American Journal of Botany 94(11): 1900–1910. 2007.

HYBRIDIZATION AND MORPHOGENETIC VARIATION IN THE INVASIVE ALIEN *FALLOPIA* (POLYGONACEAE) COMPLEX IN BELGIUM[1]

MARIE-S. TIÉBRÉ,[2,5] JEAN-P. BIZOUX,[2] OLIVIER J. HARDY,[3] JOHN P. BAILEY,[4] AND GRÉGORY MAHY[2]

[2]Laboratory of Ecology, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium; [3]Behavioural and Evolutionary Ecology Unit - CP 160/12, Université Libre de Bruxelles, 50 Av. F. Roosevelt, 1050 Brussels, Belgium; and [4]Biology Department, University of Leicester, University Road, Leicester, LE1 7RH, UK

The invasive alien knotweeds, *Fallopia* spp. (Polygonaceae), are some of the most troublesome invasive species in Europe and North America. Invasive success in *Fallopia* may be enhanced by multiple hybridization events. We examined the pattern of hybridization and its evolutionary consequences in Belgium with a concerted analysis of ploidy levels (chromosome counts and flow cytometry), morphological variation, and genetic variation (RAPDs). At least four taxa with different ploidy levels were part of the pattern of invasion in Belgium. Hybrid *F.* ×*bohemica* with various chromosome numbers restored the genotypic diversity that was lacking in the parental species. Hybrid genotypes were mainly assigned to a specific genetic pool and not to a mixture between the genetic pools of the putative parental species as would be expected for hybrids. Parental species and hexaploid hybrids differed significantly for a set of well-defined morphological characters, enabling future researchers to distinguish these taxa. On the basis of our results, the importance of hybridization has probably been underestimated in large parts of the adventive range of alien *Fallopia* species, pointing to the need for concerted molecular and morphological analyses in the study of the evolutionary consequences of hybridization.

Key words: assignment test; Belgium; chromosome counts; *Fallopia*; flow cytometry; hybridization; morphology; Polygonaceae.

Biological invasions are considered the second cause of biodiversity loss worldwide and have attracted much attention in the last decade (Vitousek et al., 1997; Wilcove, 1998; Gurevitch and Padilla, 2004; Didham et al., 2005). Interspecific hybridization is now recognized as a major mechanism of evolution in the plant kingdom, and such hybridizations between introduced or related species have been implicated as a driving force of evolutionary processes in invasions (Abbott, 1992; Ellstrand and Schierenbeck, 2000; Vilà et al., 2000; Eunmi, 2002; Hänfling and Kollmann, 2002; Callaway and Maron, 2006). Hybridization may increase genetic diversity in introduced taxa and provide the genetic material on which selection and genetic drift may act to promote population differentiation. Both genotypic and genomic alterations may stimulate invasiveness of newly formed species (Ellstrand and Schierenbeck, 2000) by inducing rapid evolution (Grosholz, 2002; Allendorf and Lundquist, 2003; Müller-Schärer et al., 2004). Human activities have provided new opportunities and new niches that may better suit the hybrids than the parents (Vilà et al., 2000), as shown for *Senecio* (Abbott, 2000), *Tragopogon* (Soltis et al., 2004), and *Spartina* (Ainouche et al., 2003).

The identification of newly formed hybrids using morphological characters, however, can be difficult and may lead to an underestimate of the true extent of species diversity processes (Petit, 2004; Hegarty and Hiscock, 2005; Lopez et al., 2005; Mallet, 2005). Identification at the subspecific level is a key feature in the studies of invasive species because it determines the number of taxa involved and enables more effective management (Child and Wade, 2000; Sakai et al., 2001; Simberloff, 2003). Combining morphological and molecular analyses may be an efficient tool for differentiating taxa within complex populations (Fjellheim et al., 2001; Persson and Gustavsson, 2001; Cattell and Karl, 2004), and RAPD analyses are useful for identifying clonal plants (Hansen et al., 2000; Torimaru et al., 2003).

The genus *Fallopia* offers an excellent opportunity to analyze the genetic and evolutionary consequences of hybridization and polyploidization in an invasive clonal plant, because it provides well-documented interspecific hybridization events (Bailey, 2003). The octoploid Japanese knotweed *F. japonica* var. *japonica* ($2n = 88$) was introduced to Europe in the 19th century as an ornamental and fodder plant, along with the related tetraploid *F. sachalinensis* ($2n = 44$). These plants have rapidly expanded in Europe and North America (Godefroid, 1996; Fojcik and Tokarska-Guzik, 2000; Hollingsworth and Bailey, 2000; Verloove, 2002; Bímová et al., 2003; Weber, 2003), with huge impacts, such as modifications of nutrient cycling rates and topsoil fertility and decreases in plant species diversity in the invaded sites (Vanderhoeven et al., 2005).

In their native range, *F. japonica* var. *japonica* and *F. sachalinensis* reproduce by a combination of sexual reproduction with hermaphroditic and male-sterile stands (Tanaka, 1966) and vegetative regeneration (Maruta, 1976). In most of its introduced range, *F. japonica* var. *japonica* is male-sterile (Bailey, 1989; Tiébré et al., 2007), and genetic studies suggest that all stands derive from a single clone by vegetative propagation (Bailey, 1989; Hollingsworth and Bailey, 2000).

[1] Manuscript received 7 June 2007; revision accepted 30 September 2007.

The authors thank B. Lagrange for technical assistance in the field and two anonymous reviewers for helpful suggestions. They also thank the Ministry of Education and Scientific Research of the Republic of Ivory Coast for funding M.-S. Tiébré. This research was supported by the Belgian Science Policy and is part of the project INPLANBEL: Invasive Plants in Belgium: Patterns, Processes and Monitoring (contract EV/11/27C).

[5] Author for correspondence (e-mail: tiebre.ms@fsagx.ac.be)

In contrast, *F. sachalinensis* has both sterile males and hermaphrodites in its adventive range (Bailey, 1994; Tiébré et al., 2007) and has higher genotypic diversity, suggesting multiple introduction events and/or in situ sexual reproduction (Bailey, 1989). Other members of the genus introduced to Europe are *F. japonica* var. *compacta* and *F. aubertii* (syn. *F. baldschuanica*). *Fallopia japonica* var. *compacta* is tetraploid ($2n = 44$) and rare, with both female and hermaphroditic stands. *Fallopia aubertii* is diploid ($2n = 20$), hermaphroditic, and rare in the nature but is now commonly cultivated as an ornamental plant (Bailey, 1994; Tiébré et al., 2007).

Hybridization of the widespread *F. japonica* var. *japonica* with *F. sachalinensis* resulted in the most troublesome hybrid, *F. ×bohemica*. *Fallopia ×bohemica*, which has various chromosome numbers from tetraploid to octoploid and both male-sterile and hermaphroditic individuals with partial to full fertility (Bailey et al., 1996; Tiébré et al., 2007). While Bailey et al. (1996) reported seed set in *F. ×bohemica* despite very irregular meiosis in the UK, Tiébré et al. (2007) found very low seed set in Belgium. Studies of these hybrids at local and country levels in the UK and the Czech Republic, using cytological and molecular approaches, show high genotypic diversity (Pashley et al., 2003; Mandák et al., 2005). This diversity may be the result of a combination of multiple hybridization events, hybrid fertility, and multiple introductions from horticulturalists (Hollingsworth et al., 1998, 1999; Hollingsworth and Bailey, 2000; Pashley et al., 2003; Mandák et al., 2005). The hybrid *F. ×bohemica* seems to be more invasive than its parents (Mandák et al., 2003; Pyšek et al., 2003). Other hybridization events may also play a role in the dynamics of the *Fallopia* complex. A significant proportion of open-pollinated seeds collected from 4× *F. ×bohemica*, *F. japonica* var. *japonica*, *F. sachalinensis*, and *F. japonica* var. *compacta* in the UK and Belgium resulted from hybridization with *F. aubertii* (Bailey, 1988; Tiébré et al., 2007). But in the UK, only a minute proportion germinated and established in nature (Bailey, 2001). Additional data from other parts of the adventive range are needed to assess the relative importance of the different hybridization possibilities within the *Fallopia* complex.

Although the variation of invasive alien *Fallopia* has been studied in the UK and the Czech Republic, we lack firm evidence on the relative roles of sexual reproduction and multiple hybridization events in shaping the genetic diversity of *F. ×bohemica* (Hollingsworth and Bailey, 2000) and on the extent of differentiation generated among geographically distant groups of hybrids as a result of independent evolution under limited gene flow. In addition, the importance of hybrid *F. ×bohemica* seems to vary among regions within the adventive range. Thus, the evolutionary processes involved may differ from region to region (Bailey and Wisskirchen, 2006; Pashley et al., 2007). New evidence may be gained by combining cytological information, molecular markers, and morphological characters in the same study.

In this study, we examined the polyploid alien *Fallopia* species and their hybrids to understand the evolutionary consequences of hybridization in an invasive plant complex. Specifically, we focused on the consequences of genetic and morphological variation. Combining chromosome counts, flow cytometry, RAPD markers, and morphological analysis, we asked the following specific questions: (1) What is the extent and pattern of hybridization in the invasive *Fallopia* complex in Belgium, and how does the pattern compare with those in other European regions? (2) Does hybridization increase genotypic diversity, and are groups of hybrids in different areas genetically differentiated? (3) Are there reliable morphological characters for separating these taxa?

MATERIALS AND METHODS

Plants and study sites—Five exotic *Fallopia* taxa have been reported in Belgium: four erect rhizomatous perennials, *F. japonica* var. *japonica* (Houtt.) Ronse De Craene, *F. sachalinensis* (F. Schmidt Petrop.) Ronse De Craene, *F. ×bohemica* (Chrtek et Chrtková) J.P. Bailey, and the rare *F. japonica* var. *compacta* (Hook. F.) J.P. Bailey; and one climbing perennial *F. aubertii* (L. Henry) (syn. *F. baldschuanica* (Regel) Holub (Lambinon et al., 2004).

