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SOIL SEED BANK AND SEED DORMANCY IN WILD POPULATIONS OF LIMA BEAN (FABACEAE): CONSIDERATIONS FOR IN SITU AND EX SITU CONSERVATION[1]

JÉRÔME DEGREEF,[2,5] OSCAR J. ROCHA,[3] THIERRY VANDERBORGHT,[2] AND JEAN-PIERRE BAUDOIN[4]

[2]National Botanic Garden of Belgium, Domein van Bouchout, B-1860 Meise (Belgium);
[3]Escuela de Biologia, Universidad de Costa Rica, Ciudad Universitaria R. Facio, San Jose (Costa Rica); and
[4]Phytotechnie des régions tropicales et Horticulture, Faculté Universitaire des Sciences agronomiques, B-5030 Gembloux (Belgium)

Seed dormancy and its impact on the soil seed bank for wild Lima bean (*Phaseolus lunatus*) populations were studied in the Central Valley of Costa Rica. Five populations were selected in contrasted environments. In all cases, distribution of seeds in the soil was limited to 3 cm depth. No innate dormancy was observed but combination of hard seed coat and hilum opening controlled by environmental conditions were responsible for an induced dormancy and the constitution of a persistent seed bank. Breaking of this dormancy was obtained by a brief elevation of temperature from 25° to 45°C. Impacts of this phenomenon concern both genetic and demographic aspects of in situ conservation of the species. Consequences on ex situ conservation are mainly related with the regeneration of the seed collection.

Key words: dormancy; ex situ conservation; in situ conservation; Lima bean; persistent soil seed bank; *Phaseolus lunatus*.

In the conservation of plant genetic resources, particular attention has been given to wild ancestral populations of cultigens with potential value as large stores of genetic variation and sources of novel variants for plant breeding. That is the case for Lima bean (*Phaseolus lunatus*, Fabaceae), a food legume originated from Central and South America and now cultivated in all tropical regions. Wild forms of Lima bean are self-compatible annuals or short-living perennials with a mixed mating system, e.g., predominantly self-pollinating but with a fair amount of outcrossing. They are essentially growing in man-disturbed habitats, namely in the vicinity of agricultural plots, and more rarely in secondary forest (Baudoin, 1991). Their high toxicity (presence of cyanogenetic components) makes the wild varieties unfit for human consumption but this character is easily suppressed by selection programmes (Baudoin, 1993).

In order to preserve the genetic diversity of *P. lunatus* in the Central Valley of Costa Rica, a conservation project was initiated in 1992 under the International Plant Genetic Resources Institute, Roma (I.P.G.R.I) supervision. This region is part of the Meso-American gene pool and a center of diversity for the species (Maquet and Baudoin, 1997). About 450 wild populations were recorded throughout the Central Valley. Because of the intensive agriculture and urbanization occurring in the region, the species is patchily distributed and most populations have few individuals. Previous studies have allowed their authors to distinguish different genotypes of Lima bean on the basis of enzyme systems (Maquet et al., 1996, 1997; Zoro Bi et al., 1996, 1997; Zoro Bi, Maquet, and Baudoin, 1997), electrophoretic mobility of phaseolin (Vargas et al., 2000), and random amplified polymorphic DNA (RAPD) markers (Fofana et al., 1997). It was also possible to determine the spatial distribution of genotypes in the Central Valley (Zoro Bi, 1999), to identify gene dispersal mechanisms (Hardy et al., 1997; Baudoin et al., 1998), and to assess the demography of wild populations (Degreef, Baudoin, and Rocha, 1997; Degreef, 1998). In the frame of the latter, we noted that some populations that were considered locally extinct (no adult individuals observed for 3–4 yr), sometimes reappeared. This finding suggested the presence of buried viable seeds of Lima bean in the soil and addressed complementary studies.

