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Biology and control of the invasive *Fallopia* taxa

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Preface

This thesis was written at the Norwegian University of Life Sciences, Department of Plant Sciences (IPV). Lab and greenhouse/garden experiments were carried out at Bioforsk Plant Health in Ås. Supervisors of the thesis are Lars Olav Brandsæter (Associate Professor at NMBU and researcher in weed science at Bioforsk Plant Health, Ås) and Helge Sjursen, (researcher in weed science at Bioforsk Plant Health, Ås). Experiment 1 was made possible through generous financial support from the Norwegian Public Roads Administration.

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Anne-Kari Holm

Abstract

Fallopia japonica and *Fallopia sachalinensis* are perennial, rhizomatous plants, native to East Asia, and introduced to Europe in the mid 1800's. Hybridization between the taxa in the introduced range has given rise to the hybrid *F.* x *bohemica*. The taxa reproduce mainly vegetatively in Europe, but sexual reproduction occurs. Disturbance and spread of rhizome and stem fragments by human activities and waterstreams are considered the most important means of spread. The taxa are among the most problematic introduced weeds in Europe and North America, especially in ruderal and riparian habitats. The taxa can greatly reduce native biodiversity and damage roads and constructions. The rhizomes have a high tolerance to both mechanical and chemical control methods, which makes eradication time-demanding and costly. The need for improved control strategies is critical. This thesis aims to increase knowledge about the biology of the taxa, and contribute to more effective control methods. Part I of the thesis is a literature study of the biology and control of the invasive *Fallopia* taxa. Part II consists of four experiments: **1)** The distribution of the taxa was assessed in five areas in Norway. Morphological and molecular methods (simple sequence repeats analysis, SSR) were used for taxonomic identification. Ploidy levels were determined by flow cytometry. Sequencing (DNA barcoding) of the *matK* region and the ITS region was assessed as a tool for identification of the taxa. **Results:** *F. japonica* was the most frequent taxon, but *F.* x *bohemica* was more frequent than previously recorded in the study area. *F. sachalinensis* was rare. The taxa could be distinguished by morphological means, and the SSR analysis supported the morphological identification. Sequencing of the *matK* and ITS region could not be used to distinguish *F. japonica* and *F.* x *bohemica*, but the ITS region appears to be useful for distinguishing *F. sachalinensis* from the other taxa. Ploidy levels were octoploid *F. japonica*, tetraploid *F. sachalinensis* and hexaploid *F.* x *bohemica*. **2)** The biomass allocation pattern in *F. japonica* and *F.* x *bohemica* was examined through harvests of above- and belowground biomass of experimental plants at different times of the growing season. **Results:** A shift in the allocation was found in June, when allocation to aboveground parts decreased and allocation to belowground parts increased. *F.* x *bohemica* had a greater aboveground and belowground biomass than *F. japonica*. **3.1)** Seasonal changes in the shoot regrowth potential of *F.* x *bohemica* was examined through single cuttings throughout the growing season. **Results:** A seasonal decline in sprouting was found from June until September, when little to no regrowth occurred. **3.2)** The effect of covering on the shoot regrowth potential was examined through covering stands with thick plastic for different time-lengths. **Results:** Three years of covering resulted in no new shoot growth.

Sammendrag

Parkslirekne (*Fallopia japonica*) og kjempeslirekne (*Fallopia sachalinensis*), også kalt de store slirekneartene, er flerårige, rhizomatiske planter, stedegne i Øst Asia og innført til Europa på 1800-tallet. Hybridisering mellom parkslirekne og kjempeslirekne har gitt opphav til hybriden *Fallopia* x *bohemica* (hybridslirekne). Reproduksjon er i hovedsak vegetativ i Europa, men frøformering forekommer. Fragmenter av jordstengler og stengler kan regenerere til nye planter, og spres bl.a. ved flytting av jord, spredning av avkapp, og med vannstrømmer. Artene er blant de mest problematiske fremmede invaderende artene i Europa, særlig i ruderale områder og langs elver og vassdrag. Et stort underjordisk nettverk av rhizomer gjør det vanskelig å bekjempe etablerte bestand, både med kjemiske og mekaniske metoder. De store slirekneartene utkonkurrerer stedegne arter og kan gjøre skade på veier og konstruksjoner. Det er et stort behov for økt kunnskap om artenes biologi og for mer effektive kontroll tiltak. Denne masteroppgaven har som mål å bidra til dette, og består av to deler: Del I er et litteraturstudie av de store slirekneartenes biologi og metoder for bekjempelse. Del II består av fire eksperimentelle forsøk: **1)** Utbredelsen av de tre artene ble undersøkt i fem ulike områder i Norge. En kombinasjon av morfologisk karakterisering og molekylære metoder ble brukt for taksonomisk identifisering. Ploiditeten til artene ble undersøkt ved hjelp av flow cytometry. **Resultater**: Parkslirekne var mest utbredt av de tre artene, men hybriden var mer utbredt enn det som tidligere var blitt registrert i områdene. Kjempeslirekne var sjelden. Ploiditeten hos artene var oktoploid parkslirekne, tetraploid kjempeslirekne og heksaploid hybridslirekne. **Forsøk 2)** Vekst og biomasse-allokering i parkslirekne og hybridslirekne ble studert gjennom et vekstforsøk med destruktive høstinger av overjordisk og underjordisk biomasse til ulike tider i vekstsesongen. **Resultater**: Allokering var størst til overjordisk vekst inntil i juni, da allokering til underjordiske deler økte. Hybridslirekne hadde en større overjordisk og underjordisk biomasse enn parkslirekne. **Forsøk 3.1)** Evnen til å produsere nye skudd gjennom vekstsesongen ble studert gjennom nedkutting og måling av gjenvekst til ulike tider i sesongen. **Resultater:** Skuddskytingsevnen avtok fra juni og utover i sesongen. Få eller ingen skudd ble produsert i september. **Forsøk 3.2)** Effekten av tildekking på evnen til å produsere nye skudd ble studert ved å dekke bestand med vevd plastduk i ulike tidslengder. **Resultater**: Tre år med tildekking førte til ingen ny gjenvekst. Avdekking tidlig i sesongen ga mer gjenvekst enn avdekking seint i sesongen.

Contents

1 Part I (Literature review)

1.1 Classification and nomenclature

The taxa with which this thesis is concerned are *Fallopia japonica* var. *japonica* (Houtt.) Ronse de Craene, *Fallopia sachalinensis* (F. Schmidt ex Maxim) Ronse de Craene, and the hybrid between them, *Fallopia* x *bohemica* (Chrtek and Chrtková) J. Bailey. The taxa are herbaceous, perennial, rhizomatous plants in the family Polygonaceae, genus *Fallopia,* section *Reynoutria* (Bailey, 1989). The taxa were previously classified to the genus *Reynoutria* (*Reynoutria japonica*, *Reynoutria sachalinensis*, *Reynoutria* x *bohemica*) and to the genus *Polygonum (Polygonum cuspidatum, Polygonum sachalinense*). Ronse de Craene and Akeroyd merged the genera *Reynoutria* and *Fallopia* in 1988 (Bailey, 1989). Arguments were the common morphological features, e.g. flower anatomy and extra floral nectaries on the petiole (Bailey et al., 2009). This classification is supported by the readily hybridization between the taxa and *Fallopia baldschuanica* (Bailey, 1989), and phylogenetic studies of the *rbcL* region (Frye and Kron 2003, cited in Bailey et al., 2007). English names for the taxa are, respectively, Japanese knotweed, Giant knotweed (Bailey, 2013) and Bohemian knotweed or hybrid knotweed (NNSS, 2014; Environment Agency, 2013). Norwegian names for the taxa are parkslirekne, kjempeslirekne and hybridslirekne (Artsdatabanken, 2012). This thesis uses the names *F. japonica*, *F. sachalinensis* and *F.* x *bohemica*, and the taxa are referred to as the invasive *Fallopia* taxa.

1.2 Introduction history

F. japonica and *F. sachalinensis* were introduced to Europe from East Asia in the mid 1800's. *F. japonica* is native to Japan, Taiwan and Northern China. It was brought from Japan to Leiden, The Netherlands, by Philipp von Siebold around the 1820's -1840's, and was made commercially available in 1846 under the name *Polygonum sieboldii*. A Chinese variety of *F. japonica* was introduced to the UK in 1825, but it did not thrive, and it is unlikely that any plants from this introduction have survived. Siebold's *F. japonica* was sent to the Royal Botanic Gardens Kew in England in 1850 (Bailey and Conolly, 2000). The introduction routes of these taxa to Norway are not known. *F. japonica* was first described in Norway by the botanist Frederik Schübeler in 1883. At this time, it had been cultivated in the Oslo region for several years, and had been spread by Schübeler to Nordland County in

northern Norway (Fremstad and Elven, 1997). The oldest herbarium specimen of *F. japonica* in Norway is from Granvin in Hordaland County, dated 1901 (Fremstad and Elven, 1997).

F. sachalinensis is native to southern Sakhalin and northern Japan. It was introduced from Japan to St. Petersburg, Russia in the early 1860's. It appears to be introduced as a forage plant and subsequently valued as an ornamental plant (Conolly, 1977). The history of *F. sachalinensis* in Norway is not well known, but the oldest herbarium specimen is from Porsgrunn in Telemark County, dated 1935 (Fremstad and Elven, 1997).

F. x *bohemica* was first described in Czechoslovakia in 1983, but herbarium specimens show that this taxon was present in a botanical garden in the UK already in 1872. A herbarium specimen dated 1911 came from a planted individual that had been grown from rhizomes provided by a plant nursery. This shows that a nursery distributed the hybrid at an early date. The first record of naturalised *F.* x *bohemica* in the UK is from 1954 (Bailey and Wisskirchen, 2004). The oldest herbarium specimen of *F. x bohemica* in Norway is from 1964, from Hareid in Møre og Romsdal County (Fremstad and Elven, 1997), but it is likely that this taxon have been introduced at an earlier time. The name *F. sachalinensis* has often been used also for *F.* x *bohemica*, which makes it difficult to know what taxon is actually discussed in the Norwegian and Nordic literature and what is the true, taxonomic identity of old herbarium specimens. Unfortunately, old herbarium specimens often consist of leaves from the upper part of the stem, which are less useable for identification (Handeland, 1991). *F.* x *bohemica* was recorded in Japan for the first time in 1997. The reason for the late finding of *F.* x *bohemica* in Japan could be that the parental species may normally not be sympatric in their native range, and if they are, the hybrid progeny may be poorly adapted. *F.* x *bohemica* has also been found in an area in Japan where the parental species were brought together due to planting of *F. japonica* as a soil stabilizer on road embankments (Bailey, 2003).

1.3 Distribution in the introduced range

F. japonica is widespread in Europe, USA and Canada (Pysek, 2006). It is also spread in New Zealand, but have a more limited distribution in Australia (Ainsworth and Weiss, 2002). *F. japonica* is cultivated as an ornamental plant in southern Chile, but not reported as invasive in natural environments (Saldana et al., 2009). *F. sachalinensis* has a distribution similar to that of *F. japonica*, but is generally distributed in lower numbers, and appears to be less invasive than *F. japonica. F. sachalinensis* is also recorded in South-Africa (Bailey, 2003; Bailey and Wisskirchen, 2004; Mandak et al., 2004; Tiebre et al., 2008). *F.* x *bohemica* is widespread in many European countries, but seems to constitute an increasing part of the invasive *Fallopia* populations the further southeast you go in Europe. *F.* x *bohemica* is widespread in the USA and Canada, where it in some areas constitutes a major part of invasive *Fallopia* populations (Bailey and Wisskirchen, 2004; Pysek, 2006; CABI, 2014; Gillies, S. L., n.d.). Gaskin et al. (2014) examined the genetic diversity of invasive *Fallopia* populations in western North-America, and found that *F.* x *bohemica* was the most common taxon, representing 71% of the sampled plants. *F.* x *bohemica* is also recorded in Australia and New Zealand, and is naturalized in southern Japan (Bailey and Wisskirchen, 2004; Pysek, 2006).

F. japonica, *F. sachalinensis* and *F.* x *bohemica* are widely spread in Norway and are listed as invasive species with a severe ecological impact on native biodiversity by Artsdatabanken (the Norwegian Biodiversity Information Centre) (Gederaas et al., 2012). *F. japonica* is the most frequent of the three taxa (Fremstad and Elven, 1997), but *F.* x *bohemica* can be locally more common than *F. Japonica* in some places (Artsdatabanken, 2012). *F. japonica* occurs in a broad belt along the coast from southeast Norway and north to Tromsø (69°N). It is less distributed in the inland parts of the country. Tromsø is also the northernmost recording for *F. sachalinensis* and *F.* x *bohemica*. *F. sachalinensis* has two main distribution areas, in southern parts of east Norway and in the fjord regions of Trøndelag, mid Norway. *F.* x *bohemica* has a distribution pattern similar to *F. sachalinensis*, but is also spread along the coast in Nordland County (Figure 1) (Fremstad and Elven, 1997).