This study was carried out in six different areas of Belgium: Brussels, Ceroux-Mousty, Comblain-au-Pont, Gembloux, Jodoigne, and Namur (Table 1, Fig. 1). Each area represented a different landscape ranging over several hundred hectares. The collection areas were chosen to cover the range of morphological variation of the *Fallopia* observed in the field in previous surveys. Because of the high potential for vegetative multiplication, a single individual (genet) of *Fallopia* spp. may cover many hundred square meters, and we expected that most *Fallopia* stands contained only a single clone. Generally, a high density of erect ramets (2–3 m tall) characterized those stands. Because we were interested in genetic variation and hybridization patterns and not in local clonality within stands, only one sample was collected per stand. When more than one ramet was needed (i.e., for morphological variation), they were chosen close to each other to ensure that they came from the same rhizome. We considered a priori that each sample represented a single unique individual.

When possible, 15–18 individuals were selected per area. Each individual was tentatively assigned to a taxon using published morphological characters (Barral, 1994; Beerling et al., 1994; Jager, 1994; Lambinon et al., 2004). Because of the scarcity of the taxa in study areas, an additional *F. sachalinensis* was collected from Kelmis. Only one individual of putative *F. japonica* var. *compacta* was found in the study areas (Ceroux-Mousty). Because this individual was found after the main sampling period, its chromosomes could not be counted. Two reference individuals of 4× *F. ×bohemica* from Cirencester (UK) with known chromosome numbers were also included. The collection comprised 40 putative *F. japonica* var. *japonica*, six putative *F. sachalinensis*, 34 putative *F. ×bohemica*, one putative *F. japonica* var. *compacta*, and six putative *F. aubertii* (Table 1, Appendix 1). From each individual, a piece of rhizome or cutting was potted and cultivated in a greenhouse at 22°C and a 16-h d; this greenhouse material was used for chromosome counts, flow cytometry, and DNA analysis. Basal leaves and stems were collected in the field, mounted, and dried for morphological studies. Of the 87 individuals used for RAPD analysis, 79 were examined for morphological characteristics, 73 were subjected to flow cytometry, and 16 were used for chromosome counts.

Determination of ploidy level—Chromosomes were counted for individuals representing the different taxa: five individuals of *F. japonica* var. *japonica*, seven *F. ×bohemica*, one *F. sachalinensis*, and one *F. aubertii*. The two individuals of 4× *F. ×bohemica* from Cirencester were also included. For each individual, chromosomes were counted for seven nuclei using fresh roots tips according to the method developed by Bailey and Stace (1992).

Flow cytometry was used for 73 individuals including 35 putative *F. japonica* var. *japonica*, 33 putative *F. ×bohemica*, two putative *F. aubertii*, two putative *F. sachalinensis*, and one putative *F. japonica* var. *compacta*. We followed the technique of Tiébré et al. (2007). A tetraploid individual *F. sachalinensis* of known chromosome number was used as an internal standard for each measurement.

RAPD analysis—To determine genetic variation and relationships within and among groups of *Fallopia* with different ploidy levels, we used RAPD markers on six individuals of putative *F. sachalinensis*, six putative *F. aubertii*, 40 putative *F. japonica* var. *japonica*, 34 putative *F. ×bohemica*, and one putative *F. japonica* var. *compacta*.

Total genomic DNA was isolated from 100 mg of fresh leaves from greenhouse plants using the CTAB method (Doyle and Doyle, 1990). For the RAPD-PCR, a reaction mixture of 25 μL per sample was used; this consisted of 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Fermentas Gmbh, Germany), 0.4 μM primer, 1 unit *Taq* polymerase (New England Biolabs), 0.2 mg/mL BSA

TABLE 1. Locality code, location, and sample sizes for the *Fallopia* individuals analyzed for variation in RAPDs, morphology, individual chromosome counts (*n*), and flow cytometry.

					No. of individuals							
Code	Locality	Latitude	Longitude	RAPDs	Morphology	Flow cytometry	*n*	*F. aubertii*	*F.* × *bohemica*	*F. japonica* var. *japonica*	*F. japonica* var. *compacta*	*F. sachalinensis*
UK	Cirencester, UK	51°42′N	1°58′W	2	0	2	2		2			
Jod	Jodoigne, Belgium	50°43′N	4°52′E	6	5	4		1	2	3		
Nam	Namur, Belgium	50°27′N	4°51′E	17	22	12	3	3	4	9		1
Gbx	Gembloux, Belgium	50°33′N	4°41′E	17	15	14	11	2	6	8		1
Brx	Brussels, Belgium	50°50′N	4°21′E	18	16	16			11	4		3
Com	Comblain-au-Pont, Belgium	50°28′N	5°34′E	18	15	18			9	9		
Mou	Ceroux-Mousty, Belgium	50°39′N	4°30′E	8	6	7	0			7	1	
Kel	Kelmis, Belgium	50°43′N	6°00′E	1	0	0						1

(Fermentas Gmbh), and 30 ng template DNA. Amplification was performed using a PTC-200 Thermal Cycler (MJ Research: Biozym) programmed for an initial denaturation at 95°C for 2 min; followed by 44 cycles of 20 s at 94°C, 1 min at 36°C, and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR products were run on a 1.8% agarose gel in TAE (Tris-Acetate-EDTA) stained with 14 μL ethidium bromide. Forty-one primers derived from Operon10-mer kit (Operon Technology, USA) were initially screened using 10 samples to test for reproducibility and polymorphism. Eight primers (A10, A19, G06, J12, M10, M15, R11, T07) that yielded reproducible and unambiguous polymorphic fragments were used for full analysis of all plants. The presence or absence of DNA fragments was scored. To check for reproducibility among PCR runs, we genotyped DNA from each individual twice. The profiles of the individuals used in the primer test were also compared with those of the same individuals in the full RAPD analysis.

Morphometric analysis—For assessing the phenotypic variability within and among groups of *Fallopia* and determining morphological characters that readily discriminate among them, 12 morphological characters were measured (Fig. 2) and their ratios were assessed on three leaves and three stems per individual. The leaves were chosen to represent the same development stage. They were the most-developed leaf of an adult stem and were generally situated in the basal part of the stem. These measurements were carried out on 41 individuals of putative *F. japonica* var. *japonica*, 28 putative *F.* ×*bohemica*, four putative *F. sachalinensis*, and six putative *F. aubertii*.

The characters examined were those most often used in the literature to identify *Fallopia* species (Barral, 1994; Beerling et al., 1994; Jager, 1994; Lambinon et al., 2004): (1) basal width, (2) central width, and (3) length of leaves; (4) length of the cord, measured from the base of the leaf to the top insertion of the petiole; (5) length and (6) width of the leaf apex; (7) number of hairs in 1 × 1 cm and (8) mean length of three hairs per leaf, measured on the back of the leaf; and (9) stem diameter measured at 20 cm from the base (Fig. 2). Three ratios were also computed: (10) cord length to leaf length, (11) leaf basal width to leaf length, and (12) apex width to apex length. Because the putative *F. japonica* var. *compacta* was only found during the course of the study, morphological analyses were not included for this taxon or for the two specimens from Cirencester.

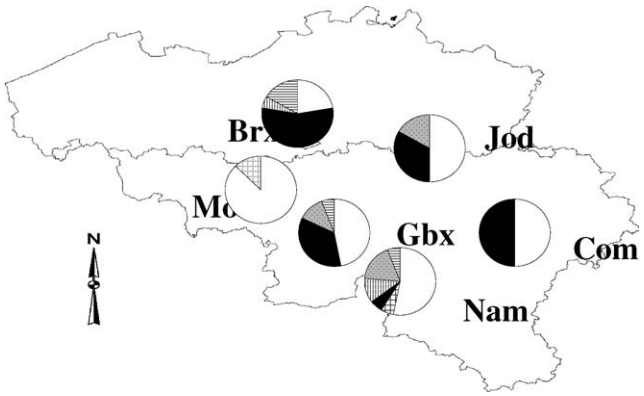

Fig. 1. Location of sampled areas in Belgium and relative importance of the different *Fallopia* taxa on the basis of combined results of morphological, cytological, and RAPD analyses. White sections represent *F. japonica* var. *japonica*. Black sections represent 6× *F.* ×*bohemica*. Gray sections represent *F. aubertii*. Horizontal hatched sections represent *F. sachalinensis*. Vertical hatched sections represent 8× *F.* ×*bohemica*. Black square sections represent 4×–6× *F.* ×*bohemica*. Light gray square sections represent a hybrid individual initially misidentified as *F. japonica* var. *compacta*. Areas are indicated by the locality code (Table 1). Scale = 1/1 584 590.

Statistical analysis—For determining whether groups of *Fallopia* taxa, identified by flow cytometry, could be classified into genotypic groups, RAPD data were subjected to a principal coordinate analysis (PCO) and a Bayesian model-based clustering method. The PCO allows visualization of genetic distance data without assuming a hierarchical topology and describes the main structures of distances matrices in the form of factor maps. The PCO was based on a matrix of between-individual Dice similarities computed from the binary RAPD data and performed using the program GenAlex 6 (Peakall and Smouse, 2006). The Bayesian-based cluster analysis was performed on the RAPD data using the program Structure 2.1 (Pritchard et al., 2000). The method uses Markov chain Monte Carlo (MCMC) to estimate allele frequencies and to assign individuals probabilistically either to distinct gene pools or jointly to two or more gene pools if their genotypes indicate that they are admixed. Because of the dominant marker used, each locus was coded as known for one copy and unknown (coded −9 as recommended in the program) for the other. To obtain data strictly from the genetic information, we did not use prior information regarding species identity. Analyses were performed under the admixture model. Four independent runs were carried out for each value of K (numbers of clusters assumed) between 2 and 8, with parameters and model likelihood estimated at over 200 000 MCMC iterations following a burn-in period of 50 000 steps. The K value associated with the maximum value of log likelihood of data [$L(K)$] was analyzed to identify the number of clusters that best described the data. However, because Evanno et al. (2005) showed that this method does not always correctly estimate K, the ΔK statistics based on the rate of change of log$K$ between successive values of K was calculated to infer the appropriate number of clusters. The value of K corresponding to the highest value of ΔK was then retained (Evanno et al., 2005). For each individual, we assessed its mean percentage of membership (q_{mean}) to each of the K genetic clusters based on the four independent runs.