The soil seed bank is constituted by all viable seeds present on or in the soil and the associated humus/litter layer (Simpson, Leck, and Parker, 1989). The presence of buried viable seeds in the soil is commonly associated with a phenomenon of dormancy, which prevents the seeds from germinating even under favorable environmental conditions. In the legumes, seed coat impermeability to water, also called hard-seededness, causes dormancy and is a major factor in promoting the formation of soil seed banks (Quinlivan, 1968; Egley and Chandler, 1983; Egley, 1989; Rice, 1989; Standifer, Wilson, and Drummond, 1989; Russi, Cocks, and Roberts, 1992). Legume seeds are generally characterized by a thin impermeable cuticle preventing the seed imbibition (Hyde, 1954; Quinlivan, 1971; Rolston, 1978; Lush and Evans, 1980; Baskin and Baskin, 1989; Thapliyal et al., 1998). For some Fabaceae, seed hydration and dehydration depend upon reversible movements of the hilum working as a hygroscopic valve that regulates the movement of water into the seed (Werker, 1980–1981).

Environmental factors are generally required to break seed dormancy in legume species (Ader, 1965; Shea, McCormick, and Portlock, 1979; Gill, 1985; Pieterse and Cairns, 1986; Auld and O'Connell, 1991; Choinsky and Tuohy, 1991; Bell, Plummer, and Taylor, 1993; Bradstock and Auld, 1995; Mucunguzi and Oryem-Origa, 1996; Teketay and Granstrom,

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[5] Author for reprint requests (e-mail: degreef@br.fgov.be).

TABLE 1. Characteristics of sites occupied by wild Lima bean populations.

Characteristic	Population				
	54TR	E25	E50	E88	J87
Coordinates	9°54′ N/83°54′ W	9°53′ N/84°07′ W	9°52′ N/84°06′ W	9°59′ N/84°09′ W	9°49′ N/84°04′ W
Habitat[a]	trail	woody	woody	trail/coffee	woody/coffee
Morphology	linear	bi-dimension	bi-dimension	linear	bi-dimension
Life zone[b]	bh-MB	bmh-MB	bmh-P	bh-P	bh-P
Soil type	Ultisol	Spodosol	Inceptisol	Inceptisol	Inceptisol
Inclination	25°	20°	40°	10°	30°
Exposure	S	NE	NE	S	NW
Altitude (m)	1473	1503	1740	1020	1385
Area (m^2)	1885	3340	1200	2400	483

[a] Trail, along trail in agricultural area; woody, in secondary regeneration forest; coffee, at the border of traditional coffee plantation.
[b] Life zone categories according to Holdridge (1967); bh-MB, tropical lower montane moist forest; bmh-MB, tropical lower montane wet forest; bmh-P, tropical premontane wet forest; bh-P, tropical premontane moist forest.

1997) but mechanisms for the breaking of hard-seededness have been seriously questioned (Ballard, 1973). Cultivated varieties of Lima bean have been specifically studied for this concern (Stienswat, Pollard, and Campbell, 1971). The authors showed that the cuticle was impermeable to water and that the arrangement of the palisade layer cells forming the hilum canal prevented the hydration, the existence of a hygroscopic valve being inappropriate in the explanation of the dormancy in this case. An action of microorganisms was also hypothesized to explain the germination of dormant Lima bean seeds (Trumble, 1937).

In this study of wild Lima bean populations, we are particularly interested in assessing the ecological significance of seed dormancy and soil seed bank underlined by many authors (see Baskin and Baskin, 1998 and references within; McCue and Holtsford, 1998; Khurana and Singh, 2001). We want to stress the buffer role played by the soil seed bank in both population dynamics and population genetics. Furthermore, we focus on the mechanisms for breaking dormancy that should be taken into account in the establishment of in situ and ex situ conservation strategies of the threatened forms of this species.

MATERIALS AND METHODS

Study sites—The present study was conducted in the Central Intermontane Valley of Costa Rica, an area of approximately 2100 km^2 (latitude range 9°47′ N–10°09′ N; longitude range 83°50′ W–84°30′ W; altitude range 400–1300 m above sea level). This is the region of the country with the highest urban development and population, but extensive areas of the Valley are still used for agriculture (Rocha, Macaya, and Baudoin, 1997).