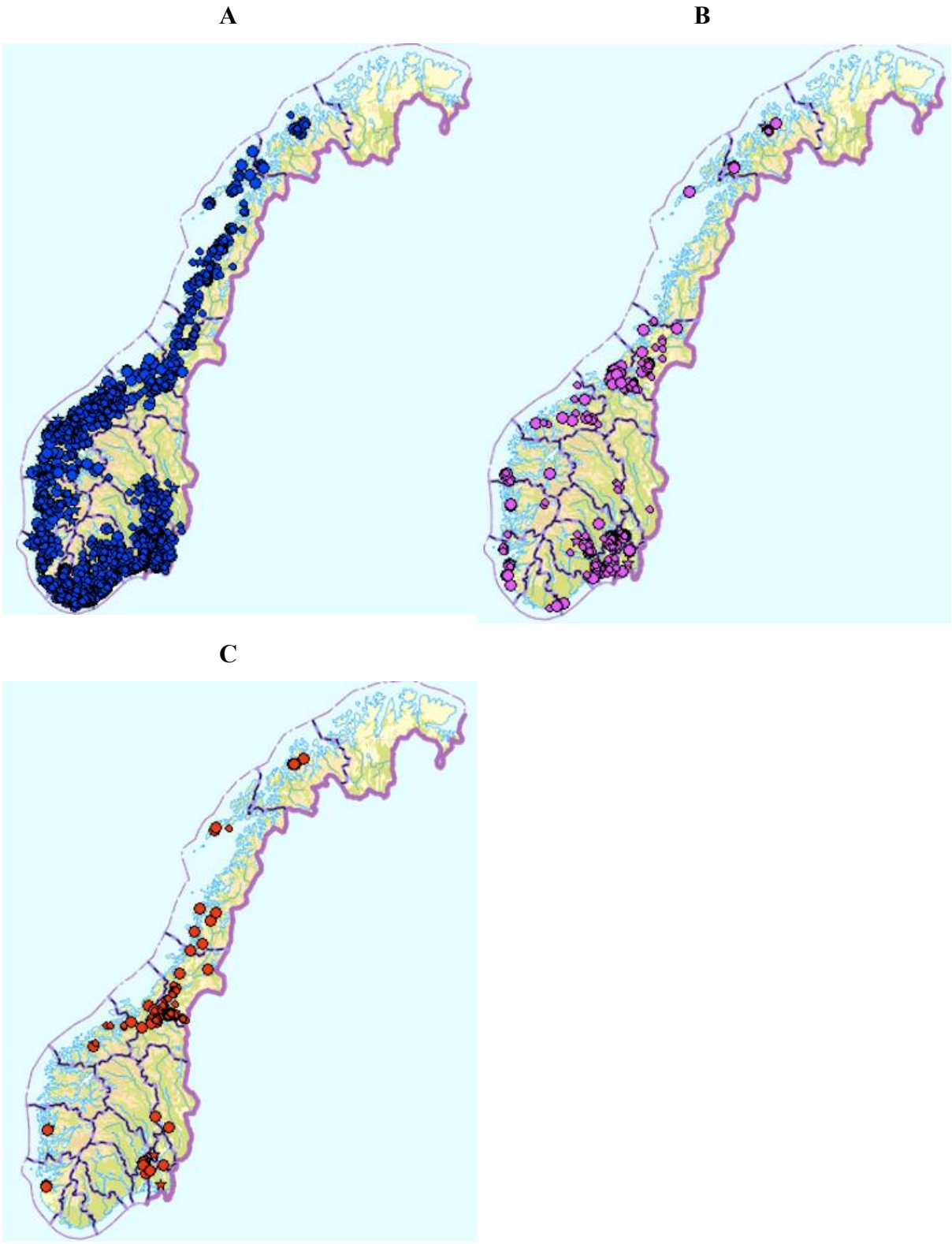


Figure 1 Recorded distribution of **A.** *F. japonica*, **B.** *F. sachalinensis* and **C.** *F.* x *bohemica* in Norway in 2014 (http://artsobservasjoner.no/vekster/, accessed 5.10.2014).

1.4 Description and identification of the taxa

1.4.1 Aboveground structure

Invasive *Fallopia* can grow in small patches, from dense, monospecific stands that may cover several acres, or 200 m long linear stands along rivers and shorelines (Beerling et al., 1994; Knotweeds IPM Profile, 2004). Shoots can reach heights from over 3 m in *F. japonica* to 4-5 m in *F. sachalinensis* in one season, and height growth up to 15 cm/day is reported for *F. japonica* (Pergl, 2001, cited in Bailey, 2009). When young shoots emerge early in the season, they have a green to red/purple colour and rolled back leaves. As the shoot extends, the leaves unfold (Figure 3) (Child and Wade, 2000). Full grown shoots are upright with arching tops and branches. Stems are hollow with distinct nodes (Figure 2), are semi-woody and contain high concentrations of lignin and hemicellulose (Callaghan et al., 1981; Child and Wade, 2000). Dead stems remain erect after shoot senescence in the autumn (Figure 2). They decompose slowly and can persist for 2-3 years (Child and Wade, 2000). Shoot density varies, but up to 70 shoots pr. m^2 at maximum shoot density in July is recorded in *F. japonica* (Callaghan et al., 1981; De Waal, 2001). Leaves and branches arise from the nodes and are alternately arranged (Child and Wade, 2000). Twigs form a zig-zag pattern on the upper branches, which reduces self-shading of individual leaves on the same shoot. Leaf length range from up to 20 cm in *F. japonica* (Handeland, 1991) to 45 cm in *F. sachalinensis*. The foliage forms a dense canopy (Figure 2) (Beerling, 1990; Child and Wade, 2000; Bailey and Wisskirchen, 2004). Papillae or trichomes are present on the nerves on the lower side of the leaves. Extrafloral nectaries are located on the underside of the base of the petiole and are reported to attract ants (Beerling et al., 1994). Flowers are small, white and borne in clusters arising from the point of the angle between the stem and a leaf (Child and Wade, 2000; Bailey and Wisskirchen, 2004). The fruit is a trigonous, dark brown and shiny achene, 2-4 mm long and 2 mm wide in *F. japonica*, and is enclosed in a winged, enlarged perianth (Beerling et al., 1994).

1.4.2 Belowground structure

Descriptions of the belowground structure and its development mostly refers to *F. japonica*, and is often based on the studies by Adachi et al. (1996) in Mt. Fuji, Japan. It has however been concluded that the native variety studied by Adachi et al. (1996) is closer to the dwarf variety *F. japonica* var. *compacta* than the *F. japonica var. japonica*, which is invasive in

Europe. The observations made by Adachi et al. (1996) may therefore not always be transferable to *F. japonica* in Europe (Smith et al., 2007). Plants originating from seeds have a deep tap root (Barney et al., 2006; Adachi et al., 1996). It grows directly downwards in or near the centre of a patch, and rhizomes grow outwards from the basal part of the tap root (Adachi et al., 1996). The rhizomes of *F. japonica* can extend 2 m deep and 7 m away from the shoots, and are 0.5 – 10 cm in diameter (Child and Wade, 2000). A rhizome depth of 2 m is also reported for *F. sachalinensis* (Marigo and Pautou, 1998).

Smith et al. (2007) found that 95% of the rhizome segments (the part of a rhizome between two branching points or between a terminus and a branching point) were 0.6 – 41 cm long, but segments up to 81 cm were found. New rhizomes are white and fleshy, while older rhizomes are woody with a reddish or dark brown coloured outside (Figure 6), and a distinct orange colour on the inside (Child and Wade, 2000). Rhizomes have a thick, hard, suburized cortex, except at its apex. The apex eventually develops into a new aerial shoot. New shoots are produced in almost the same position every year, and a cluster of shoots, called a "shoot clump" or "crown", is formed (Figure 4 - Figure 5) (Adachi et al., 1996; Bashtanova et al., 2009). Not every rhizome produces a shoot clump (Smith et al., 2007). Dormant buds, called "lateral buds" or "rhizome buds", are located at most nodes along the rhizomes. In addition, there are latent buds within the cortex of the rhizomes (Figure 5). Adachi et al. (1996) reported that rhizome buds remain dormant until the shoot-clump ceases to produce shoots and die. In contrast, Dauer and Jongejans (2013) observed new shoots connected to shoot-clumps in all investigated stands. It is not known exactly how long a shoot-clump lives, but 6 years or more in their native range and far longer than 5 years in their introduced range is reported (Adachi et al., 1996; Smith et al., 2007). Shoot clump density is found to be 1-1.5 shoot clumps pr. m^2 in *F. japonica* (Smith et al., 2007), but this may differ between taxa. *F. japonica* has quite large shoot clumps connected by long, thin rhizomes, while *F. sachalinensis* has smaller shoot clumps that are more closely connected and grow in rows. *F.* x *bohemica* is intermediate with smaller shoot clumps than *F. japonica* and longer rhizomes than *F. sachalinensis* (Bailey et al., 2009). The native variety studied by Adachi et al. (1996) has a rhizome growth pattern that leads to decreased shoot density in the centre as a patch expands (Adachi et al., 1996). This pattern is generally not found in the introduced range (Dassonville et al., 2007; Smith et al., 2007). Mummigatti (2007) observed many dead shoots in the centre of a large patch, and suggests central die-back may be the reason.

Figure 2 Aboveground structure**.** Upper picture: A dense canopy shades out other vegetation. *F. japonica* in Bergen, July 21, 2013. Lower picture: Tall, semi-woody, hollow shoots. *F.* x *bohemica* in Drammen, September 28, 2013 (left). Dead shoots remaining from last season, *F.* x *bohemica* in Ås, April 29, 2012 (right). Photo: Anne-Kari Holm.

Figure 3 Early shoots of *F. japonica* (left) and *F.* x *bohemica* (right). *F.* x *bohemica* has larger leaves with more cordate leaf bases. Pictures taken in Lørenskog. May 9, 2014. Photo: Anne-Kari Holm.

Figure 4 Belowground structure. *F. japonica* shoot clumps connected by rhizomes. New, white rhizome extends from shoot-producing shoot clump. Picture is taken in June, 2011. Photo: Anne-Kari Holm.

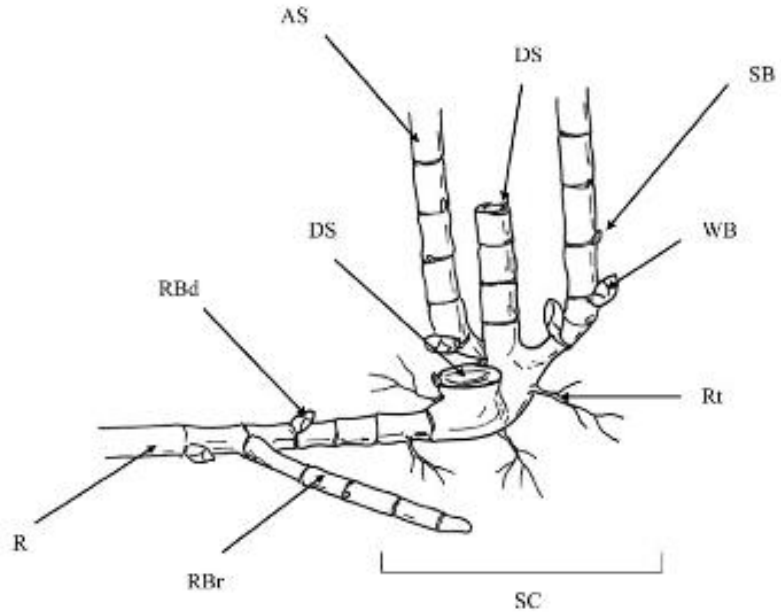


Figure 5 Structure of shoot-clump with rhizome branches. From Bailey et al. (2009). SC = shoot clump, AS = annual aerial shoot, DS = dead shoot, R = rhizome, RBr = rhizome branch, SB = stem bud, WB = winter bud, RBd = rhizome bud, Rt = root.

Figure 6 *F. japonica* rhizomes. Upper picture: Old, woody rhizome with aboveground shoot. Lower picture: New, fleshy rhizomes. The person in the picture is Lars Olav Brandsæter, main supervisor of this thesis. Photo: Anne-Kari Holm.

1.4.3 Morphological characters for identification

The main morphological characters used for distinguishing the three invasive *Fallopia* taxa are leaf size, shape of leaf base and leaf apex, and the morphology of the thricomes or papillae on the lower leaf surface (Handeland, 1991; Fremstad and Elven, 1997; Bailey and Wisskirchen, 2004). The best leaves to use for identification are from the lower part of the shoot (Child, 1999). A summary of some important diagnostic characters is found in Table 1.

Table 1: Some important characters for identification of the invasive *Fallopia* taxa. References: Bailey and Wisskirchen (2004), Fremstad and Elven (1997), Handeland (1991)

Character	***F. japonica***	***F.* x *bohemica***	***F. sachalinensis***
Shoots	1.5 – 3 m tall, densely redbrown spotted	2.5 – 4 m tall, redbrown spots	4 – 5 m, without spots
Leaf shape	Broadly ovate	Broadly ovate to narrow ovate. More similar to *F. japonica* than *F. sachalinensis,* but can be more oblong than *F. japonica*	Narrow ovate to elliptic oblong, evenly narrowing to apex
Leaf structure	Thick and leathery	Strong, but not leathery	Thin and soft
Leaf surface	Even	More similar to *F. sachalinensis* than to *F. japonica*	Dented/wrinkled
Leaf length	10 - 20 cm	20 - 35 cm	30 - 45 cm
Length:Width ratio	1 – 1.5	1 – 1.8	Ca. 1.5
Leaf base	Straight (truncate) or almost straight. Often with “sharp corners”	Straight (truncate) or cordate, weakly to moderately rounded at the base	Deeply cordate, ”lyre-shaped”
Leaf apex	Cuspidate	Cuspidate to acute	Short and acute
Lower leaf surface	Glabrous, but with short or elongated, blunt, sometimes rough and tooth-like, single-celled papillae	With short, stout hairs up to 0.5 mm long and 5-10 as long as wide.	With long, multi-celled, flexous hairs up to 1 mm long
Extrafloral pit nectaries	1 underneath the attachment site of leaf petiole	1 underneath the attachment site of leaf petiole (may also have + 1-4 smaller ones on both sides of the stem at node position)	1 underneath the attachment site of leaf petiole + 1-4 on both sides of the stem at node position
Inflorescence	Initially erect, but drooping at maturity, creamy white flowers	With medium sized branches, spreading stout to different directions (male-sterile) or with long, strictly upright branches (male-fertile) with white flowers	Short and nodding branches with greenish white flowers (male-sterile) or medium-sized branches with white flowers (male-fertile)

1.4.3.1 *Fallopia japonica*

Stems can reach over 3 m in height, are branched and with reddish spots. Leaf shape is broadly ovate with cuspidate apex and straight or almost straight, truncate base (Figure 10). Length of leaf blade is 10-18 cm (Bailey and Wisskirchen, 2004), or usually shorter than 20 cm (Handeland, 1991). Length:width ratio is 1-1.5 (Child and Wade, 2000). Leaf colour is green to yellow-green or glaucous (Beerling et al., 1994; Fremstad and Elven, 1997). Leaves are thick with a leathery feel to them and with an even surface (Beerling et al., 1994; Fremstad and Elven, 1997). The lower leaf surface is glabrous, with short or elongated, blunt, sometimes rough, single-celled papillae (Beerling et al., 1994; Fremstad and Elven, 1997; Bailey and Wisskirchen, 2004) (Figure 10). Cuticle surface on the lower side of leaf is smooth (Bailey et al., 2009). Only male-sterile plants are known in Europe (Bailey, 2013). Inflorescences are initially erect, but drooping at maturity (Figure 10). Flowers are creamy white, 2-3 mm in diameter with 5 tepals and 8 stamens, born on clusters in panicles (Beerling et al., 1994). A small stand of F. japonica is shown in Figure 7.

Figure 7 *Fallopia japonica*. Picture taken in Frogn, September 30, 2012. Photo: Anne-Kari Holm.