The morphometric data were subjected to a principal component analysis (PCA) to assess the level of phenotypic variability within and among groups of *Fallopia*. Moreover, to identify morphological characters that fully discriminate between groups of *Fallopia* taxa, we compared taxa (only those with a sufficient number of samples) for mean measurements of the morphological characters with a one-way ANOVA followed by the Tukey honestly significant difference (HSD) post hoc test. Before analysis, data were transformed as follows: $\log_{10}$ for length of hair, stem diameter, basal width of leaf, length of the cord, and width of the leaf apex; arcsine for the ratios of cord length to leaf length and leaf basal width to leaf length. We used the Kruskal–Wallis test followed by the nonparametric Mann–Whitney test for analyzing central width of leaf, length of leaf, length of the leaf apex, the ratio of apex width to apex length, and number of hairs because the distributions of these variables could not be normalized. These analyses were performed using XLSTAT 6.0 (Addinsoft, France).

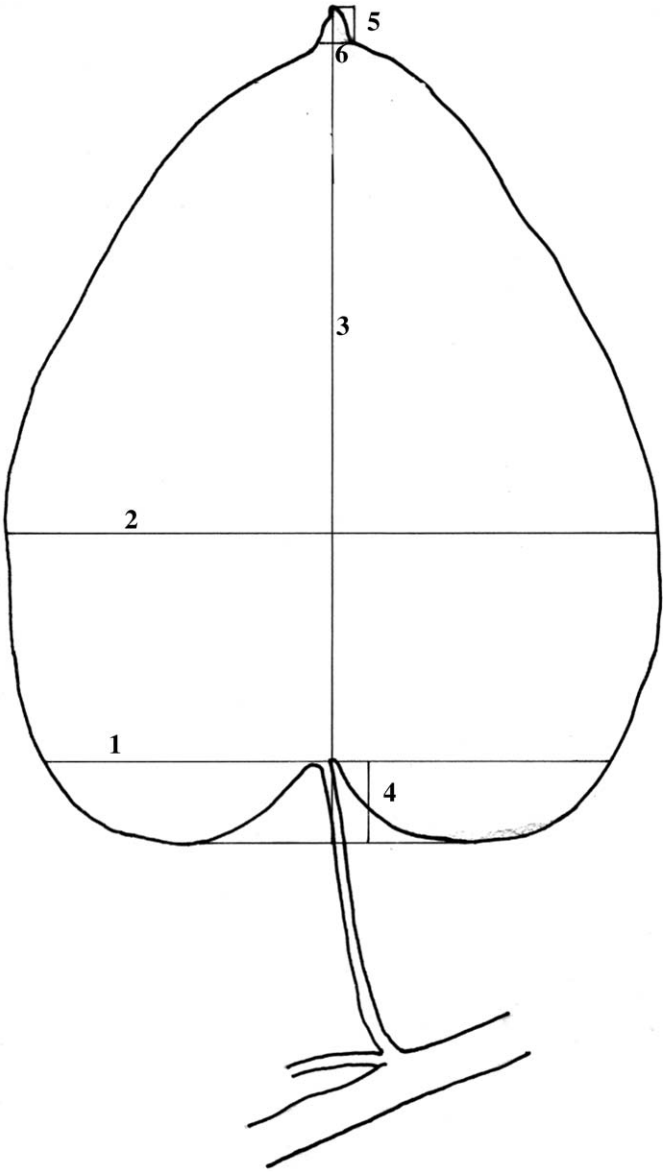

Fig. 2. Morphological characters analyzed in the invasive alien *Fallopia* in Belgium. (1) Leaf basal width; (2) leaf central width; (3) leaf length; (4) leaf cord length; (5) leaf apex length; and (6) leaf apex width.

RESULTS

Ploidy levels—Five chromosome numbers were found in the 16 individuals examined: 20 ($N = 1$), 44 ($N = 3$), 44–67 ($N = 1$), 66 ($N = 4$), and 88 ($N = 7$). Clear discontinuities in the frequency distribution of fluorescence peak ratios allowed the estimation of the ploidy levels of the individuals sampled by reference to an individual that had both a known chromosome number and a count by flow cytometry. *Fallopia aubertii* had a fluorescence peak ratio of 0.72 ($N = 2$), corresponding to diploid individuals with 20 chromosomes. The fluorescence peak ratios of the *F. japonica* var. *compacta* ($N = 1$), tetraploid *F.* ×*bohemica* ($N = 2$, from Cirencester), and *F. sachalinensis* ($N = 2$) were the same, suggesting that all were tetraploid ($2n = 44$). The peak ratios of the *F. japonica* var. *japonica* accessions

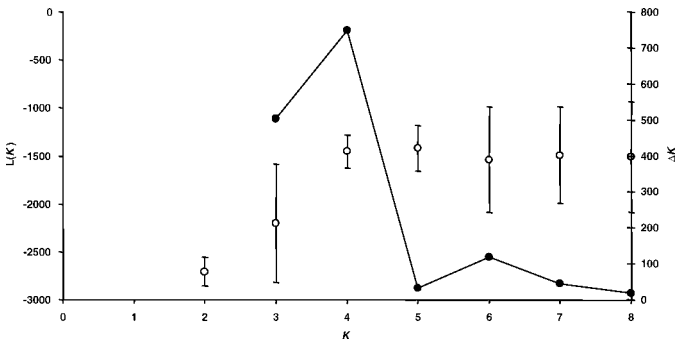

Fig. 3. Inference of genetic clusters (K) of *Fallopia* individuals using the model-based clustering method of Pritchard et al. (2000). Mean (±SD) of log probability of data [$L(K)$] based on four independent runs (○) as a function of the value of K and the rate of change in the log probability of data ΔK between successive values of K (•) for the *Fallopia* data set ($N = 87$).

ranged from 1.96 to 2.23 ($N = 35$), corresponding to reference octoploids ($2n = 88$). The ploidy levels of the individuals tentatively classified as *F.* ×*bohemica* were more variable. Most of them had a fluorescence peak ratio between 1.54 and 1.67 ($N = 26$), corresponding to reference hexaploids ($2n = 66$). One individual had a peak ratio of 1.38 and an aneuploid chromosome range from 44 to 67. Finally, three plants identified as *F.* ×*bohemica* were octoploid ($2n = 88$) and had peak ratios between 1.96 and 2.28, corresponding to the range observed for *F. japonica* var. *japonica*.

RAPD variation—Of the 134 polymorphic RAPD markers scored from the analysis of the eight primers on 87 individuals of *Fallopia*, 97.8% were polymorphic (131 fragments). The monomorphic markers were removed from the analyses.

The level of genotypic diversity varied tremendously among the different putative taxa. All the octoploid individuals identified as *F. japonica* var. *japonica* ($N = 40$) shared the same multiband RAPD phenotype, suggesting that they belong to the same genetic clone. The same situation was found for *F. aubertii* ($N = 6$). Two different RAPD phenotypes were found for the six *F. sachalinensis* individuals examined, one corresponding to male-sterile individuals ($N = 4$), the other to the male-fertile individuals ($N = 2$). In contrast, individuals identified as *F.* ×*bohemica* had a high genotypic diversity, with 28 RAPD phenotypes of 29 individuals in the case of hexaploids *F.* ×*bohemica*, three RAPD phenotypes of three individuals for octoploids, and two RAPD phenotypes of two individuals for tetraploids. The minimum pairwise difference in band presence or absence between two individuals with different RAPD phenotypes was nine, indicating that over-interpretation of small differences was not the cause of this diversity. The two pairs of hexaploid *F.* × *bohemica* sharing the same RAPD phenotype grew close to each other in the field.

The Bayesian analysis using Structure indicated the presence of four distinct genetic clusters in the sample of *Fallopia* (Fig. 3). The likelihood of the data gradually increased from $K = 2$ ($\ln = -2705.2$) to a maximum at $K = 5$ ($\ln = -1419.9$). However, the highest value of $\Delta K = 749.4$ was observed at $K = 4$. Consequently and according to Evanno et al. (2005), the value of $K = 4$ was retained and analyzed for individual assignments.