Five wild populations of *P. lunatus* with a high number of individuals were selected in a way to cover the whole range of environmental conditions in the study area (Holdridge, 1967) and among populations. Labelling of these populations follows an alphanumeric code standardized for all studies conducted in this project. They are located in very disturbed sites along trails (54TR), at the border of coffee plantations (J87) or both (E88), or in more natural sites as at the edge of secondary regeneration forests (E25 and E50). Other characteristics of the five studied populations are summarized in Table 1. In this area flowering begins in November and ends in February. Fruit maturation occurs at the end of the dry season, i.e., from late March to early May.

Soil seed bank—In order to determine the amount and the distribution of seeds aged at least 1 yr in the soil, we collected leaf litter and soil samples from each population in mid-April 1997. Soil sampling was conducted just before the period of seed dispersal. Preliminary observations (random sampling up to 5 cm depth) never revealed the presence of seeds deeper than 3 cm. Samples were taken from 1 × 1 m grids. Seed bank was estimated in randomly chosen squares in the grid (52–72 samples per population) by extracting and sieving the leaf litter layer and three successive soil samples, each 1 cm deep. Lima bean seeds in the samples were separated by hand from the soil material.

Innate dormancy assessment—The occurrence of innate seed dormancy was investigated in three replications of 100 fresh seeds collected directly from mature dry pods before the pod-opening period (end of April 1997). These seeds were collected in our five field sites. They were then placed on moistened filter paper in closed Petri dishes at 25°C. Germination was recorded daily for 3 mo. At the end of this period, the seed coat of all ungerminated seeds was clipped with a scalpel and the germination was recorded for 1 mo.

Seed coat impermeability—Impermeability of seed coat to water was tested on seeds collected in the five studied populations. The hilum of 100 fresh seeds was sealed with nail polish. After this treatment, seeds were placed on moistened filter paper in Petri dishes and kept in an incubator at 25°C. As control, 100 freshly collected seeds were placed under the same conditions. Germination of treated and control seeds was recorded daily for 3 mo as described above. Three replications of this experiment were conducted.

At the end of the experiment, we tested all ungerminated seeds for germination after clipping the seed coat with a scalpel. Seeds were then placed in the same conditions as previously described. Germination was recorded daily for 1 mo.

Seed moisture content and dehydration—Moisture content of seeds was determined in freshly collected seeds from the five experimental populations in April 1997. Four groups of 100 seeds were weighed and placed in an incubator at 105°C for 72 h to determine their dry mass. Moisture content of the seeds was the difference between the fresh and dry mass. The same procedure was conducted on two groups of 100 seeds that were collected directly from the seed bank of the five populations at the end of April 1997. The difference between freshly collected seeds and seeds from the seed bank indicate the level of dehydration that seeds might experience in the soil during the dry season.

In order to simulate the levels of dehydration that seeds experience on the soil surface at the end of the dry season, six groups of 100 seeds (collected from pods at end of April in 1997), were placed in an incubator at 45°C for 3 d. This treatment lowers the moisture content of the seeds to levels comparable to those observed in seeds collected from the seed bank at the end of the dry season.

Hydration mechanisms—We conducted an experiment to test if seed coat impermeability plays an important role in the germination of Lima bean seeds. The germination of fresh seeds was examined under the following conditions: (1) One hundred artificially dehydrated seeds, following the method described above, were surface sterilized with a 24-h exposure to UV radiation. This

TABLE 2. Distribution (as number of seeds per square meter) of Lima bean seeds in soil according to depth (N: number of squares sampled; SD: standard deviation; Min–max: minimum and maximum of range). No seeds are found below 3 cm depth.

