask myself why the situation hadn’t been anticipated. I realise that it is another difficulty that needs to be overcome in order to narrow down the object of research.

Based on their past experience and collaboration, the *Croqueurs* and Véronique had formulated an open question at the onset of the project. They were driven by a concern over *living with* plant diseases, more than by a pre-defined demand for research. In the aim of addressing this concern, the field experiments were set up. These experiments are artifices intended to activate the *recalcitrance* of those who are involved - seed growers, senior bacteriologists and officers of plant health inspection. Initiated by such an open question, the research process is unpredictable. By linking up their questions and co-evolving, the researchers - my PhD supervisors and I - and the research partners progressively flesh out the question, the object of research. Bringing to bear the risks each party feels capable of taking, while taking into account institutional positions, forms part of the research process (Mougenot, 2011; Stassart et al., 2011). By confronting the risk of detecting CBB in the field experiments, a further step was made in defining the question each party is willing to work on. Although CBB made the concern of *living with* plant diseases visible at the onset of the project, it does not turn out to be a viable model for the PhD research on this approach to plant health. Confronted with the uncertainty of the consequences that studying CBB in farmers’ fields might entail, I turn to another model - one that is *diplomatic* in the sense of Isabelle Stengers (2006).

As described in subsection 2.1, the field trials located further South (Aquitaine and Umbria), where CBB may occur, are either given up or reduced to a single variety. The experimental sites further North, in Brittany and Luxembourg, are areas where CBB is not known to occur. However, halo bacterial blight (HBB) occurs there, particularly in Luxembourg. HBB is another bacterial blight of common bean. During the project's development, different elements concur to take my focus off CBB and on to HBB. In the following two subsections, I will trace how the focus of the field experiments first shifted to HBB before realigning on general plant health.

3.2 HBB, a diplomatic plant disease?

In the previous subsection, I have discussed that studying CBB in seed growers' fields reveals to be problematic. This difficulty is translated in the research device. We will see in this subsection that the translation in the field experiment occurs in two steps. First, another bacterial blight, HBB, is taken as a diplomatic model for the *Croqueurs'* management of bean diseases. Once diplomacy has permitted settling on a pertinent research device, it is then oriented towards general bean health.

Halo Bacterial Blight (HBB) is a seed-borne bacterial blight with a similar disease cycle as CBB. It causes symptoms which are hardly distinguishable from CBB with the naked eye. In fact, *Croqueurs* members do not distinguish between CBB and HBB in their fields, they generally speak of "blight" (*graisse* in French), as discussed in Chapter III (see Figure 3.1). Common recommendations for control of HBB in Europe are the same as for CBB: mainly to use non-contaminated seed, as well as resistant varieties if available. A major difference, however, is that HBB occurs in cooler climates. Also, unlike CBB, HBB is not concerned by European plant health regulations; it is not a "quarantine pest".

In the field experiment, HBB agents are detected on seed lots from all three sites, with particularly high contamination rates in Luxembourg. Subection 3.1 has shown how it progressively becomes clear that studying CBB in the field experiment on seed growers' farms is problematic. As the research project develops, three elements concur to suggest HBB as a *diplomatic* model (Stengers, 2006; Mélard, personal communication) to study the *Croqueurs'* management of bean diseases in the field experiment. Firstly, HBB is not a regulated pest, as opposed to CBB. Whereas *living with* HBB in seed crops does not concern Plant Health regulations and seed inspection authorities, *living with* CBB is not tolerated (see also subsection 2.2 of Chapter III). Therefore, studying the interactions of bean plants with HBB in on-farm trials does not jeopardise the research project and the seed growers as studying CBB would. Secondly, the artisan seed companies among the *Croqueurs* do not differentiate between both bacterial blights - CBB and HBB - in their common bean crops. According to their approach to bean health, both blights can be managed the same way. Differentiating between the two diseases is made necessary by Plant Health regulations, but not by their own approach to bean health management. Thirdly, the field trials located further South, where CBB can occur, are either given up (Umbria) or reduced to a single variety (Aquitaine). In the remaining field trials (Aquitaine, Brittany and Luxembourg) HBB occurs. HBB does not only occur in the experimental sites, but also concerns more seed growers than CBB does. HBB thus appears as a disease which is both more illustrative of *living with* bean diseases and more *diplomatic*, as it does not put at risk the different parties involved in the research project.

The field experiment is thus reoriented to focus on HBB as a *diplomatic* model for *living with* bean diseases. Studying the interactions of the *Croqueurs'* bean varieties with HBB is to reveal the biological base of their bean health management. Therefore, the main focus of seed testing is set on the detection of HBB on the experimental seed lots. However, we will see in the following subsection that this focus on HBB leads to a misunderstanding within the research project. In this misunderstanding, the research project is confronted with a competing translation, namely the requalification of plant health management as genetic disease control.

3.3 It's *general* plant health you're after!

After four years of field experimentation, the tested bean varieties appear more or less susceptible to HBB. In particular, variety 'Flageolet Chevrier' appears to be resistant to HBB: despite high disease pressure, plants never expressed blight symptoms and seeds were never infected with HBB. 'Flageolet Chevrier' thus attracts my attention, as well as that of the senior bacteriologist hosting me for seed testing. To shed light on the seemingly resistant variety, the senior bacteriologist proposes to conduct additional tests. These tests aim at (i) determining the races of HBB agents[14] we are dealing with and (ii) identifying genetic resistances in the bean varieties. In plant pathology, these analyses are the basis for setting up a genetic control strategy with resistant plant varieties.

Despite my curiosity to pursue the question, I have doubts on the relevance of the additional tests for the PhD project. Based on the interviews conducted meanwhile, I am no longer convinced that the genetic properties of the *Croqueurs'* bean varieties constitute a main pillar of their bean health management. I refer the question to my supervisory committee[15] shortly after. The committee acknowledges that the proposed tests constitute a basic procedure for genetic disease control within the discipline of plant pathology. However, the committee agrees that creating this knowledge would not contribute to the plant health management of the *Croqueurs*.

A few weeks after the decision has been taken not to pursue the supplementary tests on races of HBB pathogens, I find myself justifying our choice in front of the senior bacteriologist who had suggested them. We are into a long debate. For her as a plant pathologist, management of HBB can consist only of genetic disease control in the absence of HBB-free seed. Despite all my (clumsy) attempts to explain that it is the bean health management of the *Croqueurs* I am studying, she does not understand why we refuse to develop a genetic control strategy. In short of the appropriate words to formulate my argument, I use the example of the bean variety 'Flageolet Chevrier' to illustrate my opinion. Although this variety had been extraordinarily healthy and exempt from HBB infection over three years of field trials, it was decimated by "black root syndrome" in Brittany the final trial year. This syndrome is caused by a viral pathogen under certain conditions. "What's the point of knowing exactly which race of HBB agents this cultivar is resistant to when another pathogen kills it so easily?", I ask. "Then it's *general* plant health you're after!" the senior bacteriologist exclaims. The root of the misunderstanding is found. Because I had discussed only the bacteriological data with her, isolated from the rest of project results, she had been misled from the start. In view of studying general plant

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[14] *Pseudomonas syringae* pv. *phaseolicola* is the bacterial species and pathovar causing HBB on common bean. Nine variants, or races, are currently known within this species (Taylor et al., 1996a). They are unequally geographically distributed and may not thrive under the same conditions. Bean varieties may have genetic resistance against one race, but not against another (Taylor et al., 1996b).

[15] The committee is composed of 2 agronomists and 2 sociologists, all of whom have experience in interdisciplinary research. One scientist in plant breeding and a seed artisan complete the committee.

health, she recommends to compile an index of general plant health based on all the symptoms scored. This idea of a general plant health index is discussed in the Introduction of Chapter IV.

Taking HBB as a model disease thus turns out to be misleading. Although the biological interactions of common bean with the HBB pathogen affect bean health management, they do not constitute the object of research as such. It’s only a diplomatic disease! By the proposition of the senior bacteriologist, the PhD project is again put to the test. In the perspective of giving the seed growers recommendations for genetic control of HBB, the bacteriologist suggests to develop the research question in a new direction. As the proposition is rejected, the object of research is reaffirmed. The field experimentation is realigned on the *Croqueurs'* governance of general bean health. This disturbance and realignment of the research device affirms the type of knowledge to be created by the research. The tests proposed by the bacteriologist aim at creating knowledge that can be generalised and reproduced: if the variety X is inoculated with HBB race Y under conditions Z, the variety is resistant to the disease. Variety X can then be recommended to any farmer who wants to grow beans without HBB race Y causing blight symptoms on her plants. In the PhD project, however, the aim is to create *situated* knowledge [16] on the *Croqueurs'* governance of

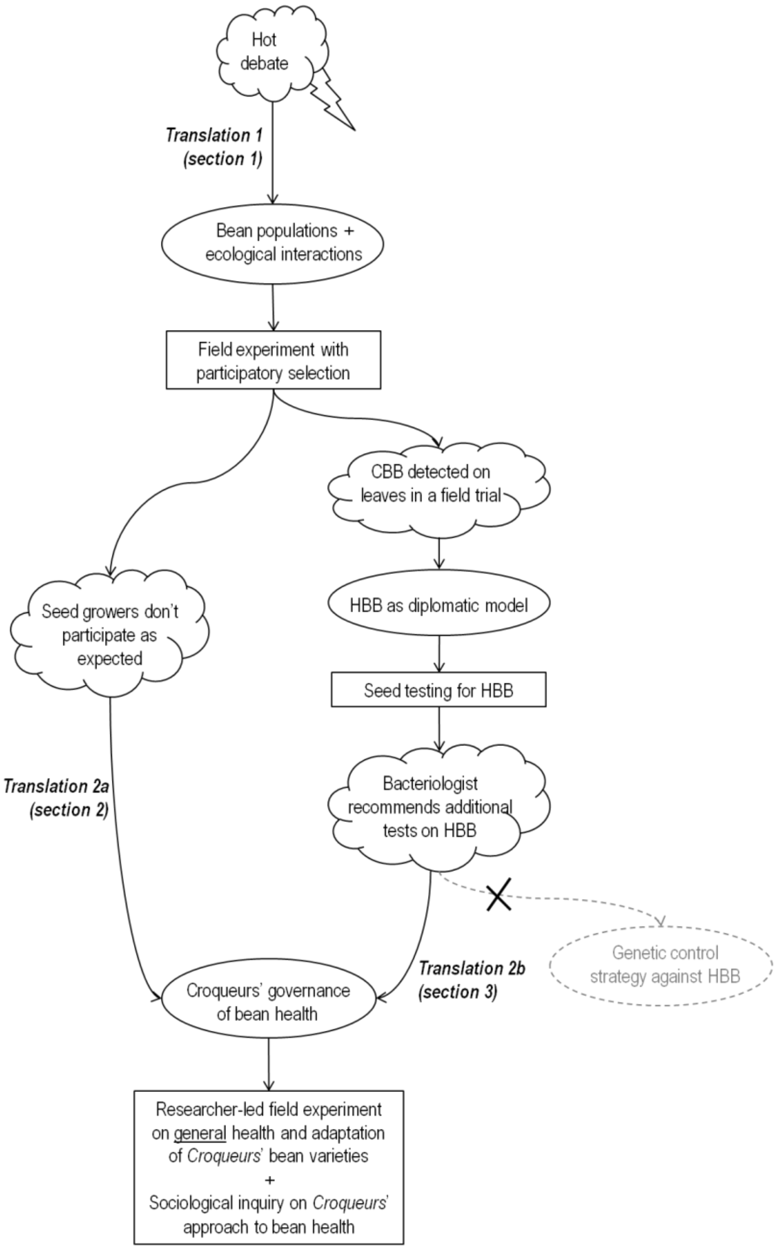

Figure 2.5: Translation 2b of the research device. Disruptions leading the object of research to shift from common bean blight (CBB) over halo bacterial blight (HBB) to the governance of general bean health. See legend in Figure 2.3.

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[16] Becoming aware this choice has been among the learnings of the PhD project, as well as learning how to communicate and justify it. The support of a supervisory committee composed of people accustomed to interdisciplinary, partnership-based research has been very valuable in this respect.

bean health, within their approach to bean health.

In summary, this section has shown how a co-evolution of the researchers and their partners is made possible by the engagement with an open question, a complex matter of concern. The object of research is progressively carved out in a research process that does not guarantee the production of a scientific result from the start. The trust I, along with my PhD supervisors, accept to put in an unpredictable research process, is a precondition for this. The focus of the field experiments meandered from CBB over HBB to the *Croqueurs'* governance of general plant health. This progressive transformation of the research device is summarised in Figure 2.5 as translation 2b. By renouncing to pursue tests in view of proposing a genetic control strategy against HBB, translation 2 - described in the previous section - is reinforced: instead of focusing on HBB as a model disease, the focus of the field experiment is widened to encompass general plant health. Beyond HBB, the interactions of bean plants with other plant pathogens and the growing environment are taken into account to assess the biological base of the *Croqueurs'* governance of bean health. Observations in the field experiment thereby complement the qualitative data collected in semi-directive interviews and participant observation.

The next section will address the connection between the field experiments and seed growers' seed crops. Hence, I will come back to the first principle of method and describe the artificial link between the research device and the "fact" that is to be construed.

4 Linking field trials and growers' seed crops

The first section has addressed the emergence of *living with* bean diseases as matter of concern. Departing from this matter of concern, the initial research device is set up as an artifice destined to activate the *recalcitrance* of the involved beings - including humans, plants and plant-association microorganisms. In sections 2 and 3, I have discussed the research process, in which the research partners co-evolve and narrow down the object of research. In this section, I will come back to considering the artificial link between the research device and the object of research, i.e. the "fact" that is to be construed by the device. This link can be traced in exchanges I have had with two seed growers hosting the field trials in Luxembourg in Brittany.

During the growing season 2013, I visit the Luxembourgish field trial approximately every two weeks. As the Luxembourgish seed grower provided the initial seed lots of two varieties for the experiment the previous year, these two varieties are grown both in the experiment and in his nearby seed crops. One day, the seed grower and I find ourselves alongside the field experiment discussing the link between the experiment and his seed crops. Derived from the same seed lot and multiplied under the same environmental conditions, the experimental plots and the seed crops of each variety should behave in the same way. Driven by a vague impression that they are not exactly the same, we begin to question their link. Indeed, the experimental plots had not been managed quite like the seed growers' regular seed crops.

During the two first years of the experiment, each bean variety is cultivated in experimental subplots of approximately $4m^2$ and replicated three times in each field trial. The rows are spaced according to the local seed grower's practices, but distances of plants within rows are wider than practiced in regular seed crops. The seed growers sow their bean seed at no more than 5 cm distance. In the trials, plants are spaced at 10 cm to ease the observation of individual plants. The Luxembourgish seed grower and I first question the management of the experiment. Do plant diseases spread in small experimental subplots as they do in much larger seed crops? What about the sowing density - wider spaced plants may dry more quickly and thus prevent the spread of diseases? Unless the plants compensate for the additional space by growing more vigorously? The seed grower adds that I enter the field experiment with the intention of observing disease symptoms, whereas he enters his seed crops hoping for the best. Might our intentions have an influence on how the plants develop?

A few weeks later, I take a break from measurements in the field experiment in Brittany to discuss with the two growers hosting the field trial there. We discuss the effect that the intermingling of varieties in the trials may have on bean health. Whereas I express the idea that the intermingling of varieties might accelerate the spread of diseases, one of the growers explains he would rather expect the contrary. On the one hand, I argue that placing a susceptible variety next to a tolerant one may constitute a source of infection that wouldn't occur in a pure stand of the tolerant variety. On the other hand, the grower argues that more resistant varieties may inhibit disease spread in susceptible varieties.

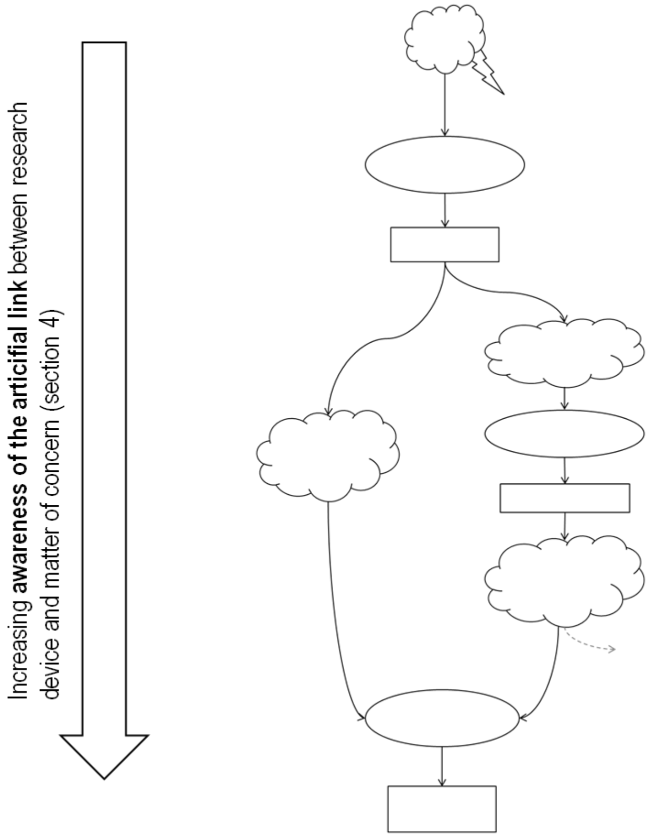

Figure 2.6: As the PhD project develops, the link between the research device and the *Croqueurs'* matter of concern is specified. As a PhD student, I become increasingly aware of the artificiality of this link and of the need to make it explicit. The translations discussed in previous sections and depicted in Figure 5 are represented only schematically.

Definitive answers to these questions are not found. They merely point to the artificiality of the link between the field experiment and seed growers' seed crops. Whether intermingling different varieties in an experiment accelerates disease spread or not - whether one believes that plants are able to react to human intention or not - it becomes clear that the link between the experiment and seed crops is an artificial one. I become increasingly aware of this throughout the research process, as illustrated in Figure 2.6. I also become aware of the necessity of defining the link by specifying research methods and their evolution throughout the research process.

In summary, the conditions in which this thesis was produced were described in this chapter. The link between these conditions and the knowledge created was questioned and discussed. In the framework of the PhD research, this is not only a precondition to situating the knowledge produced. It is also a learning opportunity for a PhD student trained in "classical" agronomic approaches, which aim at producing *universal* knowledge. In this chapter, the translations undergone by the research project are described as successive steps. Although these steps come in a logical order as the project develops, they don't necessarily correspond to the precise chronology of events. The translations traced are intermingled in the chronological succession of events. Some translations are triggered by a very specific event, others are derived from longer processes. Also, the research device consists of two parts, the field experiments on one hand and the sociological inquiry on the other. These parts can be attributed to the disciplines of agronomy and sociology, respectively. However, events that triggered translations in one or the other part of the research device are not so clearly attributed to one or the other discipline. Conversations and ideas cross disciplinary boundaries. Disentangling the essence of the transformations undergone by the research device has been one of the major learnings of this PhD project.

5 Conclusions

Taking into account the interplay of numerous actants behind this thesis, this chapter has traced the events that put the research device to the test. Accounting for the process of research, as I have done in this chapter, is essential to situate and understand its outcome - the thesis. The role played by the PhD supervisors and myself - somewhere between participants, facilitators, translators and secretary - at first sight appears to be unclear. The four principles of method for intervention research (Stassart et al., 2011) have allowed clarifying the role played by the researchers throughout the unpredictable course of project development. In the description of the research process given in this chapter, four aspects appear as crucial elements to understand the project's development.

Firstly, the research process was - and remains - full of uncertainty. At the hot debate in Marseille, *living with* bean diseases emerged as a *matter of concern*. As the research was triggered by this complex concern rather than a clearly formulated research question, the further course of the project could not be defined in advance. Departing from this uncertainty, research took shape, driven by unexpected events and obstacles. The research device was set up to activate a process which allowed narrowing down the research question. Returns in the form of scientific knowledge were progressively fleshed out from the process. These results are unfinished and incomplete, however. They open more doors than they close and formulate perspectives for future developments, rather than taking the form of final statements. While the course of the research was unpredictable, the researcher was able to design and maintain a space of trust (see 4th point of conclusion).

Secondly, the research process might be described as a socio-technical one. The research process involved a large number of actants beyond humans, including seed growers and artisans, a few of their bean varieties, a large number of microorganisms, European plant health regulations, a bacteriologist and many pieces of scientific literature - to name only a few prominent ones. Human and non-human actants coevolved around the subject of bean health management. Considering the diverse nature of actants, modes of communication were diverse: absence of seed growers in the field trials, presence of hungry animals in the trials, semi-directive interviews, telephone calls, written text, the amount of bacterial colonies in Petri dishes, symptom scores inscribed on a sheet of paper, graphs, tables and observations jotted down in a notebook were among the most common modes of communication. This coevolution of human and non-human actants implies that the definition of the frontier between the social and the technical is no longer a real stake, because what matters is the interplay. Although the methods forming the research device - the sociological inquiry and the field experiment - can univocally be attributed to the disciplines of sociology and agronomy, respectively, they mutually shaped each other during the research process. It is important to keep this in mind as we proceed through the thesis chapters: results are discussed in separate chapters according to the disciplinary focal point that produced them, but they form part of one and the same picture.

Thirdly, the research device progressively emerges from the co-evolution of all elements which compose the project. One basic premise as researchers and their partners co-evolve is not to put other parties at risk, nor to jeopardise their identity. Hence, the research device must mobilise a *diplomatic* actant, which allows the researchers to approach the research question without putting their partners at stake. In our case, the project's temporary focus on HBB may appear as a detour, but reflects the search for a way to deal with the *Croqueurs'* concern with CBB without putting any of the partners - artisanal seed companies, researchers and plant health officials alike - in danger . HBB thus appears as *diplomatic* plant disease at one point in the research process, because it does not come with the same strings attached in terms of biology (HBB develops under cooler conditions in Northern Europe) and plant health legislation (HBB is not a *quarantine* pest). It allows speaking of a problem in *another language*, thereby *casting off the stakes at the root of conflicts and antagonism* (Stengers, 2002). In the later course of the project, discussions with a senior bacteriologist reveal HBB as an unsatisfactory, misleading model of the *Croqueurs'* bean health management and the entire project finally turns to general plant health. Nevertheless, HBB operates as a *diplomatic* plant disease for some time, because it entails consequences that are acceptable to the partners of the research project. It allows advancing on the *Croqueurs'* approach of bean health without jeopardising the identities of the research partners.

Fourthly, the development of the research project was enabled by the trust the researchers - my PhD supervisors and myself - put in the research process. As discussed in section 3, the researchers are confident, from the start, that the dynamics of the project will yield material for the production of scientific knowledge, despite uncertainties linked with the project. However, the research process presented here - as a PhD research project - has a dual objective: to produce scientific knowledge and to train a PhD student. In addition to the trust they place in the research process as such, the PhD supervisors must also place trust in the apprentice researcher. For instance, the senior agronomist supervising the PhD research (Véronique) had intuitively deducted from her long experience with the research partners that the *matter of concern* addressed by the project emanated from opposing concepts of plant health (subsection 1.3). Although this was clear to her from the onset of the project, she acknowledges that her PhD student does not have the same experience. For want of this experience with the research partners, the PhD student builds upon her previous training in plant breeding and orientates the PhD project to focus on bean populations and their ecological interactions. Nevertheless, the supervisor is confident that the PhD student, with the guidance of her supervisors, will produce relevant scientific knowledge as she coevolves with the research partners.

As the outcome of the research process, this thesis deals with the bean health management of artisanal seed companies forming the association *Croqueurs de Carottes*. Its author has no intention of placing their approach in the much wider context of "official" or "mainstream" plant health protection, nor to treat opposing plant health approaches symmetrically. Although representatives of official institutions concerned with bean seed health were interviewed, these interviews are called upon to shed light on the bean health management of the *Croqueurs* by comparison. Relations between the *Croqueurs* and official institutions are brought in only to contrast the two approaches to plant health. The objective is to flesh out the *Croqueurs'* approach to plant health management.

In terms of method, ANT serves as a main pillar for this thesis, in an attempt to keep the assemblage constituting the *Croqueurs'* bean health management together. Nevertheless, research results are decomposed according to the research device that produced them. Chapter III reports the results of the sociological inquiry. The following chapters concern results of the field experiment. Chapter IV begins by specifying the methods employed in the field experiment. Chapter V then reports results concerning general bean health, departing from the idea of an index of general bean health. Chapter VI reports results on the interaction of bean plants with beneficial microorganisms of the soil. Chapter VII reports results on the microbial communities associated to experimental seed lots. Finally, Chapter VIII reports local adaptation of bean varieties revealed by the experiment. A graphic overview of the chapter set-up is given in Figure 1.1 (p. 23). Wherever possible, cross-references between chapters point to the interdisciplinary nature of the reported results.

Chapter 3: ***In situ* plant health management revealed by tensions about protective measures**

In the previous chapter, I have shown how the object of research evolved. The project began by focusing on the ecological interactions between bean plants and their growing environment and evolved to question the rationale behind the plant health management of the *Croqueurs de Carottes*. The *hot* debate at the origin of the PhD project concerned the contamination of bean seeds with seed-borne bean pathogens at the end of a research project, with Common Bacterial Blight (CBB) in particular. CBB is regulated as a so-called "quarantine pest" in the EU, implying that protective measures are prescribed by plant health regulations to prevent the plant disease from entering and spreading. The debate about CBB is considered indicative of a more general discrepancy. The underlying hypothesis is that different rationales operate behind the plant health management practices of the artisanal seed companies on one hand and behind the protective measures against CBB on the other hand. The aim of the present chapter is to characterise the rationale of plant health management practiced by the artisanal seed companies by addressing the following questions: <u>Which are the specificities of bean health management practiced by artisanal seed companies among the association *Croqueurs de Carottes*? On which interactions between bean plants and their growing environments is this plant health management based?</u>

Data was collected by means of literature review, semi-structured interviews and participant observation. Seed and plant health legislation was studied, complemented by gray literature regarding the management of "quarantine" pests, especially the responsible EU and national governing bodies. To better understand the matter, selected scientific literature on the international regulation of plant pests was consulted. Between August 2014 and June 2016, a total of 21 people were interviewed in 15 interviews, with up to 3 people per interview. Durations ranged between 51 min and 2h 23 min, for approximately 300 pages of interview transcription in total. The thematic guide used for the interviews is presented in Annex 1. Interviewees were chosen according to the snowball sampling technique starting from 5 artisanal seed companies members of the *Croqueurs* association, of which four are based in France and one in Belgium. Two seed companies not forming part of the *Croqueurs* association were interviewed in the objective of specifying the bean health management practices of the *Croqueurs* by contrast. In addition, a representative of the umbrella organisation RSP was interviewed in order to put the *Croqueurs'* practices into the wider context of a network that goes beyond vegetable seed growing. In France, the study was extended to persons in charge of implementing "quarantine" measures on bean seed. This was done in the *Pays de la Loire* region, a region renown for seed growing and faced with CBB in common bean. To account for the science and procedures on which EU protective measures against CBB are based, a phytopathologist was interviewed, who has participated in a working group in charge of revising the regulatory status of CBB agents. A detailed overview of the affiliation and role of each interviewee, as well as the groups of people interviewed together, is given in Annex 2. The first three letters of the interviewee code employed for interview citations indicate the role of the interviewed actor, as follows (Table 3.1).

Table 3.1: Three letter code (first three letters of interview code) indicating the role or affiliation of the interviewee.

Three letter code	Role or affiliation of interviewee
CRO	Member of the association *Croqueurs de Carottes*
NRI	National agronomical research institute
NGO	Non-governmental organisation for peasant seed
PIA	Plant inspection authority
SOS	Small organic seed company in Germany
MSC	Multinational seed company based in France

The data collected in the interviews was complemented by data gathered in the form of participant observation. The coordination and realisation of the field trials offered numerous opportunities for participant observation. For instance, geographically nearer farms were visited every 2-4 weeks for the observation of bean disease symptoms. Other experimental sites, located at a greater distance, implied that I spend several days on site, hosted by the seed grower hosting the trials. Also, several events organised or co-organised by *Croqueurs* members or by the RSP were attended, as listed in Annex 3. Lastly, a feedback meeting was organised in February 2016, where research results were reported and discussed with 16 participants. In the aim of extending the discussion beyond the *Croqueurs* to the users of their seed, the meeting was organised with the thematic group concerned with vegetable seeds within the RSP. The discussions at this feedback meeting mainly feed into the identification of research perspectives.

The chapter takes form of a narrative following actor-network theory (ANT), also termed 'sociology of associations' (Latour, 2005) or 'sociology of translation' (Callon, 1986b). Rather than squeezing cases into categories predefined by theoretical frames chosen by the social scientist, he proposes to study the assemblages behind (or rather around) the actor which allow the actor to act. The actor is enabled to act by the actor-network, but also generates the network. Because the actor-networks behind the actors tend to become invisible once they have stabilized, ANT suggests the description of controversies from their deployment to their settlement, to study how particular socio-technical configurations are assembled. Social assemblages are understood as networks of human and non-human *actants*, connecting and interacting[17]. In order to allow for new social assemblages to emerge, ANT encourages social scientists to *follow the actors* and what they tell in the form of narratives. This

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[17] Latour argues that sociology as it has been practiced in the past, by applying its social theories to the subjects it studies, actually erases the specificities of each situation in order to squeeze the actors into theories and concepts (see 'Reassembling the Social', p. 234). Research in sociology may thus have contributed to the reproduction of social assemblages that had been stabilized beforehand by social theory. While this may have been appropriate as long has sociology had given itself the mission of modernisation and emancipation, the challenges humanity faces today require the emergence and formalisation of new assemblages.

implies doing away with the distinction between Nature and Society[18], a frontier that has been fostered by the separation of the natural and social sciences. To deploy ANT as a method, the following three instructions, or rather proscriptions, are given (Latour, 2005).

(i) The social scientist writing the narrative shall never 'jump to the global'. Rather than referring to some unexplained global or contextual forces acting in local situations, she shall describe the vector, or mediator, by which this action is transported from one site to another.

(ii) The scientist shall not be lured into believing face-to-face interactions merely consist of two people representing their own interest. Instead, these actors are to be regarded as mediators made to act by chains of human and non-human mediators around them. By tracing the network of connectors that assemble a socio-technical configuration, the illusion of 'the global' and 'the local' is done away with. The social scientist collects statements, in written documents or in interviews conducted with the actors, in order to trace the connectors by means of the actors' own descriptions.

(iii) It is not the scientist's role to fill in the blanks and uncertainties left by the network, but to stick to the actors' statements she has collected.

ANT is employed as a heuristic frame to analyse the actor networks behind the plant health management of the *Croqueurs* and their interactions with current plant health legislation. Amongst the body of literature related to ANT and aiming at integrating non-humans in sociological analyses, the analysis of expertise by Callon and Rip (1992) is called upon. The authors propose to regard expertise as a process regulating between three domains of requirements. The socio-political domain is constituted of human actors, who each have their competencies, interests and expectations. The scientific and technical domain is concerned with the construction of knowledge on nature or on artefacts. The third domain, that of rules and regulations, is composed of the directives and recommendations guiding the experts in their work. Expertise is thus the process of aligning these three domains. It results in what Callon and Rip call a socio-technical norm. The alignment of the three domains implies questioning the resistances and oppositions of each domain. Implicitly, the following questions are addressed concerning the domains of socio-political scope, of scientific knowledge, and of rules and regulations, respectively. To what extent are the demands and interests of the concerned social actors negotiable? Are the scientific facts robust or disputable? Are existing rules and regulations absolutely binding or can they be infringed? Reconfiguring a socio-technical norm implies seeking and modulating the domain which presents the least resistance and opposition. European plant health regulations, which are based on the reports of expert groups, are an example of such a socio-technical norm.

This chapter is divided into three sections. In the first section, the plant health management of the *Croqueurs* is specified. We follow the actors in their descriptions of the assemblage that enables them

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[18] Lélé and Norgaard (2005) have described agriculture as situated at the frontier between the social-natural divide. In their paper about the practice of interdisciplinarity, they recommend that the disciplinary assumptions about the "other" half of the system constitute simplistic models that must be abandoned and replaced by more complex ones. ANT seems an interesting take at doing without both the ontological divide between nature and society and the disciplinary divide between natural and social sciences.

to manage the health of their seed crops, especially their bean crops. The *Croqueurs* are contrasted with two other seed companies to point to specificities in their plant health management. First, the *Croqueurs'* bean health management practices are contrasted with those of a multinational seed company, considered by *Croqueurs* members as positioned at the opposite of their own practices and values. Secondly, a contrast is drawn with a small-scale organic seed company in Germany, which is considered as a partner by some of the *Croqueurs* members. The second section focuses on protective measures against the bacterial agents of CBB, in order to specify the rationale on which it is based. Based on the report of an expert group in charge of giving an opinion on the categorisation of plant pathogenic bacteria, protective measures against CBB will be elucidated according to three domains of expertise. In the aim of understanding interactions between plant health regulations and French *Croqueurs* members, the implementation of protective measures in France will be described, in particular. Having specified the two approaches to plant health management in the preceding sections, the third section tackles the tensions between them. It addresses both the questions of how protective measures intervene in the plant health management of the *Croqueurs* and of means employed by the *Croqueurs* to contest the protective measures. I will discuss how the *Croqueurs* put into question the protective measures against CBB by attempting to unlock each of the three domains of expertise.

1 *Croqueurs de* Carottes: Managing plant health in situ

The *Croqueurs de Carottes* association and their concern for bean health management, which triggered this research, has been described in the previous chapter. In the following subsection (1.1), the bean health management practices of the *Croqueurs* is described. Specificities of the *Croqueurs'* approach are highlighted by contrasting them with the practices of two other seed companies in subsection 1.2.

1.1 *Croqueurs de Carottes*: Artisanal seed companies

The members of the *Croqueurs* qualify themselves as *artisans semenciers* in French, which can be translated to "artisanal seed company". Their association is in turn member of the French *Réseau Semences Paysannes*, which can be translated to "peasant seed network". The individual *Croqueurs* members differ somewhat in their translations of the qualifications "artisanal" and "peasant" into their individual practices and organisation schemes (see Chapter II, subsection 1.2). Nevertheless, they converge in that they exclusively grow and sell seed of open-pollinated varieties from the public domain. They thus sell seed that is biologically and legally reproducible by any home or market gardener. The seed marketed by the artisan seed companies interviewed is also multiplied and selected exclusively under organic growing conditions.

> "Among us, *Croqueurs de Carottes*, there is also: No protected variety, no F1 hybrid variety, no genetically modified variety. So, we stay with open-pollinated varieties that are not protected [...] And another thing that is very important, too, is that it's not just about the legal minimum requirements, which is to produce organic seed from conventional seed. Our basic seed is organic, too. That is, our varieties have been organic since 5, 10, 15, 20 years. So, with time, one may think that the variety is impregnated by its experience in organic farming in those ten or twenty years [...] it hasn't been proven scientifically, by the methods of scientific research, but we find that the plants become pretty hardy." [CRO-190515d] [19,i]

It is crucial to understand what they consider truly organic seed. It is not enough to grow seed of any variety organically for one generation, as required by the EU regulation on organic farming[20]. Continuous selection of the variety under organic growing conditions for several plant generations is regarded as essential. The understanding of bean "lineages" as determined by the interplay between plant population, field environment and seed grower is discussed in division 1.1.1. Division 1.1.2 describes how plant health management practices of the *Croqueurs* integrate the triangular system behind bean lineages. Division 1.1.3 addresses the consequences for the role of the collective in managing plant health.

1.1.1 *Lineages*

The Croqueurs continuously multiply and select vegetable varieties under organic conditions in the understanding that the seeds they offer are not only determined by the crop variety, but also by the environmental conditions under which the seed is grown and by the farmers growing and selecting the

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[19] Citations are all translated to English. The original citations are given in endnotes at the end of the document.

[20] Article 12 of Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labeling of organic products and repealing Regulation (EEC) No 2092/91

seed crop. The "lineage" of a plant variety (*souche* in French) is an expression of the interplay of three actants - plant variety, natural field environment and seed grower. The term natural environment refers to the abiotic (physical environment) and biotic factors that form part of a plant's growing environment and act upon the crop in the fields. The fields as such are of course thoroughly anthropogenic environments. The identification of the seed grower of each seed sachet reflects that the origin of the seed is considered relevant. The reasoning in terms of lineages is also well illustrated by the *Spicilège* website (Réseau Semences Paysannes, 2016), which was launched by the RSP. On the website, crop lineages are described in terms of name and origin of the plant population, grower and multiplication site. This description can be regarded as an alternative to the criteria of distinctiveness, uniformity and stability (DUS) used for the determination of varieties in official variety testing.

The plant is shaped by its environment: the environment acts upon the crop, which reacts to the growing conditions it encounters. The *Croqueurs* describe their crops in terms of "hardiness" (*rusticité*) and adaptability. Hardy crops are "weaned off" the intensive use of inputs and able to thrive and yield in more challenging growing environments. They are able to resist stress factors, for example by producing phytochemicals. Adaptability refers to the ability of a crop to evolve, to adapt to the environmental conditions in which it is multiplied over several years. According to this point of view, organic, low-input environments select for plants that are able to thrive in those environments through "natural selection", as reflected by the citation below. In conjunction with the selective breeding of the seed grower, a "lineage" of a variety develops. Population varieties, which contain several plant genotypes rather than being a pure line with a single genotype, evolve and adapt more readily. In the following citation, a seed artisan describes that this adaptation sometimes leads to difficulties when going through official seed testing for varietal type.

> "Just as if you put a conventional variety, if you thrust it into organic conditions, well, it wouldn't respond the same. Especially modern varieties, which have been selected to respond to inputs, to high amounts of inputs: you put it into organic conditions, where it will be weaned off a bit from all that, you won't really recognize it, you know. So, it's the same problem in conventional trials. Our organic varieties react differently and they tend to tell us: your variety... it does not conform with the type we expect at all, you know. It doesn't happen all the time, but... [...] under organic conditions they do [conform], and above all, they are well adapted, they are hardy for organic conditions. If it has a bit longer leaves or a bit more anthocyanines, it may be because... well, it's natural selection that wanted it that way. And they don't like that. [...] It's impossible, especially with populations, they evolve. And precisely, they are in conditions where it evolves, under organic conditions they are fine, you know. So generally, when that happens, they tell us: Your variety is not suitable. In general, we keep it anyway, because these are lineages that have gone through 20 years of organic cultivation. They would want us to restart from scratch with a conventional lineage, which has been stored in a fridge, which hasn't moved in 20 years, and all the work we have done since would be lost. So, that's not possible. And that's a topic where we cannot make ourselves understood." [CRO-190515s] [ii]

The appearance of a crop is not considered static or fixed in time and space. On the contrary, the ability of the plants to adapt to variable environments is perceived as a quality and an element of plant health which seed artisans must tend to. The ability to adapt to new environments, but also to variations within an environment must be sustained. Adaptability can also be described as the ability of a variety to interact with a given environment in ways which meet the expectations of the grower. Adaptation to a new environment does not happen instantly: it is common practice among the *Croqueurs* to observe a lineage they are not familiar with for 2 or 3 years. They give the plant population time to find its place among the weather conditions, the soil, the plant-associated microorganisms and the management practices of the new environment. The aim is to allow for it to adapt to the new growing conditions, but also to take time to get familiar with the unknown plant population and learn how to observe it.

> "For me, with the little experience I have, when a variety has never been cultivated here, the first year of cultivation, well, they always, I mean the beans always appear as if... you get the impression that they have a virus. And later there is no more of that, at all. And for all crops in general. Tomatoes for example express it differently, it's the strength of the plant. I mean, when I take a tomato that comes from elsewhere, well, the first year, it adapts - what I call adaptation - and the second it starts to... you see, it was really symptomatic when we tested the 250 tomato varieties of INRA, it was a joke, you know. The first year, they were puny, the second year, they were much better, and the third year, it was... they started looking nice, you see? It wasn't the same plant at all. One could almost say: it's another variety." [CRO-260814b] [iii]

By stressing the plants and applying natural selection pressure to them, the environment compels the plant populations to evolve by changing their genetic makeup and modifying the expression of that genetic makeup. Isolated from their growing environment, the genetic information conveyed by a crop thus doesn't mean much. Recent scientific publications on epigenetics and on transfers of genetic information between different species by viruses have attracted the attention of some *Croqueurs* members. They bear scientific explanations for adaptation processes which they observe in their crops and which go beyond mere genetics and take into account the communication between crops and their environments. The *Croqueurs* grow their seed and develop their savoir-faire at the intersect between the crop's adaptation capacity, environmental conditions and human needs. This is also true for their management of plant health.

> "In the crop, well, it's an agronomical approach, that is, the soil, the climate, the plant and I - because I form part of it, he! And those four or five of us - I can't remember - must find a compromise that is the least poor, or the best, for the three or the five, well... the weather doesn't care at all, the soil cares a bit more, the plant is really concerned and I am, too. So, the two major actors are the plant and I. We try to make the soil our ally and the weather, we have to live with it..." [CRO-290116d] [iv]

The *Croqueurs* thus reason the plant populations they multiply in terms of lineages determined by the triangulation of the plant variety, multiplication environment and seed grower. The next division will explicate the integration of this triangular system in their bean health management.

1.1.2 *"Avoiding disease won't ever favour health" - In situ management of bean health*

The health of a bean lineage and its management are embedded in the plants' growing environment. Interrupting the communication between crop and environment by eradicating plant pathogens from the crop's environment is neither healthy for the crop, nor sustainable for humans depending on the crop. Plants have to be left the opportunity to adapt to and cope with the local environment - including local pathogens - to be regarded as truly and lastingly healthy. Using the words of warfare and of ANT, the observation of bean populations *in situ*, i.e. in their local growing environment, constitutes an 'obligatory passage point' (OPP) for the *Croqueurs*. Based on the work of Callon (1986b), an OPP is defined as a key setting - be it geographical, institutional or organisational - which actors have to go through to pursue their goals according to their problematisation (for an explanation of the term 'problematisation', see the introduction of Chapter II, p.28). The general problematisation of the *Croqueurs* is to offer seeds of bean lineages based on the adaptation of crop varieties to particular growing environments, directed by the selection of the seed growers. The lineage can't be dissociated from the triangular system. Regarding plant health, seed crops have to go through the threefold interaction to obtain hardy, adaptable and durably healthy lineages. In the citation below, the expression of a variety in a given growing environment with a given seed grower's management practices are termed "local variety", which may here be understood as a synonym of "lineage".

> "It's really the question of our understanding of what is sanitary. All microbial or fungal life around plants is a signature of the *terroir*. And of cultivation practices, not only of the *terroir*. There are always microbes on a local variety. Hence, we are the only ones thinking in terms of local varieties." [NGO-260915k] [v]

Plant-associated microorganisms, including plant pathogens, are considered an integral part of plant populations and seeds. They have their role to play in the field ecosystem. For instance, diseases causing spots on common bean pods at the end of the bean growing season may be regarded as "maturation diseases", beneficial for seed maturation by their defoliating effect. Severe plant diseases that develop too early in the season may reveal problems in the crop's environment and its management. Bacteria, fungi or viruses are not pathogenic *per se*, but *potentially* pathogenic according to the state of the environment. Microbial life in the soil plays an important role by suppressing plant pathogens, be they seed- or soil-borne. The seed growers care for and rely on the soil as their ally to support plant growth. Providing the best growing conditions possible to foster plant vitality and prevent plant diseases is the base of plant health management. This includes choosing the field plots that correspond to the needs of each crop, careful soil preparation and, for some, applying fermented plant juices or biodynamic preparations to soils and crops in the aim of acting upon microorganisms and fortifying crops. Seed growers' practices aim at maintaining the environment sound, especially the soil. Conversely, the occurrence of plant diseases require that the seed grower asks himself if his crop management has been appropriate.

Bean health management is based on what can be observed *in situ* in the bean crop (Figure 3.1) and on the harvested bean seed. Identifying the causal agents of plant diseases only serves to inform whether the pathogen is seed-borne and, as a consequence, affects seed quality. For seed artisans faced with both CBB and HBB, identifying which one of the bacterial blights is affecting a given seed crop is of little practical importance, as both are seed-borne, have very similar infectious cycles and cause almost identical symptoms. Both diseases would entail the same management practices if it weren't for the protective measures against CBB set down in the EU plant health directive.

Figure 3.1: This seed grower has found a climbing off-type in his bean seed crop and is about to pull it out of the soil to prevent it from seeding. He notes that blight symptoms are developing in the bean crop, which pertains to a susceptible variety. He will come back in a couple of weeks to select the healthiest plants on which to harvest his basic seed. Knowing whether the blight symptoms are caused by regulated CBB agents or by HBB agents does not matter. We can already see now that blight symptoms on the leaves (necrotic spots surrounded by a yellow halo) also confound with other symptoms which may be attributed to viral diseases or nutrient deficiencies (mosaic-like discolorations, cupping). He will select those plants that look the healthiest, while taking into account their earliness and the effect it may have had on disease development. What matters for his selection is what he sees.

All bean lineages don't undergo positive selection for plant health like the one in Figure 3.1. The intensity of selection depends on the seed growers approach, the pressure of plant diseases on her farm and on the disease susceptibility of the bean variety. Some bean lineages undergo less intensive, negative selection for plant health, which consists of removing plants with disease symptoms. In yet other cases, seed growers leave selection in the seed crop entirely up to natural selection, relying on the fact that the fittest plants will yield the largest number of seeds. Although beans are not always actively selected in the fields, all seed artisans select for variety type and health on the bean seeds. As each bean seed lot is sorted manually at least once crosses and other off-types recognized visually are removed. In the same manual sorting step, seeds with visible spots or blemishes caused by diseases, humid conditions or mechanical damage are removed from the lot. The seed sorting step thus implies indirect selection for plant health and partial sanitation of seed lots: Removing blemished beans from the seed lots is a way of selecting against susceptible plants, but also a way of reducing inoculum of seed-borne diseases.

Managing plant pathogens as parts of the seed production system is about learning to live with them. This requires judgment on behalf of the seed growers on whether the seed crop is healthy *enough*, taking into account the growth stage of the crop and the susceptibility of the variety, among other things. In other words, the seed grower knows how much he can expect from a given variety. "Living with" plant pathogens also entails respecting nature's ways and accepting yield losses and even failure in years with weather conditions that favour disease development. This means that bean varieties susceptible to the bacterial blights may be marketed only in "good years". The limits of plant selection and plant health management of an individual seed grower are reached when she judges that a crop variety does not fit to the environmental conditions of her farm. She makes the environmental conditions of her farm and her own management practices available to the plant variety. If the plant variety is able to adapt to the site and thrive, the seed grower can multiply its seed, develop her own lineage of the variety and make a living. Practices can be adapted to some extent if the crop indicates that something is not right, *via* disease symptoms for example. However, the plant variety with its predispositions and inherent properties also sets limits to the range of environments and growers' expectations it can adapt to. Despite selection efforts on behalf of the seed grower, the variety can fail to adapt according to her expectations. In that case, the grower gives up and searches for another seed grower willing to try to accommodate the variety. This is also what happened for the variety 'St Esprit', which was produced by a seed grower in Luxembourg and formed part of the field experiment in the framework of this research: This variety was strongly affected by symptoms of viral plant diseases (as discussed in Chapter V based on results from the field experiment). After several years of unhealthy looking crops and poor harvests, the Luxembourgish seed grower gave up on growing 'St Esprit' in his seed garden. However, the variety was not discarded. It was passed on to another seed grower producing for the same seed company. This observation corresponds to several others concerning other varieties, others crop species and other seed growers: a variety that does not work in a given environment is not discarded, but passed on to someone else in the network. The network of seed growers takes the relay, making plant health management a collective endeavour.

In summary, we have seen that understanding bean populations in terms of *lineages* implies that bean health, too, be managed in the threefold interaction between crop variety, growing environment and management practices. Observing the evolution of bean lineages and selecting them *in situ* is vital to this approach. Crop pathogens form integral part of a crop's growing environment. The soundness of the growing environments is a precondition for *in situ* management of plant health. However, this approach also implies that a given variety may fail to adapt to a given seed grower's fields and expectations. This is when the collective beyond the individual seed growers come into play. In the next division, I will discuss the role of the collective of actors in the *Croqueurs'* management of plant health.

1.1.3 *Collective Management*

Taking the approach of "living with" plant pathogens, plant and seed health management have to be reasoned on the scale of the collective. The collective governance of bean health can be subdivided into three levels. The actors at the three levels must be aligned on the *in situ* approach to plant health for it to function. In the following, I will specify how the actors at each level align and which roles they play.

Individual seed growers form a network of seed growers around each seed company. Individual companies with their seed grower networks thus constitute a first level of the collective. Together, the seed growers of a network ensure the multiplication of *lineages* proposed by the seed company. By joining and staying with a seed company, they implicitly align with the values of the *Croqueurs* (open-pollinated varieties from the public domain, organic farming) and the *in situ* management of plant health. When a seed grower finds that a bean variety won't adapt to his farm, the seed company relies on the network of growers to take the relay. As described above in division 1.1.2, a "picky" variety is seldom abandoned completely, but rather consigned to another seed grower who might provide growing conditions which suit that variety. The seed production, and thus survival, of a given vegetable variety is ensured by the network of seed growers. Collective management of plant health within a seed company is about matching varieties with appropriate environments. In the aim of ensuring the seed production and guarding against occasional yield failures, some companies attribute the seed production for a given variety to several seed growers. In that case, several lineages of a variety may be maintained on different farms.

The seed users, or customers, of the artisanal seed companies represent the second level of the collective. Their implication in plant health management stems from the understanding the *Croqueurs* members have of their profession as artisanal seed companies: They provide market and home gardeners with reproducible and adaptable bean lineages, so that the customers can in turn let the plants adapt to their own growing environments.

> "We seed artisans, we exist, because, well, the gardeners cannot multiply the basic seed for all their varieties. That's a very different case from that of the farmer-bakers. A farmer-baker can... he is himself master of his basic seed. They are really at the opposite, you see, on the one hand the farmer-baker that is completely master - and you would never have seed artisans for those species [cereals]. And at the opposite, we are there to provide them with lineages; so that the lineages continue to exist. But then - and common bean is a typical example for this - there is the adaptation to the *terroir*, and that's not the seed artisans. It's the gardeners who take care of that." [CRO-280814c] [vi,21]

Indeed, whereas farmers producing cereal grains can easily multiply their own seed without additional work, this is not the case for market gardeners. Diversified market gardeners, who produce a multitude of vegetable species, are generally not able to grow the entire range of the vegetable seeds they require. In addition, some vegetable crops are bi-annual or allogamous, which makes seed growing more complicated. Market gardeners can thus fall back on the artisanal seed companies to purchase reproducible seed whenever needed. As an annual, mainly autogamous species, common bean is among the species which are frequently reproduced by market gardeners for several years, or even indefinitely. The *Croqueurs* consider that the bean lineage then adapts to the new environment; a new lineage may emerge with time. This also implies that the role of the customers goes beyond the mere purchasing of seeds. The seed users also play role in the management of bean health and align on the seed companies' plant health management in three respects.