The individuals of *F. japonica* var. *japonica*, *F. aubertii*, and

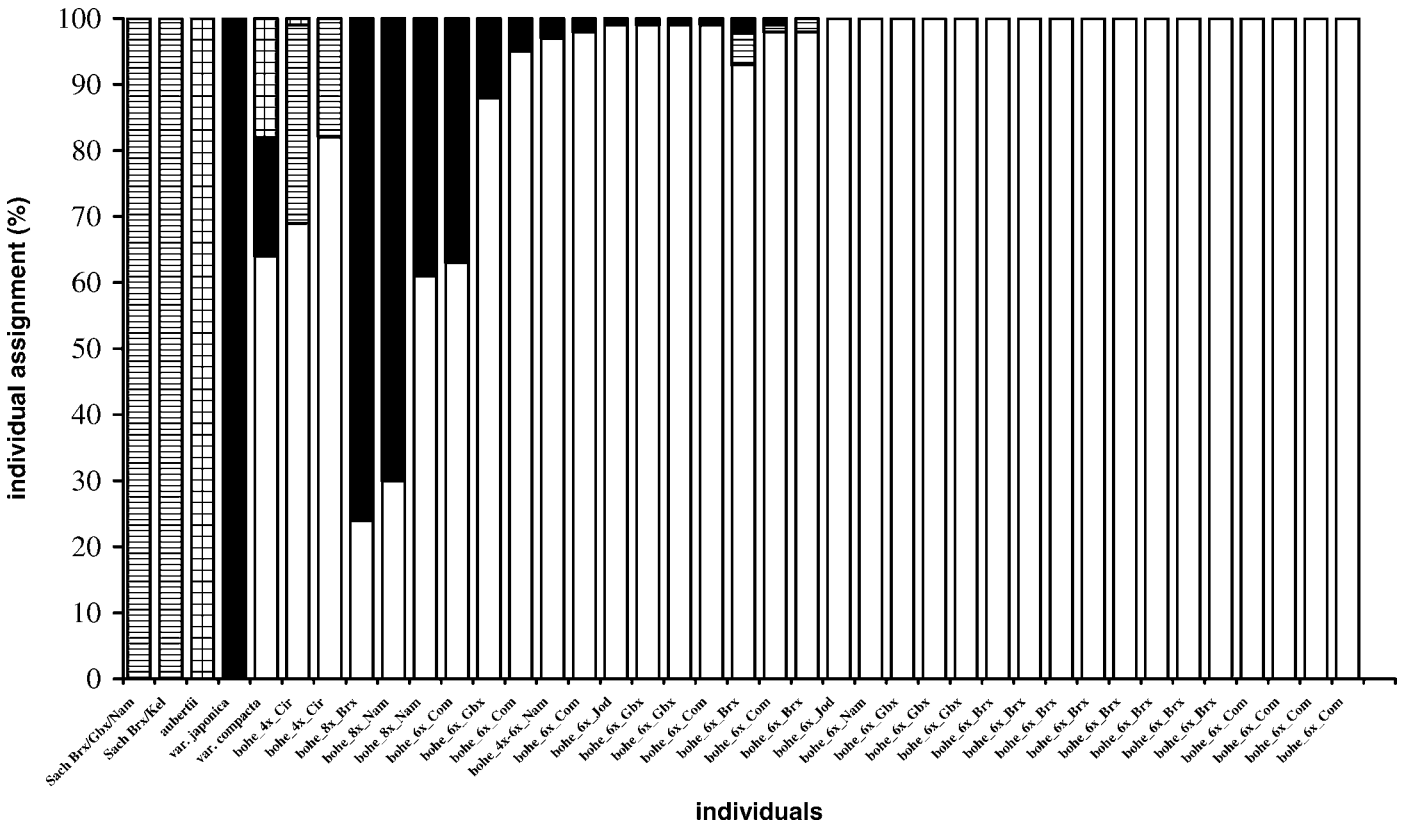

Fig. 4. Results of test to assign the 87 sampled individuals of *Fallopia* taxa into four genetic clusters (K) detected using the model-based clustering method of Pritchard et al. (2000). Individuals with the same RAPD phenotype are represented only once. Gray sections represent *F. sachalinensis* ($N = 6$). Dotted sections represent *F. aubertii* ($N = 6$). Black sections represent *F. japonica* var. *japonica* ($N = 40$). White sections represent *F.* ×*bohemica* ($N = 35$) including the putative *F. japonica* var. *compacta*. Individuals are indicated by the first letters of the putative taxon, the ploidy level in the case of *F.* ×*bohemica*, and the locality code (Table 1).

F. sachalinensis were all assigned to a single genetic cluster ($q_{mean} = 1.00$), which differed between taxa (Fig. 4). The majority (17 of 29) of hexaploid *F.* ×*bohemica* individuals were totally ($q_{mean} = 1.00$) assigned to a unique genetic cluster different from the parental species. The other hexaploid *F.* ×*bohemica* individuals (including the aneuploid individual) (12 of 29) had different degrees of admixture, with the highest percentage of assignment to the group typical of *F.* ×*bohemica* (q_{mean} ranging from 0.63 to 0.99), various percentages of assignment to the cluster characteristic of *F. japonica* var. *japonica* (q_{mean} ranging from 0.01 to 0.37), and a negligible percentage to the cluster of *F. sachalinensis* (q_{mean} ranging from 0.01 to 0.05). The three octoploid individuals tentatively identified as *F.* ×*bohemica* ($N = 3$) were admixed individuals with a more significant contribution of the genetic cluster characteristic of *F. japonica* var. *japonica* for two of them (q_{mean} ranging from 0.70 to 0.76) and a more significant contribution of the cluster typical of *F.* ×*bohemica* for one of them ($q_{mean} = 0.61$). The two tetraploid *F.* ×*bohemica* from Cirencester were also admixed individuals closer to hexaploid *F.* ×*bohemica* but with a more significant contribution from the *F. sachalinensis* cluster than other hybrids ($q_{mean} = 0.18$ and 0.30). The putative *F. japonica* var. *compacta* ($N = 1$) was also an admixed individual assigned to three clusters, with the main contribution from the cluster typical of *F.* ×*bohemica* and similar contributions of the clusters of *F. japonica* var.

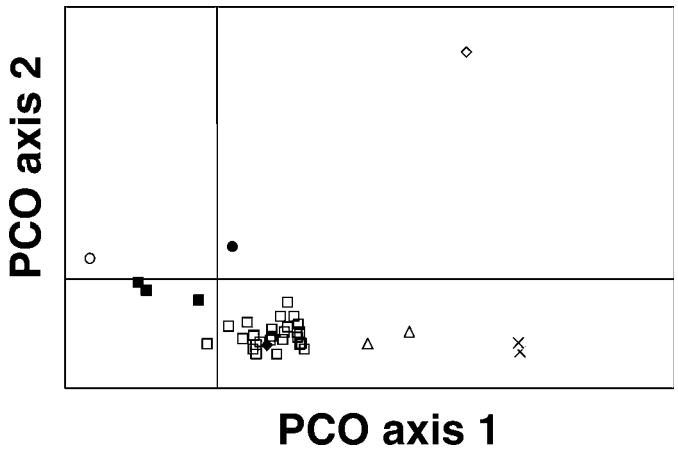

Fig. 5. Principal coordinate analysis (PCO) of the 87 individuals of *Fallopia* species sampled, based on 131 RAPD markers. The analysis was performed using a matrix of between-individual Dice similarities computed from the binary RAPD data and the GenAlex 6 program (Peakall and Smouse, 2006). The first two axes accounted for 80.3% of the total variation (axis 1: 52.9%, axis 2: 27.4%). (◇) *F. aubertii*; (Δ) 4× *F.* ×*bohemica*; (♦) 4×–6× *F.* ×*bohemica*; (□) 6× *F.* ×*bohemica*; (■) 8× *F.* ×*bohemica*; (○) *F. japonica* var. *japonica*; (•) putative *F. japonica* var. *compacta*, and (×) *F. sachalinensis*.

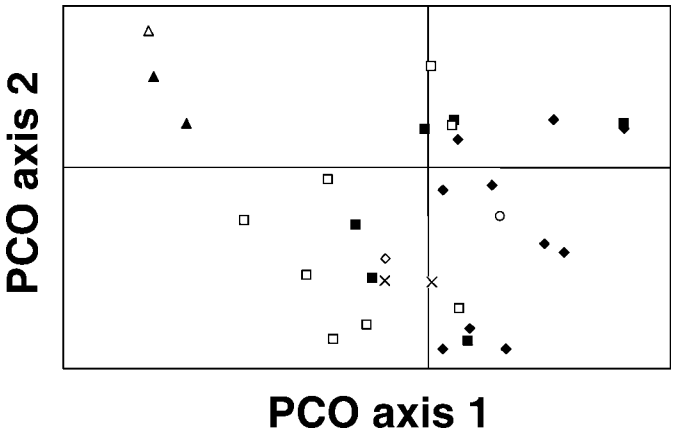

Fig. 6. Principal coordinate analysis (PCO) of the 34 individuals of *Fallopia* ×*bohemica* in relation to the sampled areas based on 131 RAPD markers. The analysis was performed using a matrix of between-individual Dice similarities computed from the binary RAPD data using the GenAlex 6 program (Peakall and Smouse, 2006). The first two axes accounted for 49.2% of the total variation (axis 1: 29.6%; axis 2: 19.6%). (+) 4× *F.* ×*bohemica* Cirencester; (◇) 4×–6× *F.* ×*bohemica* Namur; (♦) 6× *F.* ×*bohemica* Brussels; (□) 6× *F.* ×*bohemica* Comblain-au-Pont; (■) 6× *F.* ×*bohemica* Gembloux; (×) 6× *F.* ×*bohemica* Jodoigne; (○) 6× *F.* ×*bohemica* Namur; (▲) 8× *F.* ×*bohemica* Namur; (Δ) 8× *F.* ×*bohemica* Brussels.

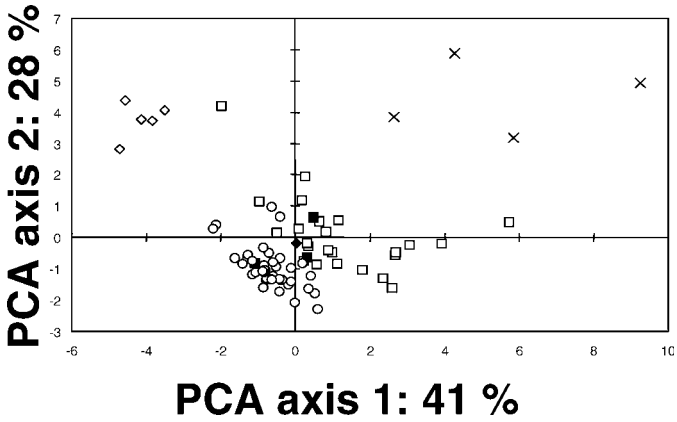

Fig. 7. Principal components analysis (PCA) of 79 individuals of *Fallopia* species sampled based on the mean measurements of 12 morphological characters and ratios. (◇) *F. aubertii*; (♦) 4×–6× *F.* ×*bohemica*; (□) 6× *F.* ×*bohemica*; (■) 8× *F.* ×*bohemica*; (○) *F. japonica* var. *japonica*, (×) *F. sachalinensis*.

japonica and *F. aubertii* ($q_{mean} = 0.18$ each). This individual was erroneously identified as *F. japonica* var. *compacta* and was probably a hybrid. The most parsimonious hypothesis on the origin of this hybrid, taking into account the results of flow cytometry and genetic assignment, is a crossing event between *F. aubertii* and an individual of *F.* ×*bohemica*. Such a hybrid would have 43 chromosomes. It is possible that flow cytometry results were erroneously interpreted as indicating 44 chromosomes.