Population	No. seeds/m²			
	Humus/litter	0–1 cm depth	1–2 cm depth	2–3 cm depth
54TR				
Mean (N = 70)	0.47	0.14	0.17	0.04
SD	1.08	0.54	0.45	0.20
Min–max	0–5	0–4	0–2	0–1
E25				
Mean (N = 72)	2.01	0.94	0.88	0.51
SD	4.31	2.40	1.72	1.20
Min–max	0–31	0–18	0–9	0–6
E50				
Mean (N = 70)	0.74	0.29	0.36	0.09
SD	1.49	0.78	0.68	0.33
Min–max	0–8	0–5	0–3	0–2
E88				
Mean (N = 52)	0.75	0.33	0.17	0.04
SD	1.88	0.54	0.45	0.20
Min–max	0–10	0–4	0–2	0–1
J87				
Mean (N = 53)	4.11	2.25	2.02	1.23
SD	6.75	4.43	3.40	1.93
Min–max	0–34	0–20	0–19	0–8

method was designed to prevent the modification of the permeability of both seed coat and micropyle that could occur by a methanol sterilization. Surface-sterilized seeds were then placed in closed Petri dishes on a sterile nutritive agar medium and maintained at a temperature of 25°C. Eventual development of microorganisms on the nutritive medium and seed germination were recorded daily for 3 mo. (2) One hundred artificially dehydrated seeds were placed on moistened filter paper in closed Petri dishes as previously described. These seeds were not surface sterilized. Germination was recorded daily for 3 mo. (3) One hundred seeds collected from each of our field sites were used as control. Germination (at 100% air moisture and 25°C) was tested as described in the previous section and recorded daily for 3 mo.

At the end of this experiment, we tested all ungerminated seeds for germination after cutting a corner of each seed with a scalpel. Seeds were then placed in the same conditions as previously described.

Seed dormancy—We examined if changes in temperature are involved in the breakage of dormancy. In order to do that, three groups of 100 artificially dehydrated seeds, following the methodology described above, were placed on moistened filter paper in closed Petri dishes at 25°C for 2 wk. At day 15 of the test, a heat treatment was applied to the dormant seeds in order to simulate the conditions encountered by the seeds at the end of the dry season. Heat treatments were determined on the basis of high temperature values recorded at the soil surface of the sites where the studied wild populations were present. Three groups of seeds were exposed for 1 h to a temperature of, respectively, 35°, 45°, or 55°C. Seeds were then replaced at 100% air moisture and 25°C for 2.5 mo. As control, we used a fourth group of dehydrated seeds that was maintained at 25°C and 100% air moisture. Germination was recorded daily for 3 mo. Two replications of this experiment were conducted.

RESULTS

Soil seed bank—The results of the surveys to determine the distribution of seeds of Lima beans in the litter/soil are shown in Table 2. In general, we found that seeds were most abundant in the upper part of the soil, particularly in the litter/humus layer. There was considerable variation in the mean number of seeds found at each litter/soil layer among the five populations studied. Overall, more seeds were found in populations E25 and J87. In contrast, we found fewer seeds in the samples taken from population 54TR than from the other four populations.

TABLE 3. Fresh mass, dry mass, and water content of lots of 100 Lima bean seeds (SD: standard deviation).

Type of seeds	Fresh mass (g)	Dry mass (g)	Water content (percentage of dry mass)
Freshly collected	7.868	7.000	12.4
	8.194	7.183	14.1
	8.097	7.156	13.1
	7.794	6.854	13.7
Mean			13.3
SD			0.7
Stored in soil up to the end of dry season	7.401	6.943	6.6
	7.656	7.082	8.1
Mean			7.4
SD			0.8
Dehydrated	7.941	7.408	7.2
	7.850	7.269	8.0
	8.096	7.559	7.1
	8.224	7.566	8.7
	7.902	7.250	9.0
	7.876	7.333	7.4
Mean			7.9
SD			0.7

Innate dormancy assessment—The germination of freshly collected seeds indicates that seeds of Lima bean do not show innate dormancy. Fresh seeds collected were observed germinating the second day of the experiment. Germinations were observed regularly during the experiment and mean cumulative germination rate reached 74% (SD = 2%) after 2.5 mo; no further germinations were observed until the end of the experiment. In addition, all seeds that failed to germinate after 3 mo germinated readily after the seed coat was clipped. These results showed that the wild Lima bean seeds can germinate readily after their dispersal.