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[21] Farmer-bakers (in French: *paysan-boulanger*) are cereal farmers who process their cereal harvest into bread and market that bread. Farmer-bakers in the RSP also reproduce the cereal varieties they grow. The citation refers to the fact that farmer-bakers, as opposed to vegetable gardeners, cultivate a limited number of cereal varieties, mostly autogamous bread wheat. They can thus readily master the reproduction and selection of all the seed they require on their own farm.

(i) Firstly, customers departing from purchased seed to reproduce it in their own farm or garden might develop their own lineage of the variety. By letting the lineage evolve in a new growing environment, they may thus contribute to maintaining the hardiness and adaptability[22] of plants.

(ii) Secondly, whether they grow their own seed or purchase seed every year, the seed users align on the triangular management of plant health which I have termed *in situ* plant health management, as reflected by the following citation.

> "The other thing we have to add to this discussion is that we sell seeds to organic peasant farmers, and that also changes everything. If we sold our seeds to conventional farmers, with the dead soils they have and all, they would maybe have a lot of diseases. But since we sell to people who work a bit like we do - most of our customers are either home gardeners, or diversified market gardeners on small surfaces - well, they also learn to work rather as we do. They rather have the same conception of life that we have, of plant health and so on. And so, well, they have less problems." [CRO-190515d] [vii,23]

Offering vegetable seeds which rely in part on sound growing environments for plant health, implies that the users of the seed manage their growing environments accordingly.

(iii) Thirdly, seed artisans are directly available to customers and rely on customer feedback on seed and plant health. Whereas they are frequently contacted in other matters, feedback concerning crop diseases is seldom obtained. This lack of customer feedback is generally interpreted as an indication that the health of their seed offer is satisfactory. To verify this interpretation, one of the artisan seed companies planned to address its customers with a short questionnaire on bean health *via* their seed catalogue and internet site. However, this project failed due to technical problems with the internet site.

Finally, the third level of the collective is formed by the *Croqueurs* association and its network of member seed companies. The association constitutes a wider network of seed growers on which the member seed companies can rely on to match vegetable varieties with appropriate environments and ensure the survival of the varieties. For instance, the companies retail seed among each other. Some companies unable to grow certain varieties themselves thus rely on other *Croqueurs* members to provide seed. Nevertheless, a given variety can also be produced by several companies, such that several lineages of that variety are maintained. This constitutes an additional guarantee for the survival of vegetable varieties. Moreover, the association plays a role in plant health management not only through the exchange of seeds, but also through the exchange of information.

> "We don't decide anything, but we initiate lots of ideas. Ultimately, it's a sort of space for the creation of ideas and arguments, I take it that way. And they are really excellent. I take things as I would on a marketplace, I bring mine, but I listen to those of the others. I confront them, we rub them against each other, compare them. Thereby, I sharpen my argument, my knowledge of the subject - because there's also one [seed artisan] who participates with a detailed knowledge of regulations, and others more with a detailed knowledge of certain topics concerning certain varieties or crop species, tomato so and so...

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[22] Adaptability is here understood at the level of the metapopulation, i.e. of the sum of all *lineages* maintained by the *Croqueurs* and their customers for a given variety. Even if adaptation to local conditions may reduce the diversity within a given *lineage*, the sum of *lineages* maintained under different local conditions constitutes the diversity growers can draw upon to adapt the variety to a new environment.

[23] The French *paysan* is here translated to "peasant farmer". It is not used to designate poor subsistence farmers as often the case in English, but rather to designate a farming style opposed to industrial agriculture.

> So: I go to the market, I take what interests me, I give mine when I find it interesting, I get run into, I fire back... in a pretty friendly way, he! At least, we try... and... it's great. But... it's not a place where we... it's not a trade union, we are not able to... we don't have the monolithism of a union, which takes a sword and cuts in two, no... that we are not able to do. [...] We are not able to get the effective side of it - or without it looking like much. I say that, but it is not completely true, because, it doesn't look like much, but the efficacy is there. Because, finally, [some seed artisans] are in panels etcetera [...] I tell them: "You say that we are not progressing, but I can tell you that the contacts I have with the FNAMS and others are changing"." [CRO-290116d] [viii,24]

In matters of plant health as in other matters of concern for the artisanal seed companies, the exchange of information among the *Croqueurs* goes in two directions. The members put forward their own practices for discussion, search for alignments among them and forge a common position. Although the aim is not to become identical, the identity of artisanal seed companies is involved through the search for common denominators. By aligning among each other and "sharpening their argument", the identity and representation of the artisanal seed companies towards external actors is strengthened. The exchange of viewpoints and practices among the association equips its members to defend their common ground (Section 3 will address the forms this takes in matters of bean health). In this task, the *Croqueurs* association is supported by the wider network of the RSP, which feeds information on legislative issues into the *Croqueurs* on one hand and takes the relay with more general advocacy for famers' and gardeners' seed autonomy on the other hand.

In summary of this subsection, the following key points can be reminded of. The understanding of lineages as the triangulation between bean variety, growing environment and seed grower leads to the PPO of *in situ* plant health management. The health of bean plants must be observed, managed and selected for in their growing environment. This approach also entails that the soundness of growing environments be taken into account. The *in situ* approach relies on the alignment of the seed growers, the seed users and the *Croqueurs* association and thus constitutes a collective approach to plant health. In the next subsection, the practices of the *Croqueurs* members will further be specified by contrasting them with the bean health management practices of two other seed companies.

1.2 Contrasting plant health management approaches

To specify the *Croqueurs'* governance of bean health in further detail, contrasts will be drawn with two other seed companies. In a first step, a contrast will be drawn with a large multinational seed and breeding company frequently cited by *Croqueurs* members as embodying the opposite of their own values and practices. This French-based multinational is criticised by the *Croqueurs* for putting intellectual property rights on vegetable varieties. Much unlike the *Croqueurs*, the multinational produces seed on a global scale. The weight of bean seed lots dealt with give a good indication of the difference in company sizes: Whereas *Croqueurs* members sometimes deal with seed lots of 5-10 kg for minor varieties, batches of 3-4 t are considered small by the multinational company. All the bean seed lots are multiplied under conventional[25] farming conditions and no organic common bean seed is produced by the multinational.

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[24] FNAMS (*Fédération Nationale des Agriculteurs Multiplicateurs de Semences*) is the French Union of seed growers.

[25] "Conventional" designates seeds or farming techniques which are not organic. This implies that synthetic chemicals may be used as herbicides, insecticides and fungicides.

In a second step, the *Croqueurs'* governance of bean health will be contrasted with the practices of a small-scale organic seed company in Germany, which is closer to the *Croqueurs* both in terms of values and company size. Some *Croqueurs* members partner with the German seed company through commercial relations, as they retail seed supplied by the German company. The German seed company aligns with the *Croqueurs* basic values, as it exclusively supplies organic seed of vegetable varieties from the public domain. Based on a network of market gardeners collaborating to produce and market biodynamic seed since the 1970's, the seed company was founded as a public limited company in 2001. It now employs about 30 people. Although this remains "tiny" as compared to large-scale breeding companies, it is a lot more than any of the *Croqueurs* members (none of the *Croqueurs* members has more than 5 employees).

1.2.1 A multinational seed company - *It's up to us to manage our genetics*

The first contrast concerns a seed company frequently cited by *Croqueurs* members in opposition to their own values and practices. Elucidating the bean health management of this multinational also sheds light on the specificities of the management approach of the artisanal seed companies forming the *Croqueurs*. Although the production manager of the multinational company is adamant that CBB agents are well established in France, he doesn't envision "living with" the bacteria the same way the *Croqueurs* do. For this seed company, "living with CBB" means keeping bean fields CBB-free despite endemic CBB agents.

In its aim of keeping bean fields free of blight symptoms, achieving genetic control of the disease through bean breeding efforts is a major objective. Resistance genes prevent disease symptoms from appearing on plants and in this regard differ from "hardiness" as it is pursued by the *Croqueurs*. Field tolerances to both CBB and HBB of most of the company's bean varieties are judged satisfactory, but the company's bean breeder pursues the aim of introducing resistance genes in the company's bean genetics. In the search for genetic resistance, breeding lines are screened under controlled conditions, either in-house or in partner laboratories. According to the disease of interest, screening methods based on molecular markers or on detached leaf testing are available. Hence, the company invests in the genetic control of bean diseases. It relies on intellectual property rights to ensure returns on this investment. By protecting its varieties with intellectual property rights, it renders their free multiplication illegal. The fact that common bean, by its biological properties as annual and mostly autogamous crop, is easily reproducible by customers or competing seed companies is regarded as a challenge: with little effort, seed users can surreptitiously multiply the company's varieties and save the royalties they owe the company. Two contrasting positions are indeed apparent: On the one hand, the *Croqueurs* consider that their customers contribute to the adaptability and health of bean by reproducing the lineages in diverse growing environments. On the other, the multinational company considers that intellectual property rights - preventing the free multiplication of bean varieties by seed users - are favourable to bean health by helping to finance genetic disease control.

> "Nowadays, concerning phytosanitary aspects we are very confident about property on plant material, because we think that it is by really putting means, research, by creating new varieties - and here we're not talking about transgenesis or taking some elephant gene, he - achieving something by simply multiplying our crosses, you know. What we notice is that the old varieties are by far not as tough as

> what we obtain. Not systematically, there are exceptions. Beans like the variety 'Talisman', one of the oldest beans that exists, generally work well all the time. But breeding for the phytosanitary side of things is nevertheless a great, great way for us, for the future, he. Because, we, at [our company], are convinced that chemicals will go down. In 10 years we will spray less than we are doing now, and already 10 years ago we were spraying more, so we have to rely on those resistances. The problem we have is that common beans are autogamous plants, they are "seed to seed", he. You sow a seed, you sow it again, and again, and again... from one generation to the next..." [MSC-150216b] [ix]

In the absence of any available treatments to control bacterial blights in bean fields, apart from copper, the management of bean health is focussed on keeping bean seed free of any blight agents, be they CBB or HBB agents. To achieve this, the production managers prefer having common bean seed grown in France, where they can visit and monitor the seed crops regularly. To limit the introduction and multiplication of CBB and HBB inoculum, registered bean seed production areas named blight-free zones (*zones hors graisse*) have been created in collaboration with other seed companies and public bodies. In these registered zones, seed growers follow strict rules to ensure the sanitary quality of seed produced, namely: absence of blight bacteria in samples of 30,000 seeds for the basic seed, a crop rotation of at least 4 years, isolation of seed production fields (any other common bean production by amateurs or professionals other than registered seed production is forbidden), prescription of cultivation practices (wide spaced hose reel passages and tractor tyre passages, no plants sown in the headlands) and prescription of 4 copper treatments. If CBB or HBB appear in the registered zones despite these measures, an insurance scheme compensates seed growers for the destruction of the seed crop. The company also systematically destroys infected seed crops when growing bean seed outside registered zones.

> "The problem we have with bean blights is that they are diseases that appear at the end of the growth cycle. These are diseases for which, if really you see them at the stage of young plants, it means you have sown a rotten lot. And I've worked for companies where I guarantee you that it has happened. For us [...] disease expressions always appear after flowering - always, systematically. So in addition, in my profession, it's a bit complicated, because 15 days before harvest, you tell [the company]: "You won't have anything"; and you tell the farmer: "Plough it over". Euhhmmm... that's it. So it's always a very sensitive issue." [MSC-150216b] [x]

Be it in or outside the registered bean seed production zones, bacterial blights have no place on seeds or in the production system and are understood in terms of economic loss. Research efforts of the multinational company are oriented towards further improving the management in view of bean seeds free of CBB and HBB. According to the experience of this company, the official method for CBB detection is not powerful enough in detecting seed infections. Therefore, its research and development department has developed more reliable sampling and testing methods. It is also testing a range of natural extracts for bactericidal and anti-stress effects on bean plants.

Contrasting the bean health management of this multinational with that of the *Croqueurs* highlights the ways in which *in situ* plant health management functions. For the *Croqueurs,* "living with" bean blight agents implies judgement on whether a bean crop is sufficiently healthy according to the intensity and timing of blight symptoms. By contrast, to the multinational company "living with" the blight agents means keeping them away from bean crops, as illustrated by "blight-free zones". Whereas the *Croqueurs* must let their bean crops go through the interactions with natural growing environments to consider them healthy, the multinational relies on laboratory methods under controlled conditions to identify resistance genes in bean plants and detect blight agents on seeds. For the former, the object of interest for plant health management is the bean crop in its growing environment. For the latter, plant health management depends on the knowledge about bean genetics and the contamination rates of seed lots.

In conclusion, this contrast has highlighted that the PPO of *in situ* plant health management is not shared by the multinational seed company. The multinational rather focuses on seed testing methods and resistance genes to manage bean health, with two main consequences: Firstly, bean seeds are the sole vehicle for plant health, while the soundness of growing environments becomes secondary. Secondly, intellectual property rights are a precondition to invest in new, resistant bean varieties. Thereby, the seed company alone is responsible for bean genetics, which become disconnected from growing environments and management practices. In the next subsection, I further highlight singularities of the *Croqueurs'* approach by contrasting it with the practices of a seed company that comes closer to them in terms of basic values.

1.2.2 *A German organic seed company - Tiny among the big*

In the objective of working out the singular practices of the *Croqueurs'* more finely, their approach is contrasted with the bean health management of a German seed company that several *Croqueurs* members consider a partner. Unlike the *Croqueurs* members, the German organic seed company is actively involved in breeding programmes for novel vegetable varieties for the organic sector. This is not the case for any of the seed companies among the *Croqueurs*, although some of them occasionally pick out and multiply off-types that have randomly occurred in their seed crops. Unlike the multinational seed company, however, genetic resistance to bacterial blights is not a major objective in common bean breeding. The tolerance of bean plants to diseases is tested for in field trials. Resistance genes are neither tested for, nor advertised.

For the management of seed-borne diseases in general, efforts of the German organic seed company are focussed on obtaining pathogen-free seed. A "seed diagnosis team" of five people is exclusively dedicated to the management of seed quality and plant health. With the means it has available as a larger company, hot water seed treatment has been developed over the past years and is now applied in an in-house facility, nicknamed the "wellness area" (it consists of a cheese vat converted by means of a whirlpool element). By soaking seeds in water of a specific temperature for a determined amount of time, pathogens are killed without diminishing the germination rate of the seed. Whereas this seed treatment is applied routinely on some vegetable species whenever the presence of a pathogen is confirmed, no appropriate treatment method has been found for bean seeds infected with CBB agents: Whatever the protocol, the bacteria survive the heat for longer than the bean seed. This has consequences for two bean varieties on which CBB agents have been detected, in particular. Multiplication and marketing of these varieties are put on halt as long as the "seed diagnosis team" doesn't find a method to obtain CBB-free seed. Despite low contamination rates and the observation that these rates seem haphazardly linked with the occurrence of CBB symptoms, "living with" the quarantine pathogen in the sense of the *Croqueurs* is not considered an option. This is explained by two interviewees in the citation below.

> "We have the problem, as I have said before, that we have already lost two varieties, for which at the moment we have seed from the breeder, where it was detected. We are working with it and trying to get it free [of CBB]. And then, once we have the method, we can go on and say: We are generally able to get basic seed free, because we can do it on limited amounts - pre-basic seed - then we can multiply. That may still be fraught with some risk, but the precondition is that the breeders and conservation breeders can work cleanly." - "Exactly. That's the alpha and omega, that the basic seed is free of these quarantine pests, of *Xanthomonas* in this case" [SOS-181215g] [xi]

The German organic seed company aims at obtaining CBB-free seed and expects "clean" work of the seed growers. The actors of this company acknowledge that plant pathogens are the natural companions of plant populations and that they have a role to play in wild ecological systems. Nevertheless, in agricultural systems, they consider it illusionary to "simply master it all with good organic cultivation" [SOS-181215g], at least at their own scale. This may be explained by the risk of spreading CBB associated to a larger company, or more generally by a different relation to norms and regulations. The German company's approach to plant health and seed quality is also related to the market segment it is supplying.

> "In our market segment, we are indeed dealing with the competition from the really big ones in the sector, comparable with Clause Tézier in France, for instance. That would be our competitor, in that case. Here, we are dealing with Bejo, with Rijk Zwaan, with Enza Zaden, with Nunhems, with Syngenta... Those are our competitors on the market. And they dictate an enormous degree of... well, how shall I say... of "health", as you might call it... visual quality, germination rates, cleanness... well, everything that is needed for today's farmers to obtain optimal results in the field with today's technology, I mean quantitatively optimal results. And this movement, our movement, was not really able to gain ground in the first twenty years, approximately, maybe the first fifteen years - it has changed since we have founded [the public limited company] - not able to gain ground due to a lack of methods, a lack of competencies, a lack of possibilities to meet these external quality criteria. Considering the question: How reliable is the seed quality? In terms of germination rates or health and so on... the farmer said: "I can't afford that" - if suddenly the lamb's lettuce has a load of mildew, because the seed already came with a lot of mildew. That was in the past, now we have that under control and of course we use all the possibilities... that we have through the modern plant diagnosis technologies, which exist." [SOS-181215g] [xii]

As comparable with *Germinance*, a French *Croqueurs* member, about 50% of the German organic seed company's seed sales are accounted for by organic market gardeners. However, while the former describe their professional clients as small scale, diversified farms engaged in local food systems, the latter see "big" market gardeners among their customers. Facing economical pressures, these "big" market gardeners have specialised their vegetable production, thereby shortening and simplifying crop rotations and enhancing nutrient levels. Whereas the French artisanal seed companies generally position their vegetable seeds in the low-input organic sector and on diversified farms, their German partner is also oriented towards the intensive organic sector.

The hot water treatments reveal some congruence between the *Croqueurs* and their German partner. By offering the *Croqueurs* the service of treating seed batches and the knowledge acquired about hot water treatments, the German company positions itself alongside the artisanal seed companies in the context of market competition. Also, the requirements of hot water treatments reveal congruence in the approach to seeds as living beings.

> "We wanted to do it on Jean-Michel's parsley which wouldn't germinate, but he tested it again and, finally, it germinated really well! (laughter) So that's why it wasn't done... Well, it's seed that is two years old, the fungus that was on it must have died or lost its vigour and the seed must have regained the upper hand since. There's that, too: a seed is alive, so..." [CRO-190515s] [xiii]

As the optimal treatment protocol has to be determined individually for each seed lot, the seed cannot be treated as a standardised product. Nevertheless, the approaches to hot water seed treatment chiefly reveal differences in approaches to plant health. While several *Croqueurs* members consider occasional thermal seed treatments for problematic seed lots, the German company routinely tests seed lots of some vegetable species and applies hot water treatments whenever the presence of a pathogen is confirmed. The German organic seed company has thus begun concentrating its bean health management efforts on bean seed, rather than on the triangular interactions between bean plants, growing environment and growers.

Both the German organic seed company and the multinational seed company demonstrate that the focus on "clean" seed signifies a rupture at two levels, as compared with the collective *in situ* management of the *Croqueurs*. Firstly, the soundness of field environments becomes secondary for the management of seed-borne blight diseases. When plant health management is oriented at obtaining pathogen-free seed through seed treatments or genetically resistant varieties, competences focus on the seed as sole vehicle of plant health. Hence, the focus of bean health management is not oriented at the triangulation of the crop, the growing environment and the seed grower *in situ*.

Secondly, a rupture takes place in the role of seed growers and customers in bean health management. For the *Croqueurs*, customers must be aligned on the management of sound environments to ensure bean health. For the German organic seed company, as for the multinational, providing "clean" seeds allow for a more lax alignment of field environments.

The break with *collective* governance of plant health is most radical for the multinational seed company: the division of labour between the seed company and the seed growers it contracts differs from the labour division among the *Croqueurs*. As the properties of a bean lineage are intimately linked to the environment in which it was grown and the seed grower's management, a bean lineage's genetic make-up can't be neatly distinguished from the environment or the seed grower for the *Croqueurs*. From the perspective of the multinational company, however, it is the seed company's job to manage bean genetics and to furnish seed growers with blight-free seed. The seed grower's job is to multiply the seed while avoiding the introduction of blight agents into the seed crop - a sound environment is one where blight agents are absent. Toward their customers, the *Croqueurs* consider it as their role to provide them with adaptable bean lineages. In contrast, the multinational seed company considers the bean varieties it develops as fixed in time and space. Genetic resistances, along with other valuable traits bred into bean varieties, are both the service offered to clients and an occasion to gain a competitive edge over other companies. The role of the customers is not to reproduce bean seed, develop own lineages and maintain adaptable crop diversity. Quite the contrary, the company's return on investments in resistance breeding depends on intellectual property rights which disallow the uncontrolled multiplication of bean seeds. By concentrating competencies of bean health management on bean seed, competencies are thus also redistributed. In this approach of plant health management, competences don't concern customers, but are distributed among specialised staff in charge of resistance breeding and seed technologies (pathogen detection, seed treatment).

Unlike the multinational, the German organic seed company does not invest in bean resistance breeding. Plants are tested for general tolerance to plant diseases in field trials, rather than for genetic resistance under controlled laboratory conditions. Like the *Croqueurs*, the German company rejects intellectual property rights on crop varieties and exclusively proposes vegetable varieties from the public domain, which are reproducible by their customers. Nevertheless, in terms of seed health management, this company has begun to concentrate its competences on bean seeds. By engaging in systematic seed testing for plant pathogens and thermal seed treatment, competences are concentrated within the 'seed diagnosis team' of the company.

In summary, this subsection has specified the main attributes of the *Croqueurs'* bean health management approach, both by tracing their practices and by contrasting them with the practices of two other seed companies. We have seen that their approach to bean health ensues from reasoning crop diversity in terms of *lineages* determined by growing environments and seed growers' practices. Along with its other properties, the health status of a bean lineage is intimately linked to the environment in which it is grown and to the seed grower's management. The observation of seed crops in their growing environments constitutes an OPP for the management of bean health, thereby coining the term *in situ* management to summarise this approach. Competences of bean health management encompass the triangular interactions between plant populations, growing environment and grower. The *in situ* management of bean health requires that the seed growers and seed users be aligned on it by accepting blight agents on seeds and in the growing environment. They align by fostering the adaptability of bean lineages on one hand and accounting for the growing environment in plant health management on the other. Managing plant health is thereby a collective endeavour. Competences for bean health management are distributed over a wide range of actors, including seed growers, seed artisans and seed users.

The previous descriptions have not, or only marginally, taken into account legal constraints on bean health management. The next subsection will address the protective measures against CBB, which are set down in the EU plant health directive. In the aim of better understanding the legal constraint encountered by the *Croqueurs* in their bean health management and the tensions that arise from it, I will unfold the protective measures on CBB.

2 Protective measures against CBB agents

In this subsection, I unfold protective measures on CBB agents as they are implemented in the EU, and especially in France. Understanding the workings of the protective measures will enable us to apprehend legal constraints faced by the *Croqueurs* members and the origin of tensions between the two approaches.

To shield its Member States from the introduction and establishment of organisms harmful to plants[26], the European Union (EU) sets down protective measures in the council directive 2000/29/EC[27]. In this legislation, rules for the control of plant pests are established[28]. CBB agents figure among the regulated pests and common bean seeds among the controlled "subjects of contamination". CBB agents are thus regulated as what is called a "quarantine pest" in phytosanitary lingo. As a consequence, common bean seed destined for European market gardeners (not for home gardeners) require a European plant passport (EPP) to circulate within the EU[29]. Be they originated from in- or outside the EU, bean seeds must meet one of two specific requirements to obtain the EPP: an official statement must confirm either that the seed was produced in an area known to be free from the bacteria, or that a representative seed sample was tested and found free from it. This differentiates "quarantine pests", regulated by the Plant Health directive, from so-called "quality pests", which are controlled in the framework of seed certification with the objective of ensuring seed quality. "Quality pests" can be accepted on seeds up to a specific threshold, whereas "quarantine pests" are not tolerated. Vegetable seeds like common bean are seldom - not to say never - certified and usually sold as "standard" seed, implying that they are not concerned by "quality pests".

2.1 The scientific opinion on the categorisation of CBB agents

At regular time intervals, the European Commission commissions the Panel on Plant Health (PLH) of the European Food Safety Authority (EFSA) to revise the list of "quarantine" pests. In 2014, the PLH formed a working group of scientific experts to emit a "scientific opinion on the pest categorisation" of CBB agents, which was published the same year (EFSA Panel on Plant Health, 2014). The working group consisted of 6 scientists working in the field of plant pathology in five countries. The "scientific opinion" emitted by the working group appears as a good starting point to unfold the rationale behind these protective measures by tracing the alliances made to justify and implement them. Based on the three domains constituting expertise according to Callon and Rip (1992), the report on the scientific opinion shall be dissected with three questions in mind: (i) Which is the socio-political and economic

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[26] Organisms harmful to plants or plant pests include insects, mites, bacteria, fungi, viruses and parasites.

[27] Council directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. To date, this directive has been amended 29 times. *Phaseolus* L., the genus to which common bean (*Phaseolus vulgaris* L.) is affiliated, is mentioned in Annexes II (Section II of Part A), IV (part A) and V.

[28] The directive is adopted by the Member States and transposed into national laws. Each Member State must adopt the protective measures set down in the EU directive, but may supplement with additional national control measures.

[29] While I myself have only a very vague memory of the days when this was also true for humans, it might remind some readers of this thesis times before the Schengen agreement. For bean seeds, this means that the requirements of the plant health directive have to be met in addition to the requirements of seed legislation.

scope of the protective measures? (ii) Which knowledge on CBB agents is mobilised? (iii) Which rules and procedures determine the work of the expert group?

The working group summarises its conclusions according to a set of criteria prescribed by international standards[30]. The first among the criteria requires that CBB agents be identified and detectable. The focus is set on the pathogenic bacteria causing CBB[31], and not on the disorder or symptoms it causes in bean plants. Protection measures are based on the knowledge bacteriologists have acquired about the bacteria, which the working group graphically summarised in the disease cycle (Figure 3.2).

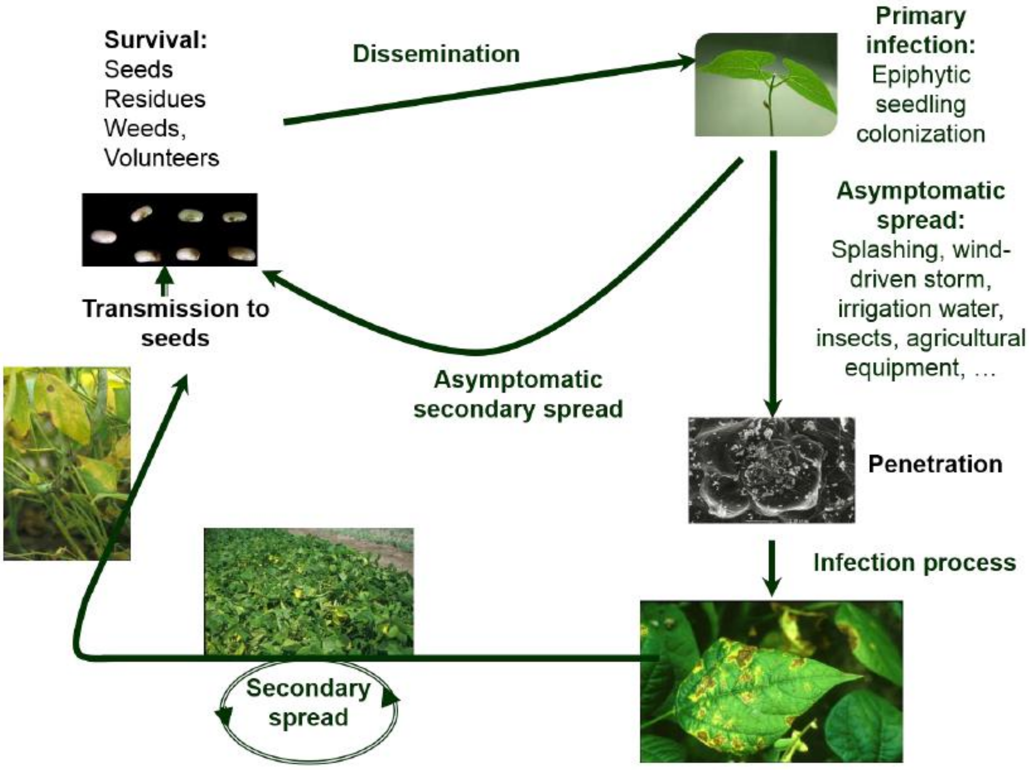

Figure 3.2: The disease cycle of common bacterial blight of common bean (source: EFSA Panel on Plant Health, 2014): In temperate regions like Europe, common bean seeds themselves are considered the only relevant source of incolum for primary infection with CBB agents. The bacteria can contaminate the seed from the inside *via* the mother plant or reach the seed surface of previously "clean" seed during the threshing and cleaning process. When the seed is sown and the bean plantlet emerges, the bacteria enter its leaves through natural openings and wounds. To multiply and induce disease symptoms, the bacteria then require temperatures of 28–32 °C (Opio et al., 1992) and a relative humidity above 80 %. This is called primary infection. Once this is done, the bacteria can proceed to secondary infection. They can move to other bean plants by water splash, wind storms, touching leaves, insects, agricultural machinery and farmers' (or other people's) boots.

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[30] International Standards for Phytosanitary Measures (ISPM) no. 11 issued by the International Plant Protection Convention (IPPC). These standards can be understood as criteria plant pathogens have to meet to be eligible for strict and costly protection measures as "quarantine pests".

[31] According to the current taxonomic nomenclature, a single disease - CBB - is caused by two causal agents, namely *Xanthomonas fuscans* subsp. *fuscans* (Xff) and *Xanthomonas axonopodis* pv. *phaseoli* (Xap). The plant health directive employs an older nomenclature which designates both these species under the name *Xanthomonas campestris* pv. *phaseoli.*

Research on the persistence of CBB agents on common bean debris, other crops and weeds has led to the conclusion that CBB agents have no other means than common bean seeds to survive from one year to the next under European climate conditions: Bean seeds are the obligatory passage point (OPP) for CBB agents to enter the EU. Detecting the bacteria on bean seeds is thus the PPO for the EU to prevent them from entering and spreading. The official method for the detection of CBB agents on seeds is set down by the International Seed Testing Association (ISTA) (2014)[32]. The risk associated to the seed trade is measured upon industrial common bean seed production schemes on a worldwide scale.

> The industrial common bean seed production scheme is currently not done on a local scale but on a worldwide scale. Two to three harvests of seeds of a given common bean cultivar adapted to a specified market can be produced each year, taking advantage of locations worldwide that are favourable for common bean production. Hence the global distribution of pathogens has consequences for the movement not only of germplasm but also of commercial seeds. The international trade in seeds is an efficient means of pathogen movement [...] (EFSA Panel on Plant Health, 2014)

The other three criteria examined by the working group comprise two basic ideas, namely that the spread of CBB agents represent an unacceptable risk for European common bean production and that it is not yet too late[33]. In assessing the risk that CBB agents represent for European economies, the working group refers to the numerous common bean plants grown in the Community. Thereby, they also point to all who would be at risk if CBB ravaged European common bean fields: seed growers, seed companies, market gardeners, bean farmers, green bean canning and freezing companies and, lastly, consumers of common bean, whether they prefer them as Luxembourgish *Bouneschlupp* (bean soup), simmered *fagioli cannellini* the Italian way, or British baked beans. The bean fields of Southern Europe are at stake, in particular, because they present climate conditions which suit CBB agents. The working group relies on reports from national plant protection organisations in concluding that CBB agents are absent from 11 Member States and present in 17 with a restricted distribution. As one of the experts of the working group explains, CBB agents are not considered endemic.

> "... it is not endemic, in any case... *phaseoli* or *fuscans* is not endemic in Europe. It may be detected occasionally, but *a priori* it doesn't seem to be endemic, that's why it is still quarantine. Apart from that, observations of *Xanthomonas*, be it *phaseoli* or *fuscans*, in Europe, date back over a long time, as the first strains of *fuscans* were observed in Switzerland - certainly on an imported lot, by that matter - it was in the years '24 or '26. [...] Anyway, it was detected in Switzerland. Apart from that, in France, the last "epidemics" in quotation marks or infected areas with *phaseoli* which have really been alerting are rare, there are a few, well, for instance at the organic farmers' in the South-West. There we were really confronted with that, but it is not so frequent. So, it doesn't seem to be an organism that is really settled in Europe, in any case." [NRI-281014Jj] [xiv]

Based on the report of the working group, the European Commission will decide upon the fate of CBB agents as regulated pests, with three possible outcomes: the Commission can directly maintain or remove the bacteria from the list of quarantine pests, or request a more thorough Pest Risk Assessment (PRA). Although the Commission has not yet decided upon the future categorisation of

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[32] It was modified recently, in 2014, because the former protocol obtained too many false positive results due to invasive pathogenicity tests.

[33] Not only are protection measures costly for the EU, but they are also examined by the World Trade Organisation (WTO) as non-tariff barriers to trade under the Agreement on the Application of Sanitary and Phytosanitary measures (SPS).

CBB agents, the report of the working group sheds light on current protective measures in view of the three questions listed above.

(i) The protective measures against CBB agents aim at protecting common bean production and all who depend on it, especially in Southern Europe (socio-political scope).

(ii) Given that CBB agents are not widespread in the European Community and common bean seeds are considered their main vector under European climate conditions, protective measures depend on the detection of CBB agents on bean seeds (science).

(iii) Common bean seeds produced in countries where CBB agents are known to occur and destined for professional users must undergo seed testing (rules and regulations).

Having identified these three conditions for the protective measures on CBB agents, I will draw the connection from the European Plant Health directive to the implementation of seed inspection in France. In view of understanding tensions between the protective measures and the *Croqueurs*, it is necessary to shed light on seed inspection as the circumstance in which they are faced with eachother.

2.2 Think global (protection), act local (inspection)

The EU plant health directive requires that common bean seed destined at market gardens in the Community come with an official statement proving that it is free of CBB agents. To become effective, requirements of the plant health directive are transposed into national law by the Member States and implemented by officers on the regional level. Seed produced in Members States known to be free of CBB, such as Belgium or Luxembourg, do not require analysis. For bean seed produced in a Member State not known to be free of the bacteria, the official statement is based on seed analysis. A comparison of how France and Germany interpret and practice this measure reveals that national provisions are proportional to the stakes the Member states have in bean seed production. In Germany for instance, the responsibility of conforming with the obligation of CBB-free seed is left to seed companies. If an unconformity with plant health requirements is found, seed companies risk being fined and made liable for the cost of the product recall.

> "But we know, that due to the low number of vegetable seed providers nowadays - most of it comes from Holland or elsewhere - this part is as good as not supplied anymore. They still go to building centres with amateur sachets and so on, they still have a lot to do there, but even that is poorly supplied. [...] We are responsible ourselves and if something was found, we would be in trouble. That is different here in Germany from what is done in France. That's why there is no official... no obligation of analysing bean seed lots for example. We don't have that. We do a lot of our seed multiplications in Germany. We analyse them voluntarily." [SOS-181215g] [xv]

By contrast, France implements more restrictive provisions for seed inspection by prescribing the means to obtain CBB-free bean seed. Every commercial seed lot produced and issued with an EPP in France must be tested according to the official ISTA method. Regional seed inspection officers check that bean seeds put on the market for sale to professionals have a negative analysis result.

> "...when I follow up the documents, I pick a random lot - I work with lot numbers - and I check that it has its analyses, at least if it's a lot of bean seed and the analysis is obligatory to conform with EPP requirements, well... I have to have a negative analysis in order to confirm that... they rightly issued the

> EPP. And if for another lot I find an EPP despite a positive analysis and the company nevertheless marketed and nevertheless issued the EPP, well then, that's a real non-compliance." [PIA-111215c] [xvi]

Formerly undertaken by public regional services for food and agriculture (*Service régional de l'alimentation* - SRAL), phytosanitary inspections on vegetable seeds have been delegated to a private actor since 2014. The inspections are now carried out by GNIS-SOC, i.e. the official service for inspection and certification (*Service officiel de contrôle et certification* - SOC) of the only French inter-branch union for seeds and young plants (*Groupement national interprofessionnel des semences et plants* - GNIS). The SOC is both fully integrated into GNIS - as it has no distinct legal form - and distinct from GNIS. SOC is accredited as independent and impartial certification body by the French accreditation body (*Comité français d'accréditation* - COFRAC). Impartiality and independence from GNIS as inter-branch union are ensured by a separate SOC national director, who is a delegated public official. The GNIS-SOC is thus distinct from GNIS in terms of missions and national director. In the regional GNIS offices, however, the distinct missions are managed and realised by the same teams of people.

> "Anyway the organisational scheme of GNIS and SOC, so the headquarters is in Paris... the headquarters is in Paris [for GNIS]... and for SOC, yes, yes. So, one has to review the history of the issue a bit, I mean: GNIS existed before inspections became obligatory in regulations. Some sectors had at that time organised to set up systems of quality control, for instance for potatoes, for maize, for cereals at the beginning. And then, at one point Brussels decided to legislate on that issue. In France there was an organisation that pre-existed in the form of GNIS and so, naturally, at that time the Ministry thought, well, we are not going to reinvent what is already functioning. And decided to entrust GNIS with the inspections, which now became obligatory [on the European level] and thus in all Member States at that time, through seed certification. And... so, here we are not talking about the sanitary issues for the moment, as these were the directives on seed marketing and the sanitary is not part of it, except for some "quality pests". But... so, I don't know if it goes back to that time, but what happened is that SOC was placed under the direct responsibility of the Ministry of Agriculture by means of an official of the Ministry of Agriculture seconded to GNIS to ensure the management of SOC. So actually one might say that GNIS is bicephalous: One part is purely interprofessional, with the GNIS director, and one part within GNIS in charge of ensuring regulatory functions, which is under the authority of an official seconded by the Ministry of Agriculture. Apart from that, when we are in the regional branches, I am in charge of a team of persons who may wear both hats." [PIA-170216p] [xvii]

Since 2014, regional GNIS branches are thus in charge of phytosanitary inspection of vegetable seed lots and reporting back to the public body in charge of phytosanitary protection, SRAL. It is in the framework of these institutional and organisational changes that artisanal seed companies in France are questioning protection measures against CBB agents, as I will describe in the next section.

In summary, the *Croqueurs'* approach to bean health management has been specified and summarised by the term *in situ* management in the first section. In this section, the conditions under which CBB agents are classified as regulated pest have been recapitulated: under the premise that CBB agents have not yet spread in Europe and to protect European bean production and consumption from their spread, only CBB-free seed is permitted to circulate. Controlling for this special requirement depends on official seed testing methods. In France, a stringent implementation of the protective measures prescribes an official seed test on any bean lot produced in France and destined at professional seed users. The French government has recently delegated seed inspections to the official certification service of a private inter-branch union concerned with seeds. Thereby, the approach of protective measures, as they are reasoned by French seed inspection based on the Plant Health directive, has been elucidated. Two approaches to bean health have been unfolded. The third and last section of this chapter will address incompatibilities between the two approaches by tracing the encounter between the *Croqueurs* members and the Plant Health directive.

3 Overrunning the frame of the protective measures

The plant health management rationale of artisanal seed companies as actor-network embedded in plant-environment-management interactions has been set out in the first section. Then, the rationale behind the protection measures against CBB agents was drawn in the second section. Particular emphasis was put on describing the rigorous seed inspection practiced in France. Seed inspection constitutes the encounter between the protective measures of the EU plant health directive and the plant health management of the *Croqueurs* members. Tensions between the two thus take shape through seed inspection. In this section, I will show how the *Croqueurs* assert their approach to plant health by overrunning the frame of the protective measures. In this aim, I will trace the *Croqueurs'* tentative to unlock the three fields of expertise upon which the plant health directive is built (Callon and Rip, 1992): the field of science and technology, the field of laws and regulations and the socio-political field. In order to do so, we will begin by following individual *Croqueurs* members in their encounter with the protective measures before tracing how individual situations are translated and relayed by the collective of the *Croqueurs* association.

3.1 Questioning the scientific presuppositions

The *hot* debate upon which this PhD research was established and the process by which the research device was progressively transformed has been discussed in Chapter II. The *Croqueurs* participate in the project in the aim of demonstrating that the "quarantine" measures on CBB agents are not compatible with their own practices. By accepting to involve in a research project with INRA, they attempt to question the science upon which the protective measures draw.

Their critique is based on the experiences of one of the seed companies among the *Croqueurs* situated in the South-West of France where both CBB and HBB occur. The company's experiences and critique have been put down in a letter cited on p.30. It is also this company which, on behalf of the *Croqueurs*, takes initiative in pushing for research on the subject. In speaking on behalf of the *Croqueurs*, the query is given weight and a wider scope. Concurrently, relay for the query is ensured by the other members. The *Croqueurs* endeavour to unsettle two components of the CBB quarantine in the scientific domain. Firstly, they challenge one of the conditions of the protective measures, namely the absence of CBB agents in Europe. Secondly, they call into question the detection method employed to implement the protective measures.

A first element of the critique of scientific presuppositions behind the protective measures concerns the endemicity of CBB agents in Europe. As shown in the previous section, the protective measures hold only if CBB agents are not yet widespread within the protected area. In the report of their "scientific opinion", the working group in charge of revising the criteria for the categorisation of CBB agents acknowledge that the bacteria are present in 17 Members States with a restricted distribution (among them France). Nevertheless, for the working group this distribution is not sufficient to consider CBB agents as widespread. It is this conclusion that is indirectly called into question by the *Croqueurs* member from the South-West of France, following an attempt to rid the company's bean lineages of CBB. Under pressure from the regional seed inspection authority[34] because of CBB agents detected on seed lots of susceptible bean varieties, the seed company had put in place informal "blight-free zone" according to the rules put in place in the official, registered bean seed production areas. As sowing CBB-free seed figured among the rules for the zones, the company had to leave aside their own bean lineages and buy commercial seed of the concerned varieties as basic seed. Although the acquired seeds had been issued with an EPP guaranteeing CBB-free seed, they were sampled and tested for CBB to make sure. Three out of eight samples were tested positive for CBB. In view of these results, the seed artisans figure that neither themselves, nor the industrial seed companies they had bought the bean seeds from, are able to rid their bean seeds of CBB despite rigorous measures. To them, it seems obvious that CBB is widespread and endemic[35] in France. This leads them to conclude that protective measures against CBB are not justified, even according to the official procedure for pest categorisation. This argument only enforces the artisanal seed company's experience of bacterial bean blights. They have learned to *live with* and manage bean blights on their bean lineages, whether they are caused by regulated CBB agents or non-regulated HBB agents. Differentiating between the bacterial species is of no practical importance to the artisanal seed company. In other words, bean blight symptoms have been "endemic" in their production regions ever since they can remember. By arguing at the level of CBB agents as bacterial species, the seed company resorts to debating in the scientific field traced out by the Plant Health directive.

The second element of critique put forward by the seed company from the South-West of France concerns the official seed testing method put down by the ISTA for the detection of CBB agents. Like the first element this one, too, stems from an attempt to rid the company's bean lineages of CBB in 2009. In parallel to setting up informal blight-free zones, the seed company tested different methods to eliminate or reduce infection rates of CBB-infected seeds of their own bean lineages. In the framework of these trials, the seed growers wanted to test the efficiency of their selection for plant health on a bean seed crop in which they observed heavy blight symptoms. In this aim, they sent one sample of seeds derived from positive selection for plant health for analysis, along with one sample from the seed harvested in bulk in the same field without applying any selection. Given the severity of symptoms observed in the fields, they were astonished to find negative analysis results for both samples - so astonished, that they sent in a second sample of the unselected seed lots to double-check. This time, the result of the analysis was positive - and cast doubt on the validity of official methods for CBB detection. On one hand, these doubts put into question the conclusions drawn about the CBB detected on commercial seed lots acquired from industrial seed companies and told in the preceding paragraph. They ensue the question: Can positive results be trusted? On the other hand, the contradictory results of the duplicate seed analyses cast further doubt on the scientific basis of the

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[34] ... which was still, at that time, a public body.

[35] In epidemiology, an infection is said to be endemic in a (plant) population when that infection is maintained in the population without the need for external inputs.

"quarantine" measures: Are the official detection methods reliable[36]? Again, the doubts cast on the scientific foundations of the protective measures support more general critique of the official method, that is based on the company's practical experience and shared by other *Croqueurs* members. The more general critique concerns the practicability of the minimum subsample size required for the official ISTA method - samples of 5000 seeds (Grimault et al., 2014) - for artisanal seed companies producing small seed lots. By questioning the validity of official detection methods, the seed company again ventures into the scientific field determined by the Plant Health directive.

It is with these arguments concerning the spread of CBB agents in Europe and the reliability of official testing methods that the seed company from the South-West of France, in its letter, underlines its recommendation to "live with" the bacteria rather than to try to eradicate it. By doing so, the seed company gives up arguments stemming from its own practice and resorts to debating in the scientific field traced by the Plant Health directive: Rather than arguing upon its own experience of *living with* blight symptoms on common bean, it directs it arguments at the prevalence of the bacterial agents and the methods employed to detect them.

In conclusion of this subsection, the Plant Health directive does not only force the seed company to align upon its OPP of CBB-free seed for bean health management. The procedures and scientific presuppositions behind the protective measures also delimit the range of arguments considered receivable in the debate about the protective measures. The artisanal seed company thus pushes its argument onto this terrain in the aim of unsettling the scientific base of the CBB quarantine. In the following subsection, I will tell how the endeavour initiated by this artisanal seed company in the South-West of France to defend their practice of *in situ* plant health management is relayed by other *Croqueurs* members. I will specify how this common intention is translated by individual seed companies in their interactions with seed inspection authorities.

3.2 Negotiating procedures and risking a legal dispute

In the first section of this chapter, in division 1.1.3, I have discussed the *Croqueurs* association as a space where the member seed companies exchange their viewpoints and practices in view of tracing out and defending their common ground. Facing the protective measures against CBB through phytosanitary seed inspections, the members attempt to align on a common position on plant health. Even if they don't all undergo the same pressure from their regional seed inspection authorities concerning CBB infections of bean lineages, the French seed companies in particular are concerned about phytosanitary regulations on seeds.

> "And I also sent an e-mail concerning the quantities, telling GNIS: 'You see, I have conducted a study on the bean productions of autumn 2013' - it was especially for beans that we had a bit of problems, or the risk of having problems - I told them: 'You see, our average lot weighs'... 25 kg, I think. 'Do you

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[36] Several hypotheses for inconsistent results in duplicate analyses have been proposed by a bacteriologist. For instance, the protocol of the official ISTA method was revised five years later (Grimault et al., 2014), because the previous method resulted in too many false positive results. Also, CBB agents at low bacterial densities near the limit of detection may be detected in one analyses, but not the other. Finally, sample sizes are about statistical calculations: If seed infection is not homogenous and only a few seeds in a lot are infected, one sample may contain infected seeds, while the other sample may not. However, the artisanal seed company doesn't go into questioning the exact reasons for the unreliable testing results.

> really consider that the regulation is suited for lots of 25 kg? Given that samples of 3 kg, or 4 , are needed to make an analysis of the content'... I mean, of presence or absence of bean blight." [CRO-190515d] [xviii]

Regarding the inspections on bean, the seed samples required for official seed tests are considered disproportional to their own activity. Given that the artisanal seed companies produce small seed lots, seed testing is proportionally more expensive for them than for seed companies producing large bean seed lots. The *Croqueurs* do not feel adequately treated by the requirement of systematic seed testing.

In addition, regarding seed inspections in general, the *Croqueurs* judge GNIS, the only French inter-branch union for seeds, illegitimate to constitute an independent and impartial seed inspection body.

> "Wait, there is a big company that produces seed in France. It's Limagrain - Clause Tézier - Vilmorin, it's the same company. It represents 80 or 90% of the market, I don't know. But they're making all the money at GNIS. The president of GNIS, I don't even remember his name, who cares, even, but he is under the thumb of Clause Tézier - Vilmorin. Thus, the partiality of that... And in addition, they have a governmental, a ministerial delegation. So, they have a power... It's a corporatist union like they don't make them anymore." [CRO-260814b] [xix]

The *Croqueurs* association does not consider the interbranch union GNIS as an adequate representative, nor its official control service GNIS-SOC, to which public authorities delegated the phytosanitary bean seed inspections. Based on the organisational structure of the GNIS, the *Croqueurs* suspect that multinational, conventional seed companies direct the interests of the inter-branch union. Some of the *Croqueurs* members also expect that these companies may profit from information gained in seed inspection. Moreover, a system of officially monitored self-controlling, which is being set-up by the GNIS-SOC to replace current phytosanitary inspections, is perceived by the *Croqueurs* as a further step towards Plant Health regulations that suit the "big" seed companies, but not the small, artisanal ones.

To begin with, the French *Croqueurs* members agree not to cooperate with the newly delegated seed inspection in the aim of conveying their discontent. However, coordinated disobedience is not practicable by the individual seed companies facing their regional seed inspectors. As most of the artisanal seed companies have already engaged in negotiations with their regional seed inspectors, they can no longer back off. Aware that they cannot keep up with the legal requirements of the CBB quarantine, they experience that arrangements can be found with the seed inspectors on the local scale. Although the legal OPP of seed testing is not negotiable, it can be adapted to the seed companies' needs. For instance, in return for implementing certain phytosanitary procedures, one company negotiates that only a proportion of bean seed lots be randomly selected and tested every year, instead of all of them. Another obtains the informal permission to test samples of 10% of each seed lot at most, even if such samples don't conform with the official ISTA methods. For the individual seed companies, bean health management is thus also about finding an interlocutor to negotiate practicable compromises in the framework of seed inspection. By negotiating sampling procedures and deviating from the official method at the local level, the objective of the Plant Health directive - eliminating CBB-infected seed from European ground - is eroded. It is transformed into negotiations about acceptable risks of spreading CBB. The question of risk is contained in the negotiations about seed samples, as the sample size is proportional to the "desired tolerance standard" (i.e. to the maximum acceptable percentage of infested seeds). Whereas the risk assessment underlying the Plant Health directive refers to the "industrial common bean seed production scheme" done "on a worldwide scale" (EFSA Panel on Plant Health, 2014), the accepted level of risk is informally adapted to artisanal seed production in the framework of local seed inspection.

In the end, only one member of the *Croqueurs* persists in its categorical refusal to cooperate with the GNIS-SOC.