The results of the principal coordinate analysis (PCO) of the individual genotypes were consistent with the results of the assignment test (Fig. 5). The two principal axes explained 80.3% of the total genetic variation. In the factorial space defined by the two first axes, there was no overlap between groups of different taxa. *Fallopia aubertii*, *F. japonica* var. *japonica*, and *F. sachalinensis* were clearly separated from the other groups, as well as from tetraploid *F.* ×*bohemica* from Cirencester and the putative *F. japonica* var. *compacta* from Ceroux-Mousty. Hexaploid *F.* ×*bohemica* accessions occupied an intermediate position between their putative parents but were closer to *F. japonica* var. *japonica*. This trend was even more pronounced for octoploid individuals tentatively identified as *F.* ×*bohemica*.

In an attempt to clarify the relationships within the *F.* ×*bohemica* complex, a separate PCO analysis was performed based on collection areas in Belgium, using a reduced RAPD data set (Fig. 6). The proportion of variance explained by the first two axes was 49.2%. Octoploid *F.* × *bohemica* tended to be isolated from hexaploid *F.* ×*bohemica*. Hexaploid *F.* ×*bohemica* individuals were little separated according to collection area.

The relative importance of *Fallopia* taxa in the studied area is depicted in Fig. 1. It shows that 6× *F.* ×*bohemica* was as widespread as *F. japonica* var. *japonica* in Belgium and that the relative abundance of these taxa varied greatly from one area to another. In Brussels for example, 6× *F.* ×*bohemica* was the dominant form, but was totally absent in Ceroux-Mousty.

Morphological variation—In the PCA analysis of total morphological data, the three first axes extracted 81% of the variation. In this analysis, the hypothesized taxa corresponded fairly well to different parts of the variation pattern, with the exception of the octoploid putative *F.* ×*bohemica* (Fig. 7). As with the genetic data, the diploid *F. aubertii* and the tetraploid *F. sachalinensis* were well differentiated from other putative taxa. Hexaploid *F.* ×*bohemica* and octoploid *F. japonica* var. *japonica* formed a continuous pattern of variation, but it was still possible to separate the two groups. *Fallopia* ×*bohemica* was morphologically more similar to its octoploid parent *F. japonica* var. *japonica* than to its tetraploid parent *F. sachalinensis*. In contrast, the positions of the putative octoploid *F.* ×*bohemica* were not fully in accordance with their earlier identification because two of the three individuals were clearly more similar to *F. japonica* var. *japonica*. In spite of their lack of RAPD variation, *F. aubertii* and *F. japonica* var. *japonica* individuals had significant morphological variation. Morphological variation was greater in the hexaploid *F.* ×*bohemica* than in its closer parent *F. japonica* var. *japonica*. Axis 1 was most strongly correlated with leaf length ($r = 0.96$), central width of leaf ($r = 0.93$), and basal width of leaf ($r = 0.91$). Apex length, length of the cord, and the ratio of cord length to leaf length were most highly correlated with axis 2 ($r = -0.75$, 0.67, and 0.93, respectively).

To identify characters that can readily discriminate between these taxa, we compared the mean values among the four main groups of individuals: diploid *F. aubertii*, tetraploid *F. sachalinensis*, hexaploid *F.* ×*bohemica,* and octoploid *F. japonica* var. *japonica*. Putative octoploid *F.* ×*bohemica* plants were excluded from the analysis because of the small number of samples. All the morphological characters differed significantly for at least one species, except for the ratio of apex width to apex length (Table 2). The three characters best able to separate all species were basal width of leaf (one-way ANOVA: $F = 46.60$, $P < 0.0001$), central width of leaf (Kruskal–Wallis test: $H = 32.34$, $P < 0.0001$), and length of leaf (Kruskal–Wallis test: $H = 36.17$, $P < 0.0001$). Moreover, the cord length (one-way ANOVA: $F = 53.80$, $P < 0.0001$),

TABLE 2. Mean measurements of 12 morphological characters and ratios of *Fallopia aubertii* (*F. aub.*), hexaploid *F. ×bohemica* (6× *F. ×b*), *F. japonica* var. *japonica* (*F. j.j.*) and *F. sachalinensis* (*F. s.*) sampled in Belgium. The same superscript letter within a row indicates no significant difference between species. For most variables, taxa were compared using a one-way ANOVA followed by the Tukey HSD post hoc test; the data were $\log_{10}$ transformed (hair length, stem diameter, leaf basal width, cord length, apex width) or arcsine transformed (the ratio of cord length to leaf length and the ratio of leaf basal width to leaf length). The Kruskal–Wallis test followed by the nonparametric Mann–Whitney test was used for leaf central width, leaf length, apex length, the ratio of apex width to apex length, and number of hairs.

	Mean measurement (SD)			
Character	*F. aub.* $N = 6$	6× *F. ×b* $N = 28$	*F. j.j.* $N = 41$	*F. s.* $N = 4$
Stem diameter (mm)	2.33 (1.13)[a]	22.81 (4.58)[b]	19.55 (4.94)[b]	21.38 (2.42)[b]
No. of hairs	0.00 (0.00)[a]	20.49 (16.20)[b]	0.00 (0.00)[a]	21.58 (10.18)[b]
Hair length (mm)	0.00 (0.00)[a]	0.66 (0.40)[b]	0.00 (0.00)[a]	3.05 (0.53)[c]
Leaf basal width (mm)	23.24 (4.80)[a]	81.82 (25.97)[b]	63.76 (14.33)[c]	148.46 (68.75)[d]
Leaf central width (mm)	28.05 (5.34)[a]	112.65 (29.40)[b]	95.22 (13.07)[c]	153.33 (34.62)[d]
Leaf length (mm)	48.91 (9.39)[a]	161.97 (42.17)[b]	127.44 (16.16)[c]	247.35 (59.48)[d]
Cord length (mm)	3.47 (1.59)[a]	3.18 (2.61)[a]	0.39 (1.05)[b]	21.51 (8.49)[c]
Apex length (mm)	4.53 (1.55)[a]	14.29 (4.47)[b]	14.69 (3.17)[b]	10.50 (5.67)[a,b]
Apex width (mm)	4.12 (0.87)[a]	13.17 (2.84)[b]	12.89 (2.17)[b]	12.79 (3.07)[b]
Cord length to leaf length	0.07 (0.02)[a]	0.02 (0.02)[b]	0.00 (0.01)[c]	0.09 (0.02)[a]
Leaf basal width to leaf length	0.58 (0.05)[a]	0.70 (0.08)[b]	0.75 (0.06)[c]	0.62 (0.07)[a,b]
Apex width to apex length	0.99 (0.29)[a]	1.04 (0.55)[a]	0.91 (0.20)[a]	1.47 (0.67)[a]

the ratio of leaf basal width to leaf length (one-way ANOVA: $F = 14.03$, $P < 0.0001$), and the ratio of cord length to leaf length (one-way ANOVA: $F = 61.90$, $P < 0.0001$) were useful in discriminating *F. japonica* var. *japonica* from 6× *F. ×bohemica*. Hexaploid *F. ×bohemica* invariably had short hairs on the back of the leaf, whereas *F. japonica* var. *japonica* had none.

DISCUSSION

Hybridization pattern and genetic variation—Interspecific hybridization and polyploidization are recognized as a central feature in the evolution of the invasive alien knotweeds *Fallopia* spp. in their introduced range (Bailey, 2003). Thanks to their large adventive range, *Fallopia* spp. provide an outstanding model to explore the diversity of patterns and mechanisms associated with hybridization. Our study adds to the information available for the UK and the Czech Republic. Comparisons among regions across the adventive range of *Fallopia* will help to establish the role of hybridization and polyploidy in the invasive success of those taxa (Bailey and Wisskirchen, 2006). In addition, as far as we are aware, our study is the first to assess the extent of the hybridization and differentiation of an invasive alien complex plant by combining morphological and molecular approaches.

We confirm that at least four taxa with different ploidy levels are taking part in the invasion of Belgium by *Fallopia* spp.: *F. aubertii* (diploid), *F. sachalinensis* (tetraploid), *F. japonica* var. *japonica* (octoploid), and the hybrid *F. ×bohemica* (various chromosome numbers, the 6× being the most frequent). The taxonomic status of the individual identified as *F. japonica* var. *compacta* was not confirmed based on assignment tests with RAPD data. Our results demonstrate a pattern of interspecific hybridization among the *Fallopia* taxa in Belgium, which in the case of *F. ×bohemica* provides increased levels of variation compared with the parental taxa.

The three putative parental taxa whose presence we confirmed in Belgium (*F. japonica* var. *japonica*, *F. sachalinensis*, *F. aubertii*) all have a low level of genetic diversity. All individuals of *F. japonica* var. *japonica* share the same multilocus genotype, indicating a high level of clonality. They nonetheless vary greatly morphologically. These results are consistent with the pattern reported from the UK and the Czech Republic (Hollingsworth et al., 1998; Hollingsworth and Bailey, 2000; Mandák et al., 2005) and demonstrate that the morphological variation observed in this species is caused by plastic responses to local environments or by a somewhat insignificant genetic variation at a small number of loci. This is the first assessment of the genotypic diversity of the introduced *F. aubertii*. This species has a single multilocus genotype probably resulting from clonal multiplication by horticulturists. *Fallopia sachalinensis* has high genotypic diversity in its native range (Pashley et al., 2007) but lower genotypic diversity in its adventive range (Pashley et al., 2007), although the diversity differs among regions. For example, the genotypic diversity of *F. sachalinensis* is less in the UK than in the Czech Republic. In our study, *F. sachalinensis* had low genotypic diversity, with two genotypes found in the six plants sampled. One could argue that this low genetic diversity results from the small sample size of *F. sachalinensis*. Nevertheless, because we sampled all the individuals of *F. sachalinensis* in the area, our data reflect the current situation of the species in Belgium. Two of the six *F. sachalinensis* examined were male-fertile and correspond to one of the two genotypes detected. This situation is similar to that in the UK (where *F. sachalinensis* mainly belongs to two widespread genotypes, one male-fertile and one male-sterile clone [Pashley et al., 2007]) and points to the role of vegetative propagation in the expansion of *F. sachalinensis* in Belgium.