Seed coat impermeability—Our results showed that the seed coats of Lima beans are impermeable to water and that water only enters the seed through the hilum. No germination was recorded during the 3-mo duration of the experiment in fresh seeds that had the hilum sealed with nail polish. In contrast, we recorded 76% germination (SD = 5%) in freshly collected untreated seeds used as control. However, we observed 100% germination rates in both control and hilum-sealed seeds, after clipping the seed coat. These findings support the notion that the seed coat is impermeable to water and that hydration of wild Lima bean seeds is restricted to the hilum.

Seed dehydration—We found that seeds may experience water losses in the soil (Table 3). On average, the water content of fresh seeds varied between 12.4 and 14.1% dry mass. In contrast, the water content in the two groups of seeds collected from the soil seed bank at the end of the dry season varies between 6.6 and 8.1% (Table 3). The levels of water content present in seeds collected directly from the soil are similar to those found in seeds dried in the oven, where after

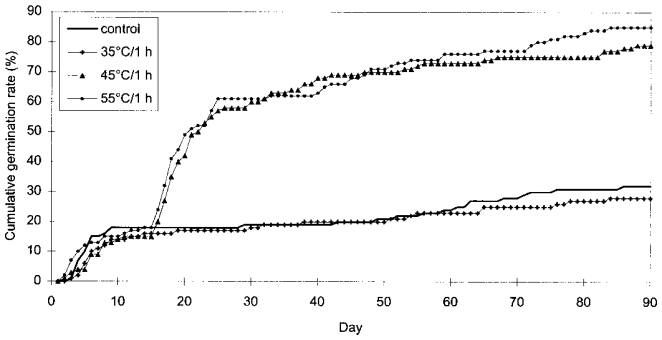

Fig. 1. Mean cumulative germination rates (two replications, each with four groups of 100 Lima bean seeds). The control group was maintained at 25°C; other groups were exposed to 1 h of heat treatment at 35°, 45°, or 55°C on day 15. All SDs ≤ 5%.

3 d (72 h) of heating treatment, the moisture content of seeds was between 7.1 and 9.0% dry mass (Table 3). These findings revealed that seeds of Lima beans experience dehydration while they stay in the soil seed bank. In addition, these data suggest that artificially dehydrated seeds had a similar moisture content than that observed in seeds in the soil seed bank at the end of the dry season.

Hydration mechanisms—Our data revealed that germination of both groups of artificially dehydrated seeds (surface-sterilized or not) occurred mainly during the first 2 wk of the experiment. In both groups of dehydrated seeds, cumulative germination rate reached only 17% and 18%, respectively, after 2 wk. Cumulative germination rates recorded after the 3-mo duration of the experiment reached 27 and 29%. Similar germination rates were observed for both sterilized and unsterilized lots of dormant seeds. Therefore, these findings indicated that microbial degradation of the seed coat may not play an important role in breaking dormancy of wild Lima bean seeds. Similar findings were reported for other cultivated legume species (Trumble, 1937; Stienswat, Pollard, and Campbell, 1971).

Cumulative germination rate in the control group seeds (76% after 3 mo) is higher than that of both dehydrated seeds (27 and 29% after 3 mo). These findings indicate that seed dehydration may play an important role in the induction of the seed coat dormancy. Germination of ungerminated fresh and artificially dehydrated seeds after clipping the seed coat reached 100% after 3 d.

Seed dormancy—To verify if a change in temperature could be responsible for the breaking of seed dormancy, we compared germination rates recorded in groups of seeds exposed to different heat treatments with those obtained for a control group maintained at 25°C. As shown in Fig. 1, no significant change in the cumulative germination rate was recorded after maintaining the air temperature at 35°C for 1 h. In contrast, we found that an elevation of temperature from 25° to 45° or 55°C for 1 h resulted in an immediate germination response. Cumulative germination rates reached 50 to 60% in the days following the heat treatment. The number of germinated seeds increased regularly until the end of the 3 mo duration of the experiment. We then recorded cumulative rates of 79% and 85% (SD = 1% in both cases) for the lots of seeds exposed, respectively, to 45° and 55°C. We suggest that a brief elevation of temperature from 25° to 45°C or more is responsible for breaking seed dormancy whereas a change from 25° to 35°C is not sufficient to bring about this phenomenon.