> "So, we are the only ones who have done strictly nothing... Plus, they have not even contacted us again, for the moment. We haven't had any contact in a year. [...] We have registered the plots. That is, last year, the only thing we did is to register the plots... as if to say: 'Anyhow, we don't have anything to hide. If you want to go and see them, go and see them, but we won't sign any contract, and we don't want to pay anything' [...] I told the regional branch: 'Anyway, you are pre-empting the European regulation.' Since the European regulation has to be published... it's not been published to date, to my knowledge. I say: 'As long as the European regulations haven't been published, I don't see why I should abide by your regulation.' " [CRO-190515d] [xx]

This company renounces to negotiating seed inspection procedures locally in order to dismiss national, if not European, plant health regulations altogether. By refusing to sign the agreement with its regional GNIS-SOC branch, the seed company is legally unable to issue EPPs and risks legal proceedings. In both cases - whether procedures are negotiated locally or cooperation refused categorically - the protective measures are undermined by unsettling their procedures.

In this subsection, I have described how the Plant Health directive and its French implementation are undermined by disputing its underlying procedures. By negotiating seed sample sizes for CBB detection, the risk assessment for protective measures is informally adapted to the scale of the artisanal seed companies. Only one seed company withstands local negotiations and refuses to cooperate with its local seed inspection authority. Although this company is "isolated" in its categorical position, this doesn't mean that it is isolated in debating the socio-political scope of the protective measures on CBB. In the next subsection, I will trace the nascent attempts - for the moment no more than ideas - to open a debate the socio-political scope of the "quarantine" measures.

3.3 Debating scopes and asserting an identity

In subsection 2.1, I concluded that the protective measures against CBB agents aim at protecting common bean production and all who depend on it, especially in Southern Europe. By protecting European bean plants from CBB, the EU legislators intend to protect seed growers, farmers and all who depend on common bean in one way or the other. In France, the GNIS-SOC, as private inter-branch union in charge of official seed inspections, also considers itself a spokesperson for seed growers, seed companies and seed users.

> "That's where I understand that the small makers, who already don't make much seed... and that one analysis constitutes this many seeds and this much money... It's not possible. So, that's where we would need to be unanimous - because there are a lot of small organic makers, and not only in France. So, their way of producing also has to be taken into account and maybe we could make things evolve as to say: It's an inspection in the crop. That could be an answer and avoid consuming seed for nothing, because I understand that it isn't possible to make an analysis on... that's it. It's just... but, the regulation exists, and that's where there is an interest in having the SOC to... relay the information and that the inter-professional union gets a move on for something." [PIA-111215c] [xxi]

The seed inspection officer cited above suggests that the GNIS serves as spokesperson for small-scale organic vegetable seed producers. Playing the role of both private union and official inspection body, the GNIS is in a position to inform legislators of the problems faced in seed testing and to push for crop inspection rather than seed inspection. The prerequisite to do so is collaboration and trust between the GNIS and the *Croqueurs*, trust which the latter refuse to give. I have mentioned in the previous section that the *Croqueurs* members consider that the GNIS-SOC lacks independence and impartiality to be legitimate as delegated seed inspection body. Beside their concern about the power structures behind the union, their lack of trust also stems from their *competing translation* of plant health. As defined in the Introduction of Chapter II (p.28), the term *competing translations* reflects that the two parties don't attribute the same meaning to plant health. The *Croqueurs'* translation of bean seed production and plant health into practice leads them to *in situ* bean health management. The translation of bean health by the Plant Health directive, and thus by the GNIS-SOC, leads to a ban on CBB-infected seeds based on seed testing.

> "Nevertheless, as far as I am concerned, [a seed artisan] who... proceeds to a form of sanitary selection, I don't accept it. [...] A seed crop in which one might have one row of bean because one has... because for instance one is doing conservation breeding and that one considers keeping the plants that resist most, but that through these resistances blight is introduced... That can be true for any other disease, no matter, it truly may be an arguable way of selecting. I don't have a position on that, I don't work in production, so... if I had to choose, I wouldn't know which [plant] to choose... but it is without a doubt very interesting. But when facing a regulated parasite, I think one has to... one has to take action and not allow this selection to be done." [PIA-111215c] [xxii]

As explained by the seed inspector in the citation above, both translations cannot be put into practice at the same time - either you attempt to eradicate CBB agents, or you let their selection pressure act upon bean plants in their growing environments. The two translations of *in situ* management on one hand and of quarantine measures on the other are indeed *competing*. As one seed artisan put it: "They want to eradicate CBB? Then they'll eradicate peasant seeds". For *in situ* bean health management, CBB agents have to be accepted among the microbial communities forming part of the bean populations. In other words, the *Croqueurs* do not feel that their bean plants are represented, nor protected, by the Plant Health directive and seed inspection. Bean seeds tested in sterile environments, isolated from the soil that will host them, are not considered legitimate spokespersons for their own understanding of plant health. These tensions involve both the professional identity of the *Croqueurs* members as artisanal seed companies and the scope the Plant Health directive.

> "... among the *Croqueurs*, we said that what needs doing, is telling them that we have another vision of plant disease, what you were saying earlier... and that we should say, well, that vision is not taken into account in the regulation, that we demand an appropriate regulation. But we haven't worked on that issue, so he haven't moved on. But it was the only way we were seriously considering. [...] That would be more in the long term, and that could perhaps be an in-depth reflection within ITAB[37], for example, to - as we have a commission on seeds [...] - it would be about saying: OK, well, facing the plant health regulation for seeds, which lines are we defending in organic agriculture, you know? Demand adaptations of the regulation. In fact, we always have two... two pillars for our demands. It's the small size, which has nothing to do with organic, he! The fact that we are in very artisanal sizes, hmmm, implies that we are not on the same planet as the big companies. We are 100 or 1000 times smaller. So, that needs to be taken into account, really. And the second thing is that, in organic agriculture, we also have a different point of view. So, that's... we always base on those two pillars, as it were, to justify that we don't agree with what is to be imposed on us. [...] it forms a whole, which is, actually, peasant seed, diversified agriculture, etcetera. Because we could be big and organic or small and conventional.

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[37] ITAB is the "technical institute for organic agriculture" in France (*Institut technique de l'agriculture biologique*)

> And here, we combine both. The fact is that I always base my argument, when I have to discuss with these people, on both these aspects. There is a combination of both." [CRO-190515d] [xxiii]

By asserting their identity as both organic and small artisanal seed producers, the *Croqueurs* question the scope of the protective measures on CBB agents. Asserting that the professional identity of the *Croqueurs* is not protected by the "quarantine" measures implies the question: Shall their identity be protected? This debate on the socio-political scope of the protective measures are far from the public agenda at the present moment. The *Croqueurs* are only just beginning to debate their needs in terms of plant health regulations among themselves. Nevertheless, one reaction to this question may be contained in the following citation of an actor within the inter-branch union.

> "... when the concept of peasant seed began to emerge, we wanted to try and find out, ultimately, what does it represent? But we were unable to find reliable data to tell us: peasant seeds represent this many hectares or that many farmers... [...] [the demands] are legitimate, but what does it really represent within organic agriculture? Because, indeed, are we not hearing only those who are expressing themselves on this issue? It's true that... it's a bit like for beekeeping, there is a fragmentation of organisations, they are often sniping at each other, and ultimately, in all that one does not know where the majority trend is and who... towards whom one should go. I am not saying that the demands are not legitimate, right! If someone, by their philosophy, wants to have organic seed of a certain variety, it's their right, but..." [PIA-170216p] [xxiv]

This actor requests that the users demanding peasant organic seed be quantified apart from other users of organic seed. He thereby suggests that this demand represents a small minority, even among organic seed users. For this GNIS officer, the small size of the artisanal seed companies does not exclude them from being represented by the GNIS. The inter-branch union has the mission to represent the seed sector in its entirety - from plant breeder to seed user, but also from organic seed to genetically modified seed. Representing artisanal seed companies is thus a question of numbers. In other words, for how many seed growers, farmers and seeds do the *Croqueurs* speak? How many seed growers, farmers and seeds are concerned by the demands of the *Croqueurs*? Based on the interests and risks the *Croqueurs'* demands represent in numbers, procedures to implement the quarantine on CBB may be developed that are acceptable to artisanal seed companies. The seed inspection body is willing to negotiate with the *Croqueurs* in terms of company sizes and risk assessment, but not in terms of diverging conceptions of plant health.

For the *Croqueurs*, however, their understanding of plant health forms part of their identity. When the *Croqueurs* members refer to themselves as very small artisanal companies, they don't only point to their companies' sizes in terms of numbers, but also to their *artisanal* practices and their identity. Conceiving crop diversity in terms of crop *lineages* and understanding plant health based on the behaviour of crops *in situ* is at the heart of the *Croqueurs'* profession as seed *artisans*. Accepting to negotiate representation by the GNIS in terms of numbers would imply abandoning their understanding, their *competing translation* of plant health. By resisting the GNIS, the *Croqueurs* maintain their practices, thereby asserting and constructing their identity of *artisanal* seed companies. The question of managing CBB on bean seed mobilises opposing understandings of plant health. In this opposition, identities are constructed, asserted and maintained. As suggested by Callon (1986b), actors are *inter-defined* by how they relate to the problem of CBB management. Identities are revealed by their non-alignment on protective measures against CBB.

This situation of lacking alignment can be contrasted with the management of a Black Sigatoka outbreak in Australia, described by McAllister *et al.* (2015). Black Sigatoka is a fungal leaf-spot disease of banana plants. Following a network analysis of emergency response to Black Sigatoka, McAllister *et al.* conclude on the critical importance of the local implementation of eradication response plans. In particular, they stress the importance of implementing plans according to local conditions and place-based knowledge. The involvement of actors at different spacial scales - from national to local - and with different roles - from public and private sectors - are considered crucial for the successful eradication of Black Sigatoka in Australia. Similarly, in our case the negotiations between seed companies and seed inspectors on the local level also shape the implementation of protective measures against CBB agents in France. At the local level, some of the *Croqueurs* members negotiate and obtain flexibility in seed sampling procedures. Thereby, they indirectly shift the scope of the "quarantine" measures from eradicating CBB agents to a negotiation about the risks of CBB spread associated to their own, artisanal form of seed production. Procedures are thereby adapted to the conditions of these companies. However, unlike the Australian case, the actors followed here do not share the same translation of plant health. They pursue different aims, with very different practices. Whereas some of the artisanal seed companies accept to negotiate seed sampling procedures, they reject the GNIS as spokesperson for their profession.

This is where we leave the *Croqueurs* in their endeavours to unlock the protective measures on CBB and override the OPP prescribed by protective measures in the aim of asserting their practice of *in situ* bean health management.

4 Conclusions

The *Croqueurs'* attempts to unsettle the quarantine measures on CBB are ongoing and I cannot report on the result of these endeavours. In the language of ANT, actor-networks with competing translations of bean health are currently rearranging on multiple levels and have not stabilised. At the EU level, the Plant Health directive is currently being reconfigured. The categorisation of CBB as quarantine pest is also under revision. At the national level in France, the newly delegated seed inspection authority has not stabilised its procedures for inspection and for the financial implication of seed companies in inspection costs. A system of officially monitored self-controlling by seed companies is currently being devised. Facing the transformations at both these levels, the *Croqueurs* struggle to reconcile their practice of *in situ* plant health management with legal phytosanitary constraints. At the same time, they make various attempts to make their own practice, their own translation of plant health management heard. Despite the unfinished character of these ongoing processes, lessons can be learnt and conclusions drawn from the road travelled up to here.

In this chapter, I have first traced the bean health management practices of the *Croqueurs*. To understand the specificities of their approach in more depth, contrasts were drawn with a multinational seed company and with a small German organic seed company (section 1). Then, the EU plant health regulation prescribing protective measures against CBB, as well as its enforcement in France, was elucidated (section 2). Finally, the *Croqueurs'* attempts to call into question protective measures against CBB were analysed according to three domains (scientific knowledge base, rules and regulations and socio-political scope). I come to the conclusion that the tensions between the *Croqueurs'* plant health management practices and the EU plant health regulation arise from incompatible understandings of plant health (section 3).

At the beginning of this chapter, the following questions were formulated: *Which are the specificities of bean health management practiced by artisanal seed companies among the association Croqueurs de Carottes? On which interactions between bean plants and their growing environments is this plant health management based?* Concerning the first question, I have termed the Croqueurs' practices in situ plant health management. In situ is to reflect that bean lineages and their health state are determined by the triangulation of crop variety, growing environment and seed grower. I have highlighted that the artisanal seed companies among the Croqueurs base their collective management of bean health on the circulation of seed. As in situ management is about finding appropriate growing environments for each crop variety, it can be reasoned at the scale of the network of seed growers. I have shown that a seed grower who finds that a given bean variety is not suited for his farm environment will pass the variety on to another seed grower. A variety that won't thrive and yield in a given environment is not discarded, but transferred to a different environment.

The *Croqueurs'* governance of bean health encompasses the care for *sound* growing environments. This has consequences on the distribution of competences.

(i) Competences are distributed across the bean cropping system. Beyond the bean plants themselves, the plants' growing environment has a role to play in the regulation of plant health.
(ii) Competences are distributed across actors, including the artisanal seed companies, the seed growers and the seed users (customers). As bean health relies on the *soundness* of growing environments, seed users must align on the *in situ* approach to plant health.

This distribution of competences contrasts with that of the multinational company and the German organic seed company with which I have compared the *Croqueurs'* approach. By focusing on bean seed as vector of plant health, competences are redistributed and concentrated within the seed companies.

The second question posed at the beginning of the chapter addresses ecological interactions between bean plants and their growing environments. As mentioned in the previous paragraph, competences are distributed across the bean cropping system. The health of a bean population is judged upon in its growing environment, i.e. in the fields. Bean crops interact with beneficial microorganisms able to regulate or suppress plant pathogens, soil microorganisms in particular. Microorganisms associated with seeds and thriving plants are considered as a signature of the growing environment (or *terroir*). A bean *lineage* can only be considered in interaction with these microbial communities. It is in interaction with local growing environments, the associated microbial communities and the seed grower that bean populations evolve and locally adapt. The adaptability of bean plants is regarded as a cornerstone of plant health, reflecting that plant health cannot be considered in isolation from the interactions with the plants' growing environment.

The *Croqueurs* attempt to unlock the quarantine measures by unsettling its presuppositions in the three domains (Callon and Rip, 1992) underlying the expertise on the categorisation of CBB as quarantine pest: (i) socio-political scope, (ii) science and (iii) rules and procedures. One might consider that the essence of the tensions concerns the socio-political stakes of the quarantine measures. Two *competing translations*, or understandings, of plant health are operating. The translations followed by the *Croqueurs* members on one hand and the Plant Health directive on the other have been described in some detail in the sections 1 and 2, respectively. In search of means to publicly assert their understanding of plant health and their practices of *in situ* management, the *Croqueurs* focus their endeavour to unsettle the quarantine measures on two other domains traced out by the Plant Health directive. In the scientific domain, they question the absence of CBB agents as endemic pests in the European Community and the validity of the official seed testing methods on which the implementation of quarantine measures is based. In the field of rules and procedures, some *Croqueurs* members locally negotiate informal adaptations of seed sampling procedures. Thereby, the accepted level of risk is adapted to the activity of artisanal seed production. To convey discontent both with the prescription of plant health management practices and with the delegation of seed inspections to the inter-branch union, one of the *Croqueurs* members refuses any cooperation with the French delegate seed inspection body. It asserts its professional identity as artisanal seed producer while taking the risk of a legal dispute.

The following chapters will address these ecological interactions through observations in field experiments, with the overall question: *What do analyses of some plant-environment interactions reveal the ecological base of the Croqueurs' bean health management?* After an overview of the field experiment's materials and methods in Chapter IV, the overall question is broken down in the following chapters in order to address specific plant-environment interactions.

Original extracts of interviews

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[i] "... au sein des Croqueurs de Carottes, il y a aussi: Aucune variété protégée, aucune variété hybride F1, aucune variété modifiée génétiquement. Donc on reste sur de variétés à pollinisation ouverte, non-protégées [...] Et une autre chose qui est très importante aussi c'est qu'on ne se contente pas du minimum réglementaire, qui est de produire une semence bio à partir d'une semence conventionnelle. Nos semences de base sont bio aussi. C'est à dire nos variétés sont en bio depuis 5, 10, 15, 20 ans. Donc à la longue on peut penser qu'il y a une imprégnation de la variété par son vécu pendant 10 ou 20 ans [...] ça n'a pas été prouvé scientifiquement, avec les méthodes de la recherche scientifique, mais on constate que les plantes deviennent assez rustiques." [CRO-190515d]

[ii] "Tout comme si tu mettais une variété conventionnelle, tu la jetterais dans des conditions bio, ben elle ne répondrait pas pareil. Surtout des variétés modernes qui ont été sélectionnées pour répondre aux intrants, à fortes quantités d'intrants: tu la mets en bio ou elle est un peu sevrée de tout ça, tu ne la reconnais pas vraiment, quoi. Donc c'est le même problème dans les essais conventionnels. Nos variétés bio réagissent différemment et ils ont tendance à nous dire: Vos variétés... elle est pas du tout conforme au type qu'on attend, quoi. Ca arrive pas tout les temps, mais... [...] En bio elles le sont, et puis elle sont surtout bien adaptées, elles sont rustiques pour la bio, quoi. Donc si elle a des feuilles un peu plus allongées ou un petit peu plus d'anthocyanes, c'est peut être parce qu'elle est... enfin, c'est la sélection naturelle qui a voulu ça, quoi. Et ça, ils n'aiment pas. [...] C'est impossible surtout en populations, ça évolue. Et justement, elles sont dans des conditions ou ça évolue, en bio elles sont bien quoi. Donc généralement, quand c'est comme ça, si ils nous disent: Votre variété, elle n'est pas bonne. En général on la garde quand même, parce que c'est des souches qui ont 20 ans de bio derrière elles. Eux ils voudraient qu'on reparte sur une souche conventionnelle, qui était stockée dans un frigo, qui n'a pas bougée depuis 20 ans, donc tout le travail derrière qu'on a fait serait perdu. Donc ça c'est pas possible. Et ça c'est un point ou on n'arrive pas à se faire comprendre." [CRO-190515s]

[iii] "Moi, le peu de recule que j'ai, c'est que quand il y a une variété qui n'a jamais été cultivé ici, la première année de culture, et bien, elles ont toujours, enfin les haricots ont une tronche... t'as l'impression qu'ils sont virosés... Et après il y en a plus, du tout. Et pour toutes les cultures en général. Pour la tomate par exemple ca s'exprime différemment, c'est la puissance de la plante. C'est à dire que, quand je prends une tomate qui vient d'ailleurs, et ben, la première année, elle s'adapte - ce que j'appelle adaptation - et la deuxième elle commence a... tu vois, ce qui était vraiment symptomatique, c'est quand on essayait les 250 variétés de tomates de l'INRA, c'était un gag, quoi. La première année; elles étaient chétives, le deuxième année, ca allait beaucoup mieux, la troisième année, c'était... Elle commençaient à être belles, tu vois? C'était pas du tout la même plante. A la limite, on aurait pu dire: C'est une autre variété." [CRO-260814b]

[iv] "En culture, et ben, c'est une approche agronomique, c'est à dire sol, climat, la plante et moi - parce que j'en fais partie, hein! Et il faut qu'on arrive, tous les 4 ou 5 là, je ne sais plus, à trouver un compromis qui fait le moins mauvais, ou le meilleur, pour les 3 ou les 5, enfin - le temps il s'en fout, le sol il s'en fout moins, la plante, elle est vraiment concernée et moi aussi. Donc les gros acteurs c'est la plante et moi, le sol on essaie d'en faire notre allié, et puis le temps on fait avec..." [CRO-290116d]

[v] "C'est vraiment la question de la conception qu'on a du sanitaire. Tout ce qui est vie microbienne ou champignons autours des plantes, c'est la signature du terroir. Et des pratiques agricoles, pas que du terroir. Une variété locale, il y a nécessairement des microbes. Si tu les enlèves tous, il n'y a plus de variété locale. Alors, il n'y a que nous qui raisonnons en terme de variétés locales." [NGO-260915k]

[vi] "C'est que artisans semenciers, on existe parce que, en faite, les paysans ne peuvent pas faire l'ensemble des multiplications de leurs semences de base. Ce qui n'est pas du tout le cas pour un paysan boulanger. Un paysan-boulanger, il peut... c'est lui qui est maitre de ces semences de base. Et c'est vraiment les deux opposés, tu vois, entre le paysan-boulanger qui est totalement maitre - et tu n'aurais jamais d'artisans-semenciers pour ces espèces là. Et nous, on est à l'opposé, on est là pour leur apporter les souches. Pour que ces souches, elles vivent. Mais, après... et le haricot en est l'exemple type... il y a une adaptation au terroir. Et ca c'est plus les artisans-semenciers. C'est la paysan, ça." [CRO-280814c]

[vii] "L'autre chose qu'il faut ajouter dans toute cette discussion, c'est que nous, on vend des semences à des paysans bio, et ça, ça change tout aussi. On vendrait nos semences à des conventionnels, avec les sols morts qu'ils ont et autres, peut-être qu'ils auraient pleins de maladies. Mais comme on vend a des gens qui travaillent un peu comme nous - la plupart de nos clients, ce sont soit des jardiniers, soit des maraichers diversifiés en petites surfaces - ben aussi ils apprennent à travailler un peu comme nous. Ils ont un peu la même conception que nous de la vie, de la santé des plantes et autre. Et du coup, ben, ils ont moins de problèmes." [CRO-190515d]

[viii] "On décide rien, mais on lance pleins d'idées. Finalement , c'est un espace de création d'idées et d'arguments, moi, je l'ai pris comme ça. Et ils sont vraiment excellents. Moi, je me sers comme dans un marché, j'amène les

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miennes, mais j'écoute celles des autres. Je les confronte, on les frotte, on les compare. Du coup, j'aiguise mon argumentaire, ma connaissance du sujet - parce qu'il y a [un artisan semencier] aussi qui intervient avec une connaissance fine de la réglementation, il y en a d'autres plus sur une connaissance fine de certains sujets sur certaines variétés ou espèces, tomates machin... Donc: Je vais au marché, je prends ce qui m'intéresse, je donne les miennes quand ça m'intéresse, je me fais rentrer dedans, je lui renvoie... de façon assez amicale, hein! Enfin, on essaie... Et... c'est génial. Mais... ce n'est pas un endroit ou on va... ce n'est pas un syndicat, on arrive pas à... on a pas le monolithisme du syndicat, qui prend l'épée et qui coupe en 2 , non... ça on ne sait pas faire. [...] On ne sait pas avoir le côté efficace - ou l'air de rien. Je dis ça, mais c'est pas complètement vrai, parce que l'aire de rien, l'efficacité elle là. Parce que, au final, [certains artisans semenciers] sont dans les commissions etc. [...] Moi je leurs dis: "Vous dites que ça n'avance pas, mais moi je peux vous dire que moi le contact que j'ai avec la FNAMS et d'autres, les choses bougent"." [CRO-290116d]

[ix] " ... à l'heure d'aujourd'hui sur les aspects sanitaires on est très confiant par rapport à la propriété du végétal, parce qu'on pense, nous, que c'est vraiment en mettant les moyens, la recherche, en créant des nouvelles variétés - et la on ne parle pas de transgénèse ou de prendre un gène d'éléphants, hein - simplement en multipliant nos croisements à arriver à sortir quelque chose, quoi. Ce qu'on remarque nous c'est que les vieilles variétés sont quand même beaucoup moins costaudes que ce qu'on sort. Ce n'est pas systématiquement, il y a des exceptions. Des haricots comme la variété 'Talisman', qui est un des plus vieux haricots qui existent, passe bien globalement tout le temps. Mais l'amélioration génétique sur le côté sanitaire pour nous c'est une grande grande voie quand même, pour l'avenir, hein. Parce que... nous, à [notre entreprise], on est persuadés que la chimie va baisser. Dans 10 ans on traitera moins qu'on ne fait là, et il y a 10 ans on traitait déjà plus, donc il faut jouer sur ces résistances-là. Le problème qu'on a, c'est que les haricots c'est des plantes autogames, donc c'est des "seed to seed", hein. Vous semez une graine, vous la resemez, vous la resemez, vous la resemez... de génération en génération..." [MSC-150216b]

[x] "La problématique qu'on a sur les graisses du haricot, c'est que c'est des maladies de fin de cycle. C'est de maladies ou, si vraiment vous en voyez au stade plantules, c'est que vous avez semé un lot pourri. Et ça, j'ai bossé dans des boites ou je peux vous garantir que ça m'est arrivé. Nous [...] c'est des expressions tout le temps après floraison - toujours, systématiquement. Donc en plus de ça, dans mon métier, c'est un petit peu compliqué, parce qu'à 15 jours de la récolte, vous dites à [l'entreprise]: "Il n'y aura rien." Et vous dites à l'agriculteur: "Tu passes la charrue". Euh... voilà. Donc c'est toujours très délicat." [MSC-150216b]

[xi] "Wir haben eben das Problem, wie ich es vorhin geschildert habe, dass wir bereits zwei Sorten verloren haben und momentan das Saatgut zur Verfügung haben, wo das festgestellt wurde, aus dem Züchterbereich. Mit dem Arbeiten wir jetzt und gucken, dass wir das frei kriegen. Und dann, wenn wir die Methode haben, dann können wir hergehen und können sagen: Die Elite ist grundsätzlich möglich freizukriegen, weil man das dann in dem begrenzten Umfang machen kann - Superelite - dann kommt die Hochvermehrung. Ist dann vielleicht immer noch mit einem gewissen Restrisiko verbunden, aber die Voraussetzung ist, dass die Züchter und Erhaltungszüchter sauber arbeiten können." - "Genau. Das ist das A und O ist, dass das Elitesaatgut halt frei ist von diesen Quarantäneschaderregern, also von *Xanthomonas* in dem Fall." [SOS-181215g]

[xii] "Wir haben in unserem Marktsegment tatsächlich mit der Konkurrenz der ganz großen in der Branche zu tun, also, in Frankreich vergleichbar mit Clause-Tezier. Das wäre dann in dem Fall unser Konkurrent. Hier haben wir es mit Bejo zu tun, mit Rijk Zwaan zu tun, mit Enza Zaden zu tun, mit Nunhems zu tun, mit Syngenta zu tun... Das sind unsere Konkurrenten am Markt. Und die geben ein enormes Maß an... ja, wie soll man sagen... an "Gesundheit" in Anführungszeichen vor... äußere Qualität des Saatgutes, Keimfähigkeit, Sauberkeit... ja, alles was so dazu gehört, damit Bauern von heute mit ihrer heutigen Technik entsprechend optimale Ergebnisse auf dem Feld haben, also quantitativ optimale Ergebnisse. Und diese, unsere Bewegung in den ersten 20 Jahren, mehr oder weniger, nicht wirklich Fuß fassen können dadurch, oder in den ersten 15 Jahren - seit es [die Aktiensgesellschaft] gibt ist das anders geworden... aber: nicht Fuß fassen können, weil es eben die Methodik nicht gab, die Kompetenz nicht gab, die Möglichkeit nicht gab, diese äußeren Qualitätskriterien zu erfüllen. Weil eben allein durch die Frage: Wie sicher ist die Qualität des Saatgutes? Im Keimbereich oder in der Gesundheit und so weiter... die Bauern an der Stelle dann gesagt haben: "Das kann ich mir nicht leisten", wenn dann da plötzlich der Feldsalatbestand einen Haufen Mehltau kriegt, weil eben schon am Saatgut ganz viel Mehltau dran ist. Das war Vergangenheit, das haben wir alles im Griff und nutzen selbstverständlich die Möglichkeiten dadurch... die wir halt haben durch die moderne Pflanzendiagnosetechnik, die es gibt." [SOS-181215g]

[xiii] "On voulait le faire sur le persil de Jean-Michel qui ne germait pas, mais il l'a retesté et, finalement, il germe super-bien! (rire) Donc c'est pour ça que ça n'a pas été fait... Bon, c'est une semence qui a deux ans, le champignon qui était autour a dû mourir ou perdre en vigueur et la semence a repris le dessus après. Il y a ça aussi, c'est vivant une semence, donc..." [CRO-190515s]

[xiv] "... il n'est pas endémique en tout cas... *phaseoli* ou *fuscans* n'est pas endémique en Europe. Il peut être détecté de manière ponctuelle, mais à priori, il ne serait pas endémique, c'est pour ça qu'il est encore en quarantaine. Après, des observations de *Xanthomonas*, de *phaseoli* ou de *fuscans*, en Europe, c'est très ancien,

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puisque les premières souches de *fuscans* ont été observé en Suisse - c'était sûrement sur un lot importé, d'ailleurs - c'est dans les années '24 ou '26. [...] Ca a été détecté en Suisse en tout cas. Après, en France, les dernières "épidémies" entre guillemets ou foyers infectieux à *phaseoli* qui ont vraiment fait l'objet d'alertes sont rares, il y en a quelques uns, ben, forcément, chez les agriculteurs bio du Sud-Ouest. Là, on avait été confronté à ça, mais ce n'est pas si fréquent que ça. Donc ce n'est pas un organisme qui semble vraiment installé en Europe, en tout cas." [NRI-281014Jj]

[xv] "Aber wir wissen, das eben auf Grund dieser dünnen Belegungsdichte von Saatgutanbietern heutzutage im gemüsebaulichen Bereich für den Profianbau - das meiste kommt ja aus Holland und sonst wo her - ist der Teil eigentlich so gut wie nicht mehr versorgt. Die gehen noch auch in die Baumärkte und die Hobbytütchen und so, da müssen sie noch viel tun, da gibt es auch noch, aber die sind auch doch relativ schwach ausgestattet. [...] Wir sind selber verantwortlich und wenn was gefunden würde gäbe es Ärger. Dann würden wir dann natürlich aufgefordert: Sofort zurückziehen, Bußgeld, was auch immer. Aber das ist eben anders hier in Deutschland als in Frankreich, deswegen gibt es auch keine offizielle... keinen Zwang der Untersuchung zum Beispiel der Bohnenpartien. Also das gibt es hier nicht. Wir haben ja ganz viel Vermehrungen auch in Deutschland. Die untersuchen wir auf freiwilliger Basis." [SOS-181215g]

[xvi] "... dans mon suivi documentaire, je vais prendre un lot au hasard - donc je fonctionne par numéro de lot - et je vais bien vérifier que il a les analyses, en tout cas si c'est un lot de haricot et que l'analyse est obligatoire pour répondre à l'exigence PPE, et bien... Il faut que j'aie une analyse négative pour que je puisse confirmer que... qu'ils ont bien apposé le PPE. Et si pour un autre lot je trouve un PPE alors que l'analyse était positive et que l'entreprise a quand même commercialisé et quand même apposé le PPE, ben là, c'est une vrai non-conformité." [PIA-111215c]

[xvii] "De toute façon l'organisation du GNIS et du SOC, donc le siège est à Paris... le siège est à Paris [pour le GNIS]... et le SOC, oui oui. Bon, il faut revoir un peu l'historique de l'affaire, c'est à dire que: Le GNIS a existé avant que les contrôles deviennent obligatoires sur le plan réglementaire. Un certain nombre de filières s'était à l'époque organisé pour mettre en place des systèmes de contrôles qualité, c'était le cas en pomme de terre, ça a été le cas en maïs, ça a été le cas au démarrage en céréales. Et puis à un moment donné Bruxelles à décidé de légiférer là-dessus. Il y avait une organisation qui préexistait en France au travers du GNIS et donc à cette époque naturellement le ministère s'est dit, ben, on ne va pas réinventer un système qui fonctionne déjà. Et a décidé de confier au GNIS les contrôles, qui cette fois devenaient obligatoires [au niveau européen] et donc dans tous les Etats Membres de l'époque, donc au travers de la certification des semences... Et... donc là on ne parle pas de sanitaire pour l'instant, puisque c'était les directives de commercialisation et le sanitaire n'est pas dedans, sauf pour un certain nombre de "parasites de qualité". Mais... alors, je ne sais pas si c'est de l'époque que ça date, mais du coup, ce qui s'est passé, c'est que le SOC a été mis directement sous tutelle de ministère de l'agriculture au travers d'un fonctionnaire du ministère de l'agriculture détaché au sein du GNIS pour assurer la direction du SOC. Donc en faite on peut dire que le GNIS est bicéphale: Il y a une partie purement interprofessionnelle avec un directeur du GNIS, et une partie en charge au sein du GNIS d'assurer la mise en œuvre des fonctions réglementaires, qui est sous l'autorité d'un fonctionnaire détaché du ministère de l'agriculture. Après quand on se trouve en région, moi je suis en charge d'animer une équipe de personnes qui peuvent avoir les casquettes." [PIA-170216p]

[xviii] "Et puis, j'ai envoyé un mail aussi sur les quantités, en disant au GNIS: Ben voyez, j'ai fait une étude sur les productions de l'automne 2013 en haricot - c'est surtout sur le haricot qu'il y avait un peu des problèmes, ou des risques de problèmes - je leur ai dit: Vous voyez, notre lot moyen, il fait... 25 kg, je crois. Est-ce que vous considérez vraiment que le réglementation est adaptée pour des lots de 25 kg? Sachant qu'il faut 3 kg, ou 4, d'échantillon pour faire une analyse de teneur... enfin, de présence ou d'absence de la graisse." [CRO-190515d]

[xix] "Attend, il y a une grosse boîte qui produit des semences en France. C'est Limagrain-Clause Tézier-Vilmorin, c'est la même boîte. Elle représente 80 ou 90% du marché, je ne sais pas. Mais c'est lui qui fait tout le fric au GNIS. Le président du GNIS, je ne sais même plus comment il s'appelle, on s'en fout, d'ailleurs, mais il est à la botte de Clause Tézier -Vilmorin. Du coup, la partialité de ça... Et en plus, ils ont une délégation gouvernementale, ministérielle. Donc, ils ont un pouvoir... C'est un syndicat corporatiste comme on n'en fait plus." [CRO-260814b]

[xx] "Donc, nous on est les seuls à n'avoir strictement rien fait... En plus on est même pas relancé, là, pour le moment. Ca fait un an qu'on a plus de contact. [...] On a déclaré les parcelles. C'est à dire l'an passé, donc, la seule mesure qu'on a prise, c'est de déclarer les parcelles... l'air de dire: 'De toute façon, nous, on a rien à cacher. Si vous voulez aller les voir, vous allez les voir, mais nous on ne signera pas de contrat, on ne veut rien payer' [...] J'ai dit à la délégation régionale: 'De toute façon, vous anticipez la réglementation européenne.' Puisque la réglementation européenne, elle doit être publiée... elle n'est toujours pas publiée à ma connaissance. Je dis: 'Tant que le réglementation européenne n'est pas publiée, je ne vois pas pourquoi je me plierai à votre réglementation.'" [CRO-190515d]

[xxixxi] "C'est là où je comprends que les petits faiseurs, qui font déjà pas beaucoup de graines... et qu'une analyse, c'est tant de graines et c'est tant d'argent... c'est pas possible. Donc c'est là ou il faudrait qu'on puisse être unanime - parce que des petits faiseurs bio, il y en a pleins, et pas qu'en France. Donc il faut aussi prendre en compte leur façon de produire et qu'on puisse, ben, peut-être faire évoluer en disant: C'est un inspection en culture. Et ça peut répondre et éviter de consommer de la graine pour rien, parce que je comprends que c'est pas possible de faire une analyse sur...voilà. C'est juste... mais voilà, la réglementation est là, et c'est là ou il y a un intérêt que le SOC puisse faire... relayer l'information et que l'interprofession se bouge pour quelque chose." [PIA-111215c]

[xxii] "Pour moi, [un artisan semencier] qui va... faire une forme de sélection sanitaire, je ne l'admets pas pour autant. [...] Une production de semence où on aurait un rang de haricot parce qu'on a... parce qu'en faite on fait une sélection conservatrice et qu'on considère qu'on va garder les plants qui résistent le mieux, mais que pour le coup dans ces résistances on aurait de la graisse qui se serait introduit... Ca peut être vrai pour n'importe qu'elle autre maladie, peu importe, c'est vrai que ça peut être une sélection qui est défendable. Moi je n'ai pas de position là dessus, je ne suis pas en production, donc... si je devais choisir, je ne saurais pas laquelle... mais c'est certainement très intéressant. Mais si on est face à un parasite qui est réglementé, je pense qu'on doit... on doit prendre des mesures face à ça et pas laisser cette sélection se faire." [PIA-111215c]

[xxiii] ".. avec les Croqueurs, on avait dit que ce qu'il faudrait, c'est leur dire qu'on a une autre vision de la maladie des plantes, ce que tu disais tout à l'heure... et qu'il faudrait dire, ben, que cette vision-là n'est pas prise en compte dans la réglementation, qu'on réclame un réglementation adaptée. Mais là-dessus, on a pas travaillé, donc on a pas avancé. Mais c'était la seule piste qu'on envisageait sérieusement. [...] Qui serait plus long-terme, et puis qui pourrait éventuellement être une réflexion de fond au niveau de l'ITAB, par exemple, de - vu qu'on a une commission semences [...]- ce serait de dire: Bon, ben, face à la réglementation pour le phytosanitaire sur les semences, quelle ligne on défend en bio, quoi? Réclamer des adaptations à la réglementation. Nous on a en faite toujours deux... deux bases de revendication. C'est la petite taille, qui n'a rien à voir avec le bio, hein! Le faite qu'on soit en tailles très artisanales, euh, fait qu'on est pas sur la même planète que les grosses entreprises. On est 100 ou 1000 fois plus petits. Donc, c'est quand même à prendre en compte. Et la deuxième chose, c'est que, étant en bio, on a aussi un point de vue différent. Donc ça, c'est... on s'appuie toujours sur ces deux piliers, en quelque sorte, pour justifier qu'on est pas d'accord avec ce qu'on cherche à nous imposer. [...] ça forme un tout, qui est, effectivement, la semence paysanne, l'agriculture diversifiée, etc. Parce qu'on pourrait être gros, et en bio, ou petit et en conventionnel. Et là, on cumule deux choses. Le fait est que j'appuie toujours mon argumentation, quand j'ai à discuter avec ces gens-là, en prenant en compte ces deux aspects-là. Il y a un cumul des deux." [CRO-190515d]

[xxiv] "... quand à un moment donné le concept de semence paysanne à émergé, nous on a voulu essayer de savoir, finalement, ça représente quoi? Mais incapables de trouver une donnée fiable qui nous dise: les semences paysannes, ça représente tant d'ha ou tant d'agriculteurs... [...] [les demandes] sont légitimes, mais qu'est-ce que ça représente réellement par rapport à l'agriculture biologique? Parce que, effectivement, est-ce qu'on entend pas que ceux qui s'expriment dans l'affaire? C'est vrai que... c'est un peu comme en apiculture, il y a une atomisation des structures, souvent ils se bouffent le nez entre eux, et finalement, on ne sait pas là dedans ou est la tendance majoritaire et qui, finalement... vers qui il faut aller. Je ne dis pas que les demandes ne sont pas légitimes, qu'on soit bien d'accord! Quelqu'un qui veut par philosophie avoir de semences bio de telle variété, c'est son droit, mais..." [PIA-170216p]

Chapter 4: ***In*-situ plant health explored in a field experiment**

In Chapter II, the evolution of the research device has been described. I have traced how the experimental setup of the field trials was modified each time it encountered resistance and how it was complemented by a social science approach. In Chapter III, results obtained by the social approach have been discussed. The plant health governance practices of artisanal seed companies have been unfolded, revealing the ways in which they are embedded in the triangulation of bean populations, growing environments and seed grower. The health of a bean population is judged upon in its growing environment, i.e. in the fields. Adaptability of plants to their local growing environment and the interactions of plants with the microbial communities of that environment are cornerstones of this approach to plant health. The "biological base" of the *in situ* approach to plant health is addressed in the following chapters. Building on the two previous chapters, results obtained in a field experiment shall be discussed in order to address the question: **Can the analyses of some plant-environment interactions reveal the biological base of the artisanal seed companies' collective bean health management?**

The present chapter presents the design of the field experiment, which was set up to study some ecological interactions between bean plants and their growing environments. Then, the overall question is broken down into the following sub-questions, each addressed in a separate chapter:

(i) How does the general health of bean plants evolve when beans are multiplied on organic farms from bean seeds provided by the *Croqueurs*? This question is linked to another, methodological one: How can *in-situ* plant health in farmers' fields be accounted for? (Chapter V)

(ii) Is the health status of bean plants correlated with their ability to interact with mycorrhizal fungi and *Rhizobia spp.*, which are both beneficial symbiotic soil microorganisms? Do bean varieties differ in their ability to interact with these symbiotic microorganisms? (Chapter VI)

(iii) Are microbial communities associated with bean seeds determined by the variety or by the growing environment? In other words: Do these communities reveal the bean variety, or the environment in which the seed was grown? (Chapter VII)

(iv) After three years of seed multiplication in contrasting growing environments, do phenotypic traits and genetic markers indicate local adaptation? In other words, do phenotypic traits and genetic markers indicate that varieties have begun to adapt to local growing environments and evolve into distinct "lineages"? (Chapter VIII)

1 Experimental design

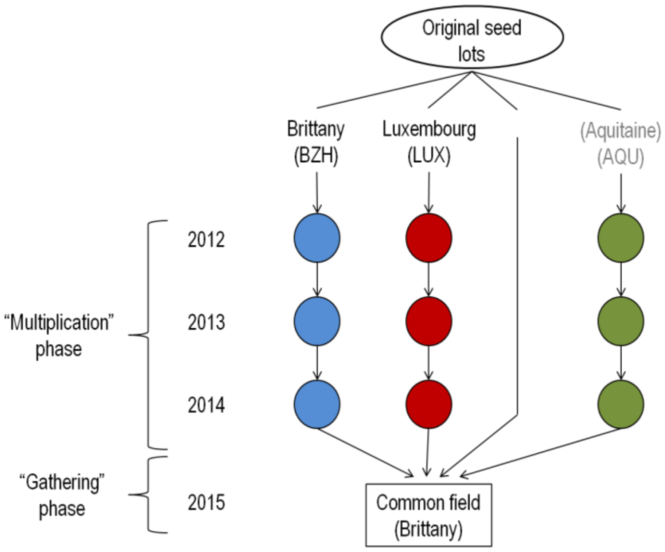

Figure 4.1: Experimental design. Original seed lots of the varieties 'Calima', 'Flageolet Chevrier', 'Rognon de Coq', 'Roi des Belges' and 'St. Esprit' were dispatched to at least two experimental sites located on organic farms. Three varieties were also multiplied in Aquitaine.

The trials comprised two phases. In the "multiplication phase", the five varieties were multiplied in two sites for three years, from 2012 to 2014, creating what has been termed 'versions' of the varieties. A 'version' is a given variety multiplied on a given site (Serpolay et al., 2011). Two sites harboured all five varieties and are considered the "core trials". Some varieties were also multiplied and observed in AQU to complement the "core trials". The fourth year (2015), in the "gathering phase", the versions of a variety obtained in the field trials were grown side by side in one environment to observe phenotypic and genetic divergence between versions of a given variety. In the following, the bean varieties included in the fields trials are briefly described (subsection 1.1). The "multiplication phase" (subsection 1.2) and the "gathering phase" (subsection 1.3) will then be specified. Materials and methods specific to each chapter are specified in the respective chapters that follow.

1.1 Varieties

The varieties included in the field trials are all bush beans with a determinate growth habit. Four varieties were chosen among the varieties produced on a regular basis by two *Croqueurs* members participating in the research project. Two varieties, 'Flageolet Chevrier' (flc) and 'Rognon de Coq' (rdc) were chosen from a producer in the Aquitaine region in South-Western France (AQU) and two, 'Roi des Belges' (rdb) and 'St. Esprit à œil rouge' (ses), from a producer in Luxembourg (LUX). All four are old varieties. The oldest (ses) was first mentioned by Vilmorin-Andrieux in 1855. The most recent variety (rdb) was bred in Belgium in the first half of the 20th century (Vilmorin-Andrieux SA, 1947). They were selected for the trial to represent a range in uses (green and shelling beans), earliness and tolerance to diseases, according to the producers' experience. In addition, the variety 'Calima' (cal) was purchased from a large-scale seed producer as commercial control. A more detailed description of the five bean varieties is given in Annex 4. Note that the initial seed of 'cal' was provided by the breeding company with a seed treatment[38]. Apart from the initial seed lot of this variety, none of the seeds sown in the experiment were treated.

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[38] I had ordered untreated seed and became aware of the seed treatment (colorless, and not indicated on the seed bag) only well after the sowing of the experiment. Given a company secret on this seed treatment, the

1.2 Multiplication phase

The five varieties were multiplied in two experimental sites during three years, from 2012 to 2014. Each year, the preceding year's harvest was sown on each site. Farms hosting all five varieties were located in Brittany, France (BZH) and in Luxembourg (LUX). The latter contributed two of the varieties included in the trial. BZH and LUX constitute the "core trials". In 2012 and 2013, the plants were grown in complete randomised blocks in three replications. Plants were spaced 75 cm between rows and 10 cm within rows. In 2014, they were grown without replications and sown mechanically according to the local seed growers' practices (distance of approximately 5 cm within rows). The total plot surface for each variety ranged from 8.4 to 12.0 m^2, with an average of $11m^2$. In addition, three varieties (flc, rdb and rdc) were also multiplied and observed in AQU to complement the "core trials". A description of the three experimental sites is given in Annex 5. All farms hosting the field trials were organic and engaged in the small-scale production of bean seed.

A variety multiplied in a given site over three years has been termed a "version" of this variety (Serpolay et al., 2011). Each version is designated by the name of the variety followed by the name of the site. In addition, the original seed lots with which the field trials were initiated in 2012 are also considered a version of each variety. These versions are identified by the code "ORI" for "original". The versions available for each variety and the end of the "multiplication phase" are recapped in Table 4.1.

Table 4.1: Versions available at the end of the multiplication phase. The thousand-seed weight (TSW) for each version is specified in grams (g).

Variety	Site/Origin	Version	TSW (g)	Comment
'Calima'	Original seed lot	**cal_ORI**	250	Harvested in Africa in 2010; seed treated with an unkown organic treatment
	Brittany	**cal_BZH**	278	
	Luxembourg	**cal_LUX**	301	
'Flageolet Chevrier'	Original seed lot	**flc_ORI**	235	Harvested in AQU in 2011
	Brittany	**flc_BZH**	244	
	Luxembourg	**flc_LUX**	256	
	Aquitaine	**flc_AQU**	244	
'Rognon de Coq'	Original seed lot	**rdc_ORI**	340	Harvested in AQU in 2011
	Brittany	**rdc_BZH**	415	
	Luxembourg	**rdc_LUX**	452	
	Aquitaine	**rdc_AQU**	439	
'Roi des Belges'	Original seed lot	**rdb_ORI**	442	Harvested in LUX in 2011
	Brittany	**rdb_BZH**	320	
	Luxembourg	**rdb_LUX**	376	
	Aquitaine	**rdb_AQU**	322	Multiplied two years only: 2012, 2014
'St. Esprit à œil rouge'	Original seed lot	**ses_ORI**	884	Harvested in LUX in 2011
	Brittany	**ses_BZH**	682	
	Luxembourg	**ses_LUX**	656	

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breeding company was unable to give me more information than ensuring that it is compatible with organic farming.

1.3 Gathering phase

In the aim of observing phenotypic and genetic divergence between versions of a given variety, the versions of all varieties were grown side by side in one environment in the "gathering phase" in 2015. This approach is inspired from 'common garden experiments' commonly practiced by ecologists (Rutter and Fenster, 2007) to study the local adaptation of plants (Bradshaw, 1984; Galloway and Fenster, 2000; Joshi et al., 2001). This second phase of the field trials was conducted on the farm in BZH (see Figure 4.1 and Annex 6). BZH was selected, because it had most favourable conditions for bean cultivation. The beans were grown in a split-plot design with bean variety as whole plot factor and version as subplot factor. The trial was sown mid-May 2015. Each subplot consisted of two rows of bean plants sown at 0.75 m distance from each other and 2.5 m long (total of 20 plants, 1.9 m^2). To ease the identification and observation of individual plants, plants were spaced by 0.25 m within rows. To compensate for germination rates and ensure that a bean plant grow at each position, two seeds were sown per position. Due to low germination rates in preliminary tests, four seeds per position were sown for the variety 'flc'. At 20 days after sowing (das), seedlings were thinned by leaving only the seedling positioned at the extreme left facing the experimenter. A plan of the plots and subplots is shown in Annex 7. The experimental design, including both phases, is illustrated in Figure 4.1.

2 Observation of plant traits

In both the multiplication and the gathering phases, morphological, phenological and agronomical plant traits were observed for each bean variety. Not all traits were observed in each site and year. Sampling procedures and missing data are specified in the following chapters. Observations of plant phenotypes were adapted from IPGRI (1982), Schoonhoven *et al.* (1987) and CIAT (1993). An overview of common bean growth stages is given in Annex 10.

2.1 Morphological traits

The length of the main stem was measured from the soil to the tip of the last flower or pod between flowering (R2) and physiological maturity (R7). Leaflet length was measured on the terminal leaflet of the third trifoliate leaf from pulvinus to leaf tip.

2.2 Phenological traits

Flowering date was recorded at the first open flower. At the end of the growth cycle, maturity on a given date was scored using 1 = 'plant green and most pods green, far from harvest maturity', 2 = 'plant and most pods yellowing, close to harvest maturity' and 3 = 'most pods dry, harvest maturity'.

2.3 Plant health

When assessing plant health in (uncontrolled) field environments, symptoms observed on plants do not suffice to establish an univocal disease diagnosis. Different causes may elicit similar symptoms which are easily confounded. For example, viral diseases such as Bean Common Mosaic Virus (BCMV) and Bean Yellow Mosaic Virus Plant (BYMV) typically lead to leaf mosaic (mosaic) and blistering (blist). However, similar leaf discolouration and malformation may be caused by nutrient deficiencies and leaf-sucking pests, respectively. Blight spots (blight.leaf) caused on leaves by CBB and HBB are visually hardly distinguishable. Dark lesions on stems and leaf veins (brown.vein) can be caused by BCMV under specific environmental conditions - a phloem necrosis called "black root" - but systemic necrosis may also be due to bacterial pathogens (Hall, 2005). White-silvery spots left on leaves by leaf-sucking and pests (feeding) cannot easily be attributed to a single species. Among the most important pests on beans are aphids, which are vectors for several viral diseases, but also indicate over-supply of N. As for leaves, the symptoms of CBB and HBB on pods are very difficult to distinguish.

Bean health was assessed on a symptom basis in the field. All of the symptoms named above were scored on a scale from 1 to 5, specified in Table 4.2. Examples are given for leaf mosaic and blistering in Annex 8. In addition, overall plant vigour was scored from 1 to 5 to reflect the general impression of the plant. The vigour score is influenced by the biomass of the plant as well as general health status (Table 4.2).