In contrast to parental species, hybrids found in Belgium have high levels of genetic diversity and a complex pattern of admixture between different gene pools. Using assignment tests with no prior information on RAPD data, we demonstrated that hybrid genotypes are mainly assigned to a specific genetic pool and not to a mixture between the genetic pools of the putative parents, as would be expected for hybrids. At least two hypotheses may explain this pattern. First, hybrids may be introduced independently of the parental species in their new range. In this case, the initial pool of hybrids may have carried its own genetic diversity. Second, recent studies show that

polyploid genome evolution appears often to be accompanied by rapid structural changes (Ozkan et al., 2001; Salmon et al., 2005) resulting in the case of dominant markers in parental fragment loss or in new fragment addition (Salmon et al., 2005). In our analysis, 16% of RAPD phenotype bands in the hybrids were hybrid specific.

Hexaploid *F. ×bohemica*, which was the most widespread hybrid in our study, had a higher level of genotypic diversity (28 genotypes from 30 plants) than that reported along river basins in the UK (Hollingsworth et al., 1998; Hollingsworth and Bailey, 2000) or in the Czech Republic, where 33 of 88 plants represented hexaploid *F. ×bohemica*, based on isozymes (Mandák et al., 2005). This difference with the Czech data possibly arises from the use of molecular markers with different levels of polymorphism, RAPDs had more polymorphism than did the isozymes. The differences from the UK situation may stem from differences in the balance between sexual and vegetative reproduction in different sites. Populations along river basins (UK) may be more prone to water dispersal of vegetative fragments following disturbance, while colonization of new sites in "nonriver" systems would rely on long-distance dispersal of seeds, which is possible for *F. japonica* var. *japonica* (Tiébré et al., 2007).

The high genotypic diversity in hexaploid *F. ×bohemica* may stem from different sources: (1) multiple in situ hybridization between parental taxa, (2) consequences of the male fertility of the hybrids (Tiébré et al., 2007) resulting in hybrid sexual reproduction or backcross with the parental species, and (3) multiple introduction by human activities. The relative importance of these different factors is debatable (Hollingsworth and Bailey, 2000; Pashley et al., 2003; Mandák et al., 2005), and our results give additional insights. Multiple in situ F1 production is not supported by the results of the assignment tests. In addition, multiple in situ F1 production would require the presence of both parents, including male-fertile *F. sachalinensis*. In two of the areas studied (Gembloux and Comblain-au-Pont), we exhaustively surveyed all *Fallopia* present and in other areas searched intensively for *F. sachalinensis*. In two of the areas where *F. ×bohemica* occurs, we did not find any *F. sachalinensis*, and in the three areas where *F. sachalinensis* occurs (albeit at low frequency), none were male-fertile. Although we cannot rule out the possibility that male-fertile populations previously occurred in the areas or their surroundings, the pattern of distribution of *F. sachalinensis* makes it unlikely that multiple in situ hybridization events are the main source of the genotypic variation of hexaploid *F. ×bohemica*. In addition to the possibility of multiple introductions, restoration of sexual reproduction in the complex, as we demonstrated (Tiébré et al., 2007), is the most likely explanation for the high genetic diversity. In that study, seeds borne on *F. japonica* var. *japonica* had a large range of ploidy levels, probably resulting from crosses with *F. aubertii* and backcrosses with more or less unreduced gametes from *F. ×bohemica*.

Such backcrosses might produce hexaploid hybrids, because irregular meiosis in *F. ×bohemica* can give rise to gametes with a wide range of chromosome numbers. Alternatively, cross fertilization between F1 (or following generations) hybrids may also give rise to $2n = 66$ individuals. One argument sustaining the possibility of backcross with parental species or interhybrid fertility is that we found one adult hybrid with an aneuploid chromosome number (44–67) that most probably resulted from a cross with *F. japonica* var. *japonica*. Besides, we also found that the tetraploid individual presumably identified as *F. japonica* var. *compacta* was probably a hybrid between *F. ×bohemica* and *F. aubertii*, confirming that *F. aubertii* may play a role in the pattern of hybridization in the *Fallopia* complex. Nevertheless, considering the existing evidence on variation of ploidy level in *F. japonica* var. *japonica* seeds (Tiébré et al., 2007), we should consider that the predominance of hexaploid hybrids in adult populations collected in the same areas is the result of a differential selection for $2n = 66$ progeny from cytological variable arrays, as suggested by Mandák et al. (2005).

The tetraploid *F. ×bohemica* is formed by crosses between *F. japonica* var. *compacta* and *F. sachalinensis* (Mandák et al., 2003; Bailey and Wisskirchen, 2006). As expected, the Bayesian-based cluster analysis and the PCO analysis of RAPD data showed that the 4× *F. ×bohemica* from Cirencester (UK) was genetically closer to *F. sachalinensis*. Unfortunately, because we did not find tetraploid *F. japonica* var. *compacta* in Belgium, we cannot comment further on the origin of the 4× hybrid.

The presence of octoploid *F. ×bohemica* with different genotypes than *F. japonica* var. *japonica* is more puzzling. These individuals have a number of possible origins (Bailey and Wisskirchen, 2006). The first scenario is the fusion of an unreduced male gamete from hexaploid *F. ×bohemica* and normal gametes from tetraploid *F. sachalinensis* (the reverse being unlikely because no male-fertile *F. sachalinensis* was present in areas). This hypothesis is not supported by the Bayesian-based cluster analysis and the PCO analysis of RAPD data because the fusion would result in an equal contribution of *F. japonica* var. *japonica* and *F. sachalinensis* chromosomes in the hybrid (44 each). In situ autopolyploidy of tetraploid *F. ×bohemica* (Mandák et al., 2003) is also unlikely because we did not detect this cytotype in the areas studied in Belgium. Because two of these clones are morphologically very close to *F. japonica* var. *japonica*, one may ask whether they are actually genetic variants of the widespread *F. japonica* var. *japonica* clone. While they differed from *F. japonica* var. *japonica* only by the absence of a few specific bands, these two accessions were also assigned for a significant percentage to the genetic cluster typical of *F. ×bohemica*, attesting to their hybrid origin.

Whatever its source, increased genetic variability may be important for the invasive success of the hybrid *F. ×bohemica* as suggested by Pyšek et al. (2003) in the Czech Republic. *Fallopia ×bohemica* has always been considered rare in Belgium, even in the most recent Flora (*Nouvelle Flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines* [Ptéridophytes et Spermatophytes], Cinquième édition) and is only described as a hybrid of horticultural origin (Lambinon et al., 2004). However, our investigations indicate that *F. ×bohemica* is widespread in Belgium and that its distribution varies between regions. This inconsistency may be due to the inability of authors to distinguish *F. japonica* var. *japonica* from *F. ×bohemica* on morphological grounds. Alternatively, it may represent a recent expansion of *F. ×bohemica,* not yet recorded in the Belgian floristic database, indicating an increase in the invasive success of the hybrid relative to that of its parents.

There is no clear regionalization of the *F. ×bohemica* clones based on spatial distribution. This absence of regionalization may be due to a common pool of parental species at the initiation of the hybridization process and to extensive gene

flow between fertile hybrids as suggested earlier or to an insufficient time for hybrid populations to differentiate under isolation by distance. A widespread survey of the genetic variability of *F.* ×*bohemica* individuals in west continental Europe would help to clarify the full extent of genetic differentiation in this complex.

Morphological characters for distinguishing all taxa of the alien Fallopia complex—In examining the distribution of *Fallopia* hybrids at the European scale, Bailey and Wisskirchen (2006) point to large areas without known occurrence. They argued that this situation may result from differential evolution among regions or from misidentification by field botanists even though keys to identify hybrids and parental *Fallopia* taxa are available in a few modern floras and synoptic tables in journals (Fojcik and Tokarska-Guzik, 2000; Kim and Park, 2000; Zika and Jacobson, 2003). This situation points to the importance of reliable characters for distinguishing the different taxa in the invasive *Fallopia* complex.

In this study, we used the morphological characters most often used in the literature to identify *Fallopia* species (Barral, 1994; Beerling et al., 1994; Jager, 1994; Lambinon et al., 2004). To buffer plasticity due to variation in environment, we used the mean measurements of three basal leaves and three stems per individual. The statistical analyses indicated several characters that could be used to distinguish between the *Fallopia* taxa and their hybrids. The most reliable characters are the leaf basal width, leaf central width, and the leaf length. *Fallopia sachalinensis* is most distinguished by its larger ovate leaf, its cordate base, and its dense long hairs. *Fallopia aubertii* is clearly different from the other taxa because of its smaller leaves (mean leaf length no more than 5 cm) and its climbing habit. Hexaploid *F.* ×*bohemica* are morphologically very close to *F. japonica* var. *japonica*. However, the presence of hairs and a somewhat cordate leaf in *F.* ×*bohemica* is sufficient to discriminate them from *F. japonica* var. *japonica*. Our results agree with studies of the morphological and chromosomal variation in the alien *Fallopia* species in Korea, Poland, and North America (Fojcik and Tokarska-Guzik, 2000; Kim and Park, 2000; Zika and Jacobson, 2003). That the octoploid *F.* ×*bohemica* could not be distinguished from *F. japonica* var. *japonica* on morphological grounds points to the value of molecular data in assessing hybridization in species complexes.

Conclusions—Clonal growth is claimed to be the major mode of reproduction of *Fallopia* species in their introduced range (Beerling et al., 1994; Child and Wade, 2000; Weber, 2003). Accordingly, *Fallopia* taxa should be characterized by a low genotypic diversity. The high level of genotypic diversity observed in the present study in the hybrid *F.* ×*bohemica* as compared to the parental species confirms the occurrence of interspecific hybridization among the *Fallopia* taxa in Belgium and the restoration of sexual reproduction by hybridization in the invasive alien *Fallopia* complex. This high genotypic diversity may increase the potential of the taxa to adapt and differentiate into new environments and contribute to the dramatic invasive success of knotweeds in their adventive range. A complete study of the hybridization status in Europe is essential and should combine morphological and molecular approaches. In our study of the situation in Belgium, we have defined valuable identification criteria that can be used to study the extent of hybridization in Europe. Our results point to the importance of hybridization and polyploidization in increasing the genetic diversity and potential invasion success of alien taxa.