1.4.3.2 *Fallopia sachalinensis*

Stems can reach over 4-5 m in height. Stems are less branched than *F. japonica* and without the reddish spots found on *F. japonica*. Leaf shape is narrow ovate to elliptic oblong, narrowing evenly to apex. Leaf base is distinct cordate and apex is short and acute (Figure 11). Length of leaf blade is up to 30-45 cm, and width is 20-25 cm. Length:width ratio is ca. 1.5 (Handeland, 1991; Child and Wade, 2000; Bailey and Wisskirchen, 2004). Leaves have a matt green colour, are softer and thinner, and the leaf surface have a more crumpled appearance than in *F. japonica*. Trichomes on lower side of leaves can appear both on and between veins (Fremstad and Elven, 1997). They vary in length, but are up to 1 mm long, multi-celled, flexious and have an even width (Figure 11) (Fremstad and Elven, 1997; Bailey and Wisskirchen, 2004). Cuticle surface on the lower side of the leaf is more crumpled than in *F. japonica* (Bailey et al., 2009). In male-sterile plants, the flowers are greenish white, and the branches of the inflorescence are short and nodding, spreading in different directions (Figure 11). Male-fertile plants have white flowers in upright inflorescences with medium-sized branches. In addition to the single extrafloral pit nectaries beneath the attachment sites of the leaves, *F. sachalinensis* has 1-4 pairs of smaller extrafloral pit nectaries on both sides of the stem at the node position (Bailey and Wisskirchen, 2004). The *F. sachalinensis* shown in Figure 8 is not of full height, perhaps due to disturbance.

Figure 8 *Fallopia sachalinensis*. Picture taken in Drammen, September 28, 2013. Photo: Anne-Kari Holm.

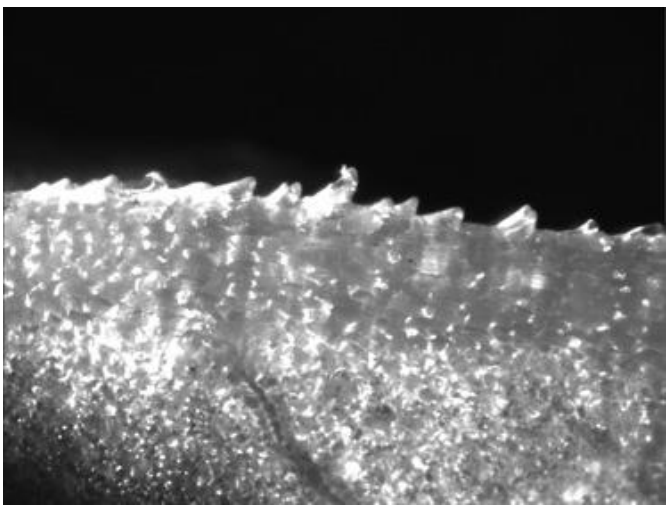
1.4.3.3 *Fallopia x bohemica*

Fallopia x *bohemica* is the hybrid between *F. japonica* and *F. sachalinensis*. Its morphology may be variable, and is intermediate compared to the parental taxa. Stems are up to 4 m high with redbrown spots. Leaf shape is broadly ovate to narrow ovate. Leaves are weakly to moderately rounded at the base, and leaf apex is cuspidate to acute (Figure 12). Length of leaf blade is 20-35 cm with a length:width ratio of 1-1.8 (Child and Wade, 2000; Bailey and Wisskirchen, 2004). Leaf colour and texture is described as being more similar to *F. sachalinensis*, while leaf shape is more similar to *F. japonica* (Fremstad and Elven, 1997). Leaves are strong, but not leathery. Trichomes on lower leaf-surface are short, stout and can be seen with a hand-lens. Trichomes can be up to 0.5 mm long and 5-10 as long as wide (Bailey and Wisskirchen, 2004). Trichome morphology can be variable, from short, blunt papillae to elongated, pointed trichomes with a wider base (Figure 12) (Fremstad and Elven, 1997, Bailey and Wisskirchen, 2004). Cuticle surface on the lower side of leaf is intermediate compared to the parental taxa (Bailey et al., 2009). Flowers are white in both sexes. Male-sterile plants have inflorescences with medium-sized branches, spreading stout in different directions, and male-fertile plants have inflorescences with long, strictly upright branches (Figure 12). Flowers are white in both sexes (Bailey and Wisskirchen, 2004).

Figure 9 *Fallopia* x *bohemica*. Picture taken in Drammen, September 28, 2013. Photo: Anne-Kari Holm.

›A

B



C

Figure 10 *Fallopia japonica* **A**. inflorescences (male-sterile), **B.** leaf, and **C.** papillae on lower leaf surface. Photo: Anne-Kari Holm.

A

B

C

D

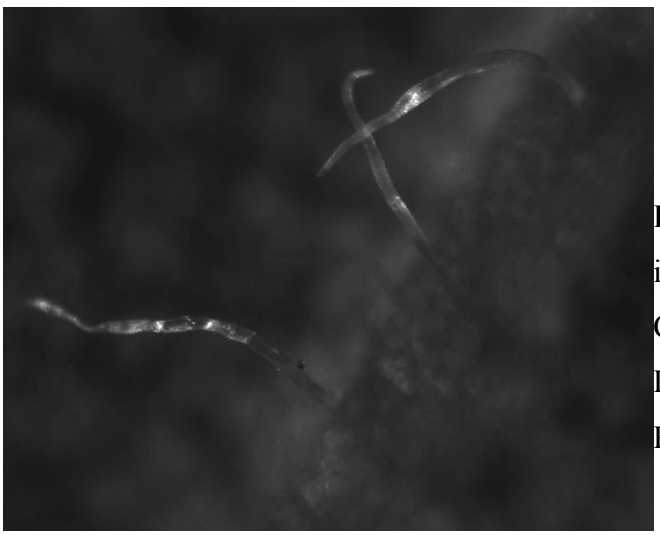


Figure 11 *Fallopia sachalinensis* A. inflorescence (male-sterile), B. leaf, C. additional extrafloral nectaries. D. trichomes.
Photo: Anne-Kari Holm

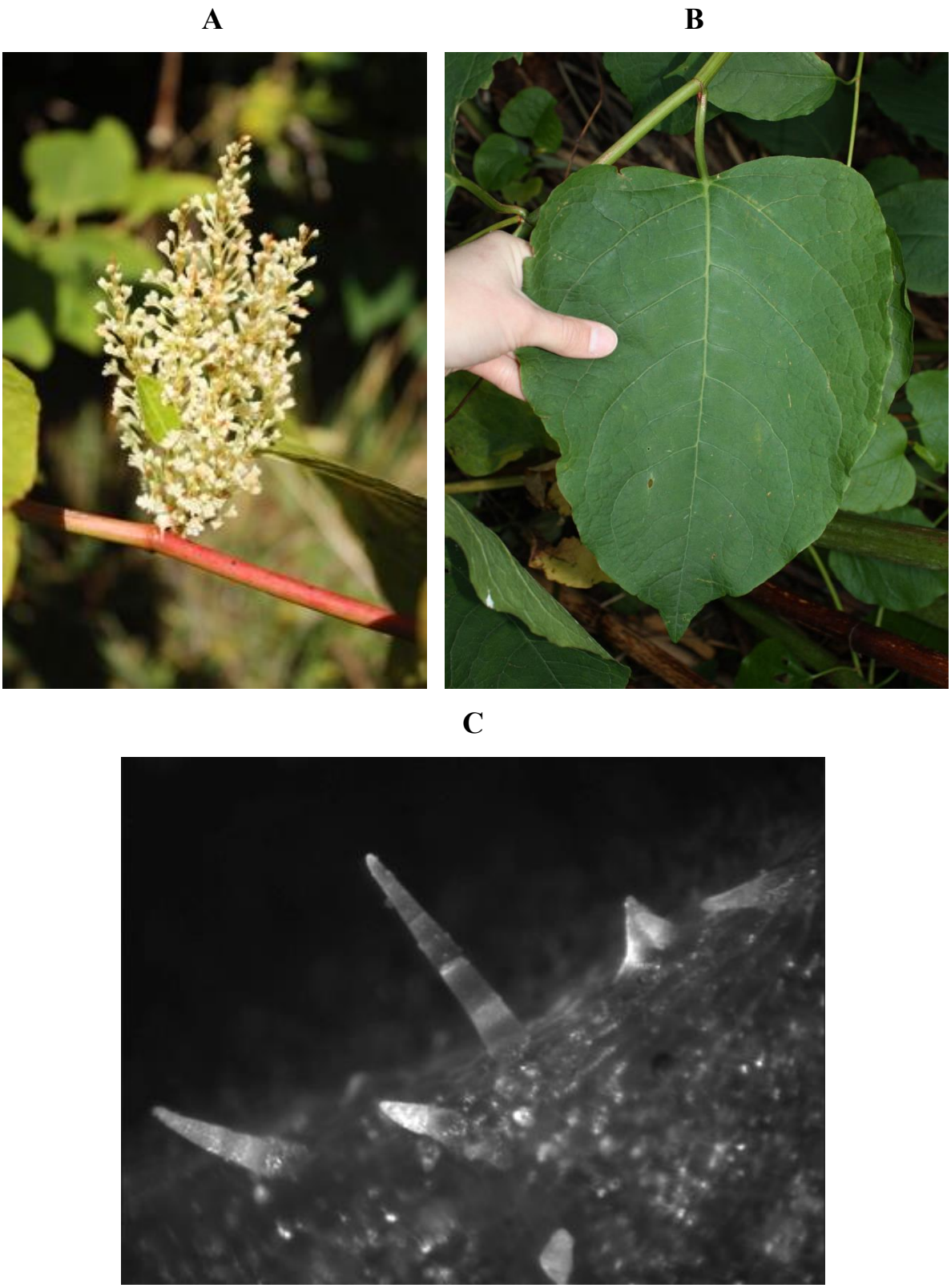


Figure 12 *Fallopia* x *bohemica* **A.** inflorescence (hermaphrodite) **B.** leaf and **C.** trichomes on lower leaf surface. Photo: Anne-Kari Holm.

1.5 Phenology

The invasive *Fallopia* taxa overwinter as roots and rhizomes in the ground, with buds just below the soil surface (Beerling et al., 1994). New shoots emerge in March-April, and growth is rapid until mid June (Price et al., 2001). As height growth reaches a plateau in June, branching of the main axis increases (Herpigny et al., 2012). Shoot density increases until June/July, and then decreases due to dieback of shoots that emerge late and remain beneath the canopy (Callaghan et al., 1981; Adachi et al., 1996). The photosynthetic canopy increases during June - August (Callaghan et al., 1981). From mid June, the allocation to aboveground growth decreases, and allocation to growth and storage in the rhizomes increases (Price et al., 2001). It is reported that production of new rhizomes starts in June or July (Dauer and Jongejans, 2013). Flowering occurs during August - October (Fremstad and Elven, 1997). Seed production is not known in Norway, probably limited by climatic conditions and availability of suitable pollen (Handeland, 1991; Fremstad and Elven, 1997). Aerial shoots produce subterranean winter buds at its base in the autumn before senescence. Small shoots and shoots that die during the growing season fail in producing winter buds (Adachi et al., 1996). Buds are also formed at the nodes along the rhizome (Child and Wade, 2000). During senescence, resources are transported from shoots to rhizomes, where they are effectively stored until the next growing season. The stored resources are remobilized to new growth the following spring (Price et al., 2001). New aerial shoots are then produced from one or more of the winter buds located at the base of last years shoot (Bailey et al., 2009).

1.6 Habitat

1.6.1 Native range

In its native range, the tall variety most similar to the *F. japonica* in Europe grows in lowland areas, and is mostly found at the edges of forests or on riversides in forests. It is also found in urban roadsides and along canalized rivers (Bailey, 2003). It can spread to man-made habitats and become one of the most problematic weeds in roadsides and pastures, especially where high amounts of nitrogen fertilizer is applied (Nashiki et al., 1986; Bailey et al., 2009). The highland variety, which is more similar to the variety *F. japonica var. compacta*, is found as a pioneer colonizer of lava fields in Japan, where it facilitates establishment of other species (Adachi et al., 1996; Bailey et al., 2009). *F. sachalinensis* in its native range is found along forest edges, along forest roads, in avalanche clearings in mountains, on coastal cliffs and

river banks and in uncultivated fields. It is also found in anthropogenic, disturbed habitats along roadsides and in human settlements. *F. sachalinensis* can also be an early successional species and facilitate establishment of vegetation on lava fields (Sukopp and Starfinger, 1995).

1.6.2 Introduced range

In their introduced range, the invasive *Fallopia* taxa are mainly found in ruderal, disturbed habitats. They are found on roadsides and railway sides, on the banks of rivers and lakes, on beaches and watersides, on waste grounds, vacant lots, within or expanding from gardens, in farmyards, parks and urban grasslands (Fremstad and Elven, 1997; Child and Wade, 2000; Bailey et al., 2009). Linear networks, like roads, railways, and rivers, seem to make up the most important habitats for these taxa (Mandak et al., 2004; Tiebre et al., 2008; Bailey et al., 2009). Tiebre et al. (2008) found that the majority of the individuals (91%) occurred within a 10 m buffer along communication routes. Stands established along rivers and roads may expand into neighbouring habitats (Palmer, 1994; Tiebre et al., 2008). In Norway, *F. japonica* is fond on beaches and shorelines, but are less recorded in river habitats than what is reported from other European countries (Fremstad and Elven, 1997). *F. japonica* and *F.* x *bohemica* are also reported to grow on beaches and in salt marshes in the UK and USA (Beerling, 1994; Richards, 2008; Walls, 2010). All three species occur in forest margins and open woodlands (Beerling et al., 1994; Fremstad and Elven, 1997). The taxa are rare on cultivated land and are not considered as agricultural weeds, but *F. japonica* is found to occupy areas in grazing pastures (Beerling et al., 1994; Beerling and Palmer, 1994; Child and Wade, 2000).

1.6.3 Environmental factors

F. japonica grows best in full sunlight and is affected by moderate shadow, but may be found in semi-shaded habitats (Beerling et al., 1994). *F. sachalinensis* and *F.* x *bohemica* are more shade tolerant than *F.* japonica, and *F. sachalinensis* is recorded growing well inside of forests (Fremstad and Elven, 1997). The three taxa can tolerate a wide range of soil-conditions. *F. japonica* is found on soils ranging from oligotrophic, acidic soils to calcareous or eutrophic soils. It can grow in loams, clays, peats, colliery spoil, alluvial soils, shingles and free-draining mineral-soils (Beerling et al., 1994; Dassonville et al., 2007). *F. japonica* can tolerate a soil pH ranging from 3 to 8.5 (Child and Wade, 2000). *F. sachalinensis* is reported

to grow on soils with somewhat higher pH than *F.* japonica (Ellenberg et al., 1991, cited in Fremstad and Elven, 1997). *F. sachalinensis* and *F.* x *bohemica* also seem to be more associated with more moist and fertile soils than *F. japonica* (Fremstad and Elven, 1997). *F. japonica* is tolerant to soil contamination of heavy metals and can grow in soils with high concentrations of Cu, Zn and Cd ions. A Cu-binding protein has been isolated (Kubota et al., 1988, cited in Beerling, 1990). It is also reported to grow on banks of rivers containing high amounts of Al, Fe, Mg and Zn (Johnson, 2007).