Table 4.2: Scales from 1 to 5 used for scoring disease symptoms and overall plant vigour

Score	... for symptom scores	... for plant vigour scores
1	no symptom	very poor vigour
2	Doubtful to weak symptom expression	poor vigour
3	Moderate to intermediate symptom expression	intermediate vigour
4	Intense symptom expression	vigourous
5	Severe symptom expression or plant death	very vigourous

2.4 Yield components

The number of pods, empty pods and seeds produced per plant were counted on a minimum of 10 plants per variety and site. 1000-seed weight was assessed.

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Chapter 5: **Evolution of general plant health**

> "In the crop, well, it's an agronomical approach, that is, the soil, the climate, the plant and I - because I form part of it, he! And those four or five of us - I can't remember - must find a compromise that is the least poor, or the best, for the three or the five, well... the weather doesn't care at all, the soil cares a bit more, the plant is really concerned and I am, too. So, the two major actors are the plant and I. We try to make the soil our ally and the weather, we have to live with it..." [CRO-290116d][39]

I have termed plant health management as it is practiced by *Croqueurs* members *in situ* management (Chapter III). The term *in situ* reflects the idea that plant health is observed and judged upon in farmers' fields. In this approach, crops as they appear to farmers in their growing environment are decisive, more so than the results of seed analyses. The effects of pathogenic microorganisms on crops are considered in their interaction with the plants' physical and living environment. The living environment includes microorganisms associated with the crop, many of them are beneficial, some potentially pathogenic. The term 'general health' is used to describe the overall health status of a crop, taking into account various stress factors - biotic or abiotic - endured by the crop. In this chapter, an attempt is made to describe the general health of the bean seed crops under field conditions.

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[39] "En culture, et ben, c'est une approche agronomique, c'est à dire sol, climat, la plante et moi - parce que j'en fais partie, hein! Et il faut qu'on arrive, tous les 4 ou 5 là, je ne sais plus, à trouver un compromis qui fait le moins mauvais, ou le meilleur, pour les 3 ou les 5, enfin - le temps il s'en fout, le sol il s'en fout moins, la plante, elle est vraiment concernée et moi aussi. Donc les gros acteurs c'est la plante et moi, le sol on essaie d'en faire notre allié, et puis le temps on fait avec..." [BR290116d]

1 Introduction

When observing plant health *in situ* - in farmers' fields or in on-farm field trials - crops can be confronted with several plant pathogens during their growth cycle, often at the same time. Interactions between different common bean diseases, soil-borne or aerial, have been investigated under controlled conditions (Paula Júnior et al., 2015 and references therein). Interactions between plant diseases can go different ways and depend on pathogen inoculum levels, inoculation timing and general growing conditions. For example, Rhizoctonia root rot (caused by *Rhizoctonia solani*) has been shown to magnify the severity of anthracnose (*Colletotrichum lindemuthianum*), but decrease severity of rust (*Uromyces appendiculatus*) (Paula Júnior et al., 2015). Under tropical conditions, the severity of Rhizoctonia root rot can be increased when plants are stressed by other plant diseases (Abawi and Pastor-Corrales, 1990). Antagonistic effects between rust and the nematode *Meloidogyne incognita* lead rust severity to be lower on bean plants attacked by the nematode (Bookbinder and Bloom, 1980). On a more general note, it has been shown that root, stem and stalk rots caused by less specialised fungi or nematodes are commonly more severe on plants infected by viral pathogens (Beute and Lockwood, 1968; Pratt et al., 1982; Chant and Gbaja, 1986, to name only researches on legumes). Concerning the interaction of aerial diseases, the interaction between rust and Halo Bacterial Blight (HBB) have been found to be dependent on disease levels: While HBB was increased in the presence of *U. appendiculatus*, heavy rust infection suppressed the occurrence of HBB lesions (Yarwood, 1969). In short, effects of interactions between root and aerial diseases cannot be anticipated. On one hand, the effect of one disease can weaken plants and predispose them for the infection with other diseases. On the other hand, antagonism among pathogens infecting plants simultaneously can be explained by competition, antibiosis and induced host plant resistance (Paula Júnior et al., 2010). Salicylic acid, jasmonates and ethylene figure among plant hormones regulating plant defence responses that may be involved in such interactions (Paula Júnior et al., 2015).

In addition, abiotic stress, such as nutrient deficiencies, can affect plant defences and thereby increase susceptibility to diseases. This is also an important mechanism by which beneficial soil microorganisms improve plant defences: by improving plant nutrition and promoting plant growth, mycorrhiza, rhizobia and some rhizobacteria improve plant health (Alström, 1991; Berendsen et al., 2012). Beyond nutritional status, soil microorganisms interacting with plant roots can suppress soil-borne diseases by competition for nutrients, niche exclusion, induced systemic resistance, and the production of antifungal metabolites (Bais et al., 2006). Moreover, many beneficial soil-borne microorganisms have been found to systemically boost the defensive capacity of the plant, priming the plant immune system for accelerated activation of defence (Avis et al., 2008; Van der Ent et al., 2009; Berg, 2009).

Organic farming practices lead to 'microbially driven systems' which affect micronutrient supplies, plant resistance and the balance of pathogenic and beneficial microorganisms in the soil (van Bruggen et al., 2016). Numerous interactions of crops with their physical and living environment imply that measuring only one type of symptom or one plant disease can be insufficient to reflect the general health status of a crop. In view of these interactions and of *in-situ* plant health management (see Chapter III), this chapter addresses the question: **how does the general health of bean plants evolve when multiplied on organic farms from bean seeds provided by the *Croqueurs*?** This question comprises another, methodological question: **how to account for *in-situ* plant health in farmers' fields?**

Concerning the second, methodological question, the idea of developing an *index of general plant health* emerged during in the course of the PhD research project (Chapter II, subsection 3.3). An attempt was made to construct such an index, but was quickly abandoned. In the 1970s, Torrance (1976) summarised attempts to develop indices of general human health in a general mathematical formulation, which could have served as model for this general plant health index. In this context, health is described as "a continuum running from dead at the one extreme" - which can be mathematically expressed as "0" - and "perfect health at the other extreme" - expressed as "1". While death is not so difficult to define and determine, defining "perfect health" is another matter. General health indices in very different domains express health as "level of functioning of an individual" (Torrance, 1976). For example, general health indices have been developed for humans (Grogono and Woodgate, 1971), for dairy sires (Boettcher et al., 1998) and oceans (Halpern et al., 2012). Focussing on the "level of functioning" of an individual allows translating health indices into economic values. However, Döring *et al.* (2012) have shown that reasoning plant health in terms of functionality constitutes only one approach to plant health among many others (see also Table II.1 in Chapter II). The following example put forward by these authors illustrates why a general plant health index based on functionality would not be appropriate to reflect plant health as it is regarded and managed by the *Croqueurs*.

> "For example, should plants be regarded as healthy when they have been sprayed with fungicides that kill the pathogens? Although the spraying would result in freedom from fungal infections and the sprayed plants would then be fully functional (e.g. in terms of photosynthesis, growth or reproduction), they would otherwise easily be infected by fungal pathogens and would fail in their functionality. A functionalist view would regard the sprayed plants as healthy. On the other hand, it could be argued that the plants are only healthy ephemerally, and that true health must be longer lasting, independent from the application of fungicides. The alternative view therefore demands that health requires a degree of resilience, i.e. the ability to maintain functionality in the face of stress." (Döring et al., 2012)

This example illustrates that reasoning in terms of functionality alone is not compatible with an approach in which plant health is understood as the result of a plant's interactions with its growing environment, *including* plant pathogens. In more general terms, expressing general plant health in a single, numerical index necessarily assumes that one concept of plant health be adopted at the expense of others. The initial idea of a general plant health index was therefore abandoned in the aim of reflecting general plant health in a way that leaves more room for different stances on plant health when interpreting the data.

2 Materials and Methods

A total of five bean varieties were multiplied on three organic farms from 2012 to 2014 ('multiplication phase', described in Chapter IV, subsection 1.2). Organic seed of four bean varieties were initially supplied by two artisanal seed companies among the *Croqueurs* and one variety was obtained as conventional seed from a larger scale conventional breeding company (see subsection 1.1 of Chapter IV and Annex 4).

Chapter IV specifies the experimental design and the methods by which field data were collected. For observations of plants in the field, 10 consecutive plants were observed in each subplot (in total 30 plants per variety and site) in 2012. The two following years, 20 random plants were observed per subplot, totalling 60 plants per variety and site in 2013 and 20 in 2014. Plant symptoms and vigour were scored as described in division 2.3 of Chapter IV. Each year at harvest, 10 plants were sampled per subplot to determine yield components. Seeds produced per plant were counted as described in division 2.4 of Chapter IV.

2.1 Statistical analyses of traits concerning plant health

In the aim of accounting for bean health on organic farms in the 'multiplication phase' over three years, several types of data were analysed by different methods. The different data analysed and methods employed allow to <u>report on plant health (i) at different geographical and time scales and (ii) in a form that leaves room for interpretation according to a range of approaches to plant health</u>. Each combination of location and year (i.e. the location*year interaction) was considered an 'environment'. An abbreviation comprising the location code (as in Chapter IV, subsection 1.2) and the two last numbers of the year designates each environment, e.g. 'Aquitaine 2012' is 'AQU:12'. Statistics were computed using the programming language and software environment R version 3.3.0 (R Development Core Team, 2016).

Firstly, multivariate analysis in the form of <u>Multiple Correspondence Analysis (MCA) was conducted on scores of symptoms</u> observed on bean leaves and stems. All environments and observation dates were included. As the numbers of observations differed strongly between sites and years, the dataset is unbalanced and some environments may influence the analysis more than others. Nevertheless, MCA may point to trends concerning plant health of the bean varieties in different environments. MCA was conducted using the R package 'FactoMineR' (Lê et al., 2008), with five types of symptom scores as active variables: (i) leaf mosaic ('mosaic'), (ii) leaf blistering ('blist'), (iii) blight spots on leaves ('blight.leaf'), (iv) brown lesions on stems and leaf veins ('brown.vein') and (v) spots left by leaf-sucking pests ('feeding'). Four factors were included as supplementary qualitative variables: variety ('var') and environment ('env'), as well as location ('loc') and year separately. Date of observation in days after sowing ('das') was included as supplementary quantitative variable. Before running the MCA, missing data were imputed with 'missMDA' package (Husson and Josse, 2016) using 5 dimensions. To examine whether the coordinates of individuals were significantly explained by categorical variables, a 1-way ANOVA was computed for each dimension, followed by F-tests to see whether the variable has an influence on the dimension and category by category t-tests (command 'dimdesc'). Blight symptoms on pods ('blight.pod') were not included in the MCA, as they were scored later in the season and scores were not paired with scores of earlier symptoms. For this symptom, a non-parametric, rank-based model was fitted with 'env', 'var' and their interaction as explanatory variables. Data was derived from one observation date per environment. Overall treatment effects were tested by rank-based ANOVA-type statistic with the R package 'rankFD' and relative treatment effects compared between treatment levels (Shah and Madden, 2004; Konietschke et al., 2016).

Secondly, leaf mosaic and blight symptoms were assessed in further detail for environment LUX:13. Local climatic conditions (see Annexes 5 and 6) and experience gained during the experiment indicate that the site LUX is favourable to the development of blight and virus-like symptoms. LUX:13 is also the environment for which most observations have been realised in terms of observation dates and plant numbers. Symptoms were scored at four growth stages (see Annex 10): V2 (2 trifoliate leaves), Vn (just before flowering), R2 (flowering) and R7 (seeds are filled, pods begin to yellow). Rank-based models were fitted to test for the overall effects of 'rep' and 'var' on leaf mosaic and blight symptom scores at each growth stage. Relative treatment effects were compared between varieties at each growth stage.

Thirdly, overall vigour was scored on individual plants around flowering growth stage (see division 2.3 of Chapter IV) in two locations and in three years. The score reflects the observer's general impression of the plant and is influenced by the biomass of the plant as well as general health status. As described for the 'pod blight' symptom above, the vigour scores were analysed with a rank-based model.

Lastly, the number of seeds produced per plant was taken as an indicator of Darwinian plant fitness (Kulheim et al., 2002). Looking at Darwinian fitness corresponds to a 'functionalist' approach to plant health (Döring et al., 2012). Given an over-dispersed count outcome variable, a negative binomial model (theta = 2.676) was fitted as follows, using the 'pscl' R package (Jackman, 2015):

$y_{ij} = \mu + \text{environment}_i + \text{variety}_j + \text{environment}_i \times \text{variety}_j + \varepsilon_{ij}$.

Overall effects of predictors where tested by sequentially adding predictors to the model and comparing models by means of likelihood ratio tests (Zeileis et al., 2015). The model was then used to predict mean seed counts and their 95% confidence intervals for each variety in each environment.

2.2 Bacteriological and virological analyses of seeds and leaves

Seed lots underwent bacteriological analyses for the detection of blight agents and determination of contamination rates. A subsample of 1000 seeds, or less according to availability, was tested for each seed lot. If tested negative, a second, equivalent seed subsample was analysed to confirm the absence of bacterial blight agents. If the second subsample resulted positive, contamination rates *p* were calculated with the formula proposed by Maury *et al*. (1985), letting Y be the number of healthy subsamples among the N subsamples analysed and n the subsample size (number of seeds per subsample):

$$p = 1 - \left(\frac{Y}{N}\right)^{\frac{1}{n}}$$

If the first subsample was positive with blight agents, a set of decreasing subsamples was analysed in order to estimate contamination rates and 95% confidence intervals according to Swaroop (1951). Seed-water extracts were prepared by soaking seed samples overnight in approximately 2 × TSW (g) of sterile water. Aliquots (0.1 ml) of the seed extracts were plated in triplicate onto semi-selective media. Milk-Tween and TSA 10% media were used for the detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomons fuscans* pv. *fuscans* (Xap/Xff), the agents of Common Bacterial Blight (CBB). Modified Sucrose Peptone was used for detection of *Pseudomonas syringae* pv. *phaseolicola* (Psp), agent of Halo Bacterial Blight (HBB). Suspensions of typical bacterial colonies at 10^6 CFU ml^{-1} were identified by PCR-amplification assays. Primer X4e (5'-CGCCGGAAGCACGATCCTCGAAG-3') was paired with primer X4c (5'-GGCAACACCCGATCCCfAAACAGG3') for the detection of Xap/Xff (Audy et al., 1996). PCR reactions were performed in an Applied Biosystems 2720 Thermal Cycler in a volume of 20 µl containing 5 µl of bacterial suspension in 4 µl of Go-Tag Buffer 5X (Promega), 160 µM dNTP, 0.5 µM each of upstream and downstream primer and 0.08 units of Go-Taq polymerase (Promega), with the following thermal profile: 94°C for 5 min, followed by 35 cycles of 94°C for 1 min and 72°C for 2 min and 10 min at 72°C in the final extension. Agarose electrophoresis (1.2%) revealed a 730 bp DNA fragment for a Xap/Xff isolate. Primer PHA19 (5'CGTCTGTAACCAGTTGATCC3') was paired with primer PHA95 (5'GAATCCTTGAATGCGAAGGC3') for the detection of Psp (Marques et al., 2000). The same thermal cycler and reaction mixture composition were used with a thermal profile as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 52°C for 45 sec and 72°C for 1 min and 7 min at 72°C in the final extension. Agarose electrophoresis (1.2%) revealed a 480 bp DNA fragment for a Psp isolate. Negative and positive controls of the PCR reaction were systematically run on sterile distilled water and a suspension of CFBP4834-R (Xff) or CFBP531 (Psp) at 10^6 CFU ml^{-1}, respectively.

In addition, leaf samples with blight symptoms were collected in LUX and AQU in 2013 for bacteriological analysis, as well as in the gathering phase in 2015 (Chapter IV, subsection 1.3). Leaf pieces were lacerated in sterile water to obtain extracts and plated on the same media as the seed extracts. Bacterial colonies suspected as Psp were confirmed by testing for fluorescence under UV light (Taylor, 1970). Colonies suspected as Xap/Xff were tested by PCR-amplification assays as for seeds. No virus analyses were conducted in the 'multiplication phase', but leaves of varieties 'rdb', 'rdc' and 'ses' grown from seeds from the three multiplication sites (AQU, BZH, LUX) were sent to the phytodiagnostic laboratory of Vegepolys *Maison du Végétal* in Angers for analyses for *potyvirus* in 2015 to confirm that the experimentators were correctly identifying viral diseases. Samples from three plants per variety and site were analysed for varieties 'rdc' and 'ses', which were the varieties showing symptoms most frequently. For 'rdb', only one sample was taken from the population coming from BZH, due to low symptom occurance in this variety. After visual inspection, samples underwent culture on selective media and identification of potyvirus by ELISA test (Porcher, 2015). The 'Poty' reagent set from the manufacturer Agdia were used for detection in a Antigen Coated Plate - ELISA. Positive and negative controls were run on infected and non-infected samples internal to the laboratory, as well as an additional negative control consisting of the extraction buffer alone. Thresholds to separate negative from undetermined samples and undetermined from positive samples were determined from the average absorbance values of non-infected samples according to the method MOA 008 (DGAL/SDQPV, 2010).

3 Results

3.1 Multivariate analysis of disease symptoms

Data available for the MCA for each environment and observation date are summarised in Annex 11. Between 100 (20 per variety) and 300 (60 per variety) plants were scored per observation date. Plants were scored on 1 to 4 observation dates per environment. Between varieties, data was balanced. No data was collected in AQU in 2014.

The first three dimensions of the MCA account for 8.6%, 7.6% and 7.2% of variance, respectively. 23.5 % of the variation in the score data is thus represented on these three dimensions. The graphs of variables for dimensions 1, 2 and 3 are shown in Figure 5.1. Variables 'mosaic' and 'blist', which are typical symptoms of viral diseases, are much linked to both the first (correlation ratios η=0.68 and η =0.64, respectively) and the second dimension (η =0.57 and η =0.49). Variables 'brown.vein' and 'blight.leaf' are both symptoms which can be attributed to bacterial blights. They are linked to dimensions 1 (η =0.2 and η =0.12) and 2 (η =0.18 and η =0.2) to a lesser extent. They are however much linked to the third dimension (η =0.48 and η =0.42, respectively). The variable 'feeding' is at the centre of both plots, so this variable is not linked to any of the three dimensions. Among the supplementary variables, 'var' appears linked to the first dimension, whereas all the others are located at the centre of the plots.

The respective graphs of categories are shown in Figure 5.2 and allow to further characterise the dimensions. Dimension 1 opposes 'mosaic_5' and 'blist_5' on one hand and 'blight.leaf_5' and 'brown. vein_5' on the other; individuals with high coordinates on this dimension tend to have virus-like symptoms, but no bacterial blight symptoms. Dimension 2 is positively related to all these symptoms, individuals with high coordinates on dimension 2 tend to have both kinds of symptoms. Dimension 3, like dimension 1, again opposes 'mosaic_5' and 'blist_5' on one hand and 'blight.leaf_5' and 'brown. vein_5' on the other. However, in contrary of dimension 1, it is linked positively to blight symptoms and negatively to virus-like symptoms; individuals with high scores on dimension 3 tend to have bacterial blight symptoms, but no virus-like symptoms.

Individuals are plotted on the first three dimensions according to variety in Figure 5.3. Given that the dimensions are built upon nuances in few variables, interpretation is not easy. Most individuals are situated at the graph centre; they cannot be differentiated on the dimensions. However, variety 'ses' forms a somewhat distinguishable group with positive coordinates on dimensions 1 and 2 (graph A). To a lesser extent, variety 'rdc' forms part of the same group with 'ses'. This indicates that 'ses' and 'rdc' expressed leaf mosaic and blustering. Dimension 3 (graph B) seperates 'ses' and 'rdc' to some extent, with a tendency of 'rdc' to adopt higher values and 'ses' lower ones. Variety 'rdc' thus more strongly expressed blight spots on leaves and brown lesions on stems and leaf veins.

Variable 'das', i.e. the date of observation in days after sowing, was very weakly, but significantly correlated with dimensions 1 ($R=0.2$, $p < 0.0001$) and 2 ($R=0.14$, $p < 0.0001$), reflecting that symptoms were stronger later in the season. The MCA does not allow to distinguish environments, locations or years that favoured the appearance of symptoms (data not shown).

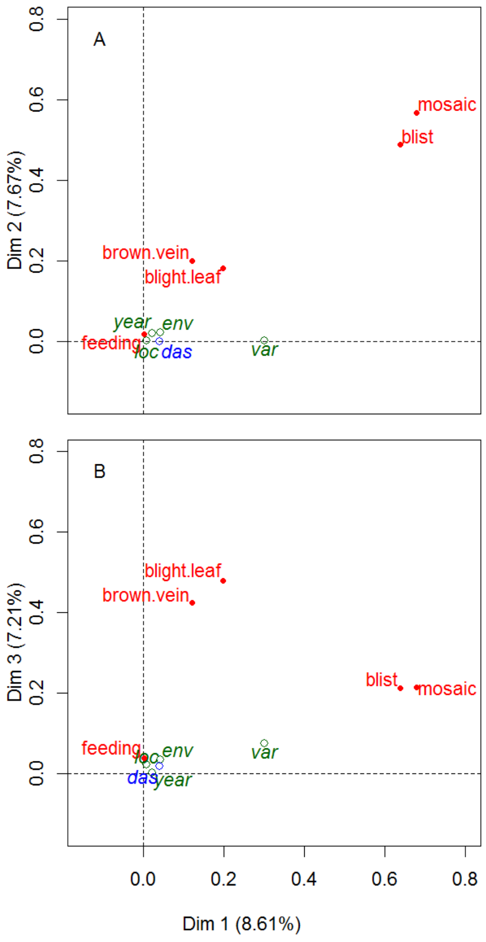


Figure 5.1: Graph of variables of the MCA on symptom scores for dimensions 1 and 2 (A) and 1 and 3 (B): active qualitative variables in red, supplementary qualitative variables in green and the supplementary quantitative variable in blue.

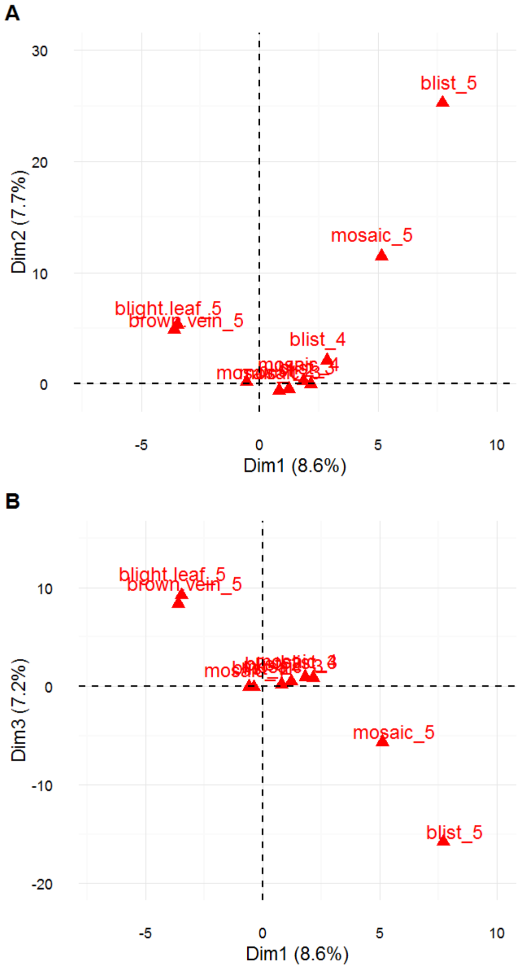


Figure 5.2: Graph of categories of the MCA on symptom scores for dimensions 1 and 2 (A) and 1 and 3 (B). The 10 most contributive categories are plotted.

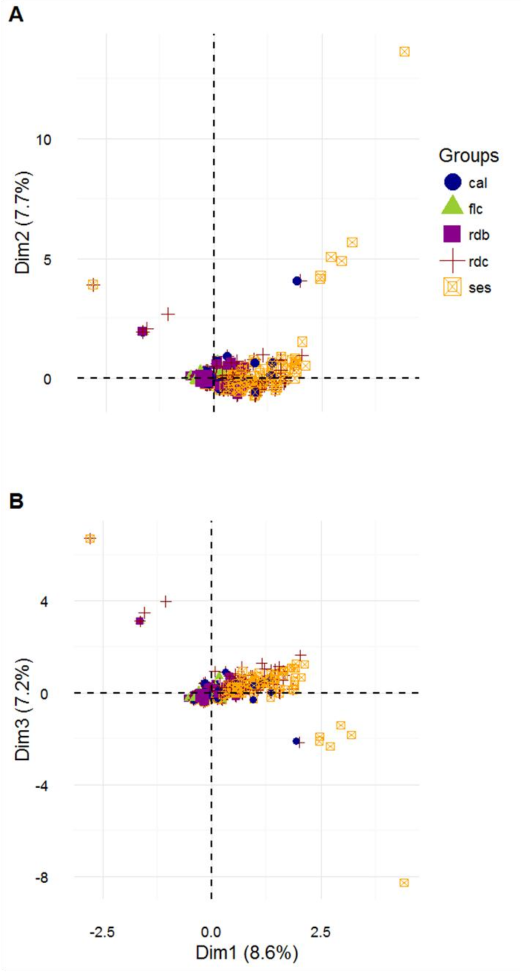


Figure 5.3: Graph of individuals of the MCA on symptom scores for dimensions 1 and 2 (A) and 1 and 3 (B). Individuals are plotted according to variety.

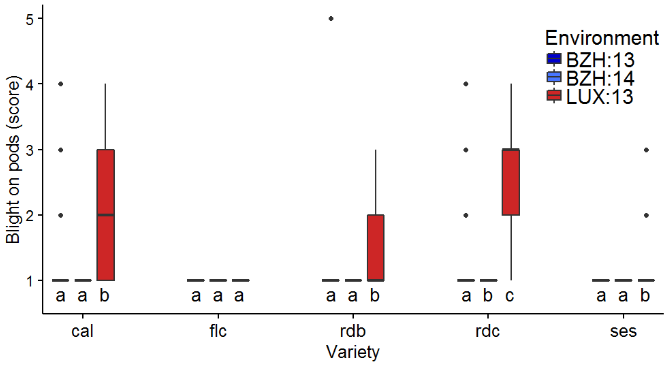


Figure 5.4: Quartiles of scores of blight symptoms on pods for each variety and environment. Within each variety, distributions with the same letter are not significantly different from each other at $p < 0.05$ according to rank-based pairwise comparisons.

Scores on blight symptoms on pods were only available for 3 environments: 300 plants were scored in BZH and LUX in 2013, respectively, and 100 plants in LUX in 2014. The number of observations was balanced between varieties. Rank-based ANOVA-type statistic resulted in a highly significant effect of variety (p<0.0001), environment (p<0.0001) and their interaction (p<0.0001) on pod symptom scores. Distributions and the results of pairwise comparisons are shown in Figure 5.4. Blight symptoms on pods mainly appeared in the environment LUX:13. Although not scored for individual plants, but only at the scale of the crop, symptoms also appeared on pods in LUX the following year (2014, data not shown). As indicated by the MCA concerning leaf symptoms (Figure 5.3), 'rdc' also expressed strongest blight symptoms on pods. Variety 'rdc' was also the only variety to have blight symptoms on pods in BZH:13 in a significant manner (Figure 5.4). In LUX:13, varieties 'cal' and 'rdb' also developed blight symptoms on pods.

3.2 Bacteriological and virological analyses

Although symptom scores give some indication of possible causal agents, disease diagnosis upon symptoms alone is difficult and uncertain. In particular, distinguishing between CBB and HBB is extremely difficult. Bacteriological seed analyses have identified only Psp, the causal agent of HBB, in all experimental sites and years (Table 5.1). Analyses of leaves confirmed that symptoms were due to Psp in LUX:13 (111 leaves positive with Psp out of 120 tested), but revealed both Psp (25 leaves positive out of 54 tested) and Xap/Xff (17 leaves positive out of 54 tested) in AQU:13.

No analyses for viral disease agents were conducted in the 'multiplication phase', but leaves of 'rdb', 'rdc' and 'ses' from various multiplication sites (AQU, BZH, LUX) were tested positive with *potyvirus* in the 'gathering phase' in 2015. This indicates the presence of BCMV (Bean Common Mosaic Virus), BYMV (Bean Yellow Mosaic Virus) and/or CYVV (Clover Yellow Vein Virus).

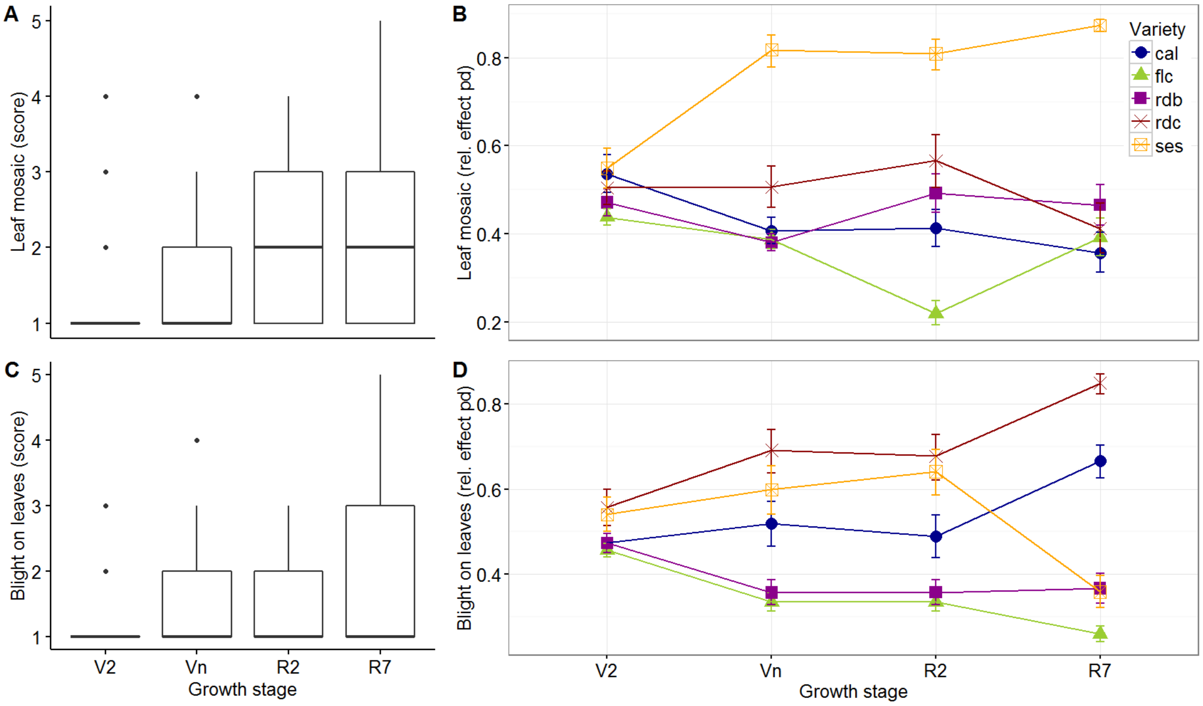
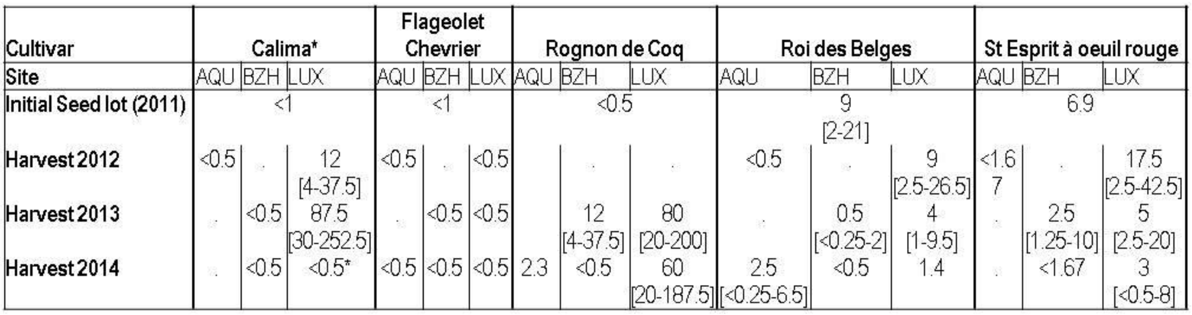


Figure 5.5: Symptom scores for leaf mosaic (above) and blight spots on leaves (below) on five bean varieties in LUX:13 at 4 growth stages: V2, Vn, R2 and R7. A and C: distributions of absolute scores across varieties for each growth stage. B and D: relative effect (pd) of bean varieties and their confidence intervals, according to a rank-based model.

Note on relative treatment effects:

The so-called relative treatment effect pd_i of variety 'i' can be regarded as the probability that a randomly chosen observation X_i results in a larger value than a randomly chosen observation from the whole data set. Specifically, if pd_i is >0.5, observations in the ith treatment tend to be larger in comparison to an independent random variable; likewise, if pd_i is <0.5, observations in the ith treatment tend to be smaller. Differences in the pd values are used to compare treatments with a rather simple interpretation: variety 'i' tends to result in (1) a smaller value than variety 'j', if $pd_i < pd_j$; (2) a larger value than variety 'j', if $pd_i > pd_j$, neither a smaller nor larger value than variety 'j', if $pd_i = pd_j$ (Brunner and Puri, 2001; Shah and Madden, 2004).

Table 5.1: Contamination rates of seed lots with Psp (number of seeds contaminated in 1000). Confidence intervals of the estimated contamination rates are indicated in brackets when available.
* The initial seed lot of variety 'Calima' (cal) was treated with an unkown seed treatment used by the breeding company to control bean diseases.



Cultivar	Calima*			Flageolet Chevrier			Rognon de Coq			Roi des Belges			St Esprit à oeuil rouge		
Site	AQU	BZH	LUX	AQU	BZH	LUX	AQU	BZH	LUX	AQU	BZH	LUX	AQU	BZH	LUX
Initial Seed lot (2011)	<1			<1			<0.5			9 [2-21]			6.9		
Harvest 2012	<0.5	.	12 [4-37.5]	<0.5	.	<0.5		.	.	<0.5	.	9 [2.5-26.5]	<1.67	.	17.5 [2.5-42.5]
Harvest 2013	.	<0.5	87.5 [30-252.5]	.	<0.5	<0.5		12 [4-37.5]	80 [20-200]	.	0.5 [<0.25-2]	4 [1-9.5]	.	2.5 [1.25-10]	5 [2.5-20]
Harvest 2014	.	<0.5	<0.5*	<0.5	<0.5	<0.5	2.3	<0.5	60 [20-187.5]	2.5 [<0.25-6.5]	<0.5	1.4	.	<1.67	3 [<0.5-8]

3.3 Leaf mosaic and blight in Luxembourg in 2013

To confirm the results of the multivariate analysis, namely that variety 'rdc' is particularly affected by blight symptoms and variety 'ses' by virus-like symptoms, the expression of 'blight.leaf' and 'mosaic' was elucidated in the environment LUX:13 in further detail. The rank-based ANOVA-type statistic resulted in a significant effect of 'variety' for every observation date. Figure 5.5 shows the distribution of absolute symptom scores for each observed growth stage (graphs A and C), as well as the relative effects of varieties (graphs B and D).

Concerning leaf mosaic (graph B), 'ses' is particularly affected across growth stages. Variety 'rdc' is more strongly affected than other varieties at the three first observation dates, but not on the last observation. Concerning blight symptoms (graph C), 'rdc' is particularly affected across growth stages. Variety 'ses' is more strongly affected than other varieties at the three first observation dates, but not on the last observation. Variety 'cal' developed blight symptoms on leaves at the end of the growth cycle, at R7. Variety 'flc' developed very few symptoms of blight or mosaic on leaves as compared to other varieties.

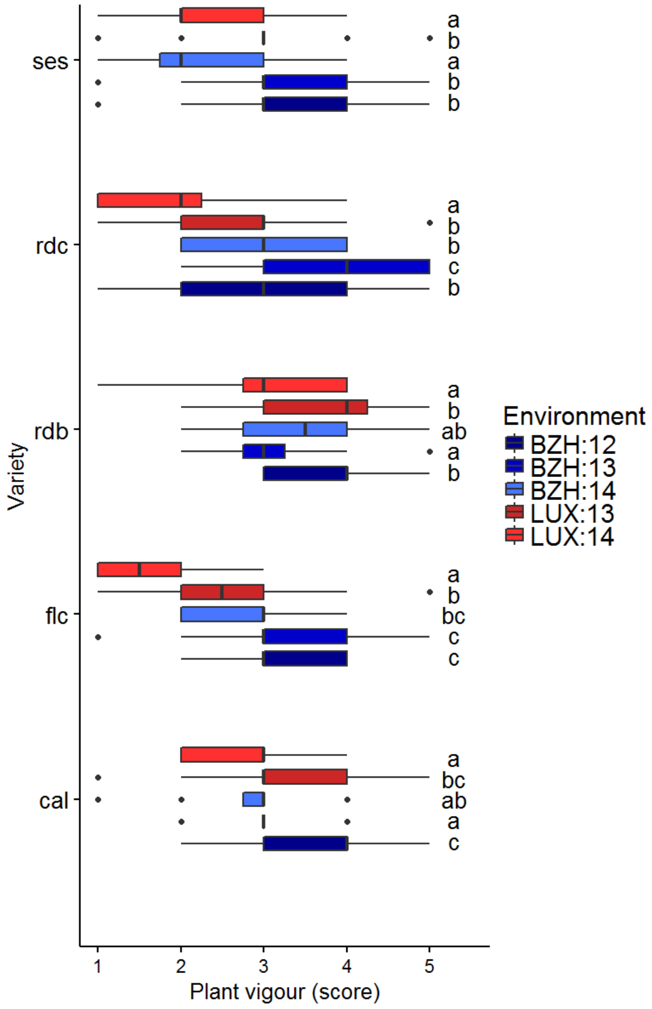


Figure 5.6: Quartiles of scores of plant vigour for each variety and environment. Within each variety, distributions with the same letter are not significantly different from each other at p<0.05 according to rank-based pairwise-comparisons.

3.4 Vigour

Rank-based ANOVA-type statistic was computed for plant vigour scored on individual plants at flowering growth stage (R6). Data was available for 5 environments, comprising two locations and three years: BZH:12, BZH:13, BZH:14, LUX:13 and LUX:14. Between 100 and 300 plants were observed per environment, with a balanced number of observations between varieties. Rank-based ANOVA-type statistic resulted in a highly significant effect of variety (p<0.0001), environment (p<0.0001) and their interaction (p<0.0001) on plant vigour. Data distributions and the results of pairwise comparisons are shown in Figure 5.6. Considering the boxes representing 1st and 3rd quartiles of the samples, i.e. the central range in which 50% of the data is located, gives an impression of the vigour generally expressed by the varieties. Variety 'rdb' was rather vigorous across environments, whereas 'ses' had little vigour. Varieties 'flc' and 'rdc' appear more responsive to environmental factors; they were generally less vigorous in LUX (in shades of red) and more vigorous in BZH (shades of blue). Variety 'rdc', in particular, expressed a very high vigour potential in BZH:13, but was not vigorous in LUX:12 and LUX:13. The commercial control variety 'cal' appears as intermediary, both in terms of its level of vigour across environments and of responsiveness to growing environments.

3.5 Seeds per plant

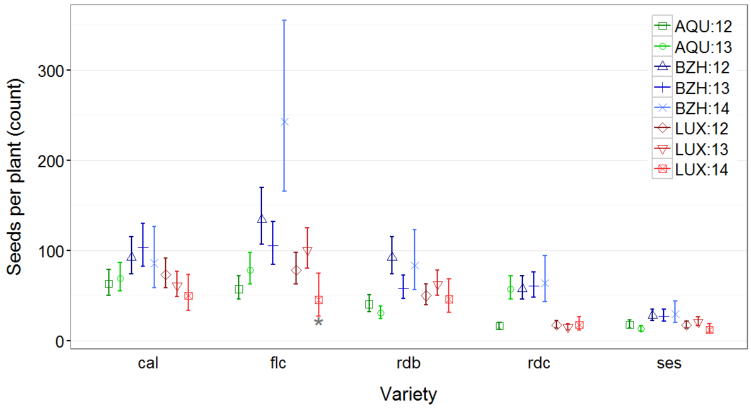


Figure 5.7: Mean counts of seeds produced per plant by five common bean varieties in 8 environments. Error bars represent 95 % confidence intervals. Different colours indicate different locations, whereas shades of these colours indicate years.
(*) The mean count of variety 'flc' in the environment 'LUX:14' was predicted with the generalised linear model, as data was missing for this subgroup.

Seeds produced per plant were counted on 30 plants per site and year in 2012 and 2013 and on 10 plants per site and year in 2014. Data as not collected in AQU in 2014 for any variety, nor for variety 'flc' in 'LUX' that same year. Seeds produced per plant ranged from 0 to 520 over all varieties and environments. Likelihood ratio tests of nested negative binomial models indicated that the effects of environment ($p < 0.0001$), bean variety ($p < 0.0001$) and their interaction ($p < 0.0001$) on number of seeds produced per plant were all highly statistically significant. In Figure 5.7, mean counts and their 95% confidence intervals are shown for each variety grown in each environment. Across varieties, the site 'BZH' generally had higher seed counts than 'AQU' and 'LUX'. Variety 'flc' was most responsive to changes in growing environments, whereas 'ses' was least responsive.

4 Discussion

Results of several analyses (Figure 5.3, Figure 5.4 and Figure 5.5) concur to show that variety 'Rognon de Coq (rdc) is particularly affected by blight symptoms. Variety 'rdc' is indeed renowned for its susceptibility to both CBB and HBB (see letter on p.30). Apart from the initial seed lot and the seed lot harvested in BZH:14, Psp, the agent of halo bacterial blight (HBB) was detected on all 'rdc' seed lots tested (Table 5.1). Contamination rates were particularly high in LUX, again indicating strong HBB pressure in this site as expected from climatic conditions (Annexes 5 and 6; EFSA Panel on Plant Health, 2014). In contrast, no Psp was detected on seed of variety 'rdc' harvested in BZH in 2014, which is in accordance with the low prevalence of blight symptoms in this site (data not shown). Contrasting growth conditions and health status of 'rdc' are also reflected by overall vigour scores

(Figure 5.6) and seeds produced (Figure 5.7), with low values for the trial site in LUX and higher values in BZH.

Results (Figure 5.3 and Figure 5.5) also concur to show that variety 'Saint Esprit' (ses) is particularly affected by symptoms of leaf mosaic and blustering. These are typical symptoms of several viral diseases (Hall, 2005), but can easily be confounded with symptoms caused by nutrient deficiencies and damage caused by aphids, respectively. For variety 'ses' the consistency of symptoms across growing environments, as well as the early appearance and rapid spread of symptoms indicate a seed-borne viral disease, such as Bean Common Mosaic Virus (BCMV). Positive tests for *potyvirus* (BCMV is a *potyvirus*) on several populations of variety 'ses' in the 'gathering phase' (data not shown) comfort this conclusion. In Figure 5.5, graphs B and D show that the two varieties 'rdc' and 'ses' express both symptoms of leaf mosaic and blight up to flowering (growth stage R2). Later (growth stage R7), leaf mosaic symptoms drop and leaf blight continues to increase for variety 'rdc'. The inverse development of symptoms is true for 'ses' (relative effect of 'ses' on blight spots decreases, relative effect on leaf mosaic increases). Two explanations are possible. (i) Antagonistic effects between HBB and (presumed) BCMV may lead to one disease taking over, as found between other aerial plant diseases (Paula Júnior et al., 2010, 2015). (ii) It also possible that blight and mosaic symptoms were confounded at early growth stages. Observations of blight symptoms are indeed recommended at later growth stages, between R6 and R8 (Schoonhoven et al., 1987). However, bacteriological leaf tests confirmed that blight symptoms were correctly identified by the evaluator, so that the first explanation seems more likely.

Multivariate analysis did not reveal any notable link of varieties 'cal', 'flc' and 'rdb' with any disease symptoms (Figure 5.3). Figure 5.4 shows that 'rdb' and the control variety 'cal' expressed some blight symptoms on pods in environment LUX:14, which was an environment with high pressure from Psp (agent of HBB). Variety 'rdb' expressed little blight on leaves, whereas 'cal' had blight symptoms on leaves at the end of the growth cycle (Figure 5.5). Concerning the control variety 'cal', a modern variety obtained from a conventional, large-scale plant breeder, it is noteworthy that HBB symptoms occurred on leaves and pods despite an HBB-resistance announced in commercial seed catalogues (Hild, 2016 p. 9). It may be that 'cal' is resistant only to certain bacterial Psp strains (Taylor et al., 1996a; b); or its resistance may have been broken since its release in 1989.

Variety 'flc' stands out due to its absence of symptoms in general and in environment LUX:13 in particular. However, in the 'gathering phase', when all versions of the varieties were cultivated in a field trial in BZH in 2015, all versions of 'flc' were all but completely destroyed by 'black root syndrome' (Jenkins, 1940), a spreading, usually lethal phloem necrosis (data not shown, see Chapter VIII). Under certain conditions, particularly high temperatures, this phloem necrosis is caused by the viral pathogen BCMV in bean varieties carrying the *I* allele, which is widely used in bean breeding as source of resistance to BCMV (Collmer et al., 2000). This explains the absence of viral disease symptoms on 'flc' in all environments the previous years. Studying the interaction between BCMV and the resistance conferred by the *I* gene in common bean, Collmer *et al.* (2000) came to a conclusion that is relevant for the difficulties of managing bean health *in situ*:

> The observations reported here contribute to a growing appreciation of the complexity of the interaction between a potentially resistant plant and a potential viral pathogen. While a predictable plant–pathogen response, whether that be extreme resistance or hypersensitive resistance, can be elicited when plant cultivar, resistance allele dosage, viral strain, and environmental conditions (including temperature, light intensity, and humidity) are carefully controlled, the variability possible when they are not can extend from one end of the resistance continuum (extreme resistance) to the other (systemic vascular necrosis and plant death).

As in Chapter III of this thesis (p.64), the authors come to consider disease agents as potentially pathogenic.

A "seed size limit" to yield potential, and thus to the number of seeds produced per plant, has been reported for common bean (White and González, 1990). This has also been reported for total biomass production (White et al., 1992). In other words, common bean varieties with larger seeds produce less biomass and fewer seeds per plant (without compensation between seed size and number). A very large range of seed sizes is represented by the varieties in this experiment (1000-seed weights range from approximately 250g to more than 800g) and somewhat illustrates this "seed size limit" to the number of seeds produced (Figure 5.7): varieties 'cal' and 'flc', with lowest 1000-seed weights, had a higher potential for seed production than other varieties, as expressed the trials site in Brittany (BZH, in blue in the figure). Variety 'ses' generally produced few seeds in all environments and was the least vigorous among the varieties (Figure 5.6). On one hand, this may be explained by the very large seed size of 'ses' (Table IV.1, p.99). On the other hand, the very low vigour and numbers of seeds produced by variety 'ses' may also be due to seed-borne viral diseases.

When comparing within a variety, plant health status is to some extent reflected by vigour scores and seed counts. Vigour and seed production were generally lower in the trial site in Luxembourg (LUX) than in Brittany (BZH). This coincides with strong pressure from plant diseases in LUX. In contrast, environmental conditions in BZH were favourable to bean production during the experimentation. The effect of disease pressure is particularly visible for variety 'rdc', with low vigour and seed counts in LUX. However, vigour and seed production in the experimental sites cannot be explained solely by plant health status, as variety 'flc' was highly responsive to environmental conditions despite outstanding plant health. High biomass and seed production in BZH may be due to weather conditions and more nutrient-rich soil, as opposed to cooler temperatures and more nutrient-deficient soil in LUX (Annex 5). Not much data was collected in the on-farm trial in Aquitaine (AQU) as compared to the other sites, for reasons elucidated in subsection 2.1 of Chapter II. From the little data available (Table 5.1, Figure 5.7), AQU appears to have had difficult to intermediary bean growing conditions in 2012 and 2013, depending on year and bean variety.

It is noteworthy that no symptoms of fungal bean diseases, such as White Mould (*Sclerotinia sclerotium* (Lib.) de Bary), Fusarium root rot (as *Fusarium solani* F. sp. *phaseoli*) or anthracnose (*Colletotrichum lindemuthianum*) were scored in any of the varieties or environments. Indeed, no typical symptoms of these bean diseases appeared in the field trials. The possible link of this non-observation with soil life, mycorrhizal fungi and Rhizobia in particular, is addressed in the next chapter.

The objective of this chapter was to represent general health in a way that allows for judgement according to actors' different approaches to plant health. Multiple correspondence analysis (MCA) was considered a promising approach to visualise the health state of bean varieties in several environments with regard to multiple types of symptoms. The aim was to reflect the general plant health situation of different varieties in different environments in a way that both allows for the comparison between varieties and environments and leaves room for different stances on plant health when interpreting the data. In this study, the interpretation of MCA on its own was not satisfactory in revealing contrasting environments with regard to disease pressure, nor in pointing to finer differences between varieties. Indeed, only very severe disease incidence was distinguishable in the MCA, such as HBB on variety 'Rognon de Coq' (rdc) or virus-like symptoms on 'St Esprit' (ses) in Luxembourg (LUX). This method of multivariate analysis may be more conclusive when more variability is represented by the data, for example when plant health is observed in more locations and more different types of symptoms are scored. Such greater variation may allow for a finer seperation of environments and varieties according to more distinctive dimensions in the MCA. In our case, conclusions were drawn by combining MCA with other analytical methods.

Further efforts would be necessary in future if the aim of rendering general plant health comparable across sites according to different stances on plant health is pursued. However, one may also question whether this aim can effectively be reached by using numerical tools alone. An alternative is to consider that the comparison of plant health according to different stances must involve actors who hold those stances. In the case of the *Croqueurs* discussed here, this might imply collectively discussing results of the analyses performed in view of action, i.e. in view of the collective *in situ* management of bean health. The supplementary information gained by symptom observation, seed analyses and statistics would then feed into the comparison and evaluation of plant health in the framework of a social learning process, which cannot be disconnected from the involved actors.

To summarise the observations presented in this chapter, the on-farm trial locations in Brittany (BZH) and Luxembourg (LUX) constitute contrasting environments in terms of climatic conditions, soil properties and disease pressure. In 2012-14, BZH presented very favourable bean growing conditions, whereas LUX was marked by very difficult ones. Variety 'flc' stood out as a variety with an excellent health status across environments. Variety 'rdc' suffered heavily from halo bacterial blight (HBB) in LUX, whereas variety 'ses' expressed virus-like symptoms across environments. The control variety 'cal' and 'rdb' can be described as intermediary in terms of plant health and seed production, although 'rdb' was most vigorous.