LITERATURE CITED

ABBOTT, R. J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology and Evolution* 7: 401–405.

ABBOTT, R. J. 2000. Hybrid origin of the Oxford ragwort, *Senecio squalidus* L. *Watsonia* 23: 123–138.

AINOUCHE, M. L., A. BAUMEL, A. SALMON, AND G. YANNIC. 2003. Hybridization, polyploidy and speciation in *Spartina* (Poaceae). *New Phytologist* 161: 165–172.

ALLENDORF, F. W., AND L. L. LUNDQUIST. 2003. Introduction: population biology, evolution and control of invasive species. *Conservation Biology* 17: 24–30.

BAILEY, J. P. 1988. Putative *Reynoutria japonica* Houtt. × *Fallopia baldschuanica* (Regel) Holub hybrids discovered in Britain. *Watsonia* 17: 163–164.

BAILEY, J. P. 1989. Cytology and breeding behaviour of giant alien *Polygonum* species in Britain. Ph.D. dissertation, University of Leicester, Leicester, UK.

BAILEY, J. P. 1994. Reproductive biology and fertility of *Fallopia japonica* (Japanese knotweed) and its hybrids in the British Isles. *In* L. C. De Waal, L. E. Child, P. M. Wade, and J. H. Brock [eds.], Ecology and management of invasive riverside plants, 141–158. Wiley and Sons, Chichester, UK.

BAILEY, J. P. 2001. *Fallopia* ×*conollyana* the railway-yard knotweed. *Watsonia* 23: 539–541.

BAILEY, J. P. 2003. Japanese knotweed *s. l.* at home and abroad. *In* L. Child, J. H. Brock, G. Brundu, K. Prach, P. Pyšek, M. Wade, and M. Williamson [eds.], Plant invasions: ecological threats and management solutions, 183–196. Backhuys Publishers, Leiden, Netherlands.

BAILEY, J. P., L. CHILD, AND A. P. CONOLLY. 1996. A survey of the distribution of *Fallopia* × *Bohemica* (Chrtek & Chrtkova) J. Bailey (Polygonaceae) in the British Isles. *Watsonia* 21: 187–198.

BAILEY, J. P., AND C. A. STACE. 1992. Chromosome number, morphology, pairing, and DNA values of species and hybrids in the genus *Fallopia* (Polygonaceae). *Plant Systematics and Evolution* 180: 29–52.

BAILEY, J. P., AND R. WISSKIRCHEN. 2006. The distribution and origins of *Fallopia* ×*bohemica* (Polygonaceae) in Europe. *Nordic Journal of Botany* 24: 173–199.

BARRAL, V. 1994. Biologie et biogéographie de *Polygonum cuspidatum*. Mémoire de Maîtrise, Université Joseph Fourier, Grenoble, France.

BEERLING, D. J., J. P. BAILEY, AND A. P. CONOLLY. 1994. Biological flora of the British Isles: *Fallopia japonica* (Houtt.) Ronse De Craene. *Journal of Ecology* 82: 959–979.

BÍMOVÁ, K. B. E., B. B. C. MANDÁK, AND P. PYŠEK. 2003. Experimental study of vegetative regeneration in four invasive *Reynoutria* taxa (Polygonaceae). *Plant Ecology* 166: 1–11.

CALLAWAY, R., AND J. MARON. 2006. What have exotic plant invasions taught us over the past 20 years? *Trends in Ecology and Evolution* 21: 369–374.

CATTELL, M. V., AND S. A. KARL. 2004. Genetics and morphology in a *Borrichia frutescens* and *B. arborescens* (Asteraceae) hybrid zone. *American Journal of Botany* 91: 1757–1766.

CHILD, L., AND M. WADE. 2000. The Japanese knotweed manual. Packard Publishing, Chichester, West Sussex, UK.

DIDHAM, R. K., J. M. TYLIANAKIS, M. A. HUTCHISON, R. M. EWERS, AND N. J. GEMMELL. 2005. Are invasive species the drivers of ecological change? *Trends in Ecology and Evolution* 20: 470–474.

DOYLE, J. J., AND J. L. DOYLE. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

ELLSTRAND, N. C., AND K. A. SCHIERENBECK. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? In variation and evolution in plants and microorganisms: toward a new synthesis 50 years after Stebbins. *Proceedings of the National Academy of Sciences, USA* 97: 7043–7050.

EUNMI, L. C. 2002. Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17: 386–391.

EVANNO, G., S. REGNAUT, AND J. GOUDET. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* 14: 2611–2620.

FJELLHEIM, S., R. ELVEN, AND C. BROCHMANN. 2001. Molecules and morphology in concert. II. The *Festuca brachyphylla* complex (Poaceae) in Svalbard. *American Journal of Botany* 88: 869–882.

FOJCIK, B., AND B. TOKARSKA-GUZIK. 2000. *Reynoutria* ×*bohemica* (Polygonaceae): nowy takson we florze Polski. *Fragmenta Floristica et Geobotanica Polonica* 7: 63–71.

GODEFROID, S. 1996. A propos de l'extension spectaculaire de *Fallopia japonica*, *F. sachalinensis*, *Buddleja davidii* et *Senecio inaequidens* en région bruxelloise. *Dumotiera* 63: 9–16.

GROSHOLZ, E. 2002. Ecological and evolutionary consequences of costal invasions. *Trends in Ecology and Evolution* 17: 22–27.

GUREVITCH, J., AND D. PADILLA. 2004. Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution* 19: 470–474.

HÄNFLING, B., AND J. KOLLMANN. 2002. An evolutionary perspective of biological invasions. *Trends in Ecology and Evolution* 17: 545–546.

HANSEN, K. T., R. ELVEN, AND C. BROCHMANN. 2000. Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). *American Journal of Botany* 87: 1466–1479.

HEGARTY, M. J., AND S. J. HISCOCK. 2005. Hybrid speciation in plants: new insights from molecular studies. *New Phytologist* 165: 411–423.

HOLLINGSWORTH, M. L., AND J. P. BAILEY. 2000. Hybridisation and clonal diversity in some introduced *Fallopia* species (Polygonaceae). *Watsonia* 23: 111–121.

HOLLINGSWORTH, M. L., J. P. BAILEY, P. M. HOLLINGSWORTH, AND C. FERRIS. 1999. Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). *Botanical Journal of the Linnean Society* 129: 139–154.

HOLLINGSWORTH, M. L., P. M. HOLLINGSWORTH, G. I. JENKINS, J. P. BAILEY, AND C. FERRIS. 1998. The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). *Molecular Ecology* 7: 1681–1691.

JAGER, C. 1994. Repartition, écologie et possibilités de contrôle de l'expansion de la renouée du Japon en Lorraine. Mémoire de Maîtrise, Université de Metz, Metz, France.

KIM, J. Y., AND C.-W. PARK. 2000. Morphological and chromosomal variation in *Fallopia* section *Reynoutria* (Polygonaceae) in Korea. *Brittonia* 52: 34–48.

LAMBINON, J., L. DELVOSALLE, AND J. DUVIGNEAUD. 2004. Nouvelle flore de la Belgique, du grand-duché de Luxembourg, du nord de la France et des régions voisines (Ptéridophytes et Spermaphytes). Jardin Botanique National de Belgique, Meise, Belgique.

LOPEZ, M. G., A. F. WULFF, L. POGGIO, AND C. C. XIFREDA. 2005. Chromosome numbers and meiotic studies in the species of *Senecio* (Asteraceae) from Argentina. *Botanical Journal of the Linnean Society* 148: 465–474.

MALLET, J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology and Evolution* 20: 229–237.

MANDÁK, B. B. C., K. B. E. BÍMOVÁ, P. PYŠEK, J. ŠTEPÁNEK, AND I. PLACKOVÁ. 2005. Isoenzyme diversity in *Reynoutria* (Polygonaceae) taxa: escape from sterility by hybridization. *Plant Systematics and Evolution* 253: 219–230.

MANDÁK, B. B. C., P. PYŠEK, M. LYSAK, J. SUDA, A. KRAHULCOVA, AND K. B. E. BÍMOVÁ. 2003. Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. *Annals of Botany* 92: 265–272.

MARUTA, E. 1976. Seedling establishment of *Polygonum cuspidatum* on Mount Fuji, Japan. *Journal of Ecology* 26: 101–105.

MÜLLER-SCHÄRER, H., U. SCHAFFNER, AND T. STEINGER. 2004. Evolution in invasive plants: implications for biological control. *Trends in Ecology and Evolution* 19: 417–422.

OZKAN, H., A. AVRAHAM, AND M. FELDMAN. 2001. Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops–Triticum*) group. *Plant Cell* 13: 1735–1747.

PASHLEY, C. H., J. P. BAILEY, AND C. FERRIS. 2003. Further evidence of the role of Dolgellau, Wales, in the production and dispersal of Japanese knotweed *s. l. In* L. Child, J. H. Brock, G. Brundu, K. Prach, P. Pyšek, M. P. Wade, and M. Williamson [eds.], Plant invasions: ecological threats and management solutions, 197–211. Backhuys Publishers, Leiden, Netherlands.

PASHLEY, C. H., J. P. BAILEY, AND C. FERRIS. 2007. Clonal diversity in British populations of the alien invasive giant knotweed, *Fallopia sachalinensis* (F. Schmidt) Ronse De Craene, in the context of European and Japanese plants. *Watsonia* 26: 359–371.

PEAKALL, R., AND P. E. SMOUSE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

PERSSON, H. A., AND B. A. GUSTAVSSON. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology* 10: 1385–1397.

PETIT, R. J. 2004. Biological invasions at the gene level. *Diversity and Distributions* 10: 159–165.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure from multilocus genotype data. *Genetics* 155: 945–959.