DISCUSSION

The goal of this study was to determine the size of the seed bank and the distribution of the seeds at different depths in five wild populations of Lima beans (*Phaseolus lunatus*) in the Central Valley of Costa Rica. We also studied the factors that cause dormancy of seeds as well as those that initiate germination in this species. We found that most seeds are located on the surface of the soil and that their numbers decline with depth. We also found that the seeds of Lima beans lack innate dormancy at the time of dispersal; however, dormancy can be induced by high temperatures and low levels of humidity in the air. Moreover, germination and breakage of dormancy are controlled by the passage of water through the hilum.

The distribution pattern and the abundance of the seeds in the soil can be explained by the following: (1) The duration of their stay in the soil—Seeds were observed moving downwards along time. Seeds found in the humus/litter layer and in the first centimeter of soil at the end of the dry season were dispersed 1 yr before. Moreover, seeds extracted from deeper soil samples were all 2- to 3-yr-old (Degreef, 1998). (2) The among-year variability of fecundity—In April 1997, populations 54TR and E50 showed greater mean seed densities in the 1–2 cm depth layer than in the humus/litter and in the 0–1 cm depth layers because high fecundities were recorded 2 yr before (Degreef, 1998). (3) The interpopulation variability of fecundity—Populations J87 and E25, the woody plants of which exhibited the highest fecundity rates, were also those in which the seed bank was the largest (Degreef, 1998). (4) The patchy distribution of plants—The size of the seed bank was highly variable (estimated ~250 to ~25 000 seeds per population) and a great within-population variability in the number of seeds stored in the soil (0 to ~60 seeds/m^2) was also observed. Plants were generally patchily distributed at the edge of each site and seed dispersal was mainly guaranteed by the explosive drought-triggered opening of pod. Indeed, seeds are too heavy to be carried by air, and since they are perfectly smooth and toxic they are not expected to be dispersed by rodents or birds, nor to be harvested by local people; therefore, the distance between the dispersed seeds and the pod-yielding plants was generally limited to ~5 m (Baudoin et al., 1998). (5) The impact of environmental conditions on both dormancy and germination—Seeds of Lima beans are typically dispersed at the end of the dry season, i.e., from March to May. At this time, mean water content of fresh seeds is ~13%, and they would readily germinate if exposed to high air moisture. Sometimes rain occurs immediately after pod opening, and seeds germinate abundantly. Rain and high relative humidity were even recorded earlier; many seeds were then observed initiating germination within the mature pods indicating that they lack innate dormancy. Most frequently, precipitation is absent at the time of seed dispersal, and the seeds remain on the soil surface where they experience high diurnal temperatures (60°–65°C), diurnal air moisture frequently below 40%, and maximal nocturnal air moisture close to 100% (Degreef, 1998). Under these circumstances the water content of the seed could be reduced to only 7.4%. This drastic change in humidity induces dormancy, and seeds can remain in that state for

several years. The induction and breakage of dormancy are regulated by the passage of water through the hilum. Unless the seed coat is mechanically or biologically degraded, drastic temperature change combined with the high precipitation typically observed at the beginning of the rainy season rapidly break dormancy.

As proposed by Dalling, Swaine, and Garwood (1997) the need for scarification in seeds of pioneer species may represent the cost of developing a persistent seed bank. This particular status could be related to the uncommon type of dormancy observed in this species: the seed coat is impermeable when the hilum opening is controlled by environmental conditions. By combining these two mechanisms Lima bean appears to respond to its environment. Under drought conditions, dehydration of the seeds is extreme and the induced dormancy is stronger. Combined with the presence of a hard seed coat, germination that could occur in case of early rains is prevented. Germination is broken off and an abundant long-term seed bank is then constituted. In wetter sites, dormancy is weaker, a smaller proportion of seeds enter the seed bank, and dormant seeds germinate more rapidly.