Implications for collective management of plant health

Varieties 'rdc' and 'ses' are particularly susceptible to certain seed-borne bean diseases. They each make a case for the collective management of bean health by the *Croqueurs* and other actors involved in bean crop diversity. Indeed, the seed grower in LUX, who had initially provided variety 'ses' for the experimentation, stopped producing this variety. He concluded that 'ses' won't adapt to the environmental conditions of his seed garden. The variety was consigned to another seed grower of his network. We see collective management (see Chapter III, division 1.1.3) of plant health at work: As 'ses' won't adapt to environmental conditions in LUX, the artisanal seed company searches for a seed grower who might provide growing conditions which suit the variety. Results presented here indicate that the lineage of 'ses' studied has low vigour and seed production potential even in favourable bean-growing environments, such as BZH. Based on the notion of *collective* plant health management, one may consider that the properties of the 'ses' lineage might be modified by natural selection in another environment, as well as conscious selection by another seed grower. In the following, Chapter VII will show how seed-associated microbial communities are impacted by the multiplication site (*terroir*) after 2 years of multiplication in BZH and LUX. Chapter VIII addresses phenotypic and genetic changes in populations after 3 years of multiplication in these trials sites.

Variety 'rdc', notoriously susceptible to bacterial blights, makes another case for collective bean health management. Table 5.1 shows high contamination rates of this variety with Psp (agent of halo bacterial blight) in LUX. In BZH, however, contamination rates passed from rather high ones in 2013 to undetected in 2014. This result was obtained without conscious selection in the experimental seed crops. Although a longer-term study including more farm sites would be necessary to confirm, these first results indicate how different farms may play different roles in the collective management of bean health. For instance, LUX may constitute an appropriate site for testing the susceptibility of bean varieties to HBB (and to cool, humid conditions) and for selecting for disease tolerance. In contrast, sites such as the farm in BZH may perhaps, in good years, be used to sanitise bean lineages carrying HBB. Evidence from the inquiry presented in the previous chapter suggests that this collective management among seed growers of artisanal seed companies is happening, implicitly. How the collective governance of bean health articulates with the management of bean diversity is addressed in Chapter VIII.

Chapter 6: **Symbiotic microorganisms of the soil**

> "And of course, since the soil also hosts lots of problems, such as weeds and pathogens and all that, hence, it is replaced, so it is replaced by something else, like stone wool or so. And here, you nevertheless have an idea of the mycorrhiza and that it builds a symbiosis with the plant as if it were a prolongation of the roots for the plant to find what is not abundant. It's like a plant that makes more roots when it is not irrigated. It's another logic than just wanting to add manure or even soluble minerals to obtain higher yield. Here, you go more into the logic of nature and it's interesting. And it might even get you away from just yield quantities, you know." [CRO-100914a][40]

In Chapter III, I have shown that the *Croqueurs* rely upon sound environments, and beneficial soil microorganisms in particular, to ensure bean health *in situ*. In this chapter, interactions of common bean with two important root symbionts - mycorrhizal fungi and rhizobia - are studied under field conditions.

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[40] "Et forcément, comme le sol est aussi hôte de pleins de problèmes, comme les herbes sauvages et les pathogènes et tout ça, donc, on le remplace, donc on met quelque chose à la place, comme de la laine de roche ou quelque chose comme ça. Et là, t'as quand même une idée de la mycorhize et qu'elle se met en symbiose avec la plante comme si elle était un prolongation des racines pour que la plante puisse encore trouver ce qui n'est pas là en abondance. C'est comme une plante qui fait davantage de racines quand il n'y a pas d'arrosage. Ca c'est une autre logique que seulement vouloir ajouter de la fumure ou même des engrais solubles pour avoir plus de rendement. Là, tu rentres plus dans la logique de la nature et c'est intéressant. Et ça permet peut-être aussi de t'éloigner de la simple quantité de la récolte, quoi." [AB100914a]

1 Introduction

The *Croqueurs* consider soil fertility and health as key for ensuring plant health. In what has been termed a tripartite symbiosis (Mortimer et al., 2008) or metasymbiosis (Garbaye, 2013) common bean, like other legumes, interacts with two important soil microorganisms: Mycorrhizal fungi and nitrogen-fixing rhizobia. Both mycorrhiza and rhizobia have been shown to enhance plant health under controlled conditions (Avis et al., 2008; Berendsen et al., 2012) and to play an important role for soil fertility and plant health in organic farming systems (van Bruggen et al., 2016).

Like about 80 % of terrestrial plants (Gianinazzi-Pearson, 1982; Wang and Qiu, 2006), common bean forms a symbiosis with arbuscular mycorrhiza (AM). Arbuscular-mycorrhizal fungi form part of the phylum *Glomeromycota* (Schüßler et al., 2001). AM fungi are biotrophic and cannot survive without plant roots. Once hyphae have begun to grow from germinated spores, they must colonise plant roots to develop and persist (Sekhara Reddy et al., 2009). In most crop plants, colonisation of plant roots by AM fungi comprises three phases (Saif, 1977). In the lag phase, spores of AM fungi germinate in the soil, hyphae grow and enter plant roots by forming an appressorium (Garbaye, 2013). Even before physical contact between the fungi and the plant is established, both partners already communicate (Weidmann et al., 2004; Oláh et al., 2005; Tamasloukht et al., 2007). Hyphae then pass the root cortex and grow within the roots. In a phase of rapid development, hyphae grow within plant roots across cell walls, forming arbuscules within plant cells. It is through these arbuscules that the plant and the fungi exchange nutrients. The increase of the number of colonised common bean rootlets has been reported to be linear (Sutton, 1973). In parallel, a dense network of hyphae extends beyond the plant's root system. In the final, constant phase, the number of infected rootlet stabilises. Inside the rootlets, the fungi produce vesicles containing spores, which will be released into the soil as root tissues decay (Saif, 1977). The main known benefits of AM for crops include nutrient mobilisation, especially of phosphorous, and improved tolerance against abiotic stresses, especially against drought (Augé et al., 2003; Parniske, 2008; Garbaye, 2013). AM is also known to improve tolerance against biotic stress factors, mainly against soil-borne pathogens (Azcón-Aguilar and Barea, 1997; Whipps, 2004). Among soil-borne pathogens of common bean, AM improves tolerance against Fusarium root rot (Dar et al., 1997; Filion et al., 2003), Rhizoctonia root rot (Abdel-Fattah, 2011) and White Mould (Aysan and Demir, 2009; Mora-Romero et al., 2015). More recently, the symbiosis has been shown to induce a mild, but effective activation of plant immune responses, not only locally but also systemically (Pozo et al., 2010; Jung et al., 2012; Cameron et al., 2013).

As a legume crop, common bean has the ability to interact with N-fixing rhizobia for symbiotic nitrogen fixation (SNF). Rhizobia are gram-negative Proteobacteria with the capacity to fix atmospheric nitrogen when they are associated with the legume's roots (Rajwar et al., 2013). Highest rates of SNF occur during the reproductive stages of common bean growth. Differences among bean varieties have not only been found in their ability to nodulate and efficiently fix N_2, but also in their capacity to remobilise N within the plant (Peña-Cabriales et al., 1993). The genetic structure of the bacterial species able to nodulate common bean is variable in different regions of the world and is probably related to the history of common bean as it spread around the globe (Laguerre et al., 1993; Martínez-Romero, 2003). It has been suggested that *Rhizobium etli* bv. *phaseoli* was introduced into Europe on bean seeds brought from the New World from 1492 on. In an extensive interspecific symbiotic gene exchange, genetic information was then transferred from American strains to bacteria pre-existing in European soils (Herrera-Cervera et al., 1999). Common bean is highly promiscuous in its relationship with different rhizobia strains (Rodiño et al., 2012), but is renown as a poor N_2 fixing plant (Graham, 1981). There appears to be large variability in nodulation and in the efficiency of native rhizobia strains, adapted to local environmental conditions. This variability may be related to the large genetic differences observed in these bacteria and to the coadaptation of cultivar and bacteria (Rodiño et al., 2012). Genotypic variability for N_2 fixing potential was found in European bean germplasm (Rodiño et al., 2005) and has led to conclude on the importance of selecting for bean genotypes able to nodulate and efficiently fix N_2 with native rhizobia (Rodiño et al., 2012). By selecting for plant vigour and plant health *in situ*, the *Croqueurs* may also be selecting for adaptation to local rhizobia strains. Rhizobia improve not only the N nutrition of legumes, but also promote plant growth and induce resistance against soil-borne and foliar pathogens (Persello-Cartieaux et al., 2003; Dakora, 2003; Deshwal et al., 2003; Avis et al., 2008; Berg, 2009; Dardanelli et al., 2010). In common bean, rhizobia have been reported to improve tolerance against root pathogens (Özkoç and Deliveli, 2001), such as *Fusarium solani* F. sp. *phaseoli* (Buonassisi et al., 1986; Dar et al., 1997) and *Sclerotinia sclerotiorum* (Lib.) de Bary (Aysan and Demir, 2009) and to induce resistance against CBB aboveground (Osdaghi et al., 2011).

In view of the intensive tripartite symbiosis and its effects reported on bean health, tracing the interactions of bean seed crops with both AM fungi and rhizobia constitutes an approach in the endeavour to reveal the biological base of the *Croqueurs'* bean health management. The first sub-question asked in this chapter is thus: **is the health status of bean plants correlated with their ability to interact with mycorrhizal fungi and *Rhizobia spp.* under field conditions?** Differences among common bean varieties in their ability to interact with both mycorrhizal fungi (Ibijbijen et al., 1996; Hacisalihoglu, 2005) and *Rhizobia* (Graham, 1981; Martínez-Romero, 2003) have been reported. Based on these past findings under controlled conditions, the second sub-question addressed in this chapter is: **do the bean varieties differ in their ability to interact with these symbiotic microorganisms under field conditions?**

2 Materials and methods

In 2013, field trials in AQU, BZH and LUX (see Chapter IV, subsection 1.2) were sampled for the quantification of the symbioses of bean plants with mycorrhizal fungi and nitrogen-fixing rhizobia in the soil. For the quantification of mycorrhiza, stained structures of the mycorrhizal fungi were directly observed under a light microscope. In view of quantifying the symbiosis with *Rhizobia spp*., scoring the number of nodules on bean root systems gave a first impression. To take into account the efficiency of the latter interaction, the percentage of N derived from the atmosphere, or percentage dependence on

N_2 fixation for growth (%ndfa) was calculated, based on the accumulation of naturally occurring isotopic nitrogen.

2.1 Soil and plant sampling

Within three days after sowing, soil was sampled at the three experimental sites for chemical analyses. Mineral nitrogen, phosphorous, potassium, calcium and magnesium contents, as well as soil textures, were analysed by the *Institut en Santé Agri-Environnement* (ISAE Combourg, France).

In growth stage R6 (flowering; see Annex 10), at 55-58 das according to site, six plants were extracted from each subplot in three field replications in each site, for a total of 18 plants per variety and site. To avoid creating random gaps in the plant rows, the samples were taken within one area of each subplot dedicated to this destructive sampling. The aerial parts of each plant were first scored for vigour and disease symptoms as described in subsection 2.3 of Chapter IV. To determine dry weights of aerial parts, they were then dried in a drying oven at 76°C for 48h and weighed. The individual root systems were identified and carefully rinsed in tap water. The number of nodules was then scored for each root system according to the scale proposed for bush beans in the 'Standard system for the evaluation of bean germplasm' (Schoonhoven et al., 1987). However, the scale was inverted so that 1 = "very poor nodulation" (less than 10 nodules) and 9 = "excellent nodulation" (more than 80 nodules). Then, roots were prepared for mycorrhiza quantification as described below (subsection 2.2).

At harvest, 106-115 days after sowing according to site, a sample of 10 plants was taken in each subplot for the quantification of %ndfa. In addition, a sample of 10 maize plants (variety 'Blanc du Monein') was harvested as non-legume reference plant (Nyemba and Dakora, 2010). The maize had been sown alongside the trial field on the same sowing date. Each sample was prepared for the assessment of %ndfa, as described below (subsection 2.3)

The set of samples collected in AQU was incomplete due to technical difficulties[41] encountered during the season in this trial site. At flowering, no samples were taken in the trial for mycorrhiza quantification. However, 18 plants of variety 'rdc' were sampled in a neighbouring seed crop on the same farm. As the farm hosting the field trial in AQU had provided the initial seed lot of rdc, this seed crop derived from the same seed lot. Whereas the samples of variety rdc taken outside the formal field trial allow for a first impression of mycorrhization rates in AQU, it was excluded from statistical inference. At harvest, varieties cal and rdc were sampled in the field trial for assessment of %ndfa. The varieties flc, rdb and ses did not allow for sampling.

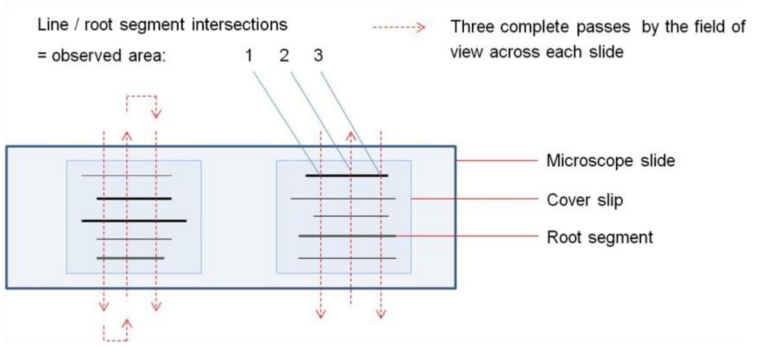

Figure 6.1: Method for the observation of mycorrhizal structures.

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[41] Damage by rabbits.

2.2 Mycorrhiza quantification

After rinsing in tap water, the fine tertiary roots of each root system were separated. The fine roots were cut into root segments of about 1 cm length and placed in a test tube for staining. A staining method based on vinegar, black ink and potassium (Vierheilig et al., 1998) was employed to stain and make visible the fungal structures of the mycorrhiza. Roots were first cleared in 10% (vol/vol) KOH at 90°C for 5 min and rinsed with distilled water. Fungal tissues were then stained in a solution of black ink (brand "Pelikan", 5 vol%) in 8% white vinegar (95 vol%) at 90°C for 3 min. Finally, the roots were again rinsed in distilled water and conserved in a 20 vol% solution of white vinegar in water.

For the observation of the mycorrhizal structure under a light microscope, the *magnified intersections method* proposed by McGonigle *et al.* (1990) was adapted.

Twenty root segments of each bean plant were mounted in water on microscope slides and covered with coverslips. Two slides were used per plant, with 10 root segments each. Roots were aligned parallel to the long axis of the slides and observed at magnification x 400 as shown in Figure 6.1. The field of view of the microscope was moved using the stage graticule to make three complete passes across each slide perpendicular to its long axis. Each time a root segment entered the field of view, the visible area was inspected for presence of mycorrhizal structures.

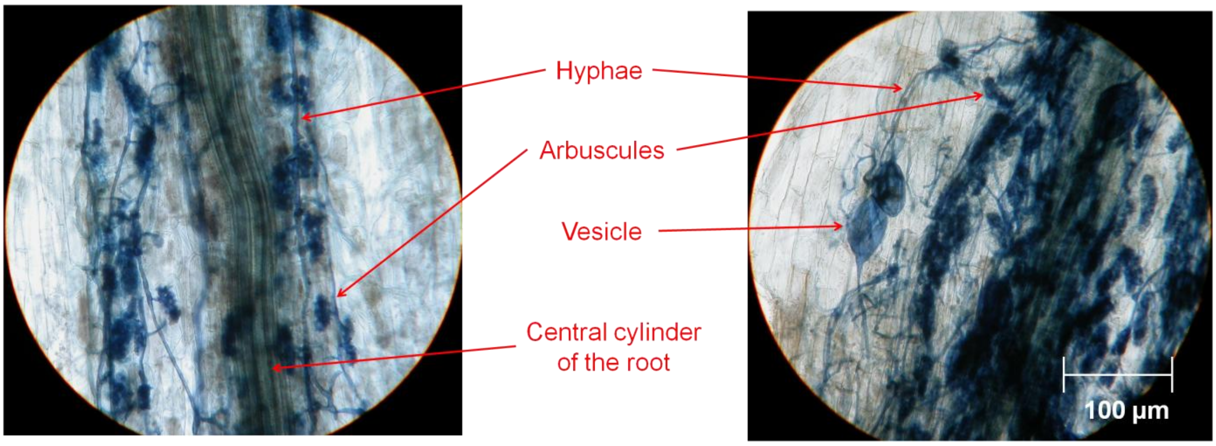

Figure 6.2: Blue-stained structures of vesicular arbuscular mycorrhiza in bean roots as seen under a light-optical microscope with 400fold magnification.

To examine each intersection, the plane of focus was moved completely through the root and a note made of the observation of any hyphae, arbuscules and vesicles. For each plant, counts of hyphae, arbuscules and vesicles encountered in 60 observation areas were thereby obtained and expressed as percentage colonisation. Figure 6.2 shows stained structures of arbuscular mycorrhizal fungi.

2.3 Quantification of nitrogen derived from air

The %ndfa is a measure of SNF. It goes beyond the quantification of root nodules and takes into account the efficiency of the symbiosis between a legume crop and rhizobia. %ndfa was quantified according to the ^{15}N natural abundance method, based on the ^{15}N analysis of a N_2-fixing plant and a non-N_2 fixing reference plant. ^{14}N and ^{15}N are the two main stable isotopes of N, with ^{14}N naturally more abundant than ^{15}N. The difference between ^{15}N concentrations of atmospheric N_2 and plant-available soil N is used to calculate %ndfa (Unkovich and Australian Centre for International Agricultural Research, 2008).

Each sample of ten plants was dried in a drying oven at 76°C for 72h or until dry and ground using a 1mm grid. Total N contents of beans and maize were determined for each site by Kjeldahl method. According to N content, between 2.7 and 6.3 mg of ground sample were fed into a EA 300 elemental analyzer (EuroVector) coupled to an IsoPrime mass spectrometer (Elementar) and analyzed for 15N/14N ratio. The 15N natural abundance (δ15N) of each plant sample was calculated as (Pate et al., 1994):

$$\delta^{15}\mathrm{N} = \frac{\mathrm{atom\%}\ ^{15}\mathrm{N\ sample} - \mathrm{atom\%}\ ^{15}\mathrm{N\ air}}{\mathrm{atom\%}\ ^{15}\mathrm{N\ air}} \mathrm{\ x\ } 1000$$

The proportion of N derived from fixation was estimated as (Unkovich and Australian Centre for International Agricultural Research, 2008):

$$\%\mathrm{ndfa} = \frac{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \delta^{15}\mathrm{N}_{\mathrm{leg}}}{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \mathrm{B}} \mathrm{\ x\ } 100$$

Where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of maize plants as non-legume, non-N-fixing reference plants, $\delta^{15}N_{leg}$ is the ^{15}N natural abundance of the common bean sample, and B represents the δ^{15}N of legume plants relying entirely on symbiotic N_2 fixation for their N nutrition. The B value incorporates the isotopic fractionation associated with nitrogenase activity during N_2 fixation and replaces the value of atmospheric N_2 (Shearer and Kohl, 1986; Pate et al., 1994). The B value of -1.97 was employed, as determined by Mariotti *et al.* (1980) for common bean in France. Negative calculated %ndfa values were set to 0.

2.4 Statistical analyses

Statistics were computed using the programming language and software environment R version 3.3.0 (R Development Core Team, 2016). In tests, null hypotheses with p-values below the significance level (α) of 0.05 were rejected.

To gain an overview of available data and their distributions, beanplots (Kampstra, 2008) were constructed using the R package 'beanplot' (Kampstra, 2015). For proportion data, i.e. percentage colonisation with mycorrhizal structures and %ndfa, a generalised linear model was fitted with a quasi-binomial variance model and deviances for each factor tested with an F-test (Hastie and Pregibon, 1992). Location, variety and their interaction were included in the model as explanatory variables. Multiple comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test (Tukey, 1949), computed by the R package 'lsmeans' (Lenth, 2016). For the %ndfa data, the effect of location was first tested on a subset containing varieties rdc and cal, in order to include AQU in the analysis with a balanced dataset. The effects of both location and variety were then tested for the sites

BZH and LUX, where all varieties had been sampled. For the mycorrhiza data, the data collected in AQU was excluded from statistical inference, because the sampling procedure differed from the other two sites (see subsection 2.1). The effects of location and variety were thus tested for BZH and LUX only.

Effects of location, variety and their interaction on nodulation scores were tested with a rank-based ANOVA-type statistic (Brunner and Puri, 2001), computed by the 'rankFD' package (Konietschke et al., 2016). In the rank-based procedure, comparing the 95% confidence intervals of relative treatment effects allows for multiple comparisons. Spearman's rank correlation coefficient was computed to test the association between percentage colonisation with mycorrhizal structures, nodulation, symptom scores and plant dry weights within each site.

3 Results

Evidence for interaction with *Rhizobia spp.* and with mycorrhizal fungi was found in all sites and varieties. The mineral nitrogen, phosphorous, potassium, calcium and magnesium contents of the soils of the experimental plots are reported in Annex 5.

3.1 Percentage colonisation with mycorrhizal structures at flowering

All three types of mycorrhizal structures were observed in the three experimental sites and on all varieties. Only variety rdc was assessed in AQU. The percentages colonisation with arbuscules found in individual samples in BZH, AQU and LUX ranged from 0 to 37 %, 22 to 50 % and 11 to 53 %, respectively. The ranges and distributions of percentage distribution with hyphae, arbuscules and vesicles are shown in Figure 6.3.

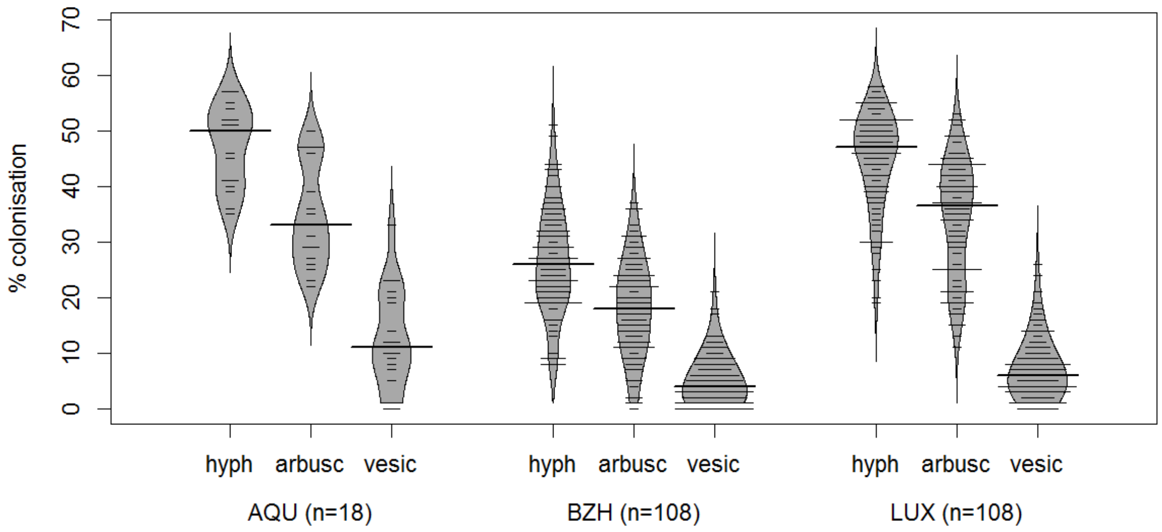

Figure 6.3: Colonisation of root segments (in % of total number of root segments observed) with hyphae (hyph), arbuscules (arbusc) and vesicles (vesic) in Brittany (BZH), Aquitaine (AQU) and Luxembourg (LUX). Lines within the plotted "bean" shapes represent individual samples. While all varieties of the field trial were sampled in BZH and LUX, only the variety 'Rognon de Coq' was sampled in AQU. Bold lines represent sample medians.

Analysis of deviance testing the effect of location, variety and their interaction on percentage colonisation with arbuscules in BZH and LUX revealed a significant effect of location only (Table 6.1). With a mean of 34.7 %, colonisation of bean roots in Luxembourg was higher than in BZH (18.5%).

Table 6.1: Analysis of deviance table testing the effect of location (loc), bean variety (var) and their interaction (loc:var) on the percentage colonisation of bean roots with arbuscules in BZH and LUX.

	Df	Deviance	F value	Pr(>F)
Null	178	1575.3		
loc	1	1548.5	111.914	<0.001
var	4	952.0	0.928	0.449
loc:var	4	939.6	0.364	0.834
Residual	169	937.6		

Table 6.2: Analysis of deviance table testing the effect of location (loc), bean variety (var) and their interaction (loc:var) on the percentage colonisation of bean roots with vesicles.

	Df	Deviance	F value	Pr(>F)
Null	178	840.8		
loc	1	688.3	8.665	0.004
var	4	777.5	7.922	<0.001
loc:var	4	669.5	0.952	0.436
Residual	169	654.8		

In contrast, highly significant effects on the percentage colonisation with vesicles were found for both location and variety (Table 6.2). The differences between locations and varieties are shown in Figure 6.4.

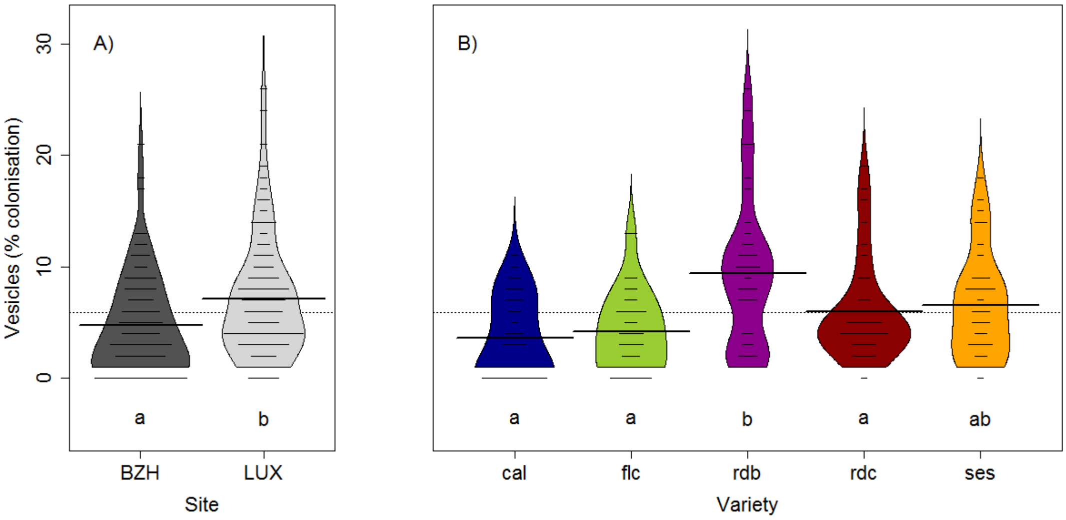

Figure 6.4: Percentage colonisation with vesicles in bean roots according to site (A) and bean variety (B). Lines within the plotted distributions represent individual samples, lines across "bean" shapes represent sample and overall means. Samples which are not marked with the same lowercase letter differ significantly at $p < 0.05$.

3.2 Nodule scores at flowering

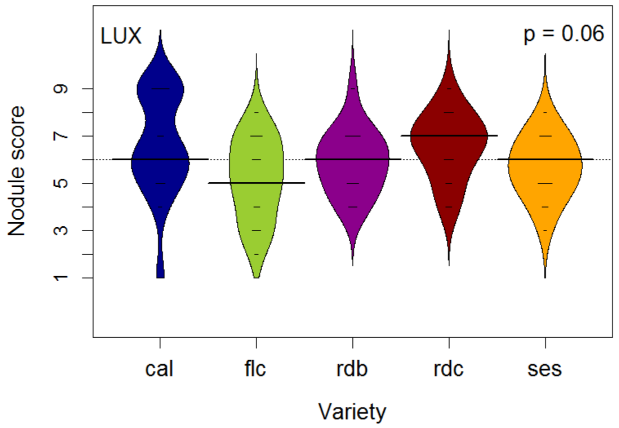

Figure 6.5: Nodule score of bean roots grown in LUX, according bean variety. Lines within the plotted distributions represent individual samples, lines across "bean" shapes represent sample and overall medians.

Nodule scores ranged from 1 to 9 in LUX and from 1 to 7 in AQU. No variation for this trait was observed in BZH, as all plants were scored 1. Rank-based ANOVA-type statistic confirmed that nodulation scores in LUX were higher than in BZH (p<0.0001). As no variation was found in BZH and the sampling procedure in AQU had not been equivalent, differences among varieties were tested for LUX only. Differences among varieties were not statistically significant (p=0.06), but represent a trend worth considering (Figure 6.5).

3.3 Correlations with vigour and disease symptoms at flowering

In LUX a significant, but weak, positive correlation was detected between the nodulation score and the score for blight symptoms on leaves (Figure 6.6, A). At the growth stage of sampling for mycorrhiza quantification, blight symptoms on leaves were not strong, with most plants scored 1 or 2.

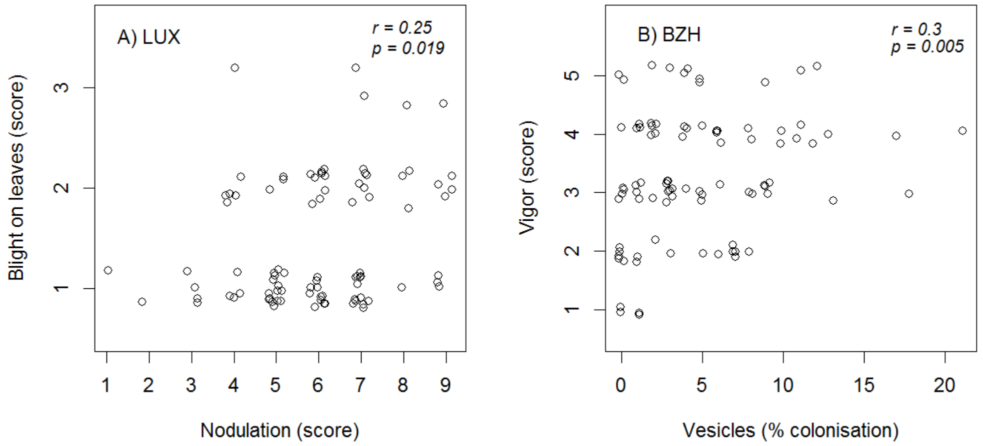

Figure 6.6: Scatter plots illustrating the correlations (A) between scores for nodulation and blight symptoms on leaves in Luxembourg (LUX) and (B) between percentage colonisation with vesicles in bean roots and vigour scores in Brittany (BZH). Points are jittered to allow to distinguish between individual points. The strength and of the correlations are indicated with Spearman's rank correlation coefficient (r), as well as the level of statistical significance (p).

For BZH, significant, but weak, positive correlations of plant vigour scores were detected with percentage colonisation with vesicles (Figure 6.6, B), with percentage colonisation with arbuscules (r=0.23, p=0.03) with percentage colonisation with hyphae (r=0.3, p=0.005) and with spots left on leaves by leaf-sucking pests (r=0.22, p=0.04).

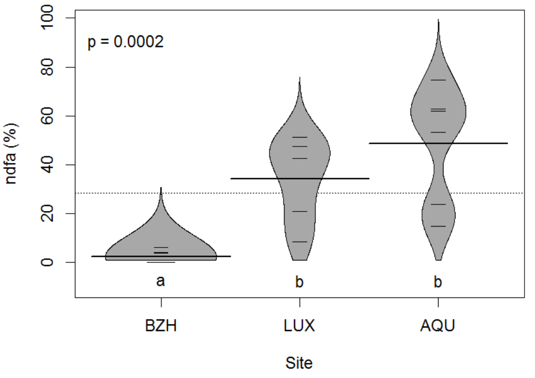

Figure 6.7: Percentage N derived from air (%ndfa) in bean plants of the varieties 'Rognon de Coq' and 'Calima' in Bretagne (BZH), Luxembourg (LUX) and Aquitaine (AQU). Lines within the plotted "bean" shapes represent individual samples, lines across "bean" shapes represent sample and overall means. Samples which are not marked with the same lowercase letter differ significantly.

3.4 Nitrogen derived from air

The %ndfa found in individual samples in BZH, LUX and AQU at harvest ranged from 0 to 8 %, 9 to 53 % and 15 to 75 %, respectively. In AQU, only varieties 'cal' and 'rdc' were assessed. Analysis of deviance on the varieties sampled in all three sites showed that bean plants harvested in BZH had derived significantly less N from the air (Figure 6.7). This result was confirmed by an analysis on all varieties in the sites BZH and LUX testing for the effects of variety, location and their interaction. Only the effect of location was found to be statistically significant ($p<0.0001$). Thus, differences in nodulation scores found among the varieties grown in LUX did not reflect the %ndfa.

4 Discussion

The ranges of percentage colonisation with mycorrhizal structures, of nodulation scores and of %ndfa in the three experimental sites correspond to the nutrient levels found in soil analyses (Annex 5). Lowest levels of interaction with both mycorrhizal fungi (Figure 6.3) and nitrogen-fixing *Rhizobia* (Figure 6.7) were found in BZH, which has the highest levels of P and mineral N in soils. The level of interaction with both symbionts were higher in AQU and LUX and corresponded to N and P levels found in soils (Pacovsky et al., 1991). For all these traits, statistically significant differences between trial sites, between BZH and LUX in particular, were found.

Differences between varieties were found for percentage colonisation with vesicles only. Variety rdb was most strongly colonised with vesicles in both BZH and LUX. Given that vesicles constitute the storage and reproduction structures of mycorrhizal fungi (Garbaye, 2013), this may be interpreted as an indication that the fungal partner profits more in the symbiosis with rdb than with other varieties. The (very) weak positive correlation found between percentage colonisation with vesicles and plant vigour in BZH (Figure 6.6 A) indicates that the strong development of vesicles did not imply a loss in plant vigour. In other words, the fungal partner of the symbiosis profits from the carbohydrates supplied by the bean plant without hindering the plant's development. Concerning the interaction with N-fixing *Rhizobia*, no significant differences were found between varieties grown in LUX, although trends are visible (Figure 6.4): variety flc formed the lowest amount of nodules and rdc the highest amount. No significant differences in %ndfa were found among varieties either, indicating that potential trends in nodulation scores did not translate to different rates of N fixation. This has to do with the efficiency of N fixation. Also in LUX, a (very) weak positive correlation was detected between nodule score and score of blight symptoms on leaves. This implies that bean plants with a higher number of nodules on roots developed stronger blight symptoms on leaves. This may indicate that the formation of root nodules hosting the N-fixing bacteria weakened plants more than it brought advantages. Or, conversely, it may indicate that aboveground bacterial pathogens may have inhibited belowground rhizobia, as reported for fungal pathogens in common bean (Ballhorn et al., 2014). However, the very weak correlation observed in only one site in one year does not allow for speculation on the threefold interaction between *Rhizobia spp*., bean plants and agents of bacterial blights. In addition, plants were sampled too early in the season to observe a large range of blight symptoms. Very few plants were given scores above 2. If further research is to study the implications of nodulation on the development of bacterial blights, a non-destructive method should be employed for mycorrhiza sampling in order to observe the development of the plants' health beyond the sampling date.

In BZH, a weak, but significant correlation was found between plant vigour on one hand and mycorrhizal structures and spots left by leaf-sucking pests on the other. The correlation between plant vigour and mycorrhization may be causal; stronger mycorrhization may have led to more vigorous plant growth. However, it cannot be excluded that differences in vigour and mycorrhization among plants both be due to differences in earliness, for example. In any case, vigorous growth was accompanied by a slight increase in visits from leaf-sucking pests, probably aphids.

Apart from the weak positive correlation between nodule scores and blight symptoms on leaves in Luxembourg, no significant correlation between disease symptoms and bean plants' ability to interact with soil symbionts was found. This may also be due to the sampling date, as bean diseases may have developed after flowering. It is noteworthy, however, that no symptoms of White Mould (*Sclerotinia sclerotium* (Lib.) de Bary) were observed in any of the experimental sites in any of the years 2012-2014, despite weather conditions that were favourable for this seed-borne fungal disease agent (Hall, 2005) in BZH and LUX (see Annex 6). Several researches under controlled conditions have shown the ability of AM and SNF to suppress White Mould on common bean, in particular (Aysan and Demir, 2009; Mora-Romero et al., 2015). Mycorrhizal structures were observed in plants sampled in the three experimental sites in 2013. Mycorrhization of bean roots may be one possible explanation of the absence of White Mould in the field trials, in accordance with the observation that most soil-borne diseases are naturally suppressed in organic farming systems (van Bruggen et al., 2016). In the interviews treated in Chapter III, none of the *Croqueurs* members mentioned this disease as a problem. This contrasts with the prominent place held by White Mould in the bean breeding program of the multinational company producing conventional bean seed interviewed (data not shown), which regards White Mould as a major challenge, especially for geographical areas in the North of France. While the data presented here does not allow for any conclusions on this matter, it does indicate a perspective for future research.

Although a second year of observation would have been necessary to confirm the results presented here, data was not collected in 2014 due to methodological uncertainties concerning the observation of mycorrhizal structures. Firstly, it was unclear whether the sampling date and the number of samples collected per treatment were well suited for the comparison of percentage colonisation between common bean varieties under temperate field conditions. A field experiment was set up in 2014 to answer these methodological questions (de la Grandville, 2014) and confirmed that both the sampling dates and sample sizes practiced in the 2013 trial were adequate. In addition, this work also revealed that mycorrhiza sampling and observation may be rendered more efficient by (i) reducing sample size to 12 plants per treatment and (ii) reducing the number of field view passes across microscope slides. Secondly, it seems likely that the percentages of colonisation were overestimated in 2013, as the entire field of vision of the microscope was inspected for mycorrhizal structures. McGonigle *et al.* (1990) propose to take into account only the intersection of observed roots with a vertical eyepiece crosshair to avoid overestimating percentage colonisation.

In summary of the data available from a single year of observation, differences in indicators of symbiosis with mycorrhizal fungi and rhizobia were mainly driven by the experimental sites. Few differences were found among varieties, including the commercial control variety 'cal': mycorrhizal vesicles were found to colonise variety rdb more strongly in BZH and LUX, whereas variety rdc showed a tendency to develop a larger number of nodules than other varieties in LUX. No variety*environment interactions were observed. In Chapter III, I have discussed the *Croqueurs'* understanding of plant health management as a practice that is intrinsically linked to the local field conditions they are managing, which constitute the plants' growing environment. With reference to this *in situ* plant health management, the interactions of seed crops with mycorrhiza and rhizobia are managed as part of the whole system by seed growers. The data obtained here indicates that their *caring* for sound soils and the level of symbiosis with mycorrhizal fungi and rhizobia they can achieve in their bean plants is mainly determined by environmental conditions, including their own cultivation practices, pointing to the interaction between grower and environment and less to the effect of bean variety. Despite numerous researches demonstrating the induction of defence mechanisms of plants by the studied root symbionts under controlled conditions (Persello-Cartieaux et al., 2003; Berg, 2009; Dardanelli et al., 2010; Pozo et al., 2010; Jung et al., 2012; Cameron et al., 2013, among others), no correlation between the intensity of the symbioses and plant health were found here under field conditions, except for a very weak positive correlation between mycorrhizal structures and overall plant vigour in one site (BZH).

However, due to methodological difficulties, the data collected does not allow to dig deeper into interpretations on the link between plant health and these symbioses. For future research into this question, the following methodological recommendations can be drawn from this research experience: (i) It is preferable to use a non-destructive method to sample bean roots for the quantification of bean roots, in order to continue observing plant health on sampled plant beyond the sampling date. (ii) The assessment of percentage colonisation with mycorrhizal structures can be rendered more time efficient according to methodological trials reported by de la Grandville (2014). (iii) The use of a vertical eyepiece crosshair as proposed by McGonigle *et al.* (1990) for the quantification of colonisation with mycorrhizal structures would avoid overestimating percentage colonisation.

Photography on previous page:
Frank Adams

Chapter 7: **Microbial communities associated to bean seeds**

> "It's really the question of our understanding of what is sanitary. All microbial or fungal life around plants is a signature of the terroir. And of cultivation practices, not only of the terroir. There are always microbes on a local variety. Hence, we are the only ones thinking in terms of local varieties." [NGO-260915k] [42]

In the previous chapter, two symbiotic microorganisms associated to bean roots were elucidated. This chapter addresses the seed microbiome. Bean seeds carry not only nutrients and the genetic information needed for the growth of a plant, but also bacteria and fungi. Seed-associated microorganisms are located within seeds, as well as on seed surfaces. The *Croqueurs'* understanding of plant-associated microorganisms as an integral part of plant populations, including seeds (Chapter III, p. 64), leads to the question: **are microbial communities associated to bean seeds determined by the variety or by the growing environment?** If microbial communities associated to seeds are determined by the bean variety, they may be considered as forming part of that variety's identity. If microbial communities associated to seeds depend on the growing environment, they may contribute to the development of 'lineages' of that variety (Chapter III, division 1.1.1). In other words, they may then represent a form of communication between bean plants and their local environment (Chapter III, 1.1.2, p. 63).

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Klaedtke, S., Jacques, M.-A., Raggi, L., Préveaux, A., Bonneau, S., Negri, V., Chable, V. and Barret, M. (2016), Terroir is a key driver of seed-associated microbial assemblages. Environ Microbiol, 18: 1792–1804. doi:10.1111/1462-2920.12977

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12977/abstract

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[42] "C'est vraiment la question de la conception qu'on a du sanitaire. Tout ce qui est vie microbienne ou champignons autours des plantes, c'est la signature du terroir. Et des pratiques agricoles, pas que du terroir. Une variété locale, il y a nécessairement des microbes. Si tu les enlèves tous, il n'y a plus de variété locale. Alors, il n'y a que nous qui raisonnons en termes de variétés locales." [PA260915k]

environmental microbiology

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Terroir is a key driver of seed-associated microbial assemblages

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Summary

Seeds have evolved in association with diverse microbial assemblages that may influence plant growth and health. However, little is known about the composition of seed-associated microbial assemblages and the ecological processes shaping their structures. In this work, we monitored the relative influence of the host genotypes and terroir on the structure of the seed microbiota through metabarcoding analysis of different microbial assemblages associated to five different bean cultivars harvested in two distinct farms. Overall, few bacterial and fungal operational taxonomic units (OTUs) were conserved across all seed samples. The lack of shared OTUs between samples is explained by a significant effect of the farm site on the structure of microbial assemblage, which explained 12.2% and 39.7% of variance in bacterial and fungal diversity across samples. This site-specific effect is reflected by the significant enrichment of 70 OTUs in Brittany and 88 OTUs in Luxembourg that lead to differences in co-occurrence patterns. In contrast, variance in microbial assemblage structure was not explained by host genotype. Altogether, these results suggest that seed-associated microbial assemblage is determined by niche-based processes and that the terroir is a key driver of these selective forces.

Introduction

Plants have evolved in association with diverse microbial assemblages, also known as microbiota, which can affect key plant traits such as metabolite production (Badri *et al.*, 2013), disease resistance (Mendes *et al.*, 2011), flowering time (Panke-Buisse *et al.*, 2015) and biomass accumulation (Sugiyama *et al.*, 2013). Owing to such important effects on plant growth and plant health, a substantial amount of work has investigated the processes involved in the assembly of the phyllosphere and the rhizophere microbiota (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Horton *et al.*, 2014; Maignien *et al.*, 2014). In contrast, other plant habitats such as the anthosphere (Shade *et al.*, 2013), the carposphere (Telias *et al.*, 2011) and the spermosphere (Ofek *et al.*, 2011) have been often overlooked. However, these plant habitats may act as reservoirs for the plant microbiota because they are associated to the reproductive stage of plants and are therefore involved in the vertical transmission of microorganisms from one plant generation to another (Aleklett and Hart, 2013).

Although transmission of microorganisms from plant to seed is the primary source of inoculum for the plant (Baker and Smith, 1966; Nelson, 2004; Darrasse *et al.*, 2010), relatively little is known about the structure of seed-associated microbial assemblages and the regulators of assemblage structure. Culture-independent surveys have revealed that seed-associated microbial assemblages are composed of 50–1000 bacterial and fungal operational taxonomic units (OTUs) (Lopez-Velasco *et al.*, 2013; Links *et al.*, 2014; Barret *et al.*, 2015). These differences in seed microbiota richness could be attributed to deterministic processes such as host genetic variations. For instance, it has been shown that some plant genotypes select certain endophytic bacterial taxa from one plant generation to another (Johnston-Monje and Raizada, 2011; Hardoim *et al.*, 2012). Moreover, a fraction of the seed microbiota is conserved among different plant lineages (Links *et al.*, 2014). However, the structure of the seed microbiota seems also driven by other deterministic processes such as field management practices, harvesting methods, seed processing and storage (van Overbeek *et al.*, 2011; Aleklett and Hart, 2013). Indeed, we have recently observed that variability of seed-associated

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bacterial and fungal assemblages could be explained, in part, by the geographic location of the production area (Barret *et al*., 2015).

Understanding the processes involved in the assembly of the seed microbiota may result in practical applications for seed quality and agriculture. For example, some seed-associated microbial assemblages may release seed dormancy through production of cytokinins (Goggin *et al*., 2015) and therefore ensure a homogenous field germination. In addition, activity of the seed microbiota may also limit the installation of exogenous microorganisms (Bacilio-Jimenez *et al*., 2001) and consequently decrease the activity of plant growth-promoting inoculants. Finally, transmission of phytopathogenic microorganisms by seed is an important mean of pathogen dispersion and therefore has been recognized as significant in the emergence of diseases in new planting areas (Baker and Smith, 1966). Altogether, these observations clearly highlight a need to improve our knowledge on processes shaping the structure of the seed microbiota.

To date, local adaptation in annual crops has been mainly studied at the genetic level (Tiranti and Negri, 2007; Pyhajarvi *et al*., 2013; Savolainen *et al*., 2013; Raggi *et al*., 2014). However, microbial assemblage may be one of the determining factors influencing local adaptation in plants (Bulgarelli *et al*., 2013). For instance, recent works have shown that soil-associated microbial assemblage is a key feature in regional variation among wine grapes (Bokulich *et al*., 2014; Zarraonaindia *et al*., 2015), therefore suggesting that soil microbiota is an important component of the terroir. According to one definition, terroir is a geographic space delimited according to a human community sharing distinctive cultural traits, know-how and practices developed over its common history based on a system of interactions between the natural environment and human factors (Prevost and Lallemand, 2010). Functioning of the terroir relies on the interactions between abiotic and biotic factors such as climate, soil and plants. While the effect of terroir on perennial species and the products derived from them is commonly recognized, its influence on the production of annual species and staple foods under field conditions has only recently become an object of research (Lhomme *et al*., 2015).

The role of the seed microbiota in the local adaptation of plants was recently questioned in the framework of a participatory research project on farm seeds (Farm Seed Opportunities), leading to the work presented here. The purpose of this work was to assess the relative influence of host genotypes and terroir on the structure of the seed microbiota. In this context, we used common bean (*Phaseolus vulgaris* L.) as a model species because common bean is a crop of worldwide importance as food crop. Many traditional cultivars exist in the Old and New Worlds and the farmers engaged in the research project produce such traditional cultivars. Moreover, common bean is concerned by several seed-borne fungal, bacterial and viral pathogens that can hinder plant health and development. Effects of seed microbiota on plant health and adaptation are thus of particular interest for this crop.

The relative influence of the host genotypes and terroir on the structure of the bean seed microbiota was assessed through metabarcoding analysis of five different bean cultivars harvested in two distinct farms. Because these two farms have their own practices (from crop management to postharvest handling) and are located in two contrasted climatic areas, they represent different terroirs. On the one hand, we found that the terroir is a key driver of the structure of seed-associated microbial assemblage and impact mainly fungal diversity. On the other hand, we found that plant cultivar is not significantly shaping the assembly of the seed microbiota of common bean.

Results

The main objective of this work was to assess the relative influence of the host genotypes and terroir on the assembly of the seed microbiota. The effect of these two deterministic processes on the structure of seed-associated microbial assemblages was studied on bean seed samples. These seed samples were obtained by multiplying five seed lots of different bean cultivars [namely 'Calima' (Cal), 'Flageolet Chevrier' (FlC), 'Rognon de Coq' (RdC), 'Roi des Belges' (RdB) and 'Saint Esprit à Oeil Rouge' (SES)] for two consecutive years in two farms located in Brittany (BZH) and Luxembourg (LUX) (Table S1).

Genetic diversity within and among cultivars

The genetic diversity of the five bean cultivars used in this study was firstly assessed through 11 simple sequence repeat (SSR) markers covering all the species linkage groups (Table S2). According to SSR data analyses, the number of analysed individuals was suitable to represent the cultivars level of genetic diversity. Indeed, mean SSR allelic accumulation curves were close to reaching the asymptote for all five cultivars (Fig. S1). A total of 38 alleles were obtained from the amplification of the 11 SSR loci selected. The majority of the loci was polymorphic because alleles with frequencies $>95\%$ were only observed at loci AG01 and BM137. Private alleles were found in all cultivars, one in RdB (frequency = 0.03), four in SES (mean frequency ± standard error = 0.76 ± 0.218), three in FlC (0.64 ± 0.294), five in RdC (0.28 ± 0.068) and two in Cal (0.53 ± 0.469).

Significant differences in effective number of alleles (*Ne*) and expected heterozygosity (*He*) were detected between RdC and both RdB and SES (Mann–Whitney test, $P \leq 0.05$), RdC being characterized by highest values (Table S3). This implies that a high level of genetic diversity was present within RdC, whereas RdB and SES had rather low levels of within cultivar diversity.

The calculated genetic distance (GD) matrix indicated that the cultivars are composed of a different number of multilocus genotypes, i.e. 16 genotypes in RdC (88.9% of total examined individuals), eight in FlC (44.4%), seven in Cal (43.7%) and five in both SES and RdB (27.8%). At individual level, the genetic relationship between cultivars was investigated by principal coordinates analysis (PCoA). The first two major axes explained 70.7% of the total variation (Fig. S2A) and this percentage reached 86.3% when the third axe was considered (Fig. S2B). The first two principal coordinates clearly separated Cal and SES from the all the other varieties, whereas the third was necessary to better separate FlC from RdB and RdC (Fig. S2).