1.7 Reproduction

1.7.1 Sexual reproduction and genetic diversity

1.7.1.1 Flower morphology

F. japonica, F. sachalinensis and *F.* x *bohemica* are gynodioicious, which means that individuals can be either male-sterile (female) or hermaphrodite (Bailey, 1989). *F. sachalinensis* and *F.* x *bohemica* are found as both male-sterile and hermaphrodite individuals in Europe, while *F. japonica* has only been found as male-sterile in Europe (Hollingsworth and Bailey, 2000a; Bailey, 2013). Male-sterile plants have small, flattened anthers that are empty and included within the perianth. Male-sterile plants can produce thousands of seeds pr. stem if compatible pollen is available (Bailey, 1994), but do not produce viable pollen (Grimsby et al., 2007; Tiébré et al., 2007b). Hermaphrodites have flowers with well developed gynoecia, in addition to large anthers and long filaments (Bailey, 1989). Hermaphrodite plants produce seeds and pollen, but seed production is much lower than in male-sterile plants (Bailey, 1989; Bailey, 1994). The hermaphrodite plants are generally self-incompatible, but rare occasions of self fertilisation in absence of normally compatible pollen occur (Bailey, 1989). A third group may be called female-sterile plants. They have poorly developed gynoecia, lack stigmatal development, and have large anthers filled with pollen on long, excerted filaments (Bailey, 1989).

1.7.1.2 Seed production and seedling survival

Flowers are insect pollinated by flies, bees and wasps, and seeds are wind dispersed (Palmer, 1994; Child and Wade, 2000; Bailey et al., 2009). Seeds may be dispersed over 16 m, but most seeds fall near the maternal clone (Tiébré et al., 2007b). Rivers, roads, and rails

facilitate dispersal over longer distances (Engler et al., 2011). Apomictic seed production is not reported in these species (Hollingsworth and Bailey, 2000b).

The invasive *Fallopia* taxa are not known to produce seeds in Norway. The autumn frost comes too early in most Norwegian regions, and although some parts of the country have milder autumns with later frosts, it is considered unlikely that mature seeds would develop under current climatic conditions. Seed production may also be limited by the availability of suitable pollen (Handeland, 1991; Fremstad and Elven, 1997). Seed production is however common in many other European countries (Hollingsworth and Bailey, 2000b; Tiébré et al., 2007b; Funkenberg et al., 2012; Bailey, 2013).

While seeds germinate readily and produce viable plants when grown under experimental conditions, seedlings are not commonly found in nature (Bailey, 1994; Bailey et al., 2007; Tiébré et al., 2007b; Engler et al., 2011; Funkenberg et al., 2012). The reasons for the rarity of seedlings are not yet fully understood, but germination and seedling survival seem to be limited by environmental factors (Bailey et al., 2007; Engler et al., 2011; Funkenberg et al., 2012). Germination and seedling establishment is negatively affected by dry conditions, late spring frost, and competition for light (Forman and Kesseli, 2003; Funkenberg et al., 2012). Forman and Kesseli (2003) suggest that seedling survival depend on the availability of resources such as light and water rather than on temperature. One theory is that seeds are destroyed by fungi infections during wet and mild winters in Europe (Bailey and Spencer, 2003; Bailey et al., 2009). A large part of the seeds may also be eaten by birds (Bailey et al., 1995; Bailey et al., 2009; Engler et al., 2011). Engler et al. (2011) suggest that seeds have a non-deep physiological dormancy that creates a time lag between the natural dispersal and germination. A stratification treatment is not required for seed germination (Forman and Kesseli, 2003; Tiébré et al., 2007b). In regions with mild and changing winter temperatures, germination could start at a time when natural conditions would not support seedling establishment (Engler et al., 2011). Although seedlings are rare, they are found occasionally (Bailey and Child, 1996; Hollingsworth and Bailey, 2000b; Pashley et al., 2003; Engler et al., 2011; Funkenberg et al., 2012). High levels of genetic variation is found within *F.* x *bohemica* compared to the parental taxa, indicating that sexual reproduction and hybridization play an important role in the development of the populations (Hollingsworth and Bailey, 2000b; Mandak et al., 2005; Tiébré et al., 2007a; Krebs et al., 2010).

1.7.1.1 Hybridization and polyploidy

Since only male-sterile *F. japonica* is found in Europe, true *F. japonica* cannot be produced from seeds (Bailey et al., 2009). Except from a recent study that claims to have found different genotypes of *F. japonica* (Bzdƒôga et al., 2012), the results from several studies indicate that only one, widely spread male-sterile *F. japonica* genotype is present in Europe. *F. japonica* can however be pollinated by *F. sachalinensis*, *F. x bohemica,* or other related species to produce hybrid seeds. *F. sachalinensis* and *F.* x *bohemica* can reproduce sexually and hybridize with related species (Bailey et al. 2009).

Other taxa that are involved in the hybridization with the invasive *Fallopia* taxa are the dwarf variant *F. japonica* var. *compacta* and the commonly grown climbing ornamental plant, *F. baldschuanica*. There is only one known record of *F. japonica* var. *compacta* in Norway, which is in the Botanical garden at Milde in Hordaland County, western Norway (Handeland, 1991). *F. japonica* var. *compacta* is termed sub-diocious, and plants can be male-sterile and female-sterile. The female-sterile plants are known to occasionally produce seeds, which suggest that ovary development is not completely suppressed in all female-sterile flowers (Bailey, 1994). Both male-sterile and male-fertile plants are present in the Botanical garden at Minde in Norway, and seed production is observed (Handeland, 1991). In the native range, there is continuous morphological variation between *F. japonica var. japonica* and *F. japonica* var. *compacta*. The clear differences between the two varieties in the introduced range is a result of bottleneck effects (Mandak, 2003). *F. japonica* var. *compacta* is much more rare in the introduced range than the other, invasive, *Fallopia* taxa (Hollingsworth and Bailey, 2000; Bimova et al., 2001; Mandak et al., 2003; Tiebre, Bizoux, Hardy et al., 2007). *F. japonica* var. *compacta* is however found to have a vegetative regeneration potential not inferior to its invasive relatives. It may be that this taxon is still in a lag-phase of invasion due to the lack of vegetative propagules (Bimova et al., 2003).

Most seeds produced by *F. japonica* in Europe result from pollination by *F. baldschuanica* or by hexaploid *F.* x *bohemica* (Tiébré et al., 2007b; Bailey et al., 2009; Funkenberg et al., 2012). The hybrid resulting from crossing between *F. japonica* and *F. baldschuanica* is named *F.* x *conollyana*, and only a few establishments in nature are known. Interestingly, one of these recordings are from Stavanger, Norway, where it has arisen spontaneously in a garden (Fremstad and Elven, 1997). The finding of *F.* x *conollyana* in Stavanger could mean

that seed production in *F. japonica* may occur under some conditions also in Norway. Another explanation could be that seeds have been unintentionally introduced from abroad. *F.* x *conollyana* has thinner stems and smaller leaves than *F. japonica*, and stems bend over, almost to touch the ground. Established plants have rhizomes, but seems to be weak competitors (Bailey et al., 2007). *F.* x *conollyana* has irregular meiosis and is probably sterile, and seed set is not reported (Bailey, 1989). *F. baldschuanica* can also pollinate other members of the *Fallopia* complex, but these hybrids are only recovered from open pollinated seeds and are not found established in nature (Bailey, 2013). Known hybridization routes within the *Fallopia* complex are shown in Figure 13.

The invasive *Fallopia* taxa are polyploid organisms, which means they have more than two multiples of the haploid chromosomeset (Bailey et al., 2007; Klug et al., 2007). Polyploidy can originate in two ways. Either through the addition of one or more extra sets of chromosomes that are identical to the normal, haploid chromosome set of the same species (autoployploidy) or through the combination of chromosome sets from different species as a consequence of hybridization (allopolyploidy) (Klug et al., 2007). *F. japonica* is only found as octoploid (8X) in its introduced range. *F.* x *bohemica* can be hexaploid (6X), tetraploid (4X) or octoploid (8X). Aneuploid individuals, individuals with uncomplete haploid sets of chromosomes (Klug et al., 2007), and a single decaploid are also found (Bailey et al., 2009, Mandak et al., 2003). The most common *F. x bohemica* is hexaploid, and is formed by pollination of octoploid *F. japonica* by tetraploid *F. sachalinensis*. Hexaploid *F.* x *bohemica* is also recorded in Japan (Mandak et al., 2003; Bailey et al., 2007). The dwarf variant *F. japonica* var. *compacta* is only found as tetraploid in both its native and its introduced range. *F. japonica* var. *compacta* and tetraploid *F. sachalinensis* may both be the male or female parent in the tetraploid *F. x bohemica.* The origins of octoploid *F.* x *bohemica* are less clear, but may involve chromosome doubling in tetraploid *F.* x *bohemica*, unreduced gametes from hexaploid *F.* x *bohemica*, or the fertilization of octoploid *F. japonica* by an unreduced gamete of tetraploid *F. sachalinensis* (Bailey et al., 2007).

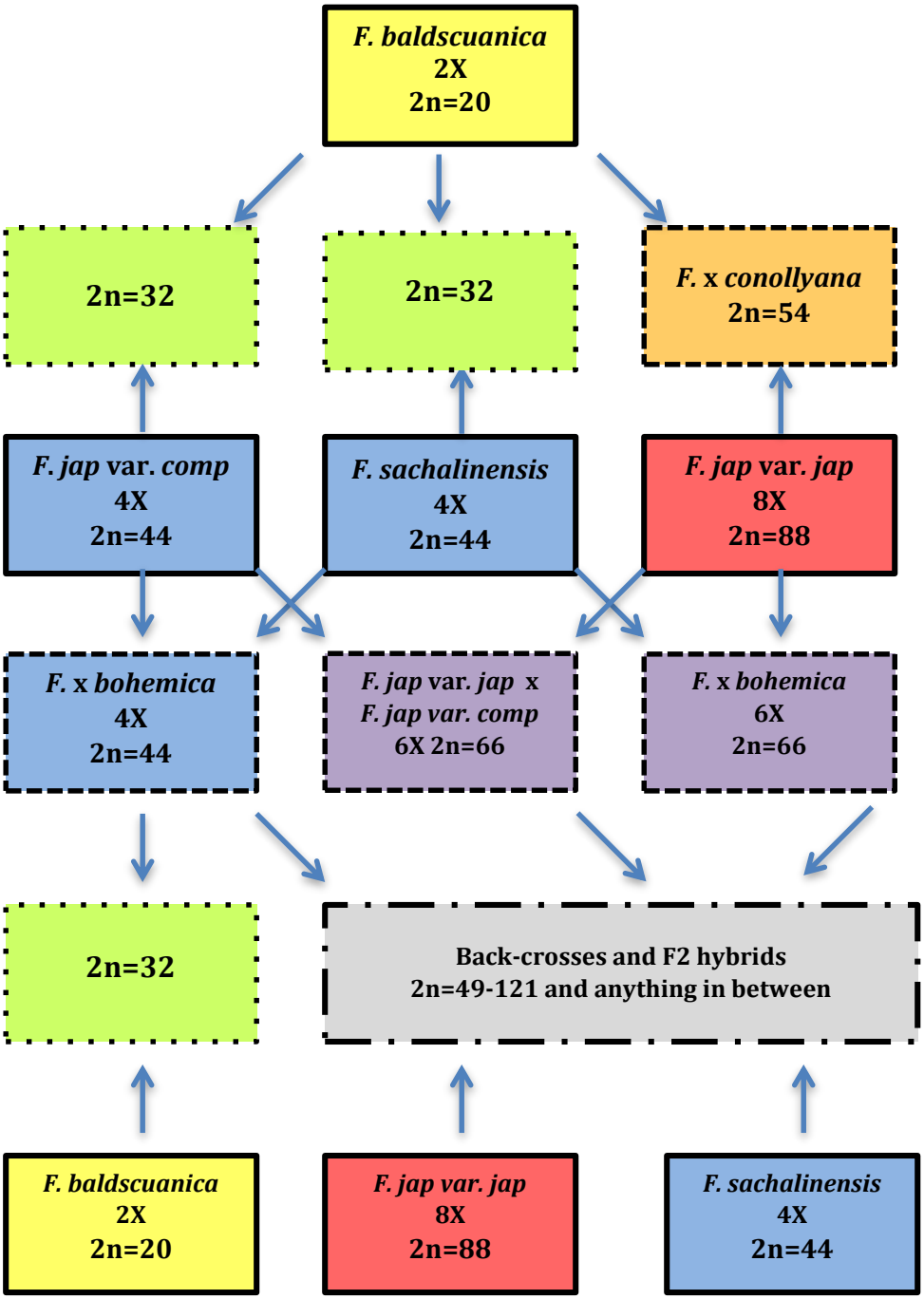


Figure 13 Simplified scheme of hybridization routes in the *Fallopia* complex in the UK, (from Bailey (2009)). Solid line: Parental taxa. Dashed line: Established hybrids. Stippled line: Hybrids found as seeds. Dot-dash line: Back-crosses and F2 hybrids, a range of aneuploids are produced here. Possible formation routes for 8X *F.* x *bohemica* and backccrossing by the intervarietal *F. japonica* hybrid are not included in the figure.

While tetraploid and octoploid *F.* x *bohemica* have regular meiosis and are completely fertile, hexaploid *F.* x *bohemica* has extremely irregular meiosis and reduced fertility (Bailey and Stace, 1992; Bailey and Wisskirchen, 2004; Bailey et al., 2007). Hexaploid *F.* x *bohemica* is still able to produce viable aneuploid or unreduced gametes (Bailey et al., 2007). Even occasional sexual reproduction contributes to evolution, because new genotypes may persist and be spread vegetatively (Ellstrand and Schierenbeck, 2000; Bailey et al., 2007; Schierenbeck and Ellstrand, 2009).

Hybridization and polyploidization are considered major drivers of plant evolution, and play important roles in the evolution of invasiveness in introduced species (Schierenbeck and Ellstrand, 2009; Soltis et al., 2010; te Beest et al., 2011). Several studies have found a positive correlation between polyploidy and invasiveness, and between polyploidy and traits that may be important for colonization (Treier et al., 2009; Pandit et al., 2011; te Beest et al., 2011). Polyploid species possess greater genetic diversity, and some polyploid introduced species may be preadapted to their new environments. Greater genetic diversity reduce inbreeding depressions and increase the potential for adaptation in small populations of introduced species. Polyploid species are often taller and more robust than diploid species, seem to tolerate a wider range of environmental conditions and are often perennials (te Beest et al., 2011). Hybridization provides genetic variation upon which evolutionary forces can act to promote adaptation and population differentiation. Some genotypes may be better suited to the environmental conditions or have improved abilities for sexual reproduction (Engler et al., 2011). Hybridization resulting in new genotypes with increased fitness compared to the parental taxa is a phenomenon known as hybrid vigour or heterosis. Indeed, some *F.* x *bohemica* genotypes express hybrid vigour, by having enhanced potential for vegetative regeneration, and faster spread compared to the parental taxa (Bímová et al., 2003; Mandak et al., 2004; Parepa et al., 2013). Hybridization may also provide an escape from sterility. Repeated backcrossing between male-fertile *F.* x *bohemica* and *F. japonica* may result in male-fertile, octoploid individuals that can replace the missing male-fertile *F. japonica* (Bailey and Wisskirchen, 2004).