Implications for in situ conservation—In situ conservation, the preservation of a plant species in its natural environment, is generally considered to be the most efficient conservation strategy. In the case of wild Lima bean in the Central Valley of Costa Rica, in situ conservation faces numerous practical problems that need to be solved to define an efficient conservation strategy. Among these problems, the diagnosis of the dynamics of the wild populations is particularly hard on the basis of field observations, and the assessment of a persistent soil seed bank is of main interest. Since 1994, monthly phenological observations have allowed us to estimate the status of about 100 populations throughout the study zone. Most populations produce adult individuals every year and are characterized by an abundant seed production allowing their number of individuals to remain stable through time. However, seed production can be severely reduced or destroyed before seed dispersal when rains fall during the pod-ripening period or with other random events like the destruction of adult plants by man or cattle. For example, in January 1996, population E107 (9°50′ N/84°04′ W) was accidentally destroyed, and seed production recorded for that year was zero. Nevertheless, 4 mo later, a great number of seedlings were observed from germination of dormant seeds. The buffer effect of the seed bank compensated for the fecundity decrease, and the population rapidly recovered its former level. But other populations may show cycles of extinction and recolonization that are longer than 1 yr, producing seeds that remain dormant for more than 1 yr and only emerge at the beginning of the following rainy season. In both cases, seed dormancy and soil seed bank provide a substantial benefit for the recolonization of the sites.

Population dynamics and consequently in situ conservation strategies are greatly influenced by the presence of a soil seed bank. Matrix population analyses showed that important divergences in the structure of Lima bean populations could have a severe impact on their demography and an equilibrium is required in the proportion of individuals at all stages (Degreef, 1998). Extremely high germination rates such those recorded with early rains are responsible for deficits in the proportion of dormant seeds stored in the soil and provoke a lack of balance in the whole population dynamics that threatens its survival. In contrast, a high proportion of dormant seeds, such as that observed in the driest sites (e.g., in E88 population, where 87% of the individuals are dormant seeds), can also slow population growth.

The presence of seed coat dormancy is also particularly important and should be considered when studying the recolonization processes in the most disturbed populations and promoting management practices. As regular clearing and weeding are observed in these sites, particular attention has to be directed to the timing of these practices. Weeding the soil surface just after the dispersal of the seeds at the end of the dry season favors breakdown of dormancy and reduces germination delays by exposing the seeds to both higher temperatures and humidity when the first rains occur. Clearing should also be promoted at the beginning of the rainy season. Indeed, our field observations show that populations that are cut or disturbed during the dry season are less likely to regrow and consequently less likely to produce seeds during the next fruiting period.

In our study zone, an equilibrium between intra- and interpopulation diversity was observed: 52% of total genetic diversity was recorded between Lima bean populations (G_{ST} = 0.519) (Zoro Bi, 1999). Similar values were noted for plant species showing comparable life history characteristics but diversity within the soil seed bank was never evaluated (Ollitrault, 1987; Barrett and Shore, 1989; Koenig and Gepts, 1989; Schinkel and Gepts, 1989; Les, 1991; Wang, Wendel, and Dekker, 1995a, b). The assertion that seed banks may act as genetic reservoirs has been rarely confirmed by experimental results and remains controversial (Tonsor et al., 1993; McCue and Holtsford, 1998). Nevertheless, one can mainly expect that genetic diversity in seed bank is higher than aboveground when reproductive system is allogamy and when seeds stay in the soil seed bank for a long time. Seeds of Lima bean mainly result from autofecondation, and the present study showed the short-lived nature of the seed bank (maximum 3 or 4 yr) making unlikely that mutations occur. Moreover, seed dispersal is so reduced that gene flow between populations is unprobable.