Analysis of molecular variance (AMOVA) results indicated that both among and within cultivar genetic diversity is significant (Table S4) with the largest proportion due to the diversity among cultivars (77.6% versus 22.4%). These results were confirmed by the values of average numbers of pairwise differences between cultivars (PiXY), which were all statistically significant. Highest pairwise differences were detected among SES and FlC (PiXY = 8.51, $P \leq 0.001$) and between RdB and SES (PiXY = 7.62, $P \leq 0.001$) (Table S5 and Fig. S3). In addition, different cultivars were characterized by a different level of within cultivar diversity as shown by PiX analysis results. PiX ranged from 0.62 (SES) to 3.43 (RdC). To a certain extent, it was similar for cultivars RdB, SES, Cal and FlC, whereas RdC showed a higher value (Table S5 and Fig. S3). According to reported results, the selected cultivars displayed a large level of both among and within genetic diversity, RdC being the most diverse.

Effect of sampling on seed microbiota profiling

The structure of the microbial assemblages associated to 27 seed samples was assessed by sequencing (i) the V4 region of 16S rRNA gene (Caporaso *et al.*, 2011) and (ii) the ITS1 region of the fungal internal transcribed spacer (ITS) (Schoch *et al.*, 2012). Overall, 3 281 571 and 5 553 827 pairs of reads were obtained for 16S rRNA gene and ITS1 sequences respectively (Table S6). Raw reads were first assembled in quality sequences and then grouped into OTUs at ≥ 97% sequence identity (see Experimental procedures section). Only abundant OTUs representing at least 0.1% of the library size were conserved for subsequent analyses (Barret *et al.*, 2015). Using this relative abundance threshold, 195 and 157 OTUs were obtained with 16S rRNA gene and ITS1 sequences respectively. These abundant OTUs represented 95.6% of all 16S rRNA reads and 96.6% of all ITS1 reads.

To investigate the magnitude of variability between seed samples of the same bean cultivar harvested on the same site, we monitored the proportion of bacterial and fungal OTU shared between field replicates. Despite a relatively high sequencing depth (> 38 000 sequences per sample, Table S6), the number of bacterial OTUs shared between field replicates was low ranging from 0.9% to 27.9% of all bacterial OTUs (Table S7). Because the bacterial assemblages associated to some seed samples were composed of only one to three abundant OTUs, the heterogeneity observed between seed samples is likely due to the presence of dominant taxa within the assemblage that masked the detection of other bacterial taxa. In comparison, composition of seed-associated fungal assemblage was more homogenous with 27.6–55.9% of OTUs conserved across all field replicates (Table S7). As the replicates were not obtained by resampling one seed lot, but consisted of field replicates obtained from a field trial sown in complete replicated blocks, this heterogeneity may be explained in part by variations within the trial field.

Next, we explored OTUs that were conserved across bean cultivars and experimental sites. A total of 22 and 16 fungal OTUs systematically associated to every seed lot were observed in BZH and LUX respectively (Fig. S4 and Table S8). Moreover, each bean cultivar was composed of 11–20 ubiquitous fungal OTUs depending on the plant genotype (Fig. S4 and Table S8). Overall, seven fungal OTUs were conserved between all the sites and cultivars suggesting that these OTUs represent the core members of fungal assemblages associated to common bean seeds. These seven OTUs corresponded to five Ascomycota and two Basidiomycota. As stated earlier, data obtained with the 16S rRNA gene sequence were more variable. Consequently, one to three OTUs were associated with different bean cultivars and only one OTU affiliated to *Pseudomonas* genus was conserved between all the experimental sites and cultivars (Fig. S4 and Table S8).

The experimental site influences the structure of microbial assemblages

The influence of experimental site and bean cultivar on bacterial and fungal richness was initially assessed by comparing the number of OTUs between treatments. According to Kruskal–Wallis test, no significant difference in bacterial richness was observed between experimental sites ($P = 0.11$; Fig. 1) nor between bean cultivars

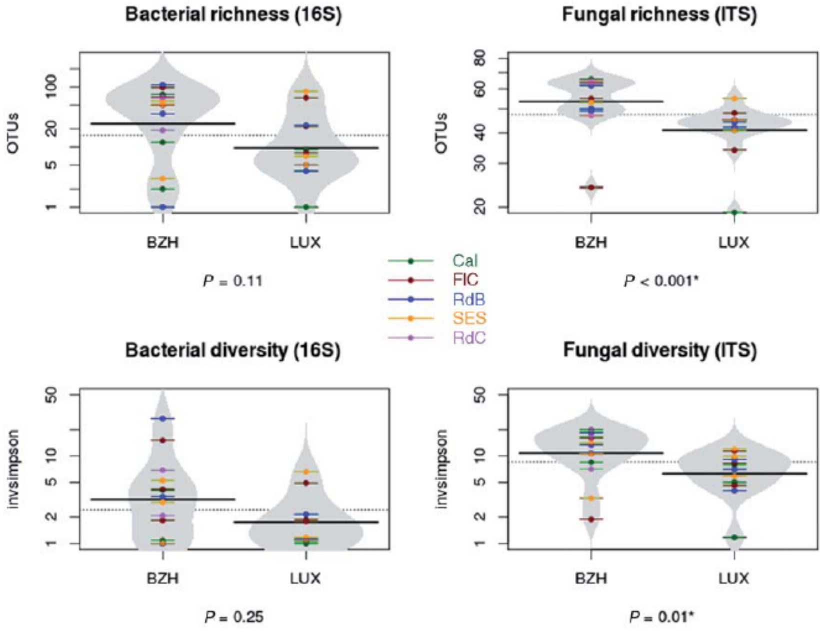

Fig. 1. Estimation of bacterial and fungal diversity. Richness (OTUs) and diversity (Simpson's inverse index) were estimated in seed samples harvested in Brittany and Luxembourg. Seed samples of each bean cultivar are represented by different colours lines, whereas the grey area represents the estimation of the distribution. Kruskal–Wallis one-way analysis of variance was performed to assess the effect of the production region on richness and α-diversity.

($P = 0.34$, Fig. S5). Moreover, α-diversity of bacterial assemblage (assessed by Simpson's inverse index) was also unaffected by experimental site and bean cultivars ($P = 0.25$ and $P = 0.29$ respectively). On the contrary fungal richness and α-diversity of fungal assemblage significantly decreased in LUX site in comparison to BZH ($P < 0.001$ and $P = 0.01$, Fig. 1), whereas no differences were observed between bean cultivars ($P = 0.61$ and $P = 0.78$, Fig. S5).

Because fungal richness and α-diversity of seed-associated fungal assemblages were affected by the experimental sites, diversity between samples (also known as β-diversity) was estimated with Bray–Curtis dissimilarity (Bray and Curtis, 1957). Hierarchical clustering of Bray–Curtis dissimilarity index and subsequent AMOVA revealed a significant effect of the experimental site on the structure of bacterial ($P = 0.032$; Fig. 2A) and fungal assemblages ($P < 0.001$, Fig. 2B). In contrast, the bean cultivar was neither significantly shaping the composition of bacterial assemblage ($P = 0.64$) nor the composition of fungal assemblage ($P = 0.81$). The relative contribution of terroir and bean cultivar on microbial β-diversity was further inspected by a canonical analysis of principal coordinates (CAP) on Bray–Curtis dissimilarity matrix. On the one hand, CAP analyses revealed that the majority of the variation in fungal diversity across seed samples is explained by the terroir (39.7% of the variation, $P < 0.001$), whereas 12.2% of variation in bacterial β-diversity is explained by this factor ($P = 0.037$). On the other hand, bean varieties did not explain variation in fungal ($P = 0.80$) and bacterial ($P = 0.78$) β-diversity. In conclusion, the terroir was the main factor driving fungal and bacterial assemblage structure.

Taxonomic composition of the bean seed microbiota

Because the structure of seed microbiota was mainly shaped by the location of the production region, we examined the variation in seed microbiota composition between seeds harvested from both experimental sites. According to 16S rRNA gene sequences, bacterial OTUs were mostly affiliated to *Gammaproteobacteria* with a relative abundance of 91% and 79% for the BZH and LUX sites respectively. *Pseudomonas* was by far the most represented bacterial genus with 48% and 77% of all reads for BZH and LUX respectively. Regarding fungal community composition, the seed microbiota of LUX was mainly composed of Ascomycota (83%), whereas ratio between Ascomycota (59%) and Basidomycota (37%) was more balanced in BZH. At the genus level, *Cryptococcus* and *Fusarium* were well represented in BZH, whereas *Alternaria*, *Eurotium* and *Fusarium* were frequently associated to LUX (Fig. S6).

Next, we investigated changes in relative abundance of OTUs between experimental sites and cultivars. Bacterial and fungal OTUs were defined as significantly enriched or depleted in one treatment at a corrected P-value < 0.001 and a $\log_2$ fold change magnitude ≥ 2. Using these criteria, we only detected significant changes in relative abundance of bacterial and fungal OTUs between experimental sites (Table S9 and Table S10). More precisely, 24 bacterial OTUs, mostly affiliated to Bacilli, were enriched

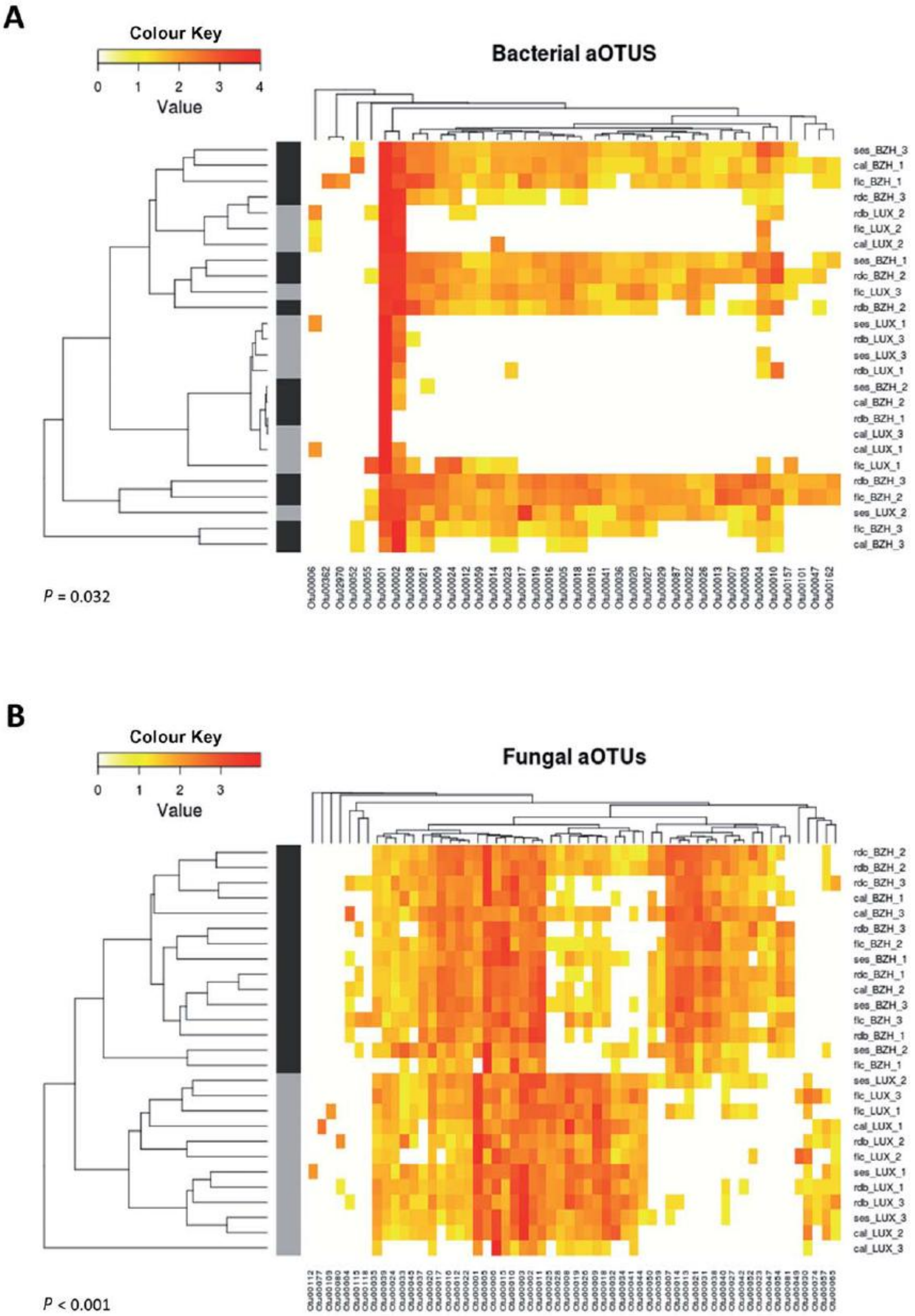

Fig. 2. Seed-associated microbial assemblages clustered primarily by experimental site. Heatmaps of bacterial (A) and fungal OTUs (B). Clustering of seed samples was performed by calculation of Bray–Curtis dissimilarity matrix. Although Bray–Curtis dissimilarity matrix was calculated on every bacterial and fungal OTU, only OTUs with a relative abundance > 1% are shown in these figures. OTUs were also clustered by co-occurrence pattern. The effect of the experimental site on the structure of microbial assemblage was assessed by analysis of molecular variance (AMOVA). Light grey and dark grey colours indicate seed samples harvested from Brittany and Luxembourg respectively.

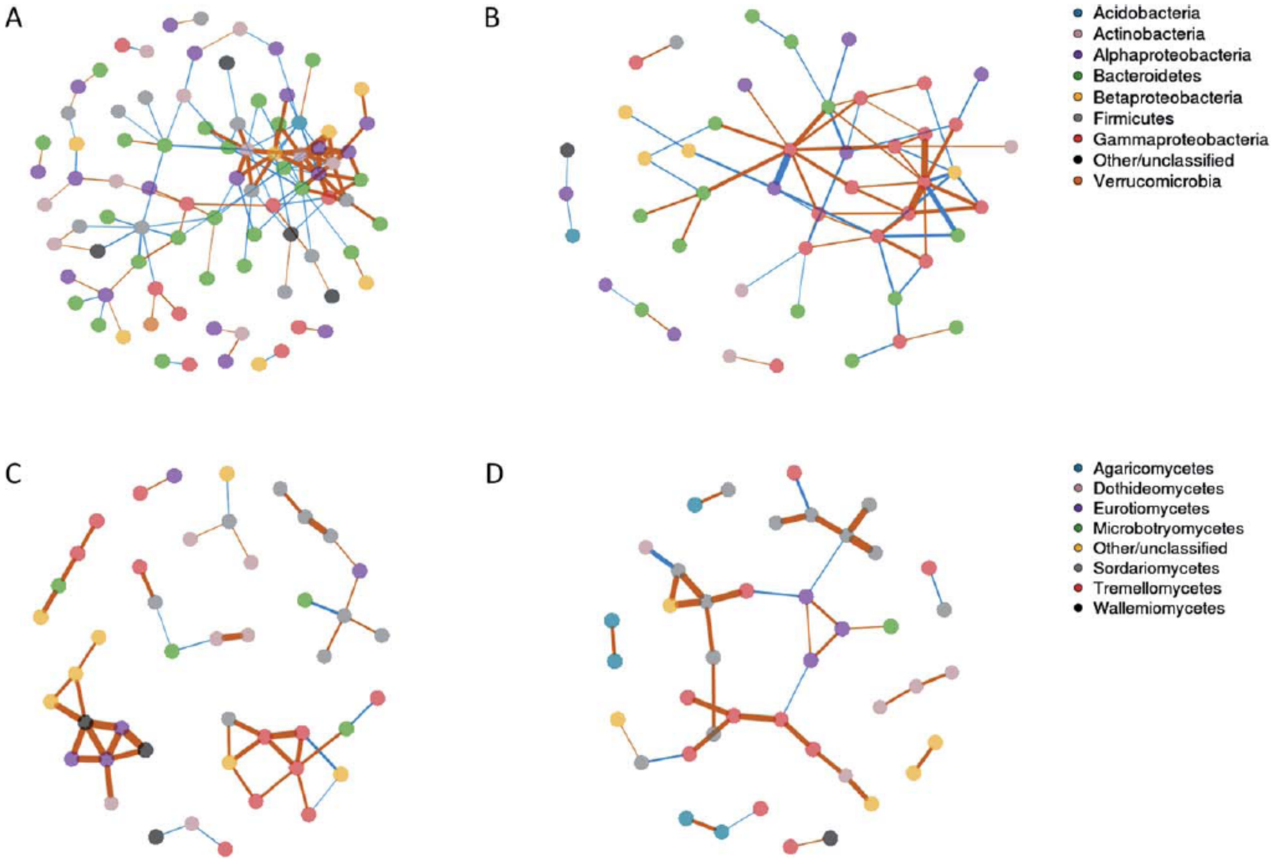

Fig. 3. Bacterial and fungal communities observed among microbial assemblages. Environment-specific correlation networks were inferred with bacterial (A and B) and fungal (C and D) OTUs associated to seeds harvested in Brittany (A and C) or in Luxembourg (B and D). Correlations between OTUs were inferred with the Sparse Correlations for Compositional data algorithm (SparCC) implemented in MOTHUR. The effect of uneven sampling was corrected by dividing sequence counts by total library size. Nodes correspond to OTUs, and connecting edges indicate inferred correlations between them. Node colour represents the different bacterial and fungal taxa. Only positive (orange) and negative (blue) correlations with pseudo *P*-value ≤ 0.001 were represented in the network using the R package QGRAPH.

in BZH in comparison to LUX (Table S9). On the contrary, 34 OTUs were enriched in LUX. Seven of these OTUs were affiliated to Enterobacteriaceae. Interestingly, members of the bacterial phyla Acidobacteria and Verrucomicrobia were also found enriched in seed samples harvested from BZH (Table S9). Numerous variations in relative abundance of fungal OTUs were also observed between the two experimental sites, confirming changes of fungal diversity observed previously. A total of 54 and 46 fungal OTUs were significantly enriched in LUX and BZH respectively (Table S10). Among these OTUs, the genera *Rhizoctonia* and *Fusarium* were mainly associated to LUX, while *Cryptococcus* and a group of unclassified OTUs were enriched in BZH (Table S10).

Interactions between microbial taxa associated to seed samples

Differences of microbial assemblage composition between seeds harvested from BZH and LUX are probably linked to changes of microbial community structure. In order to predict microbial interactions within seed-associated assemblages, we explored positive and negative associations between entities of these assemblages by generating environment-specific correlations networks with Sparse Correlations for Compositional data algorithm (SparCC; Friedman and Alm, 2012). Considering only inferred correlations with pseudo *P*-values ≤ 0.001, we identified 260 and 132 associations between 85 and 48 bacterial OTUs associated to seeds harvested in BZH and LUX respectively (Fig. 3A and B and Table S11). The lower number of nodes in the network inferred with seed-associated bacterial assemblages from LUX could be explained by the high prevalence of OTU0001 (affiliated to *Pseudomonas*) in this site. Indeed the increase in relative abundance of this OTU ultimately results in decrease of relative abundance of others OTUs, and consequently caused difference in co-occurrence patterns.

Overall, both bacterial correlation networks were fragmented into one major module and some minor clusters consisting of two to three OTUs (Fig. 3A and B). Nodal degree (number of association between nodes) was rather low for each correlation network with a median number of two inferred correlations between bacterial OTUs (Table S11). The highest degree nodes of BZH bacterial network (a.k.a hubs) were related to taxonomically diverse OTUs including *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Bacteroidetes* (Fig. 3A), whereas hubs of LUX bacterial correlation network were mostly affiliated to *Gammaproteobacteria* (Fig. 3B). According to edgeR analysis, these latter entities are part of OTUs significantly enriched in the LUX site, whereas the relative abundance of BZH hubs was not significantly different between sites.

In comparison with bacterial networks, correlations networks inferred from seed-associated fungal assemblages harvested in BZH (Fig. 3C) and LUX (Fig. 3D) were composed of less edges and nodes (Table S11), suggesting fewer interactions between entities of these assemblages. The topology of BZH fungal networks was clearly different from the topology of other fungal and bacterial networks with multiple modules composed of two to eight OTUs (Fig. 3C). The highest degree nodes of each environment-specific correlations network were related to fungal OTUs significantly enriched in BZH (Eurotiomycetes and Tremellomycetes) or LUX (Sordariomycetes). However, the number of connections that these hubs shared with other nodes was quite low in comparison with bacterial networks with a maximal degree of 5 and 4 for BZH and LUX respectively.

Discussion

Seeds have evolved in association with diverse microbial assemblages that may influence plant growth and health (Nelson, 2004; Darrasse *et al.*, 2010; Goggin *et al.*, 2015). To date, studies concerning the composition of these seed-associated microbial assemblages are scarce in comparison with other plant habitats such as the phyllosphere and the rhizosphere (Vorholt, 2012; Philippot *et al.*, 2013). Results obtained in this work indicate that terroir is a key driver involved in the assembly of the seed microbiota. The variable farm sites were taken as a proxy of the terroir, which is a combination of human practices and environmental conditions (Prevost and Lallemand, 2010). Indeed the two farm sites targeted in our study explained 12% and almost 40% of the variation in bacterial and fungal β-diversity respectively. Interestingly, a significant regional pattern has also been observed in the grape microbiota, especially for grape-associated fungal assemblages (Bokulich *et al.*, 2014), suggesting an important effect of terroir on habitats associated to the reproductive stages of the plant. Because terroir is the result of the interactions between a human community and the environment (Prevost and Lallemand, 2010), it is somewhat difficult to assess the relative contribution of each factor in the composition of seed-associated microbial assemblages. Nevertheless, according to our results it seems that the plant genotype does not significantly explain variation of microbial diversity across seed samples. This finding is in agreement with a previous study performed on multiple seed-associated assemblages of various plant species (Barret *et al.*, 2015). However, some entities of seed-associated assemblages can be conserved across different plant lineages (Links *et al.*, 2014) and from one plant generation to another (Johnston-Monje and Raizada, 2011; Hardoim *et al.*, 2012).

An important fraction of variability in seed microbiota composition is explained neither by environmental structuring factors nor by host genotype. This is especially true for seed-associated bacterial assemblages where only 12.2% of variation in bacterial β-diversity is explained by the farm site. Although seeds of various origins shared a common microbiota at higher taxonomic rank, seed-associated bacterial assemblages are extremely different at the OTU level (Lopez-Velasco *et al.*, 2013; Links *et al.*, 2014; Barret *et al.*, 2015). Indeed, only one abundant bacterial OTU affiliated to *Pseudomonas* is conserved across all seed samples. This high variability between seed lots is probably not due to insufficient sequencing effort because each seed sample contained a minimum of 38 000 high quality sequences. The lack of apparent shared OTUs between seed samples could be explained by within-individual heterogeneity of seed traits such as seed size (Halpern, 2005). Additionally, neutral processes such as assembly history may determine the structure of seed-associated bacterial assemblages (Aleklett and Hart, 2013). In other words, the colonization order of the seed habitat during its development and maturation could have a decisive impact on community assembly, as it has been previously shown for other plant-related habitat such as the phyllosphere (Maignien *et al.*, 2014).

Early seed colonizers are probably transmitted from plant to seed by two major pathways: (i) internal transmission through the vascular system or (ii) floral transmission by the stigma (Maude, 1996; Darsonval *et al.*, 2008). Then, other microbial entities are subsequently incorporated within the microbiota during seed development through contact of the seed with microorganisms present on fruits, flowers or residues (Maude, 1996). At harvest and post-harvest, the seed is also in contact with tools used for harvest and threshing and with the locale in which it is stored. Therefore, the structure of seed-associated microbial assemblage is probably shaped by the spatio-temporal dynamics of microbial interactions. In order to detect potential interactions within bacterial and fungal communities associated to seeds, we have analysed co-occurrence networks of OTUs among the seed samples examined in our study using SparCC (Friedman and Alm, 2012). These analyses revealed differences in the topology of bacterial and fungal co-occurrence networks, with more inferred correlations within bacterial networks than within fungal networks. This might suggest less interactions between fungal entities of microbial assemblages as results of compartmentalization of fungal populations within different locations (from the embryo to the testa) of the seed habitat (Singh and Mathure, 2004; Pochon *et al.*, 2012). Investigation of co-occurrence networks also highlighted frequent co-occurrence of OTUs related to the same bacterial or fungal class, which is in agreement with previous network analyses (Toju *et al.*, 2014; Barret *et al.*, 2015). However, hubs of bacterial network were related to taxonomically diverse OTUs, which may indicate that these hubs possessed different functional potentials. However, this hypothesis has to be validated with experimental models in order to determine whether these distinct bacterial taxa are not functionally similar entities.

In comparison with seed-associated bacterial assemblages, fungal assemblages are less diverse across seed samples harvested from the same bean cultivar at the same experimental site (27.6–55.9% of shared fungal OTUs), confirming previous observations on other seed-associated fungal assemblages (Barret *et al.*, 2015). Although fungal assemblages of seeds of the same bean cultivar harvested on the same production region are relatively similar, we only detected seven conserved fungal OTUs across all genotypes cultivated on both experimental sites. These core OTUs are affiliated to ubiquitous fungal genera such as *Alternaria* (Woudenberg *et al.*, 2013) and *Cladosporium* (Bensch *et al.*, 2012), which are frequently associated to various plant habitats including seeds (Links *et al.*, 2014; Barret *et al.*, 2015). The relatively small proportion of fungal members systematically associated to all seed samples is explained by a strong effect of terroir on fungal diversity. Hence, seed-associated fungal assemblages are, unlike bacterial assemblages, driven by deterministic processes, which is in accordance with biogeographic patterns observed in various soil-associated fungal assemblages (Meiser *et al.*, 2013; Bahram *et al.*, 2015). Therefore, it seems highly plausible that a majority of fungi associated to bean seeds are primarily derived from the local environment (e.g. soilborne or airborne fungi) rather than from one plant generation to another (seedborne). The taxonomic affiliation of fungal OTUs significantly enriched in the BZH or LUX sites promotes this hypothesis. For instance, OTUs related to soilborne fungi such as *Fusarium* (Roncero *et al.*, 2003) and *Rhizoctonia* (Okubara *et al.*, 2014) are significantly enriched in the experimental site located in LUX. Therefore, the dispersion of seed in different production regions (or terroirs) affects seed-associated fungal assemblages and may consequently impact seed fitness. Despite the use of genetically diverse cultivars for this analysis, no cultivar effect on fungal assemblages was detected.

Overall, the present work suggests that seed-associated microbial assemblage is determined by niche-based processes and that the terroir is a key driver of these selective forces. Farmers engaged in small-scale, local seed production thus seem to be safeguarding not only the seed and genetic diversity of traditional cultivars (Negri & Tiranti, 2010), but also the seed-associated microbial assemblages specific of their terroirs. Although we cannot conclude on the specific implications of particular terroir-bound microbial assemblages on plant health and fitness, it is tempting to speculate that these assemblages may play a role in the local adaptation of crops to their production site. Further research on the functional properties of seed-associated microorganisms will give further insight into this role.

Experimental procedures

Field trials

The effect of terroir and cultivar on the structure of seed-associated microbial assemblages was assessed by analysing seed samples obtained in two different farms located in BZH and LUX. Seed samples were obtained by multiplying five initial seed lots for two consecutive years in both farms. Both farms are organic and engaged in small-scale seed production of traditional cultivars. According to the definition given in the introduction, terroir is the result of the interaction between a human community and the environment, including its biotic and abiotic components. Under real-life conditions, the bean crop interacts with the abiotic, biotic and human environment in the field and post-harvest. Therefore, crop management (Table S1), seed harvest and post-harvest cleaning and sorting were conducted according to the practices of the local farmers to account for all the components of terroir. The two experimental sites are characterized by differences in average temperature and rainfall (Table S1). Moreover, soil analyses revealed variations in calcium, nitrogen, potassium and phosphorous content as well as differences of pH (Table S1).

Four of the seed lots consisted of farm seeds of traditional cultivars representing a range in tolerance to diseases and climatic factors, namely FIC, RdC, RdB and SES. Seed of the commercial cultivar Cal obtained from Hild Samen GmbH was used as a commercial control.

Field trials were organized as three complete replicated blocks per cultivar with plot sizes ranging from 5.6 to 9 m^2 according to site and year. Plants were harvested and threshed by stomping. A first cleaning and sorting step was carried out by a traditional wind separator. A second sorting step was done by hand according to the practices of the local farmers. A total of 27 seed samples were harvested in 2013 after the initial seed lots had been exposed to contrasting biotic and abiotic environments for two consecutive years. There were 15 seed samples obtained in BZH, whereas only 12 seed lots were harvested in LUX because the cultivar RdC failed to yield sufficient seed for analyses.

Plant DNA extraction and SSR genotyping

The diversity of morphological and agronomic traits among the bean cultivars employed suggested a large genetic diversity, suited to test the effect of host genotypes on seed-associated microbial assemblages. In order to verify and explore the genetic diversity existing among and within the five common bean cultivars, a total of 88 bean plants were genotyped using 11 SSR molecular markers. These included 18 plants from each one of the four traditional cultivars (FIC, RdC, RdB and SES) and 16 from the commercial cultivar Cal. In 2014, the plants were grown in a greenhouse from the seed lots used to implement the field trials and 50 mg of fresh leaf tissue were collected from each individual plant. Genomic DNA was extracted using the TissueLyser II (Qiagen) and the extraction kit DNeasy® 96 Plant Kit (Qiagen) according to protocols provided by the manufacturer. The DNA quality and concentration were assessed through spectrophotometry using NanoDrop 2000 (Thermo Scientific).

The molecular characterization was carried out using 11 SSR markers covering all the species linkage groups (Table S2). For each primer combination, the forward primer was 5′ end-labelled with one of the fluorescent tags 6-FAM, VIC, NED and PET (Applied Biosystems). Multiplex reactions were prepared using primers with similar annealing temperature and taking into account amplicon sizes. All the polymerase chain reaction (PCR) reactions were performed in an Applied Biosystems 2720 Thermal Cycler in a volume of 12 μl containing the following: 20 ng of template DNA, 1 × Type-it® Multiplex PCR Master Mix (Qiagen) and 0.4 μM of each primer and using the following thermal profile: 94°C for 4 min, followed by 12 cycles of 94°C for 30 s, x°C for 30 s, 72°C for 30 s, 25 cycles of 94°C for 10 s, x'°C for 15 s, 72°C for 20 s and 20 min at 72°C in the final extension where x was equal to the annealing temperature and x' to the same temperature decreased by 1°C (Table S2). The amplification products were combined with highly deionized formamide and a size standard (GeneScan™ 500 LIZ®, Applied Biosystems) and separated on a ABI3130xl automated sequencer. Sample profiles were scored manually using the GENEMAPPER® Software version 4.0 (Applied Biosystems).

SSR data analysis

A moment-based richness estimator was used to calculate allelic richness up to the number of individuals present in each cultivar using the R function for allelic richness estimation (ARES) from Van Loon *et al.* (2007). Mean accumulation curves of estimated allelic richness were produced. The total number of polymorphic loci was calculated following the criterion of 95% of major allele frequency. For each cultivar and SSR locus, number (*Na*) and effective number (*Ne*) of alleles per locus, observed (*Ho*) and (*He*) expected heterozygosity and number of private alleles (*P*) were calculated. A Mann–Whitney (MW) test was performed to assess the cultivar effect on the above-mentioned diversity parameters in pairwise comparisons. Because of the high degree of selfing of common bean, fixation index and deviation from Hardy–Weinberg equilibrium were not calculated.

A pairwise, individual-by-individual (N × N) GD matrix was calculated for co-dominant data according to Peakall *et al.* (1995) and Smouse and Peakall (1999) and used to perform a PCoA. PCoA results were plotted using the GGPLOT2 package (Wickham, 2009) in R software. AMOVA was used to study the partition of molecular variance components among and within cultivars. Statistical significance of obtained results was tested using 999 permutations with GENALEX software 6.3 version (Peakall and Smouse, 2006). Within cultivar, diversity was worked out as average number of pairwise differences between individuals (PiX). Genetic differentiation between cultivars was also assessed as the average number of pairwise differences between cultivars (PiXY). Finally, the corrected number of average pairwise differences between cultivars was worked out as [PiXY – (PiX + PiY)/2]. These parameters were calculated using ARLEQUIN ver 3.5 (Excoffier and Lischer, 2010).

Microbial DNA extraction, amplicon library construction and sequencing

Approximately 200 bean seeds of each sample were transferred in a sterile blender bag containing 250 ml of phosphate buffer saline supplemented with 0.05% (v/v) of Tween® 20. Samples were incubated overnight at 4°C under constant agitation. Suspensions were centrifugated (6000 × *g*, 10 min, 4°C) and pellets were re-suspended in approximately 2 ml of supernatant and transferred in microtubes. Total genomic DNA was extracted from 27 different samples using PowerSoil® DNA isolation kit (MO BIO) following the manufacturer's protocol (Table S6). In addition, an artificial community sample was prepared by mixing equal amounts of genomic DNA of 15 bacterial strains belonging to 13 distinct bacterial families (Barret *et al.*, 2015). This artificial community sample is a useful indicator of sequencing error rate (Schloss *et al.*, 2011).

Amplicon libraries were constructed following two rounds of PCR amplification. The first round was performed with the PCR primers 515f/806r (Caporaso *et al.*, 2011) and ITS1F/ITS2 (Buee *et al.*, 2009), which target the V4 region of 16S rRNA gene and ITS1 respectively. Forward and reverse primers carry the following 5′-CTTTCCCTACACGACGCTCTTCCGATCT-3′ and 5′-GGAGTTCAGACGTGTGCTCTTCCGATCT-3′ tails respectively. All PCR reactions were performed with a high-fidelity polymerase (AccuPrime™ *Taq* DNA Polymerase System; Invitrogen) using the manufacturer's protocol and 2 μl of environmental DNA (approximately 10 ng). Cycling conditions for 515f/806r and ITS1F/ITS2 were adapted from Caporaso and colleagues (2011) and Buee and colleagues (2009). Reactions were held at 94°C for 2 min, followed by 30 cycles of amplification 94°C (30 s), 50°C (60 s) and 68°C (90 s) with a final extension step of 10 min at 68°C. All amplicons were purified with the Agencourt® AMPure® XP system and quantified with QuantIT™ PicoGreen®. The second round of amplification was performed with 5 μl of purified amplicons and primers containing the Illumina adapters and indexes. PCR cycling conditions were: 94°C (2 min), followed by 12 cycles of amplification [94°C (1 min), 55°C (1 min) and 68°C (1 min)] and a final extension step at 68°C (10 min). All amplicons were purified and quantified as previously described. The purified amplicons were then pooled in equimolar concentrations and the final concentration of the library was determined using a qPCR NGS Library Quantification Kit. Amplicon libraries were mixed with 5% PhiX control according to Illumina's protocols. Sequencing run was performed with MiSeq Reagent Kit v2 (500 cycles).

Clustering MiSeq reads into OTUs

Raw reads were analysed using the steps described in Barret and colleagues (2015). Briefly, 16S rRNA gene sequences were aligned against the 16S rRNA gene SILVA alignment using MOTHUR V1.33 (Schloss *et al.*, 2009). Chimeric sequences were detected with UCHIME (Edgar *et al.*, 2011) and subsequently removed from the data set. Taxonomic affiliation of 16S rRNA genes was performed with a Bayesian classifier (Wang *et al.*, 2007) (80% bootstrap confidence score) against the 16S rRNA gene training set (v9) of the Ribosomal Database Project (Cole *et al.*, 2009). Unclassified sequences or sequences belonging to *Eukaryota*, *Archaea*, chloroplasts or mitochondria were discarded. Sequences were assigned to OTUs at 97% identity.

The variable ITS1 regions of ITS sequences were extracted with the Perl-based software ITSX (Bengtsson-Palme *et al.*, 2013). Then sequences were processed using the QUANTITATIVE INSIGHT INTO MICROBIAL ECOLOGY (QIIME V1.7.0) software (Caporaso *et al.*, 2010). Sequences were clustered at a 97% identity cut-off using UCLUST (Edgar, 2010) and taxonomic affiliation was performed with a Bayesian classifier (Wang *et al.*, 2007) (80% bootstrap confidence score) against the UNITE database (Abarenkov *et al.*, 2010).

Microbial community analyses

Only abundant OTUs representing at least 0.1% of the library size were conserved for microbial community analyses (Barret *et al.*, 2015). Both α and β diversity indexes were calculated with MOTHUR V1.33 (Schloss *et al.*, 2009). Richness was assessed with the number of observed OTUs and diversity with Simpson's inverse index. Kruskal–Wallis one-way analysis of variance by ranks (Kruskal and Wallis, 1952) was performed to assess the effect of the different factors on α-diversity. The effects of cultivation site and cultivar were tested across cultivation sites, whereas the effect of field replications was tested within each site. Beta-diversity was assessed using Bray–Curtis dissimilarity index (Bray and Curtis, 1957). AMOVA (Excoffier *et al.*, 1992) was performed to assess the effect of the different factors on the microbial community structure ($P < 0.05$). Moreover, CAP was conducted to measure the relative influence of terroir and the bean cultivar on the microbial β-diversity. CAP analyses were performed with the function capscale of vegan and significances of the factors were calculated with ANOVA-like permutation tests using the function permutest. Differences in taxonomic abundance were highlighted with Krona radial space-filling display (Ondov *et al.*, 2011).

Differences in relative abundance of OTUs between the different factors were assessed with the R package EDGER (Robinson *et al.*, 2010). Sequence counts were first normalized with the Relative Log Expression method (Anders and Huber, 2010), which is implemented in EDGER. Exact binomial tests corrected for multiples inferences with the Benjamini–Hochberg method (Benjamini and Hochberg, 1995) were then performed to detect differences in relative OTUs abundance between factors. OTUs were defined as significantly enriched or depleted in one treatment with a corrected P-value < 0.001 and a $\log_2$ fold change magnitude ≥ 2. Correlations between OTUs were calculated with the SparCC (Friedman and Alm, 2012) implemented in MOTHUR. The effect of uneven sampling was corrected by dividing sequence counts by total library size (proportion). Statistical significance of the inferred correlations was assessed with a bootstrap procedure (100 replications). Only correlations with pseudo P-value ≤ 0.001 were represented in the network using the R package QGRAPH (Epskamp *et al.*, 2012).

All sequences have been deposited in the ENA database under the accession number PRJEB8866.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Mean allelic accumulation curves.
Mean allelic accumulation curves of the five common bean varieties calculated on the SSR dataset.

Fig. S2. PCoA of genetic diversity among common bean individuals.
PCoA scatterplot of the 88 studied common bean individuals according to (A) PC1 and PC2 and (B) PC1 and PC3. Individuals are represented by a single dot colored according to their cultivar.

Fig. S3. Heatmap of genetic diversity between and within varieties.
Heatmap of (i) average number of pairwise differences between varieties (green, upper half of the matrix), (ii) average number of pairwise differences within variety (orange, diagonal) and (iii) corrected average pairwise difference between the varieties (blue, lower half of the matrix). The corresponding legends to the color codes are on the right side.

Fig. S4. Bacterial and fungal OTUs conserved across experimental sites and bean cultivars.

Fig. S5. Estimation of bacterial and fungal diversity.
Richness (OTUs) and diversity (Simpson's inverse index) were estimated for seed samples of the different bean cultivars. Kruskal–Wallis one-way analysis of variance was performed to assess the effect of the production region on richness and α-diversity.

Fig. S6. Composition of seed-associated microbial assemblages.
Krona radial space-filling showing mean relative abundances of bacterial and fungal taxa in seed samples harvested in Brittany and Luxembourg.

Table S1. Location and characteristics of the experimental sites.

Table S2. SSR loci list.
List of the 11 SSR loci used to characterize the five common bean varieties. For each marker, locus name, linkage group (LG), primer sequence and annealing temperature in °C (Ta) are reported.

Table S3. SSR genetic diversity indexes.
Mean diversity indexes of the five studied varieties calculated on the SSR dataset.

Table S4. AMOVA results.
Analysis of molecular variance (AMOVA) showing the partitioning of genetic variation within and between varieties.

Table S5. Variety average pairwise differences.
The (i) average number of pairwise differences between varieties (upper half of the matrix), (ii) average number of pairwise differences within variety (diagonal) and (iii) corrected average pairwise difference between the varieties (lower half of the matrix) are reported.

Table S6. Seed-associated microbial assemblages sequenced in this study.
The number of paired-reads, quality sequences, operational taxonomic units (OTUs) abundant OTUs (OTUs, representing at least 0.1% of the library size) and abundant quality sequences are indicated for each seed sample. Samples with less than 1000 sequences were discarded in this analysis. The cultivar 'Rognon de Coq' did not yield sufficient seed for sampling in Luxembourg (na : not available).

Table S7. Effect of sampling on seed microbiota profiling.
The number and proportion of OTUs shared between field replicates are indicated for the 16S rRNA gene and ITS1 sequences. The cultivar 'Rognon de Coq' did not yield sufficient seed for sampling in Luxembourg (na : not available).

Table S8. List of bacterial and fungal OTUs conserved across bean cultivars and experimental sites.
The OTUs conserved across experimental sites (BZH or LUX) and bean cultivars (Cal, Flc, RdB and Ses) are indicated with the letter C. The cultivar 'Rognon de Coq' (RdC) did not yield sufficient seed for sampling in Luxembourg. Consequently OTUs conserved within this cultivar have not been assessed.

Table S9. List of bacterial OTUs significantly enriched at each experimental site.
Differences in relative abundance of OTUs between the different experimental sites were assessed with the R package edgeR. OTUs were defined as significantly enriched or depleted in one treatment with a corrected P-value < 0.001 and a log2 fold change magnitude ≥ 2.

Table S10. List of fungal OTUs significantly enriched at each experimental site.
Differences in relative abundance of OTUs between the different experimental sites were assessed with the R package edgeR. OTUs were defined as significantly enriched or depleted in one treatment with a corrected P-value < 0.001 and a log2 fold change magnitude ≥ 2.

Table S11. Properties of environment specific networks.

Chapter 8: **Phenotypic and genetic adaptation**

> "... we ought to really care for the adaptive capacities of plants." [CRO-190515d][43]

The *Croqueurs'* management of crop diversity and governance of plant health is based on crop *lineages* (Chapter III). The term *lineage* reflects the idea that the plant population evolves in a given environment and under certain management practices. The plant population is shaped by its growing environment and by the seed grower. This process is termed *adaptation*. For instance, two bean populations of the same variety that have evolved on two different farms for several years are no longer quite the same. Taking the variety 'Rognon de Coq' as an example, the seed artisans then speak of "Philippe's Rognon de Coq" and of "François' Rognon de Coq", according to the name of the respective seed grower. Giving crops the opportunity to evolve in and adapt to their growing environment is considered essential for plant health.

To elucidate whether bean lineages can be made visible in an agronomical field trial, this chapter addresses the question: **after three years of seed multiplication in contrasting growing environments, do phenotypic traits and genetic markers indicate local adaptation? In other words, do phenotypic traits and genetic markers indicate that varieties have begun to adapt to local growing environments and evolve into distinct "lineages"?**

This section is built upon a poster presented at the annual congress of the Italian Society of Agricultural Genetics (SIGA) in Catania, Italy (13-16 September 2016):

Caproni, L., Raggi, L., Klaedtke, S., Chable, V. and Negri, V. (2016): On-farm Evolution of Genetic Diversity of Four Old Varieties of Phaseolus vulgaris L. Proceedings of the 60th annual congress of the Italian Society of Agricultural Genetics (SIGA) in Catania, Italy (13-16 September 2016). http://www.geneticagraria.it/attachment/SIGA_2016/2_07.pdf.

The poster is shown in Annex 9.

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[43] "...on a intérêt à vraiment veiller sur les capacités adaptatives des plantes." [BA190515d]

1 Introduction

Lineages are at the base of the *Croqueurs'* management of crop diversity and governance of plant health. Giving common bean varieties the opportunity to adapt to different environmental conditions, including management practices, is considered as crucial to maintain bean diversity. Concurrently, in this approach to plant health, a bean population is considered truly healthy only if it remains healthy in the interaction with its growing environment. Different *lineages* appear when different seed growers among the *Croqueurs* cultivate the same variety. The members of the *Croqueurs* exclusively market varieties from the public domain, which are reproducible. Thereby, they also offer their customers the possibility to develop their own lineage by growing their own seed in their home or market gardens over several plant generations. Beyond the artisanal seed companies themselves, the customers thus represent a second level at which bean lineages can form. Common bean, as a mainly autogamous annual crop, is easily and frequently multiplied by home and market gardeners. This is not done only for financial reasons, but also to allow for the adaptation of bean lineages to local growing conditions. We are thus considering a seed system - between formal and informal (Almekinders et al., 1994) - in which artisanal seed companies provide seed of old varieties from the public domain, meant to be multiplied locally by their customers. Regarding plant health, we have seen in Chapter III (division 1.1.3) that this seed system also involves a collective form of governance of bean health.

Evidence for the local on-farm adaptation of crops has been brought by former research. In Western Europe, three years of on-farm selection by farmers has been shown to affect populations of the old common bean variety 'Flageolet Chevrier' (flc) on the phenotypic and genetic levels (Serpolay et al., 2012). Concerning another mainly autogamous legume, local adaptation of lentil has been asserted in Germany after six years of natural and conscious selection, at the phenotypic level (Horneburg and Becker, 2008). Regarding an allogamous vegetable crop, local adaptation at the phenotypic level has been reported for spinach (Serpolay et al., 2011). The genetic structure of sub-populations of a population-variety of bread wheat was found to be consistent with the structure of seed diffusions and seed saving within a network of farmers (Thomas et al., 2012). However, population dynamics can be expected to differ between vegetable and cereal crops by the sizes of populations, in terms of numbers of individuals. The processes certainly differ between autogamous and allogamous crops. Even for crop species which are known to be predominantly autogamous, the rate of outcrossing which occurs in a species or variety influences the dynamics of population structures. In a study on the generation and maintenance of variability in common bean landraces in Malawi, natural outcrossing was found to be an important factor (Martins and Adams, 1987). The mean outcrossing rate of common bean was reported to be 7 % in a field experiment conducted in California, but was highly dependent on environmental factors (Ibarra-Perez et al., 1997). Given these specificities - according to crop species, mating system and environmental factors - elucidating the local adaptation of bean varieties produced by *Croqueurs* members appears to be relevant.

Theoretical considerations of Allard (1975) provide a clear and useful description regarding the genetic adaptation of predominantly autogamous species such as common bean. For these species, adaptation does not only occur independently at the level of individual loci, but also through highly structured genotypic frequency distributions at the population level. In other words, Allard suggests to consider that the entire populational genotype is organized into "a sort of giant supergene". Thereby, the frequency of genotypes conferring high fitness in a given environment is increased in the plant population. At the same time, free genetic variability and recombinational potential within inbreeding populations remain substantial and allow for a response to natural selection in new or changing environments. In addition, the recombination of genetic information can be accelerated by increased outcrossing in stressful environments (Allard, 1975).

***Lineages* are the product of the interaction between a bean variety, a growing environment and a seed grower**. Apart from genetic adaptation, plant phenotypes also depend on interactions with the growing environment. In this domain, ecologists have studied the adaptive properties of environmentally induced maternal effects (Roach and Wulff, 1987; Lacey, 1998; Galloway, 2005; Sadras, 2007). A maternal effect is "the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution from each parent" (Roach and Wulff, 1987). The transfer of cytoplasmatic genetic information from "mother" plant to offspring is one type of maternal effect, but maternal effect can also be environmentally induced. For example, the temperature of parental reproductive environments have been reported to affect seed properties, fitness and cold responsiveness of progenies in thale cress (*Arabidopsis thaliana*) (Blödner et al., 2007). Such environmentally induced maternal affects are considered adaptive if they increase the probability of reproductive success of an offspring phenotype relative to others in a population (Lacey, 1998).

Moreover, seed-borne plant diseases are also transmitted from a mother plant to its progeny. Plant diseases are generally considered as non-adaptive, as they don't increase progeny fitness. Nevertheless, such plant diseases contribute to shaping bean *lineages* (Chapter III, division 1.1.1), as they influence the expression of plant phenotypes. In the much longer term, plant diseases exert selective pressure on plant populations, which may lead to genetic adaptation of the plant population (Summers et al., 2003). This applies to the co-evolution of bean populations in their centres of diversity (Geffroy et al., 1999), but may also apply to on-farm maintenance and breeding of crop diversity (Maxted et al., 1997; Brown, 2000). Beyond genetic adaptation, plant diseases have been shown to interact with (Stokes, 2002; Lu, 2003; Akimoto et al., 2007) and even drive (Richards, 2006, 2011; Niehl and Heinlein, 2009; Witzany, 2009) epigenetic mechanisms in plants.

The objective of this chapter is to elucidate how bean lineages and *in situ* bean health management (Chapter III) emerge from complex interactions between bean varieties and their growing environments in terms of phenotypes and their respective genotypes. Do phenotypic traits and genetic markers indicate that varieties have begun to adapt to local growing environments and evolve into distinct "lineages"? Four old varieties of common bean and one commercial control variety were tested for local adaptation after three years of multiplication on two organic farms. Phenotypic traits and genetic markers are considered.

2 Materials and methods

The local adaptation of five bush bean varieties were studied. The varieties are described in Chapter IV, subsection 1.1 and in further detail in Annex 4. Four of them were old varieties provided by artisanal seed companies members of the *Croqueurs* association: 'Flageolet Chevrier' (flc), 'Rognon de Coq' (rdc), 'Roi des Belges' (rdb) and 'Saint Esprit à oeil rouge' (ses). One was a modern variety obtained from a large scale plant breeding company: 'Calima' (cal). In 2012, each of the varieties was sown on (at least) two organic farms and reproduced within each site for three years, up to 2014 ("multiplication phase" described in Chapter IV, subsection 1.2). Experimental design and phenotypic traits were observed as described in Chapter IV. A variety multiplied in a given site over three years has been termed a "version" of this variety (Serpolay et al., 2011). Each version is designated by the name of the variety followed by the name of the site. In addition, the original seed lots with which the field trials were initiated in 2012 are also considered a version of each variety. For this study of local adaptation, three 'versions' of each variety were considered: the original (ORI), the multiplication from a farm in Brittany (BZH) and the multiplication from a farm in Luxembourg (LUX). In 2015, all versions were grown in a split-plot experiment on the farm in BZH ("gathering phase", Chapter IV, subsection 1.3). Twelve bean plants were identified for phenotypic observation in each subplot, i.e. a total of 36 plants per population. A subset of the same plants were sampled for molecular analysis (see subsection 2.2).

2.1 Phenotypic traits

Concerning phenology, flowering date was recorded for each plant at the first open flower. Flowering date is an indicator of earliness. It is also considered an indicator of local adaptation (Tiranti and Negri, 2007). In addition, maturity was scored at 95 das on a scale from 1 (all pods are green) to 3 ('most pods dry, harvest maturity). Concerning morphology, the length of the middle leaflet of the fully developed third trifoliate leaf was measured at 43 das. The length of stems was measured at flowering growth stage. Throughout crop development overall vigour and disease symptoms were scored on a scale from 1 to 5, namely at development stages R2, R4, R6 and R7 as appropriate (see growth stages in Annex 10). At flowering and mid seed fill growth stages, overall plant vigour was scored on a scale from 1 (very little vigour) to 5 (very vigorous). Finally, after harvest, the number of pods, empty pods and seeds produced per plant were counted. Observation and scoring methods, especially on the scoring of plant disease symptoms, are described in more detail in Chapter IV, section 2.