F. japonica and related taxa are considered ideal model systems for the study of evolutionary processes such as hybridization and polyploidization. This is due to, among other things, the absence of male-fertile individuals in *F. japonica*, their effective vegetative regeneration, widespread distribution, ability of hybridization and the possibility to detect its occurrence,

the variation in ploidy levels, and the ongoing ploidy differentiation (te Beest et al., 2011; Bailey, 2013).

Climatic changes, combined with further adaptation through sexual reproduction and hybridization, may increase seed production and seedling survival in Europe in the future (Engler et al., 2011). While seed production in *F. japonica* is common in the UK today (Bailey et al., 2009), seeds were only observed occasionally in the 1970's (Conolly, 1977). Milder autumns, increased availability of pollen and more attraction of honeybees may explain the increased seed production (Bailey et al., 2009). Sexual reproduction was also considered rare in the USA until Forman and Kesseli (2003) showed that seed production and seedling survival in the field were more common than previously found (Forman and Kesseli, 2003). In USA, both male-sterile and male-fertile *F. japonica* are present (Forman and Kesseli, 2003) and sexual reproduction, including hybridization and back-crossing, result in genetically and morphologically diverse populations (Gammon et al., 2007; Grimsby et al., 2007; Gammon and Kesseli, 2010).

1.7.2 Vegetative reproduction

Vegetative regeneration is considered the main mean of reproduction of the invasive *Fallopia* taxa in their introduced range (Bailey, 2013), and the only mean of reproduction for these taxa in Norway (Fremstad and Elven, 1997). The *F. japonica* genotype known from Europe is also found to be widespread in the USA (Hollingsworth and Bailey, 2000a; Grimsby et al., 2007; Richards et al., 2008). This demonstrates the strong potential of vegetative regeneration in *F. japonica*, and the widespread male-sterile clone is given the nickname "the world's largest female" (Pysek, 2006).

Patches of the invasive *Fallopia* taxa expand laterally through rhizome growth. Patch expansion of several meters per year is reported for *F. japonica* (Child and Wade, 2000). The rhizomes can grow 2 m deep and extend 7 m away from the parent plant (Child and Wade, 2000). The rhizomes can grow in any direction and can circumnavigate walls and building foundations (Smith et al., 2007).

Fragments of rhizomes and stems can regenerate and give rise to new plants (Brock and Wade, 1992; Brock et al., 1995; Child, 1999; De Waal, 2001; Pyšek et al., 2003; Sásik and

Pavol, 2006). Dispersal of vegetative fragments by floods and human activities are the most important mean of spread in the introduced range (Bailey et al., 2009).

1.7.2.1 Factors affecting regeneration success of vegetative fragments

1.7.2.1.1 Fragment size

Generally, larger fragments regenerate better than smaller fragments (Child, 1999; Sásik and Pavol, 2006; Colleran and Goodall, 2013), and produce taller shoots with more rapid leaf production (Child, 1999; Sásik and Pavol, 2006; Colleran and Goodall, 2013). Child (1999) however found that the optimal rhizome fragment length for regeneration in *F. japonica* was 4 cm. The 4 cm fragments had regeneration rates comparable to that of 8 cm fragments, but 4 cm fragments regenerated faster. Sásik and Pavol (2006) conducted two experiments, and found that larger fragments performed better than smaller fragments in the first experiment, but not in the second experiment. A positive correlation between larger fragment size and height of the generated aboveground shoots was found by Colleran and Goodall (2013) in their study of regeneration of fragments spread by flooding. Although the regeneration potential of fragments seems to increase with fragment size, the regeneration potential in smaller fragments is also very high. Child (1999) found that 47% of 1 cm long rhizome fragments produced shoots and 90% produced adventitious roots. Brock and Wade (1992) showed that rhizome fragments of 0.7 g fresh weight could give rise to new plants. Regeneration from rhizome fragment weighing 0.48 g, fresh weight, is also reported (Rennocks, 2007). The minimum rhizome fragment weight for regeneration found in *F. x bohemica* is 0.89 g fresh weight (Child, 1999).

1.7.2.1.2 Burial depth

The regeneration success of buried fragments decreases with increased burial depths (Francis et al., 2008). A minimum depth of 5 m is recommended to prevent regrowth (Environment Agency, 2006). However, the effect of burial on fragment regeneration seem to be sparsely studied (Francis et al., 2008). Francis et al. (2008) found that increasing burial depths from 5 to 15 to 25 cm gave significant reduction in regeneration, but these shallow burial depths were not sufficient to prevent regeneration (Francis et al., 2008). Locandro (1978) reported that rhizome fragments can produce shoots from 1 m depth. Deep burial does not necessarily kill the rhizomes even when regeneration is temporarily prevented. Rhizomes may become

dormant when buried deeply, and unconfirmed observations suggest that rhizomes can be dormant for more than 20 years (Environment Agency, 2013).

1.7.2.1.3 Seasonal effects

The regeneration potential of *F. japonica* stem fragments vary between seasons (Brock et al., 1995; De Waal, 2001). Stem fragments seem to be more vulnerable to desiccation in spring, which may be due to a lower lignin content making them less tolerant to environmental stresses (De Waal, 2001). De Waal (2001) found that stem fragments had higher regeneration rates and produced taller shoots and more leaves in the summer than in the spring and autumn. Brock et al. (1995) found the highest regeneration rates in the autumn, but height growth and leaf production were lower in the autumn than earlier in the season. They also found that the generation of adventitious roots from stem fragments was lowest in the spring and highest in the autumn. The seasonal variations in regeneration and growth throughout the season may be due to seasonal changes in growth regulators, which may be controlled by day length (Brock et al., 1995; De Waal, 2001). Different studies of rhizome regeneration have been carried out at different times of season, in late May and from August to late November (Brock and Wade, 1992; Child, 1999; Bímová et al., 2003; Pyšek et al., 2003; Sásik and Pavol, 2006; Parepa et al., 2013). Rhizome fragments were found to regenerae at all these times (Locandro, 1973, cited in Child, 1999), reported that no significant seasonal changes were found in the viability of *F. japonica* rhizomes between May, June and September.

1.7.2.1.4 Variations between taxa

The regeneration potential can vary between taxa and between different genotypes (Child, 1999; Bímová et al., 2003; Pyšek et al., 2003). *F. x bohemica* is found to have more successful regeneration from rhizome fragments than *F. japonica* and *F. sachalinensis,* with higher regeneration rates and more rapid shoot growth and leaf production. *F. sachalinensis* is found to have lower regeneration rates from rhizome fragments than the other taxa (Bímová et al., 2003) and is also found to produce smaller shoots (Child, 1999). Pyšek et al. (2003) did not find a significant difference between the taxa, but found variation between different genotypes within *F. sachalinensis* and within *F.* x *bohemica*. The regeneration potential varied most within *F.* x *bohemica*, and genotypes genetically intermediate between the parental taxa regenerated better than those closer related to either parent. Parepa et al.

(2013) did also not find a significant difference between the three taxa in terms of regeneration rates, but *F.* x *bohemica* had a final biomass almost three times greater than the parental taxa when grown in an experimental community of native plants, and had the greatest negative impact on the native species in the experiment. The same study found that regeneration success varied between genotypes of *F. sachalinensis* and *F.* x *bohemica*, but also between genetically uniform *F. japonica* grown from fragments from geographically separated localities. Environmental differences between the locations where the rhizomes were collected and epigenetic effects are suggested explanations for this variation.

The taxa are found to have different regeneration success depending on the type of fragment (stem or rhizome) and growth media. Bímová et al. (2003) found that *F. japonica* regenerated best from rhizome fragments completely buried in soil, while *F. sachalinensis* regenerated best from stem fragments in water. *F. sachalinensis* was the taxon with the lowest regeneration rates from rhizome fragments, while *F. japonica* was the taxon with the lowest regeneration rates from stem fragments, with regeneration only in water and not in any of the soil treatments. In contrast, Child (1999) found that *F. japonica* regenerated better than *F. x bohemica* from stem fragments. Brock et al. (1995) found that *F. japonica* stem fragments regenerated well in both soil and water, but stems were vulnerable to desiccation in the soil treatments. Stems with any aerial exposure had lower regeneration rates, and no regeneration occurred in stems placed on the soil surface. The contrasting results obtained by the different studies may have several explanations, e.g. variation in moisture levels, seasonal effects (Brock et al., 1995; De Waal, 1995), genetic variations and epigenetic effects (Pyšek et al., 2003; Richards et al., 2012; Parepa et al., 2013).

1.8 Consequences of the invasive *Fallopia* taxa

1.8.1 Environmental consequences

1.8.1.1 Suppression of native species

The invasive *Fallopia* taxa are strong competitors that can replace existing vegetation and greatly reduce species diversity (Child and Wade, 2000; Maerz et al., 2005; Dassonville et al., 2007; Gerber et al., 2008; Urgenson et al., 2009; Aguilera et al., 2010). The suppression of other species may involve multiple interacting mechanisms, and the effect on other plants is species-specific. The dense canopy of the invasive *Fallopia* taxa restricts light availability to the ground flora and reduces establishment and growth of other species (Siemens and Blossey, 2007; Urgenson et al., 2012). Shading may have the greatest impact on light-dependent, early-seral species, while shade-tolerant, late-seral species may be more affected by belowground processes (Urgenson et al., 2012). Invasive *Fallopia* taxa produce secondary compounds, mainly phenolic compunds, with allelopathic effects on the germination and growth of other plants (Siemens and Blossey, 2007; Murrell et al., 2011; Urgenson et al., 2012). Some compounds have antimicrobial and antifungal effects, and allelopathy may act indirectly by affecting other plants mycorrhizae (Urgenson et al., 2012).

1.8.1.2 Modification of soil conditions

Invasive *Fallopia* taxa can alter soil condition by reducing soil pH and soil moisture, and through modification of soil nutrient levels (Dassonville et al., 2007; Dassonville et al., 2011; Urgenson et al., 2012). Dassonville et al. (2007) found that invaded sites had 3 – 13 times higher biomass production compared to uninvaded sites, and even though the aboveground biomass of *Fallopia* has a high C/N ratio and low nutrient concentrations, the greater biomass production results in higher standing nitrogen in invaded sites compared to uninvaded sites. The nitrogen translocation from shoots to rhizomes during senescence is very effective in these taxa, and little nitrogen goes back to the environment with the litter (Dassonville et al. 2008a; Dassonville et.al 2008b; Urgenson et al., 2009). Dead stems and litter decompose slowly due to the high C/N ratio, resulting in increased litter depth and N-immobilization.

F. japonica can modify nitrification/denitrification processes in the soil. The secondary compounds produced by the roots and rhizomes of *F. japonica* may have allelopathic effects

on nitrifying/denitrifying organisms (Dassonville et al., 2011). Dassonville et al. (2007) found that the magnitude and direction of the effect on nitrification activity depended on the site conditions. Nitrification activity was positively affected in sites where uninvaded plots had low nitrification activity, and negatively affected in sites where uninvaded plots had high nitrificaiton activity. Generally, *F. japonica* decreased denitrification activity, but the effect was greatest in sites where denitrification activity in uninvaded plots was high. *F. japonica* seem to promote nitrogen retention in the ecosystem by reducing nitrogen loss to the environment through nitrate leaching and denitrification. Dassonville et al. (2007) found that *F. japonica* can affect the availability of mineral nutrients in the topsoil. The availability of P and cations (K, Cu, Mn, Mg and Zn) was generally higher in invaded plots, probably due to an uplift of nutrients from deeper soil layers by the roots. The effect varied between sites and followed a clear pattern. Sites with high values of a nutrient element in uninvaded plots had lower values of the same element in invaded plots, while the opposite was found in sites where uninvaded plots had low values of a nutrient element. This regulation of nutrient levels results in homogenization of the soil conditions in invaded sites (Dassonville et al., 2007). The invasive *Fallopia* taxa may be performing a kind of niche construction by modifying the nitrogen cycle and soil conditions to its own advantage (Dassonville et al., 2011).

1.8.1.3 Ecosystem effects

Replacement of native plant species and altering of soil conditions by invasive *Fallopia* taxa have consequences on an ecosystem level. Generally, *Fallopia* invasions causes a shift from plant-based to detritus-based food chains. The abundance and diversity of the soilfauna, microflora and invertebrates are reduced, with effect on higher trophic levels (Maerz et al., 2005; Kappes et al., 2007; Dassonville et al., 2008a; Gerber et al., 2008; Topp et al., 2008). When *Fallopia* invades riparian habitats, the lower quality of the litter reduces nutrient input to the water, affecting aquatic food-webs (Urgenson et al., 2009). *Fallopia* invasions can alter riparian habitats structurally by suppressing the regeneration of riparian trees that provide shadow, organic matter and serve as aquatic habitat (Claeson and Bisson, 2013). Although the invasive *Fallopia* taxa have an extensive root system, they have few fine roots, and a poor soil-stabilizing capacity. The aboveground shoots of the nearly monocultural stands die back in the winter, and the soil is left bare and vulnerable to soil erosion. River bank erosion and increased sediment load can lead to broader, shallower and warmer waterways, and affects the habitat value for fish and other organisms (Soll et al., 2006)

1.8.2 Other consequences

The invasive *Fallopia* taxa can cause construction damages with high associated costs. Rhizomes can penetrate foundations, walls, drainage works and flood defense structures. The shoots can grow through asphalt and tarmac and can push through weaknesses and cracks in concrete surfaces, damaging roads (Figure 14**)**, pavements, parking lots, buildings and archeological values Their tall shoots reduce sight along roadsides, railways and bike/walking paths, and may also block desirable view of landscapes (Beerling and Palmer, 1994; Palmer, 1994; Child and Wade, 2000). *Fallopia* may in extreme cases grow through walls and floors of houses, but this normally only occurs when house footings are weak (Payne and Hoxley, 2012). Where redevelopment or landscaping affects infested areas, the required treatment or removal of the plant can increase costs and delay project progress. Excavated infested masses cannot be used elsewhere, and disposal options may be limited or costly (Child et al., 1998; Child et al., 2001). Dense stands growing on riverbanks, and dead shoots that are washed into rivers, can clog waterways and increase flooding. Little groundflora establishes within *Fallopia* stands, and the soil is left bare and exposed to erosion when shoots die back in the winter (Child and Wade, 2000). Invasive *Fallopia* can be

Figure 14 *F. japonica* spreads from the other side of the wall, and damages the asphalt layer in a street in Moss. Picture taken in September, 2013. Photo: Anne-Kari Holm.

problematic weeds in gardens, parks, graveyards and other green environments. Stands spread laterally through rhizome growth and can extend into lawns, paths and plantings. The homogeneous, dominating vegetation and the remaining dead stems may reduce the aesthetic value and character of the landscape. Stands in urban areas may become used as waste and litter dumps and collects wind blown litter. Dense stands along rivers and lake margins can reduce accessibility and reduce the recreational value of the site (Scott and Marss, 1984; Child and Wade, 2000).