A main challenge in determining the genetic diversity consists in designing optimum strategies to sample the whole gene pool of a species or a population and has important implications in the conception of core collections (Yonezawa et al., 1996; Brown, Brubakker, and Grace, 1997). A preliminary study with Lima bean (Zoro Bi et al., 1998) showed that the success of the sampling procedure was closely linked to the number of collected plants rather than to the number of seeds collected per plant. Differences in the estimations of intra- and interpopulation genetic diversity of Lima bean in similar studies conducted in the same region at different periods (Maquet et al., 1996; Zoro Bi, 1999) could be explained by the sampling procedure adopted or by the stochastic variations in both fecundity and germination rate. Seed dormancy could indeed affect specific genotypes more than others (Evans and Cabin, 1995), and consequently the frequency of the first ones could be underestimated during the sampling of mature plants at the surface if environmental conditions have favored the induction of dormancy. Therefore, we particularly promote to extend the sampling to the seed bank in the most disturbed populations of Lima bean where aboveground individuals are regularly cut before producing seeds and where the risk of extinction of such specific genotypes is the highest.

An alternative bet-hedging strategy against environmental variability, consisting of the storage of dormant seeds, has been developed by Lima bean for maximizing its fitness and

reducing the likelihood of extinction. This temporal dispersal behavior is frequent (Kalisz, Horth, and McPeek, 1997) in species like Lima bean whose metapopulation functions are impacted by geographical isolation (Rocha, Macaya, and Baudoin, 1997) and that cannot rely on spatial dispersal. For *Phaseolus lunatus*, as for many wild legume populations, the role of seed coat dormancy in maintaining genetic diversity over time is probably similar to that played generally by gene flow in other species in which hard-seededness delays the population response to selection and counteracts the negative effects of genetic drift.

Implications for ex situ conservation—Even if priority must be assigned to the in situ conservation of a plant species, ex situ conservation can be seen as a complementary method, being part of a global strategy for genetic preservation. In some situations, a species or a genotype is so threatened in the field that the preservation of its genetic diversity is only safe in seed collections and ex situ conservation is consequently adopted. For Costa Rican Lima beans, ex situ conservation is chosen in some particular cases. Some populations that are known as owning unique or rare alleles are submitted to an emergency preservation plan in which seeds are rapidly collected on plants, dehydrated, and conserved ex situ. This procedure also applies to main threatened populations, the genetic pattern of which is unknown in the majority of the cases. Use and availability of this wide diversity requires a rigorous management of the collections. Moisture content of seeds is lowered and maintained at 5%, then samples are packed in hermetic bags made of plastic-aluminum laminated foils. Conservation at low temperature (-20°C) ensures a long-term preservation (about 100 yr) (Vanderborght and Baudoin, 1998). The drying of the lots of seeds to a 5% moisture content results in the induced dormancy described before. The material needs to be regenerated from time to time and part of the seed collection is regularly checked for its potential to germinate. For curators of ex situ collections, dormancy of Lima bean seeds constitutes an important impediment because part of the seed coat has to be removed mechanically before putting the seeds in optimal germination conditions in Petri dishes. Germination tests under specific environmental conditions comparable to those conducted with Costa Rican forms of Lima bean have to be conducted with other wild varieties of this legume to test for their efficiency and could then be applied by curators to seed collections.

The future of wild Lima bean conservation in the Central Valley of Costa Rica will probably rely on the design of synthetic populations in which dynamics will be monitored and as much genetic diversity as possible will be preserved. Such genetic conservatories have been established already under environmental conditions in 1998. Circular patches and linear populations were designed in protected sites throughout the whole region and seeds collected in different wild populations were sown. The first field observations are encouraging in the sense that it seems that the main genotypes could be safeguarded in this manner. Nevertheless, preliminary demographic results also confirm that a careful management is required to break seed dormancy. Rainy season weeding had to be applied to speed up the colonization process and to ensure the establishment of these populations in the synthetic sites (Meurrens et al., 2001). For the future, a priority must be the evaluation of genetic diversity in the seed bank by enzyme markers that could confirm the role of seed dormancy in influencing the evolutionary potential of Lima bean populations and that will allow us to fit the in situ and ex situ conservation strategies to wild populations of this important food legume.

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