2.2 Analysis of SSR markers

The molecular analysis of 22 SSR markers was conducted in the "Dipartimento di Scienze Agrarie, Alimentari e Ambientali" of the University of Perugia (Università degli Studi di Perugia). Genomic DNA was extracted from: (i) 30 individuals from each original population (ORI) and (ii) 32 individuals from each evolved population multiplied in BZH and LUX, respectively. A total of 470 samples where thereby obtained. 35 SSR neutral markers, selected from literature, were initially tested on a subset of samples and 22 of them were chosen (Gaitán-Solís et al., 2002; Blair et al., 2009; Córdoba et al., 2010). There were two markers per linkage group. Fluorescent PCR amplicons were analysed on an ABI3130xl sequencer.

2.3 Data analysis

For phenotypic traits, statistics were computed using the programming language and software environment R version 3.3.0 (R Development Core Team, 2016). In statistical tests, the null hypothesis (the values are not statistically different) is rejected at a significance level (Type 1 Error rate) of 5% (i.e. the p-value is below 0.05).

Interval and ratio type data (days to flowering, leaf length, stem length, 1000-seed weight, number of seeds per plant) were analysed with linear mixed effects models. Linear mixed effects models permit data analysis with hierarchical structure through the inclusion of random effects in the model. Unlike the count data generated in field expreiments designed as complete randomised blocks in the "multiplication phase" and discussed in Chapters V to VII, count data generated by the common garden experiment with a split-plot design in the "gathering phase" confronts the analyst with two challenges: in addition to non-normality, they present random effects. No method of analysis proposed to date provides an exact solution to this problem. According to Fang and Loughin (2004), proper modelling of random effects is by far more important than exactly matching the parent distribution of the data in an analysis. The count data, namely number of seeds per plant, was thus analysed by the same linear mixed effect model without adapting for parent distribution. Sub-plots (version) were nested in main plots (variety) in a split-plot design. Also, several plants were observed per subplot, leading to pseudo-replication. Nesting and pseudo-replication were taken into account in the model by setting appropriate random effects. Firstly, the overall effect of variety, version and variety*version interaction was tested in a model including the data over all varieties. Secondly, the effect of version was specified within each variety by subsetting the data and building the linear mixed effects model with only version as fixed effect. Linear mixed effects models were computed using the R package 'nlme' (Pinheiro et al., 2016). Least square means were computed with the package 'lsmeans' (Lenth, 2016), as well as Tukey's Honestly Significant Difference (HSD) test (Tukey, 1949) for multiple comparisons.

For ordinal variables (score data), rank-based ANOVA-type statistic (Brunner and Puri, 2001; Shah and Madden, 2004) was computed by the 'nparLD' (Noguchi et al., 2012) and 'rankFD' (Konietschke et al., 2016) R packages. Relative effects pd were also computed for appropriate two-way comparisons (see note on p.115 for more detail on the application of relative effects pd). For traits scored several times along the growth cycle (overall vigour, leaf mosaic, leaf blustering, systemic phloem necrosis and blight on leaves) 'nparLD' was employed to calculate the overall effect of variety and version over all observation dates. This method allows calculating the ANOVA-type statistic for longitudinal data in nested factorial designs. Any subject with missing data for the trait of interest was removed from the data subset for the respective analysis. For traits scored only once (stunting at R2, maturity at 95 das, streaks on pods at R7, symptoms on pods at R7), the overall effects of variety and version were tested using 'rankFD'. The nesting of data in the split-plot design was taken into account by calculating ANOVA-type statistics in 2 steps. Finally, the effect of version within each variety was tested. To do so, a rank-based model including replication, version and their interaction was applied to the respective data subset and the effect of version tested via rank-based ANOVA-type statistic. For longitudinal data, this was done for each observation date.

The main plot containing 'rdb' in field replication 1 was excluded from analysis due to technical difficulties. Also, variety 'flc' was damaged by 'systemic phloem necrosis' from flowering onwards and all but completely destroyed by the end of the experiment. Linear mixed effects model calculations and subsequent least square means can cope with missing data and, more importantly, missing cells. However, the rank-based model calculations cannot. Therefore, replication 1 was excluded from the tests for overall effects of variety and versions on the score data. For those traits and observation dates for which no data was collected concerning variety 'flc', this variety was also excluded from analysis.

Concerning the genetic markers, SSR data analysis was conducted in the department "Dipartimento di Scienze Agrarie, Alimentari e Ambientali" of the University of Perugia (Università degli Studi di Perugia). The evolution of the studied populations was assessed through the calculation of pairwise population fixation indices (F_{st}) using Arequin 3.5 software (Excoffier and Lischer, 2010). Analysis of Molecular Variance (AMOVA) was employed to assess the variance among populations (a population being a version of a given variety), among individuals and within individuals. In addition, Principle Coordinates Analysis (PCoA) was conducted on standardised data via a co-variance matrix. PCoA and AMOVA were carried out using GenAlEx 6.5 (Peakall and Smouse, 2006). Expected heterozygosity (He) was also calculated.

3 Results

Overviews of phenotypic differences found between varieties, versions across varieties and versions within varieties are given in Table 8.1, Table 8.2 and Table 8.3. Table 8.1 covers quantitative traits analysed by linear mixed effects models, whereas Table 8.2 covers ordinal score data analysed by rank-based models. Table 8.3 summarizes this information further by indicating the sum of phenotypic traits for which significant differences were found between versions of each variety.

Table 8.1: Effects of variety, version and their interaction found in the complete models for quantitative phenotypic data, as well as effects of version found within each variety.
* indicates that a significant effect was found at $p < 0.05$; ./. indicates that the trait was not analysed.

		Overall effect in complete model			Effect of version within variety…				
Type of trait	**Trait**	**Variety**	**Version**	**Variety* Version**	**cal**	**flc**	**rdb**	**rdc**	**ses**
Morpho-phenological	Flowering date	*	*		*	*		*	*
	Leaf length	*	*	*	*	*		*	*
	Stem length	*		*		./.	*	*	
Yield components	1000-seed weight	*	*			./.		*	
	Seeds / plant	*				./.			

Table 8.2: Effects of variety and version found in the complete models for ordinal phenotypic data, as well as effects of version found within each variety.
* indicates that a significant effect was found at $p < 0.05$; ./. indicates that the trait was not assessed.

		Overall effect in complete model over growth stages		at growth stage	Effect of Version for each growth stage within variety…				
Type of trait	**Variable**	**Variety**	**Version**		**cal**	**flc**	**rdb**	**rdc**	**ses**
Morpho-phenological	Vigour	*		R2	*			*	
				R6		./.			
	Maturity	*	*		*	./.		*	
Disease symptoms	Leaf mosaic	*	*	R2				*	*
				R4		./.		*	
				R6		./.		*	
	Leaf blustering	*	*	R2				*	
				R4		./.		*	
				R6		./.		*	
	Blight on leaves	*	*	R2					
				R4	*	./.		*	
				R6	*	./.		*	
	Phloem necrosis	*		R2					
				R4					
				R6					
	Stunting (R2)	*	*	R2				*	
	Blight on pods (R7)	*	*	R7	*	./.			

Table 8.3: Numbers of phenotypic traits for which significant differences were found between versions of a given bean variety, for different types of traits and in total.

Type of trait	**Number of variables assessed per trait type**	Number of variables for which a significant effect of version was found within variety…				
		cal	**flc***	**rdb**	**rdc**	**ses**
Morpho-phenological	5	4	2	1	5	2
Yield components	2	0	./.	0	1	0
Disease symptoms	6	2	0	0	4	1
Total	**13**	**6**	**2**	**1**	**10**	**3**

*Note: Given that variety 'flc' was almost completely destroyed before harvest, the total number of traits assessed for this variety was reduced.

An overall effect of variety was found for all phenotypic traits assessed, reflecting that different varietal types were represented in terms of morpho-phenological and yield component traits, as well as tolerance to diseases. Varietal differences concerning seed size and number of seeds produced per plant had already been found in the 'multiplication phase', as reported in Chapter IV (Table IV.1, p.99) and Chapter V (Figure 5.7, p.117), respectively. Trends in susceptibility to a range of diseases were also reported in Chapter V. These differences were again found in the 'gathering' phase'. In addition, variety flc was all but completely destroyed by a systemic phloem necrosis between flowering and pod-filling growth stages. This had not been observed in the 'multiplication phase' the previous years.

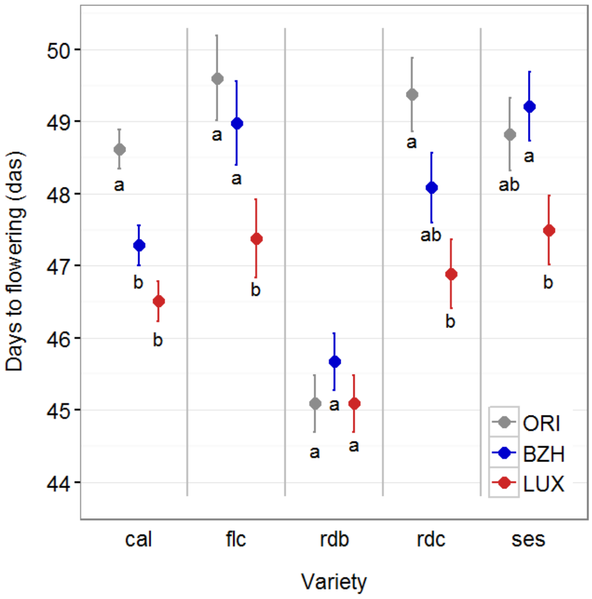

Figure 8.1: Effect of 'version' on days to flowering within each variety. Error bars represent standard errors of the means. Within each variety, samples which are not marked with the same lowercase letter differ significantly.

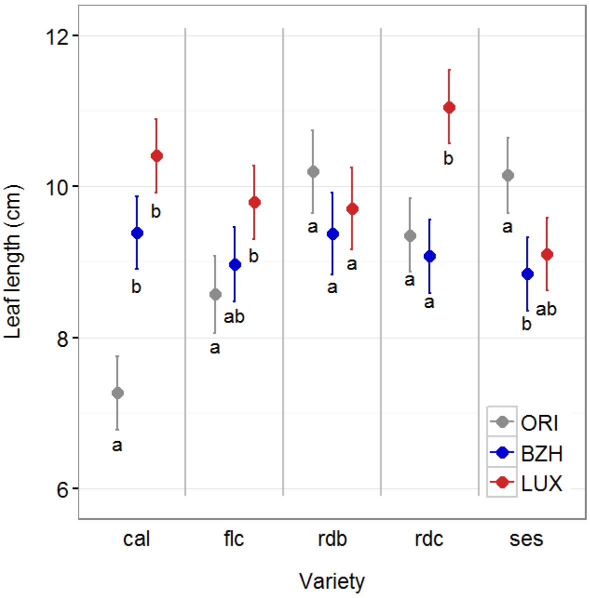

Figure 8.2: Effect of 'version' on leaf length within each variety. Error bars represent standard errors of the means. Within each variety, samples which are not marked with the same lowercase letter differ significantly.

Concerning morpho-phenological traits, an overall effect of version was found for 'days to flowering', 'maturity' and 'leaf length'. Overall, populations from LUX flowered 1.2 days earlier than populations from BZH ($p < 0.0001$) and 1.6 days earlier than original populations ($p < 0.0001$). When looking into the effect of version within each variety, effects were found for all varieties but 'rdb' (Figure 8.1). Phenological differences between versions were confirmed by an overall effect of 'version' on maturity scores at the end of the growth cycle: populations from LUX obtained significantly higher maturity scores (95% confidence interval of relative effect pd: 0.54 - 0.60) than populations from BZH (0.44 - 0.50) and original populations (95% confidence interval of relative effect pd: 0.43 - 0.49). However, within individual varieties the effect of 'version' was significant only for 'cal' ($p = 0.002$) and 'rdc' ($p < 0.0001$).

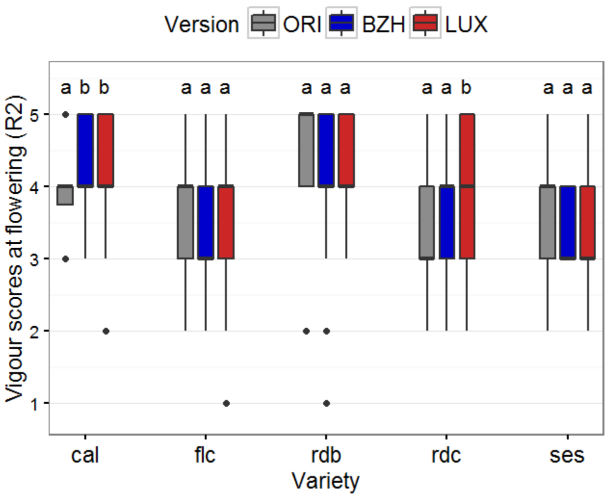

Figure 8.3: Effect of 'version' on vigour scores at flowering within each variety. Within each variety, samples which are not marked with the same lowercase letter differ significantly. Note: For variety 'rdb', only field replications 2 and 3 were taken into account.

For the morphological trait 'leaf length', a significant interaction of variety and version was found (p<0.0001). Comparisons between versions for each variety resulted in different trends within the varieties, shown in Figure 8.2. No significant differences were found between versions of 'rdb'.

ANOVA-type statistic on the overall rank-based model for vigour scores over 4 varieties and two growth stages (R2 and R6) did not result in a significant effect of 'version'. However, an effect was found for varieties 'cal' (p = 0.0002) and 'rdc' (p = 0.001) at flowering growth stage (R2) when testing within individual varieties for each growth stage (Figure 8.3).

Concerning yield components, a significant overall effect of 'version' was found for seed weight. Seeds produced by populations from LUX were 13.7 g heavier than those of original populations (p=0.046). However, seed sizes differed strongly between varieties, as already discussed in Chapter V. When looking into individual varieties, a significant effect of version was found for variety 'rdc' only (Figure 8.4).

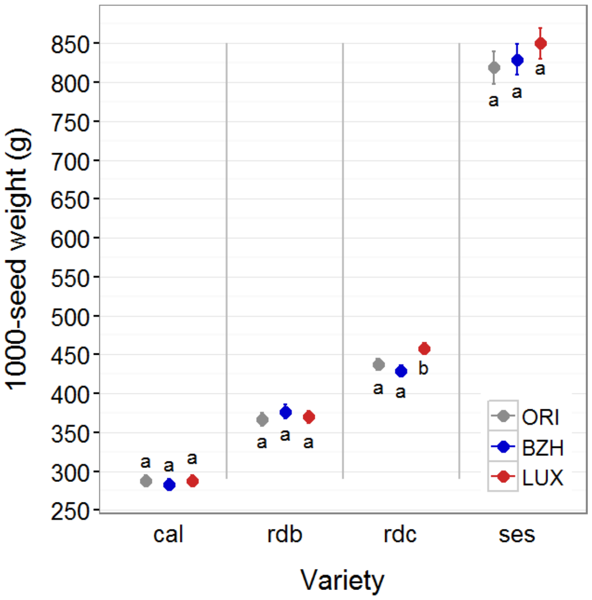

Figure 8.4: Effect of 'version' on 1000-seed weight within each variety. Error bars represent standard errors of the means. Within each variety, samples which are not marked with the same lowercase letter differ significantly.

Regarding symptom scores, version had a significant effect on all symptoms but 'phloem necrosis' (Table 8.2). When looking into the effect of version on typical symptoms of viral diseases (leaf mosaic and blustering, stunting) within each variety, the effect of version was found in variety 'rdc' for all symptoms. Apart from 'rdc', a significant effect of version was found only in variety 'ses' for 'leaf mosaic' symptoms at flowering (R2). Varieties 'rdc' and 'ses' have been identified as susceptible to viral diseases in Chapter V. Figure 8.5 shows the distributions of 'leaf mosaic' scores at flowering for varieties 'rdc' and 'ses', as well as the result of multiple comparisons between versions within these two varieties. The trend observed within each of the varieties is not the same: whereas the version 'LUX' of variety 'rdc' had lowest scores, version 'LUX' of variety 'ses' had highest scores for leaf mosaic symptoms.

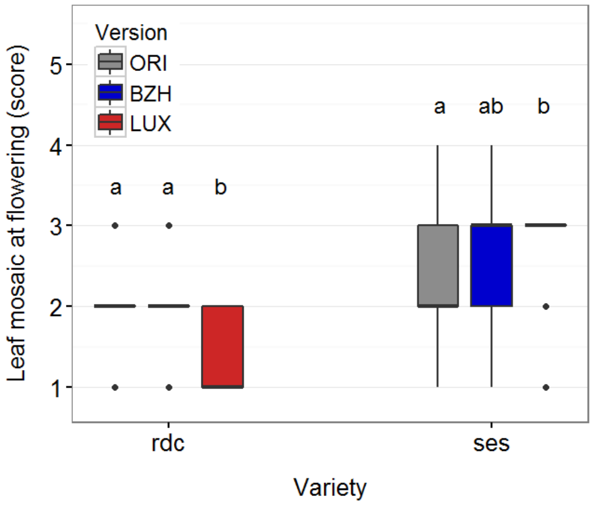

Figure 8.5: Effect of 'version' on 'leaf mosaic' scores at flowering within the varieties 'Rognon de Coq' (rdc) and 'St Esprit' (ses). Within each variety, samples which are not marked with the same lowercase letter differ significantly.

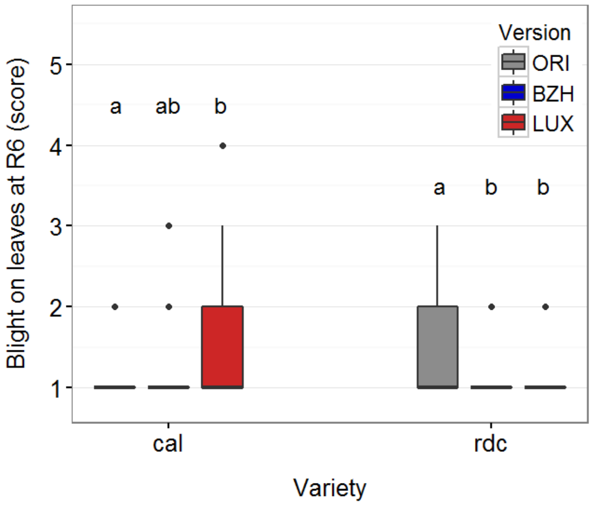

Figure 8.6: Effect of 'version' on scores for blight symptoms on leaves at flowering within the varieties 'Calima' (cal) and 'Rognon de Coq' (rdc). Within each variety, samples which are not marked with the same lowercase letter differ significantly.

When looking into the effect of version on <u>blight symptoms</u> within each variety, an effect of version was found in varieties 'cal' and 'rdc' for leaf symptoms. For 'cal', version also had a significant effect on pod symptoms. Figure 8.6 shows the distributions of scores for blight on leaves at growth stage R6 for varieties 'cal' and 'rdc', along with the results of multiple comparisons between versions within these two varieties. For variety 'cal', the version from LUX had highest scores for blight symptoms on leaves. For variety 'rdc', the original seed lot (version ORI) had highest scores for this symptom. The contamination rates of the seed lots with the bacterial agent of halo bacterial blight (HBB) are indicated in Table V.1, p.115. Seeds were also tested for the bacterial agent of common bacterial blight (CBB), but none were detected.

<u>Genotyping</u> resulted in the production of about 20K data points. A total of 83 different alleles were identified in 16 polymorphic loci. Number of alleles per locus ranged from two (markers BMb293, BM156, BMd-44) to18 (marker BMd-43) with a mean value of 5.2 alleles per polymorphic locus. AMOVA showed that 76, 20 and 4% of total diversity is among populations, individuals and within individuals, respectively (Figure 4 of Annex 9). The first two axes of the PCoA explained 69,7 % of total diversity and clearly distinguished the five varieties (Figure 8.7). On this plane of the PCoA, the LUX versions of varieties 'flc' and 'rdc' demarcate from the other two versions (BZH and ORI) of the respective variety.

Pairwise populations F_{st} analyses confirm that the 'flc' population multiplied in LUX differs significantly, but moderately, both from the original (ORI) population (F_{st}= 0.142, $p< 0.001$) and from the population multiplied in BZH (F_{st}= 0.093, $p< 0.01$). Similarly, the 'rdc' population from LUX differs significantly, yet moderately, from both the original (ORI) population (F_{st}=0.167, p< 0.001) and from the population multiplied in BZH (F_{st}=0.1931, p< 0.001).

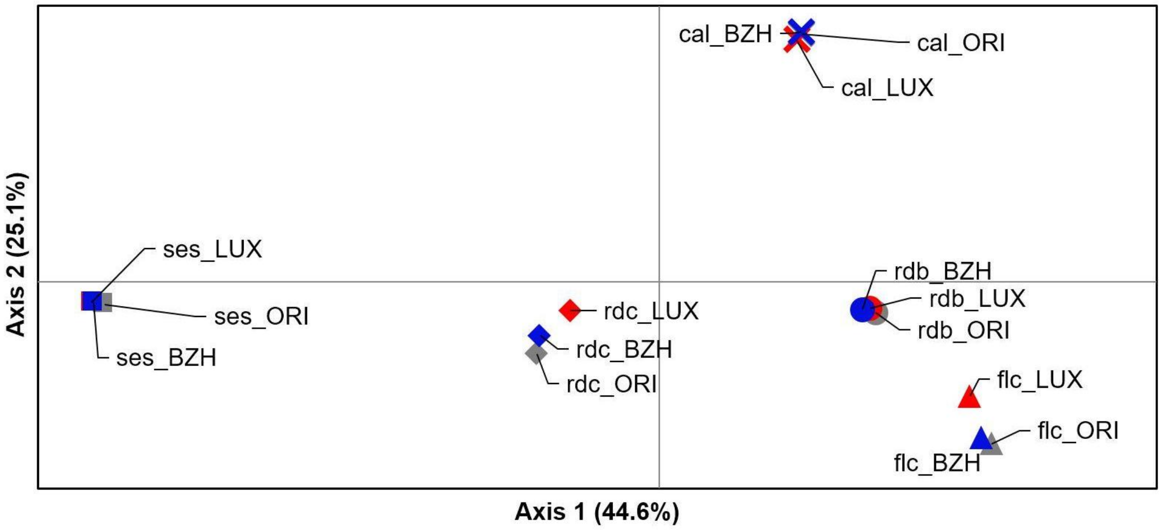

Figure 8.7: Graph of populations on axes 1 and 2 of the Principal Coordinate Analysis (PCoA)

Genetic diversity within the original population (ORI) of each variety was estimated by calculating expected heterozygosity (He). Diversity was highest within the initial populations of variety 'rdc' (He= 0.26). Initial populations of 'rdb' (He = 0.14) and 'flc' (He = 0.1) had intermediary intra-population diversity. Initial populations of the commercial control 'cal' (He = 0.04) and 'ses' (He = 0.02) had lowest intra-population diversity.

4 Discussion

For four out of five varieties, plant vigour of original populations (ORI) at flowering was equal to the vigour of one (variety 'rdc') or both populations (varieties 'flc', 'rdb' and 'ses'), which were multiplied in BZH and LUX over three years (Figure 8.3). This indicates that storage of the original populations over three years did not affect plant growth, at least not in a manner that persisted throughout the growth cycle. For the commercial control variety 'cal', the original population ORI was less vigorous than the populations BZH and LUX. This may be due to the age of the seed, as the seed provided by the breeding company was one year older (2010) than the seed provided by the *Croqueurs* members for the experiment. However, the original seed lot also underwent an unknown seed treatment (Chapter IV, subsection 1.1). As a consequence, it is not possible to distinguish between effects of this seed treatment and other effects when comparing the initial 'cal' population with the (untreated) populations multiplied in BZH and LUX. Comparisons between the original population of 'cal' and the other two populations therefore cannot be interpreted. Only the two multiplied populations BZH and LUX can be compared with each other.

Local adaptation indicated by adaptive phenotypic traits: Flowering date and leaf length

After three years of seed multiplication on different organic farms in contrasted environments (BZH and LUX), flowering date and leaf length differed between versions of all varieties except 'rdb' (Figure 8.1 and Figure 8.2). Both 'days to flowering' (Tiranti and Negri, 2007; Serpolay et al., 2011; Serpolay-Besson et al., 2014) and leaf size (Joshi et al., 2001; Leimu and Fischer, 2008) are considered as indicators of local adaptation. It may be deducted that selective pressures in the multiplication sites caused the bean populations to adapt locally.

Varieties 'Flageolet Chevrier' (flc) and 'Rognon de Coq' (rdc) were originally provided by a seed grower in Aquitaine, in the South West of France. For these varieties, populations multiplied in LUX differed significantly from the original population (ORI) both in terms of flowering date and leaf length. According to variety and trait, populations of 'flc' and 'rdc' multiplied in BZH had either an intermediary expression of flowering date and leaf length, or remained at the level of the original population. This indicates that selective pressure in cool and humid Luxembourg was stronger than in BZH, causing adaptive changes in the bean populations from warmer Aquitaine within three years. This results in populations from LUX flowering earlier and forming longer leaves. As compared to LUX, populations from BZH generally followed a similar trend: although differences are not significant, 'flc' and 'rdc' from BZH flowered earlier than the original populations. This result may reflect climatic conditions in BZH which are cooler than in Aquitaine, but not as harsh as in LUX (see Annex 5).

Varieties 'Roi des Belges' (rdb) and 'St. Esprit' (ses) were originally provided by the same seed grower in Luxembourg who multiplied the LUX versions in the 'multiplication phase' of the experiment. For these varieties, populations multiplied in LUX remained in the environment which had provided the original population ORI. Therefore, it is not surprising that versions ORI and LUX do not differ in phenology and leaf morphology. For 'rdb', no significant differences among versions were found at all in terms of flowering date or leaf length. Phenology and leaf morphology of 'rdb' populations thus appear to have remained stable in both multiplication sites. However, for variety 'ses', the population from BZH flowered later than the population multiplied in LUX. Also, the population from BZH developed smaller leaves than the original population ORI. These results indicate that some adaptation of the 'ses' population to local conditions in BZH took place on the phenotypic level, despite low initial intra-varietal diversity.

In general, environmental conditions in the Luxembourgish site seem to select for earlier flowering and longer leaves. In variety 'rdc', earlier flowering was associated with earlier maturity. These results are considered as an example of the emergence of bean *lineages* at work. They were obtained within three years of multiplication in contrasting farm environments and without conscious selection in the crop (harvested seed lots were sorted, thus undergoing conscious selection at this stage). *Lineages* of a given variety may thus begin to emerge within three years of cultivation in a new farm environment. Despite differences in mating systems (bean is a mainly autogamous species) and cropping systems (arable/vegetable crops), this converges with findings on allogamous spinach (Serpolay et al., 2011) and maize (Serpolay-Besson et al., 2014), as well as autogamous bread wheat (Dawson et al., 2013).

Local adaptation at the genetic level

Flowering date is considered to be strongly determined by plant genotype in common bean (Raggi et al., 2014). Evidence from molecular analysis (Figure 8.7) indeed indicate that the phenotypic differentiation of populations multiplied in LUX are due to processes of genetic adaptation. There are two possible explanations why genetic changes are significant in varieties 'flc' and 'rdc' only. Firstly, both these varieties were originally provided by a seed grower from Aquitaine, a region with a warm climate and rather long growing season. The transfer to the cool and humid environment in LUX may have led to strong selective pressure. This is especially true for 'rdc', as this variety was heavily affected by HBB in LUX during the 'multiplication phase' (Chapter V). Secondly, 'flc' and 'rdc' had intermediate to high levels of genetic diversity within initial populations. This initial genetic diversity may have facilitated genetic adaptation.

The level of genetic diversity of the initial populations can also be put into relation with phenotypic adaptation. Indeed, differences among versions of 'rdc' were found for 10 phenotypic traits (Table 8.3), which concurs with its fairly high level of initial genetic diversity. In contrast, differences among versions were found for only 3 traits in variety 'ses', which had the lowest level of initial diversity. This link between initial genetic diversity and phenotypic adaptability does not seem to hold for 'rdb': this variety had intermediate initial genetic diversity, but showed phenotypic differences between versions for only one trait (stem length). Conclusions on the phenotypic stability of 'rdb' must be drawn with caution, as tests performed for effects of version within variety 'rdb' were less powerful due to the exclusion of one field replication from data analysis. Nevertheless, 'rdb' appeared to be less variable than other varieties across traits, such as for example flowering date (Figure 8.1) or plant vigour (Figure 8.3). For variety 'flc', only a very low number of traits were assessed, because the subplots were lost to a systemic phloem necrosis known as 'black root syndrome' (Jenkins, 1940)[44]. Therefore, we could not verify whether the genetic differentiation of the 'flc' population multiplied in Luxembourg results in phenotypic differentiation in a high number of traits.

Environmentally induced maternal effects potentially shape bean *lineages*

In Chapter III, a bean *lineage* has been defined as the product of the interaction between bean variety, local environment and seed grower's practices. Indeed, phenotypic differences between populations cannot be explained by genetics alone. Many traits are expected to be strongly dependent on interactions between plant genetics and their environment. This is the case for plant vigour, seed weight and disease symptoms, in particular. For common bean, small-seeded genotypes have been shown to have higher potential for vigour (biomass production) and grain yields (White et al., 1992). Nevertheless, seed weight depends not only on the plant genotype, but also on the growing environment. For a given variety, seed harvested in LUX in 2014 had generally been larger than seed harvested in BZH the same year (Table IV.1, p.99), with the exception of 'ses'. These seeds were

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[44] 'Black root syndrome' is a spreading, usually lethal phloem necrosis. Under certain conditions, particularly high temperatures, this phloem necrosis is caused by BCMV in bean varieties carrying the *I* allele, which is widely used in bean breeding as source of resistance to BCMV (Collmer et al., 2000).

used to establish the field trial in BZH in the 'gathering phase' (Figure 4.1, p.98). For variety 'rdc', the LUX population grew more vigorously (Figure 8.3) and in turn yielded larger seeds (Figure 8.4) than the other two populations, ORI and BZH. The data obtained does not enable us to determine whether the greater seed weight of the LUX population is due to an environmentally induced maternal effect or due to genetic adaptation. An environmentally induced maternal effect is considered *adaptive* if it increases the probability of reproductive success of an offspring phenotype relative to others in a population (Lacey, 1998). Seed weight and plant growth have been discussed as examples of adaptive maternal effects in plants in former studies (Roach and Wulff, 1987; Lacey, 1998; Galloway, 2005; Sadras, 2007). In this study, no differences were found among versions of any variety for the number of seeds per plant (Table 8.1), which is an indicator of reproductive success and plant fitness (Galloway and Fenster, 2000). Nevertheless, these considerations on adaptive environmentally induced maternal effects illustrate that a bean population's genetics are not the only pathway through which the growing environment (including seed growers' management practices) shapes a bean *lineage* and its phenotypic expression.

Health of bean lineages amidst complex interactions

Moreover, the interaction of bean populations with plant-associated microorganisms play an important role in shaping phenotypes. The previous chapter has shown that the growing environment, or *terroir*, influenced seed-associated microbial communities in BZH and LUX within 2 years of multiplication. Among the seed-associated microbial communities, seed-borne plant pathogens can play a particular role in shaping plant phenotypes by causing plant diseases. An effect of version was found on leaf mosaic symptoms for varieties 'rdc' and 'ses' at flowering growth stage (R2). Both these varieties were identified as susceptible to viral diseases in Chapter V. For 'rdc', the population multiplied in LUX expressed significantly less leaf mosaic symptoms than the populations ORI and BZH (Figure 8.5). On the contrary, the 'ses' population from LUX expressed more leaf mosaic symptoms than the other populations, although the difference was significant only with the original population. Unfortunately, symptom expression cannot be related to contamination rates of seed with viral disease agents, as no such seed analyses were conducted. This result nonetheless shows that the influence of multiplication site on seed and plant health is complex and depends on bean variety, the multiplication history of previous plant generations and environmental conditions during plant growth, among other factors. The same is true when considering blight symptoms expressed on leaves of the 'rdc' populations. All seed lots were analysed for bacterial blight agents. No CBB agents were detected on any of the seed lots, but HBB agents were detected on some (Table V.1, p.115). Variety 'rdc' from LUX had particularly high contamination rates with HBB. Discrepancy between symptom expression (Figure 8.6) and detected contamination rates can have many reasons. For instance, the race of HBB agents carried by the seed from LUX may have been poorly adapted to the environmental conditions in BZH in 2015. In fact, environmental conditions were generally not favourable to the development of HBB (Annex 6). Also, 'rdc' populations ORI and BZH may have been contaminated at rates below the detection limit with blight agents that were better adapted to the environmental conditions. In addition, inoculum in surrounding bean fields may have led to secondary infection with blight agents. In any case, all these putative reasons again point to the complexity of interactions behind plant health under field conditions.

Bean lineages - Articulating governance of plant health and management of crop diversity

To conclude, the objective of this experiment was to elucidate how bean lineages and *in situ* bean health management (Chapter III) emerge from such complex interactions between bean varieties and their growing environments. Although I am not able to disentangle the interactions that lead to the development of bean *lineages* in seed growers fields, results show that differences between versions of a given variety can be found after three years of multiplication in contrasting farm environments. This finding is congruent with a practice of seed growers which consists of observing a new bean variety for one or two growing seasons to see how the variety adapts to their farm environment (Chapter II, section 2.1; Chapter III, division 1.1.1). Phenotypic differences are driven in part by genetic adaptation, but probably also by environmentally induced maternal effects such as seed properties and certainly by interactions with plant disease agents.

By considering the differences between versions in this field experiment as the emergence of bean *lineages*, we also gain an insight into the interrelations between the management of bean diversity and the governance of bean health practiced by the *Croqueurs*. I have discussed in Chapter III that governance of *in situ* bean health is a collective endeavour (division 1.1.3 therein). Managing bean health is in part about finding the *right* growing environment to multiply a given variety. Therefore, bean diseases contribute to structuring the distribution of bean varieties among the seed growers of an artisanal seed company. Results presented in this chapter attest that *where* a bean population is multiplied influences its genetic makeup and phenotypic expression. From there, perspectives can be derived - for research and maybe also for the management practices of artisanal seed companies. The roles played by seed growers in the collective management of bean health may differ according to the environmental conditions on their farm. For instance, farms with harsh environmental conditions and high pressure from diseases may serve to test and select for disease tolerance. In particular, sites such as LUX allow to reveal which varieties cope well with halo bacterial blight (HBB) and which are sensible (Chapter V). However, one can also ask whether strong selective pressure in sites such as LUX might reduce the genetic diversity within bean populations. The range of diversity found in the initial populations (ORI) indeed raises questions about what causes these differences in initial diversity: is it dependent on the variety, or does the multiplication environment play a role in maintaining a wide diversity within bean lineages (not to mention conscious selection by seed growers)? May some farm environments in a seed grower network contribute to collective bean health management by maintaining a wide diversity within bean *lineages*, thereby maintaining the ability to adapt to new growing environments? Some first indications concerning these questions may arise from further analysis of the data obtained in this experiment.

Chapter 9: General discussion

The questions addressed by this thesis were formulated as follows in the General Introduction (Chapter I). *Which are the specificities of bean health management practiced by artisanal seed companies among the association Croqueurs de Carottes? On which interactions between bean plants and their growing environments is this plant health management based?*

Actor-network theory (ANT) has been employed as the basic approach to these questions. One of the founding principles of ANT is to do away with the separation between the "social" and the "natural" (Latour, 2005). This case study has gained in pertinence and precision by breaking down the artificial distinction between the "social" and the "natural" and following the associations of humans and non-humans. This is not only true for the social science approach. Indeed, ANT was deployed in the analysis of the data collected through interviews and participant observations, as I followed the actors in the alliances they make with non-humans - bean plants, microorganisms associated with bean plants or soils, cultivation media used to test for the presence of bacteria etc. However, the deconstruction of the barrier between the "natural" and the "social" was also taken as a basic principle in the field experiments. When studying bean seed crops on farms as has been done here, the distinction between human and non-human research objects may not be so relevant. The crop we are studying consists of bean plants, which are non-human (of course), but bean seed crops are nevertheless deeply human systems, not only in the sense that they are created by human activity, but in the sense that they are deeply linked to values held by humans. Although the on-farm experiments addressed apparently non-human objects - such as bean populations, their genetics and their plant diseases - they still relied on humans - seed growers, bacteriologists, seed inspectors - to object to questions, or hypotheses, that they did not judge pertinent. In other words: the artisanal seed companies would not be quite what they are if it weren't for the bean populations they multiply. And: the bean populations would not be quite what they are if they weren't multiplied by the artisanal seed companies.

Studying the *Croqueurs'* governance of bean health in this thesis implied navigating in between the two disciplines of crop ecology and sociology. Health and disease are indeed interdisciplinary objects (Caplan et al., 1981; Cabaret and Nicourt, 2011). Beyond clinicians and psychologists, human health is also addressed by social scientists (Laplantine, 1993; Annandale, 1998) and philosophers (Canguilhem, 2013; Foucault, 2015). Although plant health has not crossed disciplinary boundaries as extensively and has mainly remained in the domain of plant pathology, approaches to plant health, beyond technical questions, come into scope (Trutmann et al., 1996; Döring et al., 2012). In this PhD research, embracing the governance of bean health as matter of concern from an agroecological perspective implied “the redefinition of scientific and social boundaries” (Stassart et al., 2012) and called for interdisciplinarity. Navigating between agronomy and sociology has permitted studying bean health and a bean health management approach jointly. I was able to study plant health without losing sight of the associated plant health management approach. Concurrently, field experiments provided concrete experiences and cases upon which the *Croqueurs'* governance of plant health was elucidated and specified.

In the previous chapters, results have been discussed in separate chapters according to the disciplinary approach that produced them - semi-directive interviews and participant observation on one hand (chapter III) and field experiments on the other (chapters IV to VIII). Nevertheless, they form part of one and the same picture: the governance of bean health as practiced by the *Croqueurs de Carottes*.

1 *In situ* governance of bean health based on ecological interactions

In this thesis, the governance of bean health practiced by the *Croqueurs de Carottes* has been termed *in situ* plant health management. In the *in situ* approach, bean health is considered as a product of the interactions between a bean variety, the growing environment in which it is grown, and the growers' management practices. The bean variety comes with its own requirements and possibilities concerning the range of environments to which it can adapt. The growing environment is marked by pedo-climatic conditions. It can favour certain microbial communities and disfavour others. The growing environment also exerts natural selection pressure and can cause plant populations to adapt. The growers growing the seed - be it for the production of seed or for consumption - influence the growing environment, especially biological soil properties, by their cultivation practices. Together, the pedo-climatic growing environment and the local cultivation practices may also be called *terroir*. In addition, seed growers influence bean health through conscious plant selection. The decision to cultivate, multiply and select a bean variety implicitly involves the expectations of a seed grower as to what is healthy or healthy *enough*. This *in situ* concept of plant health has three important consequences.

Firstly, potentially pathogenic microorganisms are regarded as forming part of the production system and of plant populations themselves. A plant population is considered truly healthy only if it is able to *live with* these microorganisms, i.e. if it is able to thrive in its growing environment which includes potential plant pathogens. Plant populations must be given the opportunity to evolve in and adapt to the environment in which they are multiplied and the adaptability of crop populations is regarded as a pillar of plant health. Plant health can thus be described as a dynamic process rather than a steady, objective state. This *move towards health* was initially described as salutogenesis in the context of human health (Antonovsky, 1996) and taken up by Döring et al. (2012) in the context of plant health. In a salutogenic view, plant health cannot be judged upon as an objective entity based on some functional performance traits. In Chapter V, an attempt was made to account for the health of bean populations in contrasting production sites over three years in ways that leave space for the interpretation of plant health according to different concepts of plant health. A multivariate analysis of leaf symptoms scores by Multiple Correspondence Analysis did not result to be satisfactory in revealing contrasting environments with regard to disease pressure, nor in pointing to fine differences in disease tolerance between varieties. Perhaps multivariate methods which are better suited for ordinal score data, such as nonlinear PCA (Leeuw and Mair, 2009) can benefit future research. However, the analyses of disease symptoms and other traits in Chapter V do show that bean varieties react differently to contrasting environments and disease pressure. This variety*environment interaction is not a novelty as such, of course, but thinking about it in terms of *in situ* plant health management imposes a new perspective, which leads us to the two other consequences: Understanding bean populations, their growing environment and their grower as a system implies considering the role of growing environments in plant health and reasoning plant health management on a collective scale.

Thus, as a second consequence, a role for plant health management is attributed to the growing environment. Within the *in situ* approach to plant health, ecological interactions between growing environment and plant populations are endowed with competences to establish and maintain plant health. This implies that plant health management encompasses the care for a sound environment, sound soils in particular. Conversely, it also implies that the expression of a plant disease may point to a disequilibrium in the field environment or a mistake in crop management. Unlike approaches which focus on bean seed as vector of plant health - as disease-free seed or as a means to propagate resistant varieties - in this approach competences for plant health management are distributed throughout the production system. Chapter VI addressed interactions of common bean populations with two important root symbionts of common bean, mycorrhizal fungi and rhizobia. These root symbionts have been established as beneficial for plant health in numerous studies under controlled conditions. However, in the field experiment, we did not find evidence of improved plant health in individual plants more strongly colonised with root symbionts within each site. Results of the field experiment indicate that the intensity of these symbiotic interactions are driven by the growing environment. The bean varieties did not differ in any of the symbiotic traits, except percentage colonisation with mycorrhizal vesicles. Both mycorrhiza and rhizobia can thus be considered as elements of a field environment. They form part of the framework in which the seed grower manages plant health, although growers can influence these soil microorganisms via crop and soil management.

The third consequence can be derived from the two former ones. On one hand, the field environment is imparted with a role in plant health management. On the other, bean varieties come with their requirements in terms of environmental conditions and cannot thrive and adapt everywhere. As a consequence, *in situ* plant health management operates at a collective scale. Within a network of seed growers, plant health management is also about attributing each variety to a suitable growing environment. As seen in Chapter V, some varieties - such as 'Flageolet Chevrier' - more easily adapt to a range of growing environments, whereas others -such as 'Rognon de Coq' - are susceptible to certain diseases and more "picky" in terms of growing environments. A variety that won't adapt to a given environment is not discarded, but passed on to another seed grower and tried in another environment. Moreover, the collective extends to the customers of the artisanal seed companies, as they must align on the management of *sound* growing environments to ensure plant health.

2 Governance of plant health and crop diversity management

The collective *in situ* governance of plant health is radically different from an approach of genetic disease control. Referring to seed-borne Halo Bacterial Blight (HBB) in subsistence agricultural systems in developing countries, where bean seed is generally grown from farm-saved seeds, Taylor (1996a) recommends that disease control be based on genetic resistance of bean varieties. He deducts this recommendation from the impossibility of producing disease-free seed and the general presence of the plant pathogen in field environments. In the case of the *Croqueurs*, basing plant health management on a genetic disease control strategy does not appear as an option, as one of their fundamental purposes is to maintain crop diversity in home and market gardens in the form of old and heirloom varieties. Rather than *a priori* choosing those varieties that are genetically resistant to a given variety or breeding new varieties in an objective of genetic resistance, varieties are taken as they are. Bean health management then consists of matching varieties with appropriate environments and letting the population adapt to local environmental conditions. This points to the intimate entanglement of plant health governance and crop diversity management.

Like plant health, bean *lineages* emerge from the triangular interaction in the *terroir*. A *lineage* is distinguishable from another *lineage* of the same variety because its appearance - its phenotypic expression - is different: Philippe's 'Rognon de Coq' is not quite the same as François' 'Rognon de Coq'. Chapter VIII has demonstrated that after only three years of multiplication in contrasting environments, phenotypic differences - *lineages* - begin to emerge. When trying to disentangle the possible sources of phenotypic differences, however, making a clear-cut distinction between mechanisms that are internal and those that are external to the plant populations is difficult. Some phenotypic differences can be attributed to genetic differences between populations. These genetic differences are driven by local environmental conditions and growers' practices. The genetic differences between lineages observed after only three years of multiplication in contrasting environments are probably far from reaching an equilibrium. Genetic adaptation is ongoing. In fact, past research on bread wheat indicates that local genetic adaptation may not ever reach an equilibrium state in the context of crop diversity management by networks of farmers. It rather appears as a perpetual dynamic process in which the genetic makeup of a plant population is shaped by yearly changes in the local environment and growers' management practices (Thomas, 2011).

In addition to the genetic information of a plant population, seed lots also come with physical and biological properties. For instance, seed size and infection rate with plant disease agents are induced by the environment in which the seed was grown. These seed properties can in turn affect the phenotype of the following plant generation, in terms of plant health and in more general terms. Chapter VII has addressed microbial communities associated with seed. These communities include microbial life associated to seeds beyond seed-borne plant pathogens. Although microbial communities associated to plants in the phyllosphere and rhizosphere have been shown to influence plant growth and health, little is known about the effect of microbial assemblages associated to seeds. Chapter VII of this thesis has shown that on the common bean seed harvested in the field experiment after two years, microbial assemblages, fungal communities in particular, were determined by the growing environment, or *terroir.* Microbial communities conveyed by the seed reflect the environment in which it was grown; in other words: *terroir* extends onto the seeds.

In short, plant phenotypes are the product of a perpetual coevolution between plant populations and their growing environment. The growing environment shapes plant genetics, physical seed properties and microbial life on the seeds. This renders it difficult to draw a boundary around plant populations: Where do the plant populations end, where does the growing environment begin? Facing the entanglement of plant populations and growing environments behind *lineages* and plant health, the term *seed system* takes on a second meaning.

The concept of *seed system* is usually used to describe the social structure by which seed is disseminated. It has been defined as “the sum of physical, organisational and institutional components, their actions and interactions that determine seed supply and use, in quantitative and qualitative terms” (Scoones and Thompson, 2011). For example, formal seed systems can be distinguished from informal or local ones, according to the actors and types of relations involved (Almekinders et al., 1994; GRAIN, 2008). Considerations on the *Croqueurs’* approach to plant health and crop diversity management highlight a set of interactions situated upstream to seed dissemination: the system of interactions that make a seed lot what it is might also be called a *seed system*. The *Croqueurs'* approach to plant health and crop diversity then constitutes an <u>*in situ* seed system</u>, in which seed cannot be considered isolated from the environment in which it was grown and the environment in which it will be grown. The boundary defining seeds isn't clear-cut. This approach differs from approaches where seeds are clearly circumscribed as vector of plant health and stable plant varieties. Before discussing seed systems in terms of supply and use, i.e. in terms of seed dissemination, considering the upstream seed system, that which produces the seed that is later disseminated, appears to be necessary.

Within the *in situ* seed system of the *Croqueurs*, crop diversity is not taken for granted, but sustained through continuous care. It implies the management of flows rather than the management of stocks. The *Croqueurs'*, and more generally the RSP’s rejection of the term crop diversity *conservation* and preference for the terms *maintenance* reflects this conception of crop diversity management. It is about maintaining crop diversity in fields and gardens, in the sense of cultivating, nurturing, and developing this diversity. Therefore, it is about managing dynamic flows - flows of plant populations from one growing season to the next, flows of information between growing environments and plant populations and flows of plant populations between growing environments. It is a quite different conception of crop diversity management than *conservation*, which rather reflects the idea of crop diversity as a given legacy for which one must ensure that stocks don’t run out.

Looking at this *in situ* seed system as the management of (ecological) flows rather than of (seed) stocks, conclusions can be drawn both in view of future research on such systems and in view of plant health regulations. Both these points are addressed in the following sections.

3 Ecopathology as a perspective for research in '*in situ* seed systems'

Research on such in situ seed systems, in which seeds, variety lineages and plant health cannot be considered isolated from their growing environments, calls for appropriate scientific approaches. In Chapter II, I have described the difficulties of linking on-farm field experiments with the situation in seed growers' seed crops. The perspectives of comparing several varieties produced by the Croqueurs in a few predetermined environments, even if they are farm environments, may be limited. Hesitations over the right sowing density and the right subplot size to apply to field trials, described in section 4 of Chapter II, illustrate this. Indeed, if no clear boundary allows separating seeds, plant phenotypes and plant health from the system in which they are situated, the most pertinent sowing density and plot size might well be those which are practiced by a given seed grower for a given lineage. As a consequence, the basic unit of plant health management would be the production system, determined by plant variety, farm environment and seed grower. Such a framework for plant health management is not acknowledged by current plant health regulations in Europe, nor by prevalent experimental approaches in agronomy. In this framework, actors don't have an ontological understanding of plant disease, which consists of regarding disease as the direct consequence of a pathogen, a "universal thing" (Hahn, 1982; Laplantine, 1993 in Cabaret and Nicourt, 2011; Canguilhem, 2013). The focus is placed on the system expressing disease. Disease is regarded as the consequence of disequilibrium in the system, a process. The solution of the problem of disease is sought by restoring an equilibrium in the system, and not so much in controlling the pathogen as such.

The ecopathology approach developed for research on animal health in animal husbandry may be relevant for research on *in situ* plant health (Ganière et al., 1991; Landais, 1991). As reflected by the term 'ecopathology', the approach acknowledges that factors affecting animal health operate in the rearing system. Instead of attempting to isolate animal health from the farming system, ecopathology allows for research on the health of animals within the herds of farmers, while involving farmers and other actors in the construction of research priorities, questions and protocols. The approach consists of replacing the concept of the "cause" of a disease by that of "risk factors". Ecopathological studies seek to reveal risk factors statistically associated with the emergence and development of pathological processes in production systems (Landais, 1991). According to the initial matter of concern, the objective may be to study a given animal health problem in diverse production systems, or to study general animal health in a certain type of production system (Ganière et al., 1991). By involving working groups of farmers and other actors of animal health in the planning phase, in data collection in their respective production system and in data analysis, the concepts of health held by participants can be taken into account and debated throughout the research process.

Adapting the ecopathology approach to the study of plant health in '*in situ* seed systems' appears as a relevant approach for future research. Based on the experiences and findings of this PhD research - described as *translations 1, 2a and 2b* in Chapter II - embedding research in seed growers' crops may be a promising perspective - *translation 3*, so to say.