1.9 Control

1.9.1 General considerations

Control of an invasion on a site should be viewed as a part of a broader management program. An effective management program should include (Child and Wade, 2000):

- Distribution surveys. Use of Geographic Information System (GIS) makes it possible to link survey data with other information held on the GIS, such as landownership, nature conservation values and development plans. The GIS data should be updated, and re-surveys are necessary.

- Prevention of spread. A policy that deals with prevention should be established. Prevention is cost-effective compared to eradication of established infestation.

- Education and awareness raising within the general public and public authorities.

- Co-ordination of different organizations and landowners involved.

- An integrated approach to control of existing infestations. The invasive *Fallopia* species can grow in a wide range of environments. Site conditions, stand size, environmental sensitivity and public access are some factors that should be considered when planning a treatment program. Using multiple methods may often be necessary and most effective.

Control of invasive *Fallopia* can be achieved through chemical or mechanical means, often used in combination. Whatever control method is used, treatment may have to be continued for several years (Child and Wade, 2000). Large stands are often more difficult to control than smaller stands, and it should be a general rule of thumb to take action sooner rather than later. Small, newly established plants may easily be removed manually, and monitoring combined with a rapid response to new plants is an effective way of preventing infestations and spread. This approach is called the Early Detection Rapid Response method (EDRR) (Colleran and Goodall, 2013). A summary of some methods that can be used to control invasive *Fallopia* taxa is found in Table 2.

When control efforts are carried out, there are some precautions that must be taken to not promote spread of the plant. These precautions are fundamental for a successfull treatment program:

- Stem and rhizome fragments must not be spread because of their strong ability to regenerate and establish new infestations (Brock and Wade, 1992; Child, 1999). Care must be taken when control is carried out close to watercourses, that fragments are not spread into waterstreams. Cut material can be piled at the site to dry out if there is no risk of fragments being spread with wind, water, traffic etc. The cut stems should be inspected regularly for signs of regrowth. Otherwise, the plant material should be burned. Soil excavated within 7 m away from the aboveground plant must not be dumped or used other places, as it may contain rhizomes (Child and Wade, 2000). Rhizome fragments as small as 1 cm are able to regenerate into new plants (Brock and Wade, 1992). It is recommended to bury infested soil masses at 5 m depth. Moving infested soil should be avoided because of the risk of spreading the plant to new sites (Environment Agency, 2006).

- All tools and vehicles used in infested areas must be thoroughly cleaned before leaving the area. This should be done over a hard surface or a root barrier membrane that allows the washed off material to be collected and disposed of safely (Environment Agency, 2006).

- All operators should know how to identify shoots and rhizomes, and how to prevent spread.

- The site should be monitored for at least three years after aboveground growth has ceased. It is reported that stands can appear dead for three years and then start producing new shoots. Rhizomes may be alive even if no aboveground growth occurs (Soll et al., 2008). It is not known how long rhizomes can persist in the soil, but unconfirmed observations suggest that rhizomes can stay dormant for more than 20 years (Environment Agency, 2006).

1.9.2 Mechanical control

1.9.2.1 Cutting

When cutting invasive *Fallopia,* it may be practical to use a brush cutter with metal blades. Cutting-strings can be torn by the though, woody shoots. When using a brush cutter, small fragments may be scattered over the area. Handheld loppers might be preferred in some situations, for example when cutting close to watercourses, to prevent scattered fragments from entering the water.

A single cutting during the growing season reduces belowground biomass, but is not sufficient for eradication (Seiger and Merchant, 1997; Bimova et al., 2001; Rouifed et al., 2011). The effect of cutting increases with increased number of cuttings during the growing season (Seiger and Merchant, 1997). To deplete the rhizomes of energy reserves, cutting should be carried out every 2-4 weeks from April/May to August/September and repeated annually (Seiger and Merchant, 1997; Child and Wade, 2000; King County Noxious Weed Program, 2008). Shoot regrowth after cutting is strongest from spring until July and decreases later in the season (Callaghan et al., 1981). More intensive cutting in the early part of the season may therefore deplete rhizomes more effectively. Cutting may be less frequent in the later part of the season as shoot regrowth ceases, but shoots should not be allowed to grow taller than 15 cm (Soll, 2004). Eradication of small stands is possible through frequent and consistent cutting over several years, but cutting used alone is not recommended for larger, well-established stands (Child and Wade, 2000; McHugh, 2006). McHugh (2006) reported

that a small stand (25 shoots) was successfully eradicated through monthly cuttings over three growing seasons.

Cutting can be used in combination with other methods. Cutting prior to chemical control reduces the plants vigour and may increase shoot density (Fløistad, 2010). Increased shoot density can provide a larger leaf area to spray on and a more effective herbicide uptake. Cutting early in the season reduces height and makes access and herbicide application on regrowth later in the year easier. Cutting later than June may however result in decreased shoot density and slow regrowth

F. japonica and *F.* x *bohemica* are found to respond differently to cutting. Rouifed et al. (2011) found that a single cutting reduced the belowground biomass in *F. japonica,* but less in *F. x bohemica* than in *F. japonica*. Cutting also increased the proportion of roots in the upper soil layer in *F. japonica*, but did not affect root distribution in *F.* x *bohemica*.

There is some evidence that cutting may lead to increased lateral growth and stand expansion (Beerling, 1990). Because of the risk of fragment dispersal and increased lateral spread following cutting, cutting should only be carried out as a part of a well-planned, long-term strategy.

1.9.2.2 Mowing

Frequent mowing can be used to control growth and prevent invasion of grassed areas (Child and Wade, 2000). A mower with a collecting box should be used, and the mowing should be left on site or disposed of safely. Flail-mowing infested areas is not recommended because of the risk of stem fragments being spread into non-infested areas (Devon County Council, n. d.).

1.9.2.3 Hand-pulling

Hand-pulling of shoots may be used for eradication of small stands or new infestations (Child and Wade, 2000). If the stand is established in soft soil or sand, it may be possible to remove some of the rhizomes along with the shoots (McHugh, 2006). Baker (1988), cited in Child

and Wade (2000), reports that a small stand (2 m^2) was successfully eradicated through continuous hand-pulling over three growing seasons.

1.9.2.4 Covering

It is reported that covering can eradicate stands <100 m^2 in 5-6 years (Nickelson, 2013), but larger stands may require more than eight years of covering for eradication (Sally Nickelson, Wildlife biologist/Watershed ecologist, Cedar River Watershed, Washington, Personal Communication). Experiences with covering as a mean to control invasive *Fallopia* suggest that the method is most suited for smaller stands in open terrain and as part of an integrated control strategy (McHugh, 2006; King County Noxious Weed Program, 2008; Nickelson, 2013)

Successful covering depends on good installation techniques and frequent monitoring. A tough type of plastic/geotextile should be used. Trials suggest that woven material allows for less shoot growth under the fabric than unwoven material (McHugh, 2006). The fabric should be laid loosely over the cut stems to prevent shoots from poking through it. The fabric must be properly secured to the ground to prevent strong shoot growth from lifting the fabric out of place. Stones and logs can be used to weigh down the fabric. Another method is staking the edges of the fabric and securing with crisscrossing chords tied at the stakes (McHugh, 2006). Ideally, the fabric should extend several meters beyond the margins of a stand, as rhizomes may put up shoots 7 m away from the parent plant (Child and Wade, 2000). This will increase material costs and may not always be possible due to site conditions. Shoots sprouting along the edges may be controlled through hand-pulling or chemical control during monitoring visits. The sprouting tends to lessen after the first year (McHugh, 2006). Stomping on the fabric every 2-4 week may increase the effect. Cutting the shoots several times prior to covering depletes rhizome reserves and may reduce plant vigour (King County Noxious Weed Program, 2008).

1.9.2.5 Excavation

The following information about excavation is extracted from Environment Agency (2013), *The Japanese Knotweed Code of Practice* (http://www.environment-agency.gov.uk/static/documents/Leisure/Knotweed_CoP.pdf). The document provides valuable instructions for excavation and use of root barrier membranes, and advises on how to manage invasive *Fallopia* infestations on developmental sites.

Excavation and removal of the whole plant may be a solution when treatment in the original location is not possible. Transporting excavated masses to other sites involves a high risk of contamination of non-infested areas. If there is sufficient room on the site, a better option is to relocate the masses on the site. Depending on site conditions, the masses can be either buried or relocated to an area on site where they can be treated with herbicides. When burying infested masses, a burial depth of 5 m is recommended. A root barrier membrane can be used to cover or enclose the infested masses before covering with non-infested masses. The burial site should be mapped and future owners of the property should be informed about its location. When relocating infested masses to an area where they can be controlled, the masses may be spread in a 0,5 – 1 m deep prepared bund. The bottom of the bund should be covered with a root barrier membrane. Since the topsoil contains more rhizomes than the subsoil, the topsoil should be spread at the surface of the bund to stimulate shoot growth. Rhizomes may become dormant if buried too deeply. Disturbance of the soil and raking rhizomes to the surface may stimulate dormant buds to sprout.

When moving infested soil on site, precautions must be taken to avoid spread during the work. Transport routes should be carefully planned and marked, and infested soil should not be moved across transport corridors, watercourses or areas of high conservation value. If non-infested areas are crossed, the ground should be protected with layers of root barrier membrane, sand and a hardcore surface layer. This covering material must be disposed of in a safe way, e.g. buried along with the infested masses. Access to the operation area should be limited to vehicles involved in moving the infested soil. Vehicles must be thoroughly decontaminated before leaving the area.

Methods that aim to reduce the amount of soil that needs to be disposed of after an excavation, such as chipping/shredding of plant material or screening of soil to remove

rhizomes, have been studied and are also in use (Rennocks, 2007; Wise Knotweed Solutions, n. d.; Japanese Knotweed Surveys, 2011). Rennocks (2007) reports that passing rhizome material through a chipping machine once, and then screen it through a 10 mm sieve, resulted in regeneration from one rhizome fragment (0.48 g fresh weight). Rhizome material that had been chipped twice or screened through a 2 mm sieve did not regenerate. Fungal infections were observed on the chipped plant material. Trials of composting shredded rhizome material showed that 100% *F. japonica* compost reached lower temperatures than compost of mixed plant material. However, all composts reached temperatures over 60°C, and no regeneration took place. Laboratory heat tests of rhizome fragments (> 1 cm) also showed that regeneration did not occur from fragments after they had been subjected to temperatures exceeding 60°C for 1 hour or longer (Rennocks, 2007).

1.9.3 Biological control

1.9.3.1 Classical biological control

In their native range, the invasive *Fallopia* species are naturally controlled through predation by various insects and fungal infections. This is in contrast to the introduced range, where few associated pathogens and predators exists (Bailey, 2003). Classical biological control of weeds is a management approach that involves the introduction of a specialist herbivore insect or fungal pathogen that reduces the growth and spread of an invasive plant (Djeddour and Shaw, 2011). The high costs and limited long-term effects of conventional control methods, often including repeated use of herbicides, has encouraged the interest in biological control as a more permanent and sustainable mean of management of the invasive *Fallopia* species (Child and Wade, 2000; Djeddour and Shaw, 2011). A psyllid, *Alfa itadori,* has been found to be a promising potential biocontrol agent. *A. itadori* is native to Japan, Korea and the Kurile and Sakhalin Islands where it is host-specific to *F. japonica* and *F. sachalinensis* (Burckhardt and Lauterer, 1997, cited in Grevstad et al., 2013). The sap sucking psyllid reduces the growth and photosynthetic ability of *F. japonica,* making it less competitive and more susceptible to control efforts. The psyllid was released at specially chosen sites in the UK, spring 2010. This represents the first official release of a biocontrol agent against weeds in the EU. An investigation of its potential use in USA and Canada revealed that different *A. itadori* populations performed differently among *F. japonica*, *F. sachalinensis* and *F.* x *bohemica*. More than one population or host-race of *A. itadori* may be needed to control

genetically diverse populations (Grevstad et al., 2013). A leafspot fungus, *Mycosphaerella* sp., is also being investigated as a potential biocontrol agent (CABI, 2014).

1.9.3.2 Grazing

Shoots of invasive *Fallopia* are palatable to sheep, cattle, horses and goats. Grazing occurs mostly in the spring when the shoots are young. Few young shoots are available after late July. Grazing can control growth and spread of the plant, but the plant will recover when the grazing regime ends (Brabec and Pysek, 2000; Child and Wade, 2000).

1.9.4 Chemical control and combination treatments

1.9.4.1 Herbicide treatment

Recommended methods for control of invasive *Fallopia* species often involve the use of a systemic herbicide that is translocated from the leaves to the rhizomes, e.g. a herbicide with glyphosate as the active ingredient (Child and Wade, 2000). The herbicide is most effectively transported to the rhizomes when carbohydrates are allocated from leaves to rhizomes (Bashtanova et al., 2009). In *F. japonica*, this occurs from mid June and increases towards the end of the growing season (Price et al., 2001). Herbicide treatments applied in July is shown to be more effective than treatments applied in May (Soll, 2004; Kabat et al., 2006). The tall plant height in July can however make access and application difficult. A treatment earlier in the season can be used to set back growth and provide a more practical plant height for treatment later in the season. The second treatment may be carried out when the plants are 1 – 1.5 m tall and sufficient leaf area is developed (Child and Wade, 2000). Some trials suggest that a single herbicide treatment in the autumn is as effective as two or three herbicide treatments during the season (Brown, 1999, cited in Rennocks, 2007).

When treatment reduces the height and density of the *Fallopia* stand, other species may quickly start establishing within the stand (De Waal, 1995; Child et al., 1998). To encourage further revegetation by native species, a more precise applicator, e. g. a weed wiper, may be considered for treatment of the remaining *Fallopia* shoots (De Waal, 1995).

It should be noted that the invasive *Fallopia* taxa are edible for humans and animals. Children may play within the stands, and may also eat the shoots, or drink the water that collects within the hollow shoots. Caution must be paid to the risk of poisoning when herbicide treatment is considered in areas with children or grazing animals.