PYŠEK, P., J. H. BROCK, K. BÍMOVÁ, B. MANDÁK, V. JAROSÍK, I. KOUKOLÍKOVÁ, J. PERGL, AND J. STEPÁNEK. 2003. Vegetative regeneration in invasive *Reynoutria* (Polygonaceae) taxa: the determinant of invasibility at the genotype level. *American Journal of Botany* 90: 1487–1495.

SAKAI, A. K., F. W. ALLENDORF, J. S. HOLT, D. M. LODGE, J. MOLOFSKY, K. A. WITH, S. BAUGHMAN, R. J. CABIN, J. E. COHEN, N. C. ELLSTRAND, D. E. MCCAULEY, P. O'NEIL, I. M. PARKER, J. N. THOMPSON, AND S. G. WELLER. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332.

SALMON, A., M. L. AINOUCHE, AND J. F. WENDEL. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Molecular Ecology* 14: 1163–1175.

SIMBERLOFF, D. 2003. How much information on population biology is needed to manage introduced species? *Conservation Biology* 17: 83–92.

SOLTIS, D. E., P. S. SOLTIS, J. C. PIRES, A. KOVARIK, J. A. TATE, AND E. MAVRODIEV. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biological Journal of the Linnean Society* 82: 485–501.

TANAKA, H. 1966. The insect visitors of *Polygonum cuspidatum* Sieb. and Zucc. *Collecting and Breeding* 28: 141–143.

TIÉBRÉ, M.-S., S. VANDERHOEVEN, L. SAAD, AND G. MAHY. 2007. Hybridization and sexual reproduction in the invasive alien *Fallopia* (Polygonaceae) complex in Belgium. *Annals of Botany* 99: 193–203.

TORIMARU, T., N. TOMARU, N. NISHIMURA, AND S. YAMAMOTO. 2003. Clonal diversity and genetic differentiation in *Ilex leucoclada* M. patches in an old-growth beech forest. *Molecular Ecology* 12: 809–818.

VANDERHOEVEN, S., N. DASSONVILLE, AND P. MEERTS. 2005. Increased topsoil mineral nutrient concentrations under exotic invasive plants in Belgium. *Plant and Soil* 275: 169–179.

VERLOOVE, F. 2002. Ingeburgerde plantensoorten in Vlaanderen. Brussels. *Mededeling van het Instituut voor Natuurbehoud* 20.

VILÀ, M., E. WEBER, AND C. M. D'ANTONIO. 2000. Conservation implications of invasion by plant hybridization. *Biological Invasions* 2: 207–217.

VITOUSEK, P. M., C. M. D'ANTONIO, L. L. LOOPE, M. REJMANEK, AND R. WESTBROOKS. 1997. Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21: 1–16.

WEBER, E. 2003. Invasive plants in the world: a reference guide to environmental weeds. CABI Publishing, Wallingford, UK.

WILCOVE, D. S. 1998. Quantifying threats to imperiled species in the United States. *BioScience* 48: 607–615.

ZIKA, P. F., AND A. L. JACOBSON. 2003. An overlooked hybrid Japanese knotweed (*Polygonum cuspidatum* ×*sachalinense*; Polygonaceae) in North America. *Rhodora* 105: 143–152.

APPENDIX 1. List of *Fallopia* records from Belgium with locality, the street of the material collection, and the collection number. An em dash (—) indicates missing information. Voucher specimens are deposited at FUSAGx = Gembloux Agricultural University.

Taxon—Locality; *Voucher number*.

Fallopia aubertii (L. Henry) Holub—Jodoigne; Rue de la chapelle, surrounding wall; *P1415*. ***F. aubertii***—Malonne; Rue de Bransart, surrounding wall; *P 1422*. ***F. aubertii***—Malonne; Fond de Malonne, surrounding wall; *P 1510*. ***F. aubertii***—Malonne; Rue les Tris, surrounding wall; *P 1512*. ***F. aubertii***—Sauvenière; Rue du Stordoir, surrounding wall; *P1435*. ***F. aubertii***—Sauvenière; Rue du Try al Vigne, surrounding wall; *P1434*.

Fallopia ×bohemica (Chrtek et Chrtková) J.P. Bailey—Jodoigne; Rue de Septembre, near the cycle track; *P1411*. ***F. ×bohemica***—Jodoigne; Rue de l'église, near the cycle track; *P1412*. ***F. ×bohemica***—Wepion; —, near the alluvial forest; *P1424*. ***F. ×bohemica***—Beez; Avenue Reine Elizabeth, roadside near the Meuse River; *P1442*. ***F. ×bohemica***—Bouge; Rue des Baliveaux, near the sports center; *P1444*. ***F. ×bohemica***—Bouge; Rue des Baliveaux, near the sports center; *P1445*. ***F. ×bohemica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River ; *P1427*. ***F. ×bohemica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River; *P1428*. ***F. ×bohemica***—Sauvenière; Rue du Trichon, roadside near the sand quarry; *P1446*. ***F. ×bohemica***—Sauvenière; Chaussée de Tirlemont, roadside near the alluvial forest; *P1448*. ***F. ×bohemica***—Sauvenière; Chaussée de Tirlemont, roadside near the alluvial forest; *P1451*. ***F. ×bohemica***—Sauvenière; Rue du Trichon, roadside near the sand quarry; *P1454*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1455*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1456*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1457*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1458*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1460a*. ***F. ×bohemica***—Bruxelles; Parc de la Heronniere, —; *P1460b*. ***F. ×bohemica***—Bruxelles; Avenue de la Houlette, near the roadside; *P1464*. ***F. ×bohemica***—Bruxelles; Avenue du Gui, near the Verrewinkel cemetery; *P1466*. ***F. ×bohemica***—Bruxelles; Avenue du Gui, near the Verrewinkel cemetery; *P1467*. ***F. ×bohemica***—Uccle; Avenue Houzeau, near the observation post; *P1470*. ***F. ×bohemica***—Bruxelles; Dreve du Rouge Cloitre, at the Massart garden; *P1471*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1473*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1476a*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1476b*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1482*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1483*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1484*. ***F. ×bohemica***—Comblain-au-Pont, near the Ourthe River; Quai de la Cité; *P1487*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1488*. ***F. ×bohemica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1489*. ***F. ×bohemica***—Ceroux-Mousty; Rue du Puits, near the roadside; *P1499*.

Fallopia japonica* var. *japonica (Houtt.) Ronse De Craene—Jodoigne; Rue de Septembre, near the cycle track; *P1413*. ***F. japonica* var. *japonica***—Jodoigne; Rue de Septembre, near the cycle track; *P1414a*. ***F. japonica* var. *japonica***—Jodoigne; Rue de Septembre, near the cycle track; *P1414b*. ***F. japonica* var. *japonica***—Salzinnes; Avenue de Woitrin, near the roadside; *P1416*. ***F. japonica* var. *japonica***—Salzinnes; Avenue de Woitrin, between Salzinnes and Flawinne; *P1418*. ***F. japonica* var. *japonica***—Malonne; Rue de Navinne, garden near the restaurant Alain Peters; *P1421*. ***F. japonica* var. *japonica***—Wepion; —, roadside near the forest; *P1423*. ***F. japonica* var. *japonica***—Wepion; Rue des Chênes, roadside near the alluvial forest; *P1425*. ***F. japonica* var. *japonica***—Jambes; —, near the Perré bank; *P1436*. ***F. japonica* var. *japonica***—Jambes; —, —; *P1437a*. ***F. japonica* var. *japonica***—Jambes; —, —; *P1437b*. ***F. japonica* var.**—Beez; Avenue Reine Elizabeth, roadside near the Meuse River; *P1440*. ***F. japonica* var. *japonica***—Beez; Avenue Reine Elizabeth, roadside near the Meuse River; *P1441*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River; *P1429*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River; *P1430*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River; *P1431*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Pont des Pages, roadside near the Orneau River; *P1432*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Trichon, near the Orneau River; *P1447*. ***F. japonica* var. *japonica***—Sauvenière; Chaussée de Tirlemont, roadside near the alluvial forest; *P1449*. ***F. japonica* var. *japonica***—Sauvenière; Chaussée de Tirlemont, at the beginning of the street near the alluvial forest; *P1450*. ***F. japonica* var. *japonica***—Sauvenière; Chaussée de Tirlemont, at the beginning of the street near the alluvial forest; *P1451*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Trichon, near the sand quarry; *P1452*. ***F. japonica* var. *japonica***—Sauvenière; Rue du Trichon, near the sand quarry; *P1453*. ***F. japonica* var. *japonica***—Bruxelles; Parc de la Heronniere, near the play area; *P1462*. ***F. japonica* var. *japonica***—Bruxelles; Parc de la Heronniere, Avenue des Gerfauts; *P1463*. ***F. japonica* var. *japonica***—Bruxelles; Avenue du Gui, near the Verrewinkel cemetery; *P1465*. ***F. japonica* var. *japonica***—Bruxelles; Avenue du Gui, near the Verrewinkel cemetery; *P1469*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1474*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1475*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1477*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1478*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1479*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1480*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1481*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1485*. ***F. japonica* var. *japonica***—Comblain-au-Pont; Quai de la Cité, near the Ourthe River; *P1486*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue des Franquenies, near the roadside; *P1490*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Monument, near the railways; *P1491*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Monument, near the railways; *P1492*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Monument, near the railways; *P1493*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Berthet, near the roadside; *P1494*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Berthet, near the roadside; *P1495*. ***F. japonica* var. *japonica***—Céroux-Mousty; Rue du Commerce, near the roadside; *P1500*.

Fallopia sachalinensis (F. Schmidt Petrop.) Ronse De Craene—Wepion; —, near the auction room; *P1426*. ***F. sachalinensis***—Gembloux; —, at the Gembloux Agricultural University near the botanical garden; *P1496*. ***F. sachalinensis***—Bruxelles; Parc de la Heronniere, —; *P1459*. ***F. sachalinensis***—Bruxelles; Avenue du Gui, near the Verrewinkel cemetery; *P1468*. ***F. sachalinensis***—Bruxelles; Dreve du Rouge Cloitre, at the Massart garden; *P1472*. ***F. sachalinensis***—Kelmis; Rue des Tilleuls, near the calamine site; *P1497*.