4 Protective measures impose a boundary around seed

This research was triggered by tensions concerning the management of CBB on bean seeds in France (Chapter II). EU Plant Health regulations prescribe *protective measures* against CBB, which exclude bean seed carrying CBB agents from European seed markets. The aim of these measures is to protect common bean production from CBB outbreaks, particularly in Southern Europe. However, this thesis shows that the protection granted by the measures is somewhat ambiguous when it comes to the '*in situ* seed system' of the *Croqueurs*. Protective measures potentially jeopardise this seed system at three levels.

(i) In the case of "quarantine" measures against CBB, seed lots infected with CBB agents are excluded from seed markets. Given that no method has yet been found to rid infected bean seed of the pathogen, the measure implies that seed lots infected with CBB agents have to be abandoned. The direct effect this may have on bean diversity managed by the *Croqueurs* is obvious: If all the lineages of a given bean variety within the seed grower network were detected positive with CBB agents, that variety would be completely lost from the network. According to the data collected in the framework of the thesis, such extreme situations have not occurred up to now. Indirect effects on the *Croqueurs'* activities may be more relevant than this direct effect on bean diversity.

(ii) By questioning rules and regulations put in place by Plant Health regulations on CBB in France (Chapter III), the *Croqueurs* point to tensions between their practices and protective measures. Seed inspection procedures do not appear to be adapted to the small bean seed lots they deal with as *artisanal* seed companies. By contesting and negotiating seed sampling procedures at the local scale, *Croqueurs* members attempt to protect their artisanal practices from inadequate seed inspection procedures. As a result of such negotiations, some *Croqueurs* members have arranged for *ad hoc* adaptations of the official sampling procedure with seed inspectors at the local scale. These adapted sampling protocols maintain a space for the *Croqueurs'* practices of artisanal bean seed production.

(iii) Nevertheless, such local, informal compromises do not imply an alignment of the *Croqueurs* on the rationale behind official protective measures against CBB. By circumscribing bean seed as vehicle of plant health, seed quality and plant health is decoupled from growing environments. This approach to plant health is in opposition with the *Croqueurs' in situ* approach to seed systems. Facing the prescription of protective measures, which impose a boundary on plant health and exclude the triangular interaction in *terroirs*, the *Croqueurs'* identity is at stake. Aligning on that rationale would imply abandoning *in situ* governance of plant health and, by the same move, *in situ* seed systems. Thus, the *Croqueurs'* identity is at stake in their refusal to endorse the French inter-branch union as legitimate seed inspection body and spokesperson. The inter-branch union has the mission to represent the seed sector in its entirety - from plant breeder to seed user, but also from organic seed to genetically modified seed. By rejecting the inter-branch union as representative, the *Croqueurs* are able to maintain their *translation* of seeds and plant health as an expression of triangular

> interactions in the *terroir* in opposition to a competing translation which decouples seed and plant health from growing environments. The *Croqueurs* are claiming respect of their own identity.

The translations made of plant health by EU protective measures against CBB on one hand and by the *Croqueurs* on the other are incompatible and raise the question of the path dependence of quarantine measures and respective inspection bodies in relation to the DUS criteria (distinctiveness, uniformity and stability) in official variety testing. Döring *et al*. (2012) have recommended that in the absence of a common definition of plant health, plant health and its management be defined by a procedural approach. This procedural definition of plant health involves actors with divergent understandings of plant health in an inclusive debate (see Chapter II, section 2.2). We have seen in Chapter III (section 3) how the *Croqueurs* association endeavours to upgrade the debate on crop health management to a more general and public scale. In view of the role played by the *Croqueurs* in safeguarding vegetable crop diversity in home and market gardens, taking into account their understanding of plant health seems crucial. Whereas the SPS agreement of the WTO (MacLeod et al., 2010) addresses the barriers that phytosanitary non-tariff measures represent for international trade, the barriers they represent for small-scale seed companies engaged in the maintenance of crop diversity at the national or regional level is seldom accounted for. With revisions of the EU Plant Health directive currently underway[45], the procedural approach proposed by Döring *et al*. appears as a timely recommendation pointing to an inclusive way forward.

By specifying the *Croqueurs'* approach to bean health and pointing to diverging conceptions of plant health, this PhD thesis may contribute to opening a debate on approaches to plant health. It has become clear that such a debate must not merely take into account practices of plant health management in terms of methods and techniques, but also the professional identities linked to these practices. The *Croqueurs'* governance of plant health and management of crop diversity are entwined and consist of managing flows rather than stocks. Taking their professional identity seriously involves taking into account the flows and ecological interactions they manage. Beyond questioning the norms and seed inspection procedures set out in plant health regulations, the debate may thus open questions as to the way the politics behind these regulations function. Thereby, a door may be opened on the distribution of political competences - among humans (will the *Croqueurs* have a place in the politics of plant health?), but also among non-humans: will bean populations and blight pathogens have a place to *speak for themselves* in the politics of plant health? Giving plants and pathogens a say implies taking into account the nuances of their ecological interactions (flows), rather than ensuring bean yields through the absence of pathogenic organisms (stocks). This transformation, which consists of bringing ecology into politics, has been discussed as *cosmopolitics* in the fields of philosophy (Stengers and Stengers, 1997; Latour, 2004) and social science (Hinchliffe et al., 2005).

Opposing concepts of plant health become particularly clear in the case of the *Croqueurs de Carottes*, because it is an association of seed companies which are subject to Plant Health regulations in view of

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[45] New plant health regulations were voted in the European Parliament in second reading on October 25th, 2016.

issuing European Plant Passports (EPP) for the seed lots they market. Within RSP, the case of the *Croqueurs* speaks for much broader network of farmers and other actors, who regard the boundary between plant population and *terroir* as a porous one. Like the *Croqueurs*, the actors federated within the RSP are safeguarding not only crop biodiversity in fields and gardens, but also knowledge and practices concerning the management of crop diversity and governance of plant health embedded in the *terroir*. Mathieu Thomas (2011) has shown that in the case of bread wheat, genetic diversity is maintained on farms at the scale of the farmer network. This thesis has demonstrated that collective dynamics of crop diversity management may be linked to a collective, *in situ* governance of plant health. They can thus be affected not only by inadequate seed market regulations, but also by plant health regulations that neither take into account the diversity of approaches to crop diversity and plant health, nor the diversity of identities these approaches imply.

Finally, this thesis has raised many more questions than it has brought answers to. Many doors have been opened, just to take a peek through them and realise that I have neither the time, nor the necessary resources (including competences) to cover the space behind those doors in the framework of this thesis (Not to mention the doors I have passed by and chosen to leave closed!). These open doors are perspectives. They are attributed to the transformative character of the thesis. Chapter II has addressed the research process behind the thesis and the *translations* it comprised. Research was initiated by a complex concern for bean health management, instead of a clearly defined question. Such a relatively open matter of concern is a precondition for a research process in which researchers and their partners co-evolve and progressively outline the object of research. As the research question is narrowed down, the research process concomitantly puts forth questions that are left aside. In the process, researchers become aware of these questions and why they are left aside. Moreover, the research process is not isolated from other processes, both scientific and societal ones. The concern for plant health management did not appear out of the blue and certainly won’t dissipate after the PhD research, but the PhD research may contribute to transform it. Opening doors upon the perspectives of the research forms part of the research process, be they perspectives for science, for plant health management practices or for plant health regulations.

Conclusions

The thesis has shown that indeed, tensions between the bean health management practices of the *Croqueurs de Carottes* and protective measures against Common Bacterial Blight (CBB) are driven by different approaches to plant health. From there, the findings of the thesis can be apprehended in three steps:

(i) What do tensions about the management of CBB teach us about the *Croqueurs'* governance of plant health?
(ii) What does the *Croqueurs'* governance of plant health teach us about their management of crop diversity?
(iii) What does the *Croqueurs'* approach to the governance of plant health and management of crop diversity teach us about plant health regulations and the politics behind them?

Firstly, the members of the *Croqueurs* association consider plant health *in situ*, i.e. in plants' growing environment. Governance of plant health is reasoned at the scale of the collective encompassing the network of seed growers, but also seed users. Bean variety, growing environment and seed grower interact and form a whole, a system. Within these triangular interactions, the boundary of the plant population cannot be drawn clearly: growing environments do not only shape the phenotypes and health of crops, but also induce seed properties and genetic adaptation of plant populations. The growing environment shapes microbial communities associated with seeds and thereby extends onto seeds. The difficulty - impossibility? - of drawing a clear boundary around plant populations, that would separate them from their growing environment, also renders it difficult to grasp and study the ecological base of this plant health management approach experimentally. For future research, methods allowing to study plant health in the environmental context of seed growers' seed crops and integrating the seed growers' approach to plant health appear to be more pertinent than on-farm trials designed by researchers. The research approach of ecopathology constitutes a promising research perspective.

Secondly: despite the aforesaid difficulties of grasping the ecological base of the *Croqueurs'* governance of bean health experimentally, its link with their management of crop diversity has been highlighted both through sociological inquiry and field experiments. The adaptability of plant populations is regarded as a pillar of plant health. Like plant health, *lineages* of a variety emerge as a result of the triangular interaction between variety, environments and grower. Lineages are the result of phenotypic and genetic adaptation of plant populations to a growing environment, which includes seed growers' management practices. Both the governance of plant health and the management of crop diversity are about managing dynamic flows - flows of plant populations from one planting season to the next, flows of information between growing environments and plant populations and flows of plant populations between growing environments. By maintaining crop diversity and managing plant health *in situ*, actors such as the *Croqueurs* are not only dynamically safeguarding crop genetic diversity, but also perpetuating their practices and asserting their identity as *artisanal* seed companies.

Thirdly, it is this identity that is at stake in the *Croqueurs'* contestation of plant health regulations. Beyond the *Croqueurs*, a wider network of farmers and other actors engaged in maintaining crop diversity and associated practices on farms and in gardens constitute the *Réseau Semences Paysannes*. A shared approach of crops and plant health as the expression of triangular interactions *in situ* may explain a shared concern over a constriction of their practices by Plant Health regulations. Recognising the range of plant health concepts that are held and practiced by actors in Europe appears to be a precondition for the negotiation of plant health regulations, if they are not to jeopardise the seed systems associated to these plant health concepts. Debating approaches to plant health involves not only plant health management practices in terms of techniques and norms, but also the professional identities of the actors involved.

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Annexes

Annex 1: Guide thématique d'entretiens (Thematic guide for interviews)

Question de recherche:

Comment la santé du haricot est-elle géré par les artisans semenciers au sein de l'association Croqueurs de Carottes?

Consignes initiales possibles :

Producteurs : *Comment gérez-vous les maladies de la semence dans votre système de production ?*

Chercheurs : *Expliquez-moi votre travail concernant la graisse du haricot.*

Autres (institutions…) : *Quel est votre rôle dans le secteur semencier par rapport à la qualité de la semence?*

Qualité de semences

Qualité d'une semence [définition, phytosanitaire, variété, critères, alternatives, attentes]

Assurer la qualité [pratiques, culture, sélection, analyses, tests, contrôles, difficultés, attentes des clients]

Systèmes de production

Agriculture [paysanne / industrielle ; conventionnelle / biologique ; relation avec société]

Production de semences [artisanale / industrielle ; adaptation ; variétés ; signification]

Maladies des plantes [Définition, signification, lutte, problématisation]

Ecosystème [sol, microorganismes, plantes, producteurs]

Législation sur la santé des plantes

Rôle de la législation [en général ; avantages et inconvénients ; nécessité]

Impacte sur la production [application, contrôles, sanctions, pratiques]

Acteurs [législateurs, application, institutions FNAMS, PV, contrôleurs, chercheurs, ONG, relations]

Difficultés [pour la pratique, dépendant du système de production, application, compréhension, information]

Compétences et pratiques

Rôle [métier, engagement, motivation]

Compétences [formation, expérience, curriculum]

Quotidien [relations de travail, lieu, taches]

Relations

Producteurs [réseaux, organisation, conseil, clients]

Institutions [PV, FNAMS, GNIS, RSP, INRA]

Recherche [entre chercheurs / disciplines, recherche et pratique]

Politique [action politique, réseau]

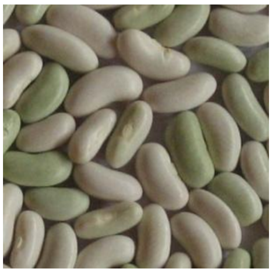
Annex 2: Affiliation and groups of interviewees

Inter-view no.	Interview	Person (code)	Affiliation	Profession/Role	Country
1	260814	CRO-260814b	Artisan seed company, *Croqueurs* member	Seed grower and artisan	FR
2	280814	CRO-280814c	Artisan seed company, *Croqueurs* member	Seed grower and artisan	FR
3	100914	CRO-100914a	Artisan seed company, *Croqueurs* member	Seed grower	LU
4	281014	NRI-281014j	National research institute	Researcher, bacteriologist	FR
5	190515	CRO-190515s	Artisan seed company, *Croqueurs* member	Coordinator of seed production	FR
5	190515	CRO-190515d	Artisan seed company, *Croqueurs* member	Seed artisan	FR
6	260915	NGO-260915k	NGO for peasant seed	Advocacy	FR
7	221015	CRO-221015a	Artisan seed company, *Croqueurs* member	Seed grower and artisan	BE
7	221015	CRO-221015m	Artisan seed company, *Croqueurs* member	Seed grower and artisan	BE
8	111215	PIA-111215c	Phytosanitary inspection body	Regional coordinator for seed inspection on vegetable crops and maize	FR
9	181215	SOS-181215g	Artisan seed company	Director for seed production	DE
9	181215	SOS-181215s	Artisan seed company	Research and development	DE
9	181215	SOS-181215r	Artisan seed company	Seed health diagnosis and management	DE
10	070116	MSC-070116v	Multinational seed company	Bean selection	FR
11	290116	CRO-290116d	Artisan seed company, *Croqueurs* member	Seed grower and artisan	FR
11	290116	FSM-290116c	Regional agency of the national federation of seed multipliers	Research and development	FR
12	150216	MSC-150216b	Multinational seed company	Production manager for peas and beans	FR
13	170216	PIA-170216p	Phytosanitary inspection body	Regional director of seed inspection	FR
14	020616	PIA-020616o	Regional body of the Ministry for Agriculture	Regional delegate for plant health, resource person for phytosanitary management plans	FR

Annex 3: Observations participantes (Participant observations)

Evènement/Activité	Organisateur	Date	Lieux
Rencontres Internationales Maisons des Semences Paysannes 2012, des modes d'organisations collectives pour gérer la biodiversité cultivée; avec excursion	Réseau Semences Paysannes	27-30/09/2012	Périgeux (FR)
Produire ses semences et plants: les droits et la réglementation avec intervention de Guy Kastler, paysan et délégué général du Réseau Semences Paysannes	Kerna ùn Sohma (association membre du RSP)	17/04/2013	Bennwhir (FR)
Let's Liberate Diversity! Annual Forum, 8th edition "From planting to plate"	ProSpecieRara in conjunction with the European Coordination: Let's Liberate Diversity!	20-22/09/2013	Basel (CH)
Portes ouvertes des jardins de Semailles	Semailles (artisan semencier)	16/09/2014	Faulx-les-Tombes (BE)
Rencontre internationale « Sème ta résistance : les semences paysannes nourrissent les peuples »	Réseau Semences Paysannes	24-26/09/2015	Pau (FR)
Echanges autours des essais aux champs		2012-2015	divers

Annex 4: Bean varieties in the field trials



The cultivar 'Flageolet Chevrier vert' (flc) is used as dry or semi-dry shelling bean. In France, it is traditionally harvested before maturity and dried for the consumption of light green colored dry grain. It was obtained near Paris in the 1870's by M. Chevrier from a genetic mutation discovered in one of his fields (Doré and Varoquaux, 2006) and introduced for the 1878 International Paris Exposition (Weaver, 2013). It has white flowers. It is considered as tolerant to a number of biotic and abiotic stresses, although somewhat susceptible to common rust (*Uromyces appendiculatus*) (Denaiffe, 1906). While this cultivar indeed appeared as very healthy and tolerant in most experimental sites and years, it was almost completely destroyed by systemic phloem necrosis ("black root syndrome") caused by Bean Common Mosaic Virus (BCMV) in BZH in 2015. This syndrome is associated to the I gene, conferring incompletely dominant resistance to BCMV under certain conditions, but can cause systemic phloem necrosis under others (Collmer et al., 2000).

'Rognon de Coq' (rdc) is also called 'Flageolet rouge' in French and 'Red flageolet ', 'Scarlet flageolet' , ' Crimson flageolet' or 'Crimson Wonder ' in English. In 1883, it is praised for its hardiness and productivity in the Vilmorin-Andrieux catalogue. Plant are described as vigourous (Vilmorin-Andrieux et Cie, 1883). In 1906, Denaiffe states that it is a universally known and cultivated dry bean. Although the quality of its wine colored dry grain is particularly reknown, its semi-dry seeds and young green pods are also recommended for consumption. Flowers are of a pale lilac color (Denaiffe, 1906). Although Denaiffe describes it as semi-early maturing, it is among the later maturing cultivars of the field trials. It is considered as very susceptible to common bacterial and halo blights by farmers. This was confirmed by symptom scores in the field trials.

'Roi des Belges' (rdb) is an old Belgian variety selected from an older cultivar, 'Noir de Belgique'. According to one source, it exists since 1920 (https://www.vreeken.nl/046065-stamslabonen-koning-der-belgen-roi-des-belges). It is cited by Vilmorin-Andrieux in 1947, where it is described as very early and productive. It is also called 'Métis noir' and 'Triomphe d'Epizy' (Vilmorin-Andrieux SA, 1947). The flowers are light purple. Its long, straight pods are consumed as French bean, but must be picked before they become too mature and become tough and fibrous (Biodimestica, 2016). The Luxembourgish seed producer who provided the seeds for the experiment in 2012 had himself multiplied it since 2003 after he was given seeds from a Belgian producer. The seed originally came from another gardening and seed-saving association in Belgium.

'Saint Esprit à œil rouge' (ses) may already have been mentioned in the catalogue of Vilmorin-Andrieux in 1885 (Vilmorin-Andrieux et Cie, 1855). The long history of this cultivar would explain the large number of names in different regions of France: 'Haricot à la Religieuse', 'Haricot à l'aigle', 'Nombril de Bonne Soeur'. Both Vilmorin-Andrieux(1855) and Denaiffe (1906) describe a semi-early maturing cultivar (120 days) with slightly blustered leaves and white flowers. However, these authors mention black or brown markings, whereas the markings of the cultivar used are wine red. Although it can be consumed as French bean or semi-dry, it is particularly recommended as dry bean for its taste (chestnut) and digestibility (Polese, 2006). The Luxembourgish seed producer who provided the seeds for the experiment in 2012 had himself multiplied it since 2003 after he was given seeds from a Belgian producer. He has since stopped the production of this cultivar due to a lack of productivity and plant health problems, probably due to virus infection.

'Calima' (cal) is a stringless snap bean released by the German company Hild Saat in 1989 (Hild saat, personal communication). It is considered semi-early maturing. It was chosen as commercial check because it is sold as highly resistant to BCMV, halo blight and anthracnose (Hild, 2016) and has been recommended for organic growing conditions (Arbeitsgemeinschaft Ökologische Gartenbauberatung, 2012).

Annex 5: Pedo-climatic characterisation of the trials sites and crop management

	Aquitaine (AQU)	**Luxembourg (LUX)**	**Brittany (BZH)**
Soil and climatic conditions			
Latitude	44°21'13.32"N	49° 42' 7.42"N	48°2'57.50"
Longitude	0°31'31.06"E	6° 2' 20.43"E	1°47'10.96"
Altitude (m above sea level)	88	259	34
Average annual:			
- minimum temperature (°C)	8.6	5.2	7.7
- maximum temperature (°C)	19.1	15.0	16.7
- average temperature (°C)	13.4	9.6	11.9
- rainfall (mm)	644	788	694
Soil type	Silty clay	Sandy clay	Clay-loam
Soil pH	8.5	8.1	6.4
Organic matter content	1.9	2.7	2.6
Mineral nitrogen* (kg N / ha)	11.1	29.6	102.9
Phosphorous (mg P_2O_5 / kg)	36	15	144
Potassium (mg K_2O / kg)	140	141	385
Calcium (mg CaO / kg)	12380	8848	1644
Magnesium (mg MgO /kg)	261	110	188
Date of soil sampling	*12/06/2013*	*04/06/2013*	*07/06/2013*
Crop management			
Soil preparation	Subsoiler every 3-4 years (60 cm) Plough (18 cm) 'Actisol' harrow (10 cm) Rotary cultivator	Rotary tiller (maximum 20 cm)	Rotary cultivator (15 cm) Shank cultivator (30 cm) Spring-tooth harrow (15 cm)
Distance between rows	60 cm	75 cm	75 cm
Distance between plants	2012: 10 cm 2014: approx. 5 cm	2012-13: 10 cm 2014: approx. 5 cm	2012-13: 10 cm 10 cm 2014: approx. 5 cm
Irrigation	overhead; to field capacity every 10 days in absence of rain	overhead; to field capacity	none
Fertilization	Composted farm yard manure every 3-4 years (75 t/ha), followed by green manure	In crop rotation: Green manure, compost Application of on-farm preparation of "effective microorganisms"	none

* Mineral nitrogen including $N\text{-}NO_3$ and $N\text{-}NH_4$

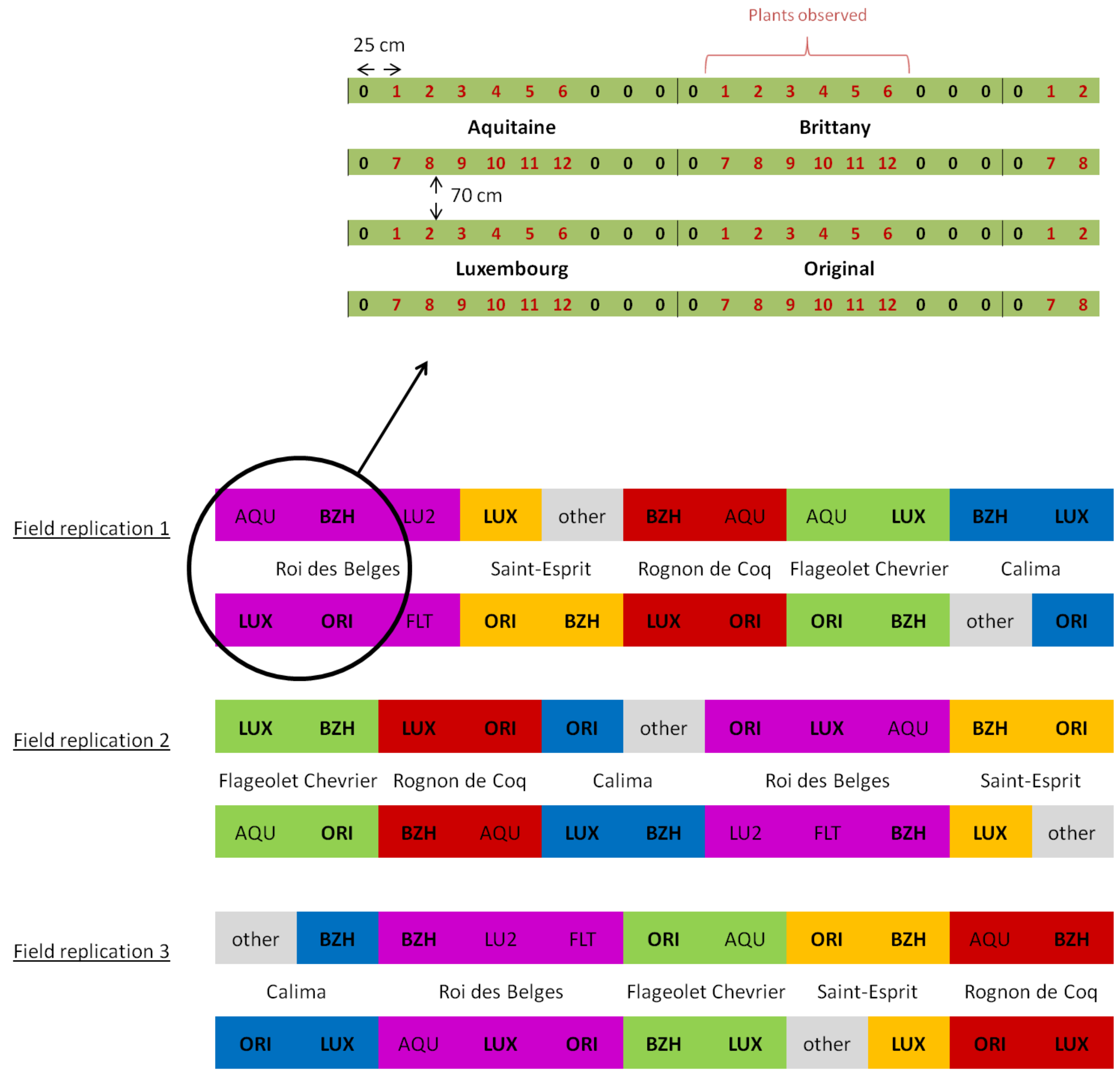
Annex 6: Yearly meteorological conditions at the trial sites

*Note:

Weather statistics are taken from the weather stations closest to experimental sites. They are located in Estillac (for AQU), in St. Jacques de la Lande (for BZH) and Koerich (for LUX). Given that different authorities are in charge of the weather statistics, according to country, comparable data is not available for humidity. Monthly humidity minima and maxima are available for France, and monthly humidity averages for Luxembourg.

Location	Year		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Aquitaine (AQU)	2012	Temp. mean (°C)	6.2	0.9	10.3	10.7	16.7	19.9	20.3	22.9	18.7	14.6	9.3	7.4
		Temp. minimum (°C)	3.3	-3.5	2.9	7.0	11.2	14.4	13.6	16.3	12.6	10.3	6.0	4.1
		Temp. maximum (°C)	9.6	6.4	18.5	15.5	22.8	26.0	26.8	30.5	25.9	20.6	13.7	11.5
		Precipitation (mm)	40	9	17	137	57	67	26	20	26	43	52	92
		Humidity minimum (%)	72	47	37	56	46	47	40	36	36	54	69	69
		Humidity maximum (%)	99	90	93	94	93	92	91	90	91	96	97	96
	2013	Temp. mean (°C)	5.2	4.8	9.0	11.8	12.9	17.5	24.1	21.6	18.4	15.6	8.5	4.8
		Temp. minimum (°C)	2.4	1.3	4.7	7.0	8.3	12.5	17.6	15.0	13.5	11.6	5.9	1.4
		Temp. maximum (°C)	8.5	9.5	14.7	17.3	18.0	22.8	31.1	28.5	24.9	21.5	11.6	9.5
		Precipitation (mm)	139	58	84	43	139	78	14	12	58	43	94	49
		Humidity minimum (%)	74	58	51	47	50	52	38	37	49	55	69	76
		Humidity maximum (%)	97	93	94	91	94	92	91	90	93	95	95	99
	2014	Temp. mean (°C)	8.1	7.9	10.0	13.5	14.8	21.1	20.9	19.7	19.7	16.4	12.1	5.8
		Temp. minimum (°C)	5.1	4.0	4.4	8.1	9.6	14.7	16.2	14.5	12.9	11.2	8.7	3.1
		Temp. maximum (°C)	12.0	12.6	16.3	19.6	20.5	27.4	26.7	25.5	27.5	24.0	17.1	9.1
		Precipitation (mm)	105	87	66	59	58	30	80	82	31	19	96	24
		Humidity minimum (%)	74	61	47	49	49	41	51	50	41	48	67	73
		Humidity maximum (%)	98	97	95	97	96	91	96	96	98	98	97	96
Brittany (BZH)	2012	Temp. mean (°C)	7.1	3.7	9.6	8.9	14.0	16.2	17.3	18.9	14.7	12.9	7.9	7.7
		Temp. minimum (°C)	4.3	-0.1	3.6	4.8	8.9	12.3	12.2	13.6	9.6	10.1	4.9	4.9
		Temp. maximum (°C)	10.3	7.8	16.1	13.9	19.4	20.9	22.7	25.0	20.6	16.1	11.6	10.9
		Precipitation (mm)	31	13	32	115	74	85	53	20	62	117	59	125
		Humidity minimum (%)	70	59	51	50	52	56	52	46	52	70	69	71
		Humidity maximum (%)	96	94	98	95	95	95	95	96	97	96	96	96
	2013	Temp. mean (°C)		4.5	6.0	9.3	11.6	15.6	20.6	18.7	16.7	14.3	8.5	6.4
		Temp. minimum (°C)	2.9	1.4	2.8	4.8	7.4	11.1	14.5	13.0	11.3	11.0	5.5	2.9
		Temp. maximum (°C)	8.1	8.2	10.1	14.5	16.2	21.1	27.4	25.1	22.8	18.7	11.5	10.4
		Precipitation (mm)	57	47	94	40	72	41	73	10	25	106	94	90
		Humidity minimum (%)		63	60	49	54	53	45	42	48	62	71	73
		Humidity maximum (%)		92	93	92	95	95	94	96	96	97	96	98
	2014	Temp. mean (°C)	7.6	7.8	8.8	11.3	13.3	17.2	19.3	16.9	17.7	14.1	10.4	6.7
		Temp. minimum (°C)	4.7	4.6	4.0	6.7	8.4	11.2	13.8	12.4	11.7	10.4	7.7	4.1
		Temp. maximum (°C)	10.9	11.4	14.5	16.7	18.4	23.0	25.4	22.2	24.1	19.1	13.7	9.9
		Precipitation (mm)	146	140	34	36	63	63	55	92	3	77	90	75
		Humidity minimum (%)	76	62	53	53	52	46	48	52	49	62	75	71
		Humidity maximum (%)	98	95	95	96	96	95	97	96	95	96	97	95
	2015	Temp. mean (°C)	6.1	4.7	8.1	12.3	13.4	17.2	18.9	18.5	14.4	12.0	11.8	10.4
		Temp. minimum (°C)	2.7	1.0	4.2	6.4	8.8	11.1	13.8	13.1	9.4	8.2	8.7	7.6
		Temp. maximum (°C)	9.7	9.1	12.7	18.2	18.4	23.3	25.1	25.0	20.3	16.6	15.4	13.3
		Precipitation (mm)	78	65	21	51	53	43	40	79	50	24	61	32
		Humidity minimum (%)	71	63	57	46	57	45	45	47	48	62	69	72
		Humidity maximum (%)	95	94	94	93	95	93	94	96	96	96	96	96
Luxembourg (LUX)	2012	Temp. mean (°C)	3.6	-1.3	7.2	8.0	14.3	15.3	16.7	17.8	12.5	9.0	6.2	3.9
		Temp. minimum (°C)	1.0	-5.2	1.1	2.9	8.2	10.8	11.5	12.0	7.1	4.5	3.4	1.5
		Temp. maximum (°C)	6.7	3.3	14.5	13.8	21.1	20.5	22.8	25.0	19.5	14.4	9.2	6.3
		Precipitation (mm)	117	13	16	93	65	122	131	39	73	83	52	146
		Humidity mean (%)*	98	84	84	81	82	90	87	86	91	96	97	98
	2013	Temp. mean (°C)	1.8	0.4	1.9	8.6	11.1	15.6	19.4	16.8	13.4	11.3	5.5	3.5
		Temp. minimum (°C)	-0.1	-2.2	-2.1	3.5	6.7	9.9	12.8	10.9	8.7	7.4	2.7	0.5
		Temp. maximum (°C)	3.8	3.4	6.9	14.1	16.1	21.5	26.9	24.2	19.4	15.9	8.4	6.3
		Precipitation (mm)	61	38	43	65	148	91	32	54	61	131	89	74
		Humidity mean (%)*	98	91	83	76	90	85	83	87	94	97	98	98
	2014	Temp. mean (°C)	4.5	4.7	6.3	11.2	12.4	16.3	18.6	15.7	14.7	12.1	7.6	3.8
		Temp. minimum (°C)	1.6	0.7	0.2	4.6	6.5	9.4	13.0	10.8	9.0	8.0	4.6	1.5
		Temp. maximum (°C)	7.4	8.7	14.5	18.2	18.8	23.5	25.2	21.3	21.5	16.5	10.6	5.9
		Precipitation (mm)	84	83	9	7	77	28	124	126	20	99	50	87
		Humidity mean (%)*	98	96	83	77	85	75	85	89	90	97	99	99

Annex 7: Plan of field trial in Brittany (BZH) in 2015 ('gathering phase')



Note:
Field replications 1-3 are shown one beneath the other in the figure, but they were actually situated one after the other in the field. The trial thus consisted of 4 rows of bean plants. The field trial was 90 m long, including « border » plants at the beginning and at the end of the trial (at least 2m, not shown in the figure).

Legend

Bean variety		Versions
🟪	**Rois des Belges (rdb)**	Brittany (BZH) Luxembourg (LUX) Aquitaine (AQU) Luxembourg 2 (LU2) Faulx-les-Tombes, BE (FLT) Original population from Luxembourg (ORI)
🟥	**Rognon de Coq (rdc)**	Brittany (BZH) Luxembourg (LUX) Aquitaine (AQU) Original population from Aquitaine (ORI)
🟨	**Saint-Esprit (ses)**	Brittany (BZH) Luxembourg (LUX) Original population from Luxembourg (ORI)
🟩	**Flageolet Chevrier (flc)**	Brittany (BZH) Luxembourg (LUX) Aquitaine (AQU) Original population from Aquitaine (ORI)
🟦	**Calima (cal)**	Brittany (BZH) Luxembourg (LUX) Original population, seed from East Africa (ORI)

Plan by Martin Dutartre (2015)

Annex 8: Examples for leaf symptoms scores

Leaf mosaic (scored from 1 to 5)

1: No symptom

5: Severe symptom expression or plant death


2: Doubtful to weak symptom expression


3: Moderate to intermediate symptom expression


4: Intense symptom expression

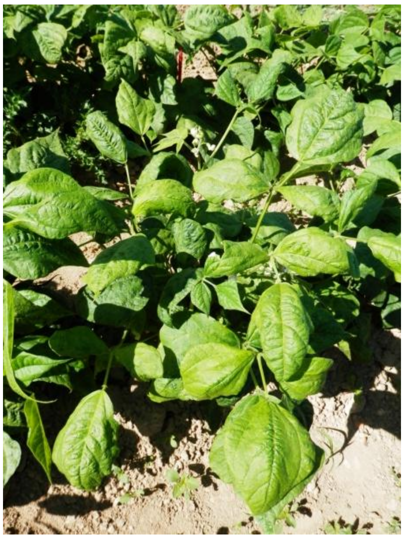
Leaf blustering (scored from 1 to 5)

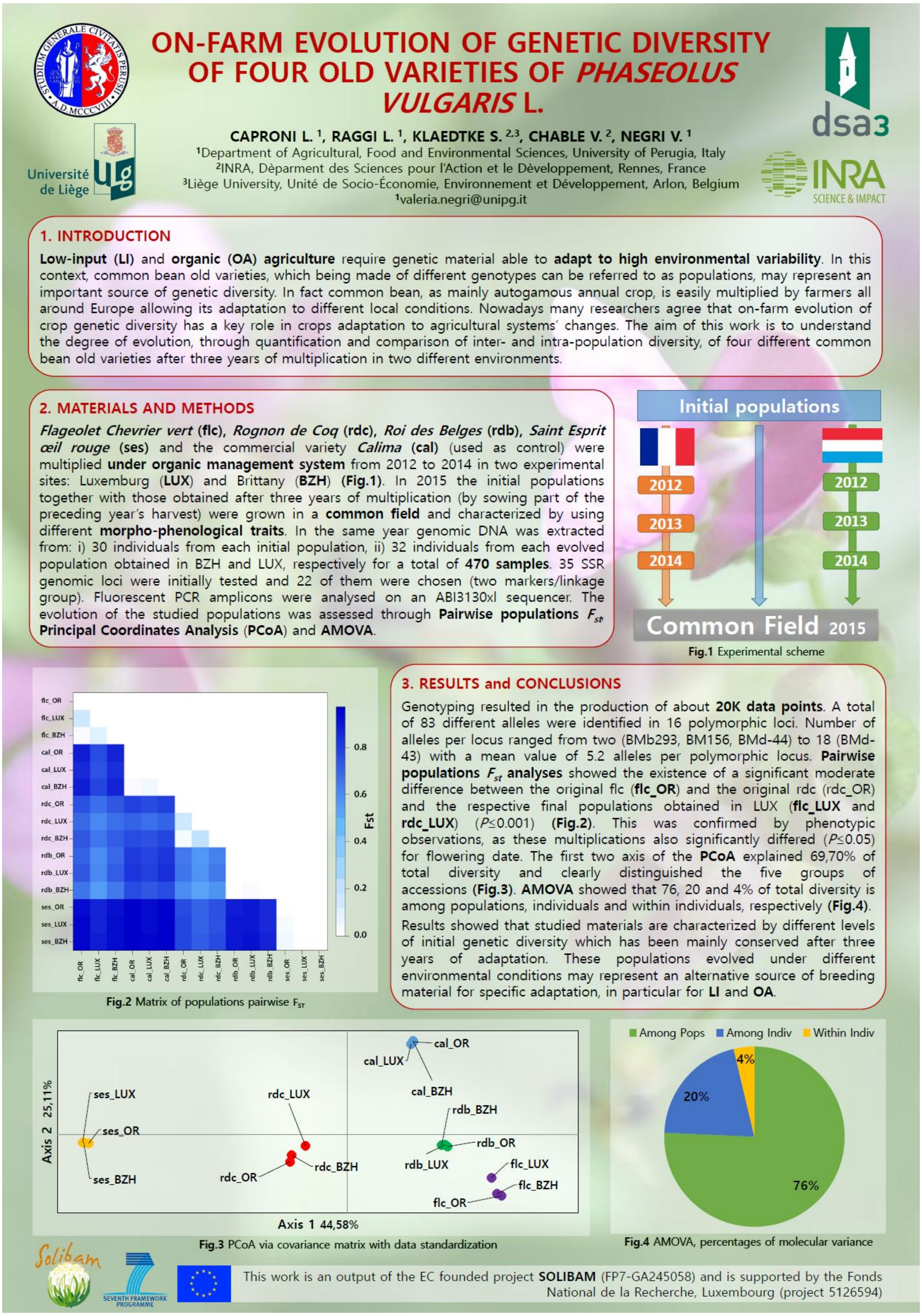
1: No symptom

5: Severe symptom expression or plant death


2: Doubtful to weak symptom expression


3: Moderate to intermediate symptom expression


4: Intense symptom expression



ON-FARM EVOLUTION OF GENETIC DIVERSITY OF FOUR OLD VARIETIES OF *PHASEOLUS VULGARIS* L.

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1. INTRODUCTION

Low-input (**LI**) and **organic** (**OA**) **agriculture** require genetic material able to **adapt to high environmental variability**. In this context, common bean old varieties, which being made of different genotypes can be referred to as populations, may represent an important source of genetic diversity. In fact common bean, as mainly autogamous annual crop, is easily multiplied by farmers all around Europe allowing its adaptation to different local conditions. Nowadays many researchers agree that on-farm evolution of crop genetic diversity has a key role in crops adaptation to agricultural systems' changes. The aim of this work is to understand the degree of evolution, through quantification and comparison of inter- and intra-population diversity, of four different common bean old varieties after three years of multiplication in two different environments.

2. MATERIALS AND METHODS

Flageolet Chevrier vert (**flc**), ***Rognon de Coq*** (**rdc**), ***Roi des Belges*** (**rdb**), ***Saint Esprit œil rouge*** (**ses**) and the commercial variety ***Calima*** (**cal**) (used as control) were multiplied **under organic management system** from 2012 to 2014 in two experimental sites: Luxemburg (**LUX**) and Brittany (**BZH**) (**Fig.1**). In 2015 the initial populations together with those obtained after three years of multiplication (by sowing part of the preceding year's harvest) were grown in a **common field** and characterized by using different **morpho-phenological traits**. In the same year genomic DNA was extracted from: i) 30 individuals from each initial population, ii) 32 individuals from each evolved population obtained in BZH and LUX, respectively for a total of **470 samples**. 35 SSR genomic loci were initially tested and 22 of them were chosen (two markers/linkage group). Fluorescent PCR amplicons were analysed on an ABI3130xl sequencer. The evolution of the studied populations was assessed through **Pairwise populations F_{st}**, **Principal Coordinates Analysis** (**PCoA**) and **AMOVA**.

Fig.1 Experimental scheme

3. RESULTS and CONCLUSIONS

Genotyping resulted in the production of about **20K data points**. A total of 83 different alleles were identified in 16 polymorphic loci. Number of alleles per locus ranged from two (BMb293, BM156, BMd-44) to 18 (BMd-43) with a mean value of 5.2 alleles per polymorphic locus. **Pairwise populations F_{st} analyses** showed the existence of a significant moderate difference between the original flc (**flc_OR**) and the original rdc (rdc_OR) and the respective final populations obtained in LUX (**flc_LUX** and **rdc_LUX**) ($P \leq 0.001$) (**Fig.2**). This was confirmed by phenotypic observations, as these multiplications also significantly differed ($P \leq 0.05$) for flowering date. The first two axis of the **PCoA** explained 69,70% of total diversity and clearly distinguished the five groups of accessions (**Fig.3**). **AMOVA** showed that 76, 20 and 4% of total diversity is among populations, individuals and within individuals, respectively (**Fig.4**).

Results showed that studied materials are characterized by different levels of initial genetic diversity which has been mainly conserved after three years of adaptation. These populations evolved under different environmental conditions may represent an alternative source of breeding material for specific adaptation, in particular for **LI** and **OA**.

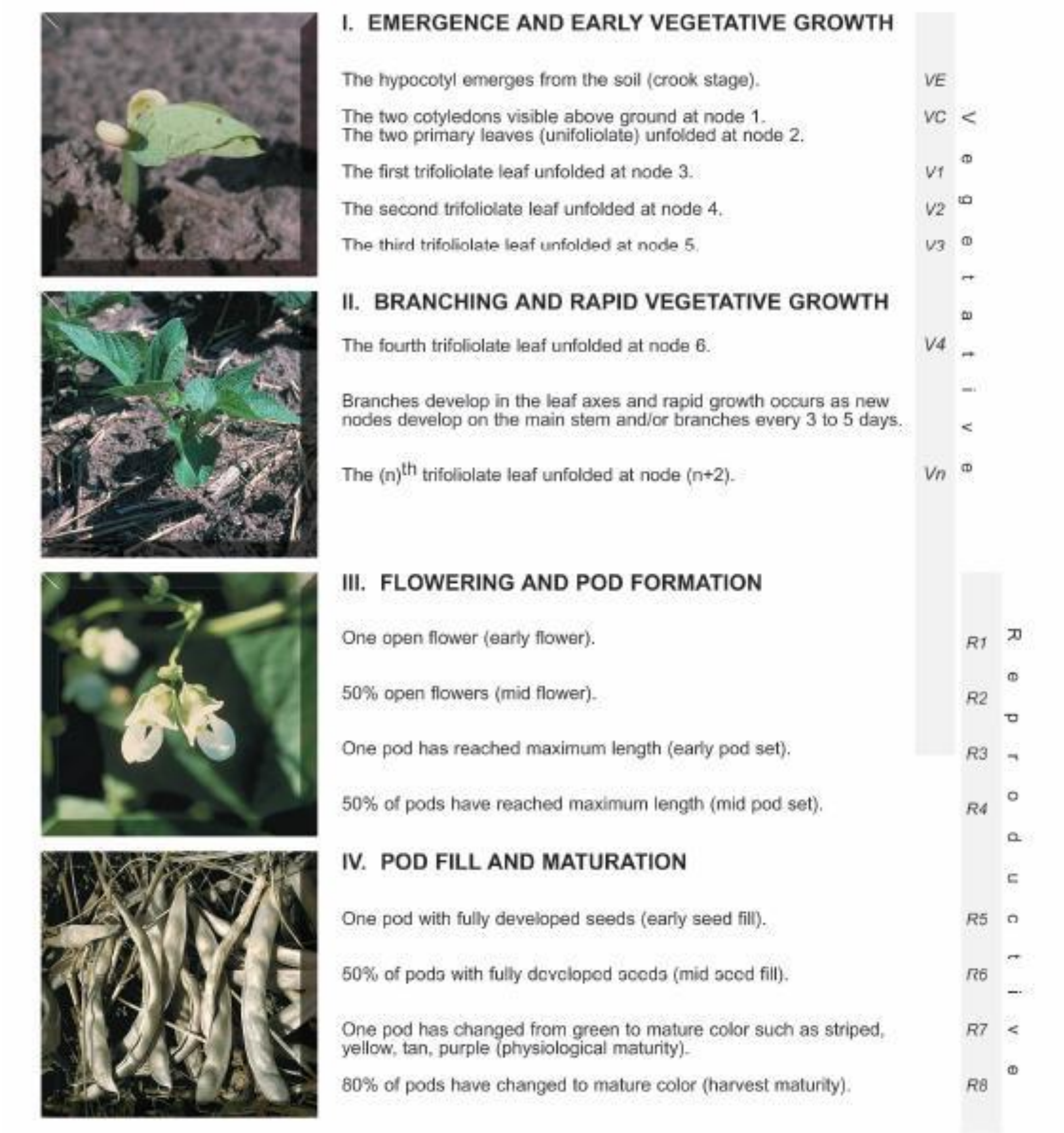
Fig.2 Matrix of populations pairwise F_{ST}

Fig.3 PCoA via covariance matrix with data standardization

Fig.4 AMOVA, percentages of molecular variance

This work is an output of the EC founded project **SOLIBAM** (FP7-GA245058) and is supported by the Fonds National de la Recherche, Luxembourg (project 5126594)

Annex 10: Growth stages of common bean



I. EMERGENCE AND EARLY VEGETATIVE GROWTH

The hypocotyl emerges from the soil (crook stage).	VE	Vegetative
The two cotyledons visible above ground at node 1. The two primary leaves (unifoliolate) unfolded at node 2.	VC	
The first trifoliolate leaf unfolded at node 3.	V1	
The second trifoliolate leaf unfolded at node 4.	V2	
The third trifoliolate leaf unfolded at node 5.	V3	

II. BRANCHING AND RAPID VEGETATIVE GROWTH

The fourth trifoliolate leaf unfolded at node 6.	V4	
Branches develop in the leaf axes and rapid growth occurs as new nodes develop on the main stem and/or branches every 3 to 5 days.		
The $(n)^{th}$ trifoliolate leaf unfolded at node (n+2).	Vn	

III. FLOWERING AND POD FORMATION

One open flower (early flower).	R1	Reproductive
50% open flowers (mid flower).	R2	
One pod has reached maximum length (early pod set).	R3	
50% of pods have reached maximum length (mid pod set).	R4	

IV. POD FILL AND MATURATION

One pod with fully developed seeds (early seed fill).	R5	
50% of pods with fully developed seeds (mid seed fill).	R6	
One pod has changed from green to mature color such as striped, yellow, tan, purple (physiological maturity).	R7	
80% of pods have changed to mature color (harvest maturity).	R8	

Source: Howard F. Schwartz, Mark A. Brick, Robert M. Harveson, and Gary D. Franc (2004): Dry Bean Production & Pest Management, 2nd Ed.; regional publication produced by Colorado State Univ., Univ. of Nebraska and Univ. of Wyoming.

Annex 11: Summary of leaf symptom score data available for multivariate analysis across locations and years

Environment	Observation date (das)	Plants scored	Plants scored per variety	Total plants scored in environment
AQU:12	59	150	30	**150**
AQU:13	31	300	60	**300**
BZH:12	75	150	30	**150**
BZH:13	49	300	60	**900**
	70	300	60	
	84	300	60	
BZH:14	62	100	20	**200**
	77	100	20	
LUX:12	50	150	30	**150**
LUX:13	30	300	60	**1200**
	47	300	60	
	54	300	60	
	85	300	60	
LUX:14	41	100	20	**200**
	67	100	20	

RÉSUMÉ

Gouvernance de la santé des plantes et gestion de la biodiversité cultivée - Le cas de la santé du haricot gérée par les membres de l'association « Croqueurs de Carottes »

De multiples réseaux d'agriculteurs et de jardiniers maintiennent la biodiversité cultivée dans le monde. Leurs pratiques de gestion de la santé des plantes demeurent peu étudiées. La thèse a pour objectif de caractériser l'articulation entre gestion de la biodiversité cultivée et gouvernance de la santé des plantes, se saisissant du cas d'une association d'artisans semenciers, les Croqueurs de Carottes. Elle développe une approche interdisciplinaire et transformatrice pour décrire et comprendre la gouvernance de la santé du haricot par ces acteurs, dans une perspective agroécologique. La théorie de l'acteur-réseau est mobilisée pour situer l'analyse à l'intersection entre approches agronomique et sociologique, reposant sur les données produites par un triple dispositif : expérimentations à la ferme, entretiens semi-directifs et observation participante.

Nous qualifions d'in situ l'approche de la santé des plantes des Croqueurs dont l'objectif est de vivre avec les agents pathogènes potentiels. Fondées sur des interactions écologiques entre plantes et terroir, les compétences contribuant à la gestion de la santé des plantes sont distribuées à travers le système de production. Que ce soit en termes de santé ou de biodiversité, un lot de semence est l'expression d'un jeu complexe d'interactions. Il est alors difficile de délimiter des populations de plantes de leur terroir de manière précise. Par conséquent, (i) la santé des plantes ne peut être jugée qu'in situ, dans l'environnement dans lequel elles évoluent et (ii) la gouvernance de la santé des plantes doit être prise en compte à l'échelle du collectif.

Mots-clefs : santé des plantes, artisans semenciers, haricot, recherche participative, agroécologie

ABSTRACT

***Governance of plant health and management of crop diversity - The case of bean health management among members of the association* Croqueurs de Carottes**

All over the globe, networks of seed growers are cultivating crop diversity in fields and gardens. Their contribution to the maintenance of this diversity has been studied, but research has widely left aside their management of plant health. The governance of bean health practiced by an association of artisanal seed companies, Croqueurs de Carottes, is approached as a case study in the objective of specifying how management of crop diversity and governance of plant health are articulated. Their concern for the governance of bean health is elucidated from an agroecological perspective, taking an interdisciplinary and transformative approach. Actor-network theory constitutes the backbone of the thesis, situated between Agronomy and Sociology and drawing upon a threefold research device: on-farm experiments, semi-directive interviews and participant observation.

The Croqueurs' approach to bean health is described as in situ approach, in which plant populations are considered healthy if they are able to live with potential plant pathogens and adapt to their growing environments. Relying on ecological interactions, competences of plant health management are distributed throughout the production system. Both for plant health and crop diversity management, a seed lot is determined by a complex system of interactions. A clear boundary distinguishing plant populations from their growing environment cannot be drawn. This implies (i) that plant health must be judged upon in situ in the plants' growing environment and (ii) that the governance of plant health must be considered at the collective scale.

Keywords: *plant health, small-scale organic seed production, common bean, participatory research, agroecology*

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