1.9.4.2 Direct application methods, stem injection

Foliar spraying can cause herbicide drift and damage to adjacent vegetation. Methods that target the *Fallopia* shoots more directly may be more suitable in environmentally sensitive areas or where invasive *Fallopia* grow close to desirable vegetation. Examples of direct application methods are:

- Stem injection: Injection of herbicide into the cavity of uncut stems is shown to be an effective method (Soll, 2004; Rennocks, 2007; Hagen and Dunwiddie, 2008; Soll et al., 2008; Barták et al., 2010).

- Cut and inject: Injection of herbicide into the cavity of cut stems (Ford, 2004; Soll, 2004; Rennocks, 2007).

- Cut and wick (wipe): Wiping herbicide onto the surface and the inside of cut stems using a sponge or a wick. The method is found to have mediocre effect unless repeated for several years (Soll, 2004).

- Wick wipe: Wiping herbicide on the leaf and stem surface of uncut plants using an applicator wand with a sponge on the end. It may be hard to get the herbicide on the leaf surface, and the method seems to increase personal contact with the herbicide (King County Noxious Weed Program, 2008).

Stem injection seems to be much used and has shown promising results (Soll, 2004; Hagen and Dunwiddie, 2008; Soll et al., 2008; Barták et al., 2010; Delbart et al., 2012). Each stem is injected with 3-5 ml of undiluted herbicide or a herbicide-water solution (Knotweeds IPM Profile, 2004; Hagen and Dunwiddie, 2008; Soll et al., 2008; Delbart et al., 2012). Hagen and Dunwiddie (2008) found that 3 ml and 5 ml injections of undiluted glyphosate had equal effect, while a 5 ml 1:1 glyphosate-water solution was less effective than undiluted

glyphosate. Delbart et al. (2012) used a glyphosate-water solution at a rate of 3.4 kg ha^{-1} acid equivalent (AE) of herbicide and achieved 100% stem volume reduction after one year and 99.9% reduction after two years. The same study also found that a stem injection and a foliar spray in Agust/September had comparable effect, a rhizome viability test indicated that the rhizomes were more affected by the stem-injection method. Stem-injection can be used independently of plant height (Child, 1999), but requires a stem diameter of minimum 1.5 cm. Other methods, such as spot spraying, may be used to treat the smaller shoots and regrowth (Hagen and Dunwiddie, 2008; Soll et al., 2008). The stem injection method can be labor-intensive and time-consuming, especially when large stands are treated (Soll et al., 2008; Barták et al., 2010), and each stem needs to be treated separately (Hagen and Dunwiddie, 2008). An advantage of the stem injection method is that it may be applied independent of the weather conditions.

Although stem injection reduces the risk of herbicide drift, the method has sometimes been observed to cause herbicide symptoms to adjacent vegetation (Crockett, 2005; Miller, 2005). The reason is thought to be herbicide leaking from the rhizomes (Crockett, 2005). Crockett (2005) reported that the problem had occurred in situations where a large number of plants had been treated and the soil was water-saturated during unusual heavy rainfall. The problem may also be predicted to occur where soil has low organic matter content with few binding sites to bind the herbicide. Problems with needle breakage and herbicide leaking from injection tools are also reported (Hagen and Dunwiddie, 2008). A special injection tool for use on invasive *Fallopia* has been developed (Crockett, 2005).

1.9.4.3 Herbicide treatment combined with cutting

An early cutting can be used to reduce plant height for a later herbicide treatment. Shoot regrowth after cutting slows down during the growing season (Callaghan et al., 1981; De Waal, 1995; Gover et al., 2005), and a late cutting can result in regrowth being to small for an effective herbicide uptake. Gover et al. (2005) reports that cutting around June 1 (Pennsylvania, USA), gave regrowth that was vigorous, but of reduced height. It may take 6-7 weeks from the time of cutting until herbicide treatment can be carried out (De Waal, 1995; Gover et al., 2005). The treatment may be carried out when the shoots are 1-1.5 m tall and have developed sufficient leaf area for effective herbicide uptake (Child and Wade, 2000).

De Waal (1995) studied the effect of two foliar sprays (early June and July) and the effect of a cutting in July followed up with a foliar spray in September. Both methods were effective and reduced the *F. japonica* cover with 95 % and 99 % respectively. Some trials have found that a single spray in August was more effective than cutting followed by spraying of regrowth. However, the cutting was performed in August, and the late timing of the cutting may have resulted in insufficient regrowth and reduced effect compared to if cutting had been performed earlier in the season (Misselbrook, 2000, cited in Rennocks, 2007). Bimova et al. (2001) found that a combination of cutting shoots in May, and spraying regrowth with glyphosate in July had excellent effect on *F. sachalinensis,* and good effect on *F. japonica* and *F. x bohemica*. Two years of treatment was however not sufficient to eradicate any of the taxa.

1.9.4.4 Herbicide treatment combined with digging

A combination of chemical control and digging is found to have good effect (Child et al., 1998; Bimova et al., 2001). Rhizome fragmentation increases shoot density by stimulating dormant buds to sprout, which may increase herbicide delivery to the rhizomes. Digging may however reduce height, shoot diameter and number of leaves. Increased shoot density and reduced shoot diameter is unpractical if the herbicide will be delivered through stem injection. Child et al. (1998) investigated the effect of combined digging and chemical control, and concluded that the combination treatment could reduce the time required for eradication compared to spraying only. The combination treatment also resulted in a greater cover of native species. Another invasive species (*Impatiens glandulifera*) established in plots that had been dug but not sprayed.

The procedure used for the combined treatment by Child et al. (1998) was:

1) Scraping of surface crowns and rhizomes into a pile (autumn - early spring)
2) Excavation to a depth of 50 cm and replacement of soil
3) Spreading crowns and rhizomes back over the area
4) Spraying with glyphosate (5 l ha^{-1} using a low water volume of 80 l ha^{-1}) when the plants have reached a height of 0.75-1 m and have sufficient leaf area for effective herbicide uptake.

This method resulted in almost total reduction of aboveground growth one year after the glyphosate treatment. The authors suggest that total control might be achieved by the addition

of a second herbicide treatment later in the season (Child et al., 1998). Bimova et al. (2001) concluded that combined digging and glyphosate treatment had excellent effect on *F. japonica* and *F. sachalinensis*. *F.* x *bohemica* seemed to be more tolerant than the parental taxa to this treatment, although the effect was good also on this taxon. Two years of treatment did however not eradicate the taxa. Bimova et al. (2001) used the following procedure:

1) Digging of soil to 50 cm (or cutting aboveground shoots) in May
2) Spraying regrowth with glyphosate in July (Roundup Biaktiv at a rate of 50 ml per 100 m^2 diluted with water volume 12 l/100 m^2).

1.9.4.5 Continuation and monitoring

Regardless of the method used, complete eradication will in most cases require treatment for 2-5 years or more (Soll, 2004; Soll et al., 2008; Devon County Council, n. d.). Herbicide treatments will often have greatest effect the first year and decreased effect the following years. Regrowth may eventually become too small and deformed to be treated effectively (Soll et al., 2008). A solution may be to excavate a large part of the rhizomes. This will remove some of the living rhizomes and may stimulate the remaining rhizomes to produce shoots with normal leaves (Soll et al., 2008). It is essential that the entire rhizome system is killed and that treatment is continued until no new shoots appear (De Waal, 1995; Child and Wade, 2000; Soll, 2004). The site should then be monitored for at least three years after shoot growth has stopped. Soll et al. (2008) observed that treated stands could appear dead for three years and then start producing new shoots. The reason for this apparent dormancy and recovery is not clear.

Table 2 Summary of methods used to control invasive *Fallopia*

Treatment	Timing	Comment	References
Cutting	May – Sept.	Cutting every 2-4 weeks during the growing season, repeated for minimum three years or as long as needed. Depletes rhizomes of energy and reduces plant vigour, but is ineffective for eradication. Risk of spreading stem fragments. Can be used in combination with other methods.	Seiger and Merchant (1997) Child and Wade (2000) Bimova et al. (2001) Soll (2004) King County Noxious Weed Program (2008) Rouifed et al. (2011)
Covering	All year	Reduces plant vigour, but eradication may require 5-6 years or more. Best suited for small, isolated stands and in combination with other methods. Good installation technique and monitoring is important.	McHugh (2006) King County Noxious Weed Program (2008) Nickelson (2013)
Digging	All year / spring	Ineffective method used alone, can be used in combination with other treatments. Increases shoot density.	Child et al. (1998)
Excavation	All year	Can be used when treatment on site is not possible. Moving infested masses should be avoided. Relocation of masses on the site for further treatment may be a better option. For burial of infested masses, a depth of 5 m is recommended. Root barrier membranes may be used to enclose buried masses and to protect uninfested areas. Rhizomes can grow 2-3 m deep and 7 m laterally.	Environment Agency (2013)
One herbicide treatment	July – Sept.	A single herbicide treatment of full-grown shoots during July – September using foliar spray, stem-injection or other method. Spraying tall plants may be difficult and increase risk of herbicide drift.	Child and Wade (2000) Hagen and Dunwiddie (2008) Delbart et al. (2012)
Two herbicide treatments	May/June + July – Sept.	Early herbicide treatment when shoots have reached 0.75 – 1.5 m in height, followed up by herbicide treatment in July – September when sufficient regrowth has developed.	De Waal (1995) Child and Wade (2000)
Cutting + Herbicide treatment	Early June + July – Sept.	Cutting shoots around June, followed up with herbicide treatment during July - September when sufficient regrowth is developed.	De Waal (1995) Child and Wade (2000) Gover (2005)
Digging + One or two herbicide treatments (or one cutting + one herb. treatment)	Autumn/spring + June – Sept.	Disturbance of rhizomes through digging in late autumn/early spring, followed up with herbicide treatment when sufficient regrowth is developed. A second herbicide treatment may be carried out in the end of the season. This combination is found to increase treatment effect.	Child et al. (1998) Bimova et al. (2001) Environment Agency (2013)

1.9.5 Revegetation

Revegetation is an important element of the control strategy, and should be determined at the start of the treatment program. The risk of soil erosion following the removal of monocultural stands should be considered. A treated site may be revegetated through natural establishment by native species or through active revegetation with selected species (Child and Wade, 2000). Native species will often start establishing as soon as the invasive *Fallopia* stand is eradicated or its competitive advantage is reduced (De Waal, 1995; Ford, 2004; Davenport, 2006; Murrell et al., 2011). Control methods that involve digging of soil surface may increase natural revegetation (Child et al., 1998). Native plants that establish within the area should be protected from herbicide and maintenance damage during the treatment and monitoring period (Davenport, 2006). Eradication of a *Fallopia* stand creates a light-open and disturbed site that is vulnerable to invasion by other introduced species, e. g. *Impatiens glandulifera* (De Waal, 1995; Child et al., 1998; Barták et al., 2010). Claeson and Bisson (2013) studied the effects of natural revegetation on species composition in a riparian habitat following herbicide treatment of invasive *Fallopia*. They found that 3-6 years after the first treatment, treated sites generally contained more introduced species than non-infested, non-treated sites, especially in highly disturbed riparian areas and along large rivers. Monitoring and rapid removal of secondary invasive species is critical for establishment of native species. Active revegetation with native species may enhance restoration of native plant communities (Claeson and Bisson, 2013).

Davenport (2006) recommends treating invasive *Fallopia* taxa for two years before planting other species. Skinner et al. (2012) found that sowing native species after two years of mowing and chemical control could not suppress *F. japonica* from recovering and grow taller than the native species. Planting of shrubs may impede monitoring and follow-up treatments and can reduce treatment options in the future. Use of grass-seed mixes may be a better solution if active revegetation is desired (Child and Wade, 2000).

2 Part II (Experimental part)

Research questions

To increase knowledge about the biology of the invasive *Fallopia* taxa and contribute to improved control strategies, four experiments were carried out. The main research questions addressed by each experiment were:

Experiment 1*:* Distribution and ploidy levels of the invasive *Fallopia* taxa in five areas in Norway

- What are the distributions of *F. japonica*, *F. sachalinensis* and *F.* x *bohemica* in the study area?
- Can morphological leaf characters be used to distinguish the invasive *Fallopia* taxa in the study area?
- Can the molecular markers simple sequence repeats (SSRs) analysis and DNA barcoding (sequencing) distinguish between the invasive *Fallopia* taxa in the study area?
- What are the ploidy levels of the invasive *Fallopia* taxa in the study area?

Experiment 2: Growth and allocation pattern of *F. japonica* and *F.* x *bohemica*

- What are the growth and biomass allocation patterns of *F. japonica* and *F.* x *bohemica* throughout the growing season?
- Do *F. japonica* and *F.* x *bohemica* differ in their growth and allocation pattern throughout the growing season?

Experiment 3.1 and 3.2: Shoot regrowth potential of *F. japonica* and *F.* x *bohemica* throughout the growing season and after covering.

- What is the seasonal pattern of the shoot regrowth potential of *F. x bohemica*?
- How long is the shoot regrowth potential retained in *F. japonica* and *F.* x *bohemica* when aboveground shoot production is prevented through covering with thick plastic?

2.1 Experiment 1: Distribution and ploidy levels of the invasive *Fallopia* taxa in five areas in Norway

2.1.1 Introduction

2.1.1.1 Distribution in Norway

The distribution of the invasive *Fallopia* taxa in Norway is previously studied by Handeland (1991) and Fremstad and Elven (1997). New findings are successively recorded in the Spieces Map Service (www.artskart.artsdatabanken.no) administered by Artsdatabanken (Norwegian Biodiversity Information Centre, www.artsdatabanken.no). *F. japonica* is the most frequent of the three taxa in Norway, but *F.* x *bohemica* can be more frequent in some areas (Fremstad and Elven, 1997, Artsdatabanken, 2012). By September 2014, *F. japonica* was represented by 4701 recordings in the Species Map Service, while *F. sachalinensis* and *F.* x *bohemica* were represented by 391 and 412 recordings, respectively. *F.* x *bohemica* has spread unnoticed in some countries, due to the identification of the hybrid as one of the parental taxa. Its distribution in some areas may therefore be underestimated (Handeland, 1991; Bailey and Child, 1996; Bailey and Wisskirchen, 2004; Mandak et al., 2004; Artsdatabanken, 2012).

2.1.1.2 Reproduction and genetic diversity

Vegetative reproduction through lateral rhizome growth and regeneration from stem and rhizome fragments are considered the main mean of reproduction for the invasive *Fallopia* taxa in their introduced range, and the only mean of reproduction for these taxa in Norway, where climatic conditions limits seed production. Only male-sterile *F. japonica* is found in Europe, and true *F. japonica* cannot be produced from seeds. The genetic variation within *F. japonica* is therefore extremely low in Europe, and several studies suggest that a single, male-sterile clone is present (Hollingsworth and Bailey, 2000a; Mandak et al., 2005; Tiébré et al., 2007a; Krebs et al., 2010). A recent study from Poland however claims to have found different genotypes of *F. japonica* by use of the molecular marker Amplified Fragment Length Polymorphism (AFLP) (Bzd*f*ôga et al., 2012). *F. sachalinensis* is present as both male-sterile and hermaphrodite individuals, and can reproduce sexually. Pollination of *F. japonica* by *F. sachalinensis* results in the hybrid *F.* x *bohemica*. *F.* x *bohemica* can be male-

sterile or hermaphrodite, and can cross with both parental taxa and with other *F.* x *bohemica* (Bailey et al., 2009). Other taxa that are involved in the hybridization complex are the dwarf variant *F. japonica* var. *compacta* and the commonly grown garden plant, *F. baldschuanica*. Only one recording of *F. japonica* var. *compacta* is known in Norway (Handeland, 1991), and the taxon is also rare in other parts of Europe (Bailey et al. 2009; Tiebre et al. 2007a). Most seeds produced by *F. japonica* in Europe results from pollination by *F. baldschauanica* and *F.* x *bohemica* (Tiebre et al. 2007b; Bailey et al. 2009; Funkenberg et al. 2011). *F. japonica* can produce high amounts of seeds, which germinate readily when grown under experimental conditions, but seedling are not commonly found in nature. Germination and seedling establishment seem to be limited by environmental conditions (Bailey 1994; Bailey et al, 2007; Funkenberg et al. 2012). *F. sachalinensis* and *F. x bohemica* are both found to be genetically variable in Europe, and the greatest variation is found within *F. x bohemica* (Hollingsworth and Bailey, 2000b; Mandak et al., 2003; Mandak et al., 2005; Krebs et al., 2010). Multiple introductions of different genotypes may in part explain this variation, but the great genetic diversity within *F.* x *bohemica* compared to within the parental taxa, and the existence of evolutionary "hot spots" where F1 and F2 hybrids are produced, indicates that sexual reproduction play an important role for the development of the populations (Pashley et al., 2003; Mandak et al., 2005; Tiébré et al., 2007a; Krebs et al., 2010). Since the taxa are not known to produce seeds in Norway, the genetic variation within these taxa in Norway can be expected to be very low.

The invasive *Fallopia* taxa are polyploid, and *F. japonica* and *F. sachalinensis* are cytologically variable in their native range. *F. japonica* is only found as octoploid in its introduced range, but *F. sachalinensis* and *F.* x *bohemica* can both be tetraploid, hexaploid or octoploid. The most common ploidy level for *F. sachalinensis* in Europe is the tetraploid, while *F.* x *bohemica* is most common as hexaploid (Bailey and Stace, 1992; Hart et al., 1997; Mandak et al., 2003; Mandak et al., 2005; Tiebre et al., 2007a; Bailey et al. 2009; Krebs et al., 2010). Aneuploid and decaploid individuals of *F.* x *bohemica* have also been recorded (Pashley et al., 2003; Tiébré et al., 2007a; Bailey et al., 2009). The relative distribution of the different ploidy levels of *F. x bohemica* differ between different parts of Europe. While the second most abundant ploidy level of *F. x bohemica* in the UK is the tetraploid, in the continental Europe it is the octoploid (Bailey and Wisskirchen, 2004). The ploidy levels of the invasive *Fallopia* taxa in Norway has hitherto not been examined.

F. x bohemica of different ploidy-levels have different degrees of fertility. The tetraploid and octoploid *F. x bohemica* have regular meiosis and are completely fertile. The hexaploid *F. x bohemica* has irregular meiosis and reduced fertility, but is still able to produce viable aneuploid or unreduced gametes (Bailey and Stace, 1992; Bailey and Wisskirchen, 2004; Bailey et al., 2007). Knowledge about the distribution of the different taxa and their ploidy levels can increase understanding of how hybridization and polyploidy affect development of the invasive *Fallopia* taxa, and other introduced taxa (te Beest et al., 2011; Bailey, 2013).

2.1.1.3 Taxonomic identification

2.1.1.3.1 Morphological characterization

Taxonomic identification of plants is traditionally based on morphological characters (Duminil and Di Michele, 2009). The main morphological characters used for distinguishing the three invasive *Fallopia* taxa are leaf size, shape of leaf base, the presence or absence of trichomes on the lower side of the leaves, and the morphology of the trichomes when present (Handeland, 1991; Fremstad and Elven, 1997; Bailey and Wisskirchen, 2004). *F. x bohemica* can be morphologically variable, and may resemble either parents (Bailey and Wisskirchen, 2004). Morphological characters for identification of the three taxa are more closely described in in Part I, Chapter 1.4.3, p. 18. Identification based on morphological characters has limitations in that morphology can be affected by environmental factors and phenotypic plasticity, and in that it depends on the availability of vegetative or reproductive parts for identification. Closely related taxa may also be morphologically very similar (Duminil and Di Michele, 2009). Tiebre et al. (2007a) found that generally, morphological characters could be used to distinguish between the invasive Fallopia taxa. The most reliable characters for identification of the taxa were found to be leaf length, leaf basal width and leaf central width. The presence of trichomes on the lower side of the leaves and the somewhat cordate leaf bases of *F.* x *bohemica* were sufficient to distinguish the hybrid from *F. japonica*. However, the same study found that some octoploid *F.* x *bohemica* could not be distinguished morphologically from *F. japonica*. Gammon et al. (2007) found continuous morphological variation between *F. japonica* and *F.* x *bohemica*, and concluded that the morphological characters used to distinguish between the taxa in Europe were unreliable for identification of the taxa in New England, USA. This may be due to that sexual reproduction, including

hybridization and introgression, result in a high level of genetic and morphological variation in the USA (Forman and Kesseli, 2003; Gammon et al., 2007, Grimsby et al., 2007).

2.1.1.3.2 Molecular characterization

DNA-based molecular markers have advantages compared to morphological characters in that they are not affected by environmental factors, and can be applied when vegetative or reproductive parts for morphological identification are not available. Several molecular markers have been used to study the genetic diversity and taxonomic compositions of invasive *Fallopia* populations, e.g. inter-simple sequence repeats (inter-SSR) (Hollingsworth et al., 1998), isoenzyme analysis (Mandak et al. 2005), Randomly Amplified Polymorphism DNA (RAPD) (Hollingsworth and Bailey 2000a; b; Tiebre et al. 2007a; Krebs et al., 2010), single nucleotide polymorphisms (SNPs) (Gammon et al. 2007), simple sequence repeats (SSR) (Grimsby et al. 2007) and Amplified Fragment Length Polymorphism (AFLP) (Bzd*f*ôga et al., 2012; Gaskin et al., 2014). Grimsby et al. (2007) developed simple sequence repeats (SSR) markers to examine the genetic diversity of invasive *Fallopia* populations in Massachusetts, USA. One of the SSR markers, KW6, amplified a fragment that was species specific to *F. sachalinensis*. KW6 amplified the fragment in all *F. sachalinensis* and the hybrid *F. x bohemica*, but not in *F. japonica* (Grimsby et al., 2007). The SSR marker KW6 can therefore be used for taxonomic identification and detection of hybridization (Gammon et al., 2007; Grimsby and Kesseli, 2010).

2.1.1.3.3 Cytological characterization, Flow cytometry

Determination of ploidy levels can be used in combination with other methods for morphological identification (Hart et al., 1998; Tiebre et al. 2007a; Krebs et al. 2010). Since *F.sachalinesis* and *F*. x *bohemica* may both be tetraloid, hexaploid or octoploid, and *F. japonica* is octoploid (Bailey et al. 2007), ploidy levels cannot alone reveal the taxonomic identity of the taxa, but can support identification based on other methods. Ploidy levels can be determined through chromosome counting or through flow cytometry. Flow cytometry is a convenient and accurate high-throughput method that measures and analyses multiple optical properties of single particles (e.g. cells or nuclei) that are usually labelled with fluorescent stains. The values can be used to determine physical and chemical characteristics of the particles, including genome size and ploidy level. The total amount of DNA is quantified and

calibrated against the DNA amount of an internal standard, a sample of known chromosome number (te Beest, 2011). Suda et al. (2010) used flow cytometry to determine the amount of nuclear DNA in the invasive *Fallopia* taxa, and found that genome size could be used as a reliable marker for identification of homoploid invasive *Fallopia* taxa.

2.1.1.4 Aims of the study

This study examines the relative distribution of *F. japonica*, *F. sachalinensis* and *F.* x *bohemica* in four areas in south-east Norway and one area in western Norway. A combination of morphological characters and the molecular SSR markers KW2 and KW6 are used for identification of the taxa. The suitability of the chloroplast DNA regions *matK* and *rbcL,* and the nuclear DNA region ITS, as genetic barcodes for identification is assessed. The ploidy levels of the taxa in the study area are determined by use of flow cytometry, performed by extern lab. The aim of the study is to increase knowledge about the distribution of the invasive *Fallopia* taxa and their ploidy levels in the study area.

It was hypothesized that: 1) *F. japonica* is the most frequent taxon in the study area, and *F.* x *bohemica* is more frequent than *F. sachalinensis*. 2) Morphological characters can be used to distinguish between the taxa in the study area, but the molecular markers SSR analysis and DNA barcoding are helpful in situations where identification is uncertain. 3) The ploidy levels found in the study area were expected to be the same ploidy levels that are most commonly found in Europe for these taxa, octoploid *F. japonica*, hexaploid *F.* x *bohemica* and tetraploid *F. sachalinensis*.

2.1.2 Materials and methods

2.1.2.1 Sample collection

121 *Fallopia* stands were sampled in five main areas (Figure 15): Moss (N=26, including 1 sample from Rygge municipality), Ås (N=19, including 2 samples from Frogn municipality and 3 samples from Ski municipality), Oslo (N=23, including 2 samples from Lørenskog municipality and one sample from Asker municipality), Drammen (N=17), and Bergen (N=35, including 2 samples from Meland municipality) A single sample was collected in Sogndal, western Norway. Samples were mostly collected in urban habitats, but some

samples were collected in more rural habitats. 19 out of 23 samples from the Oslo area were collected in the riparian habitat along the river Akerselva in central Oslo. The GPS coordinates of each sampled stand was recorded with a Garmin eTrex 10 GPS. Some of the sampled stands had been recorded previously by others, and their locations were found in the Species Map Service database (http://artskart.artsdatabanken.no/).

Some of the largest leaves from the mid and lower part of the shoots were collected for morphological characterization. These leaves were transported in plastic bags back to the lab, where they were stored in 4°C. Young leaves without signs of fungal infections or herbivory were selected for molecular and cytological analyses (SSR and flow cytometry). Samples for DNA extraction were transported in zip locked bags back to the lab where they were put in sealed plastic tubes, flash frozen using liquid nitrogen and stored in -20°C. For most stands, an additional sample was dried in silica gel. Samples for flow cytometry analyses were dried in silica-gel.

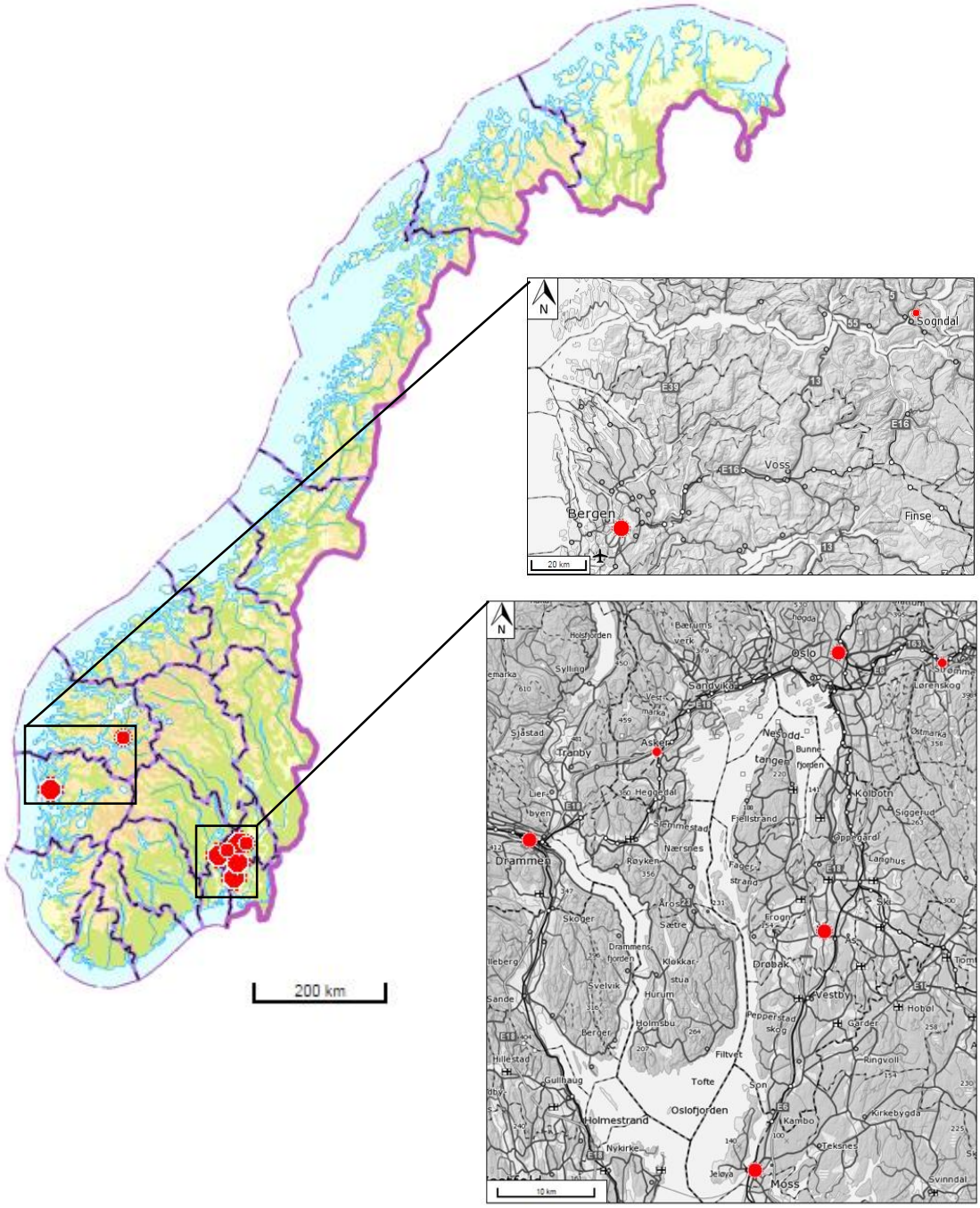


Figure 15 Samples were collected from five main areas (marked with red circles): Bergen in western Norway (A) and Drammen, Oslo, Ås and Moss in southeast Norway (B). A few samples were collected from areas outside the main areas (smaller circles: Sogndal in western Norway, and Asker and Lørenskog in southeast Norway (map generated at **www.kartiskolen.no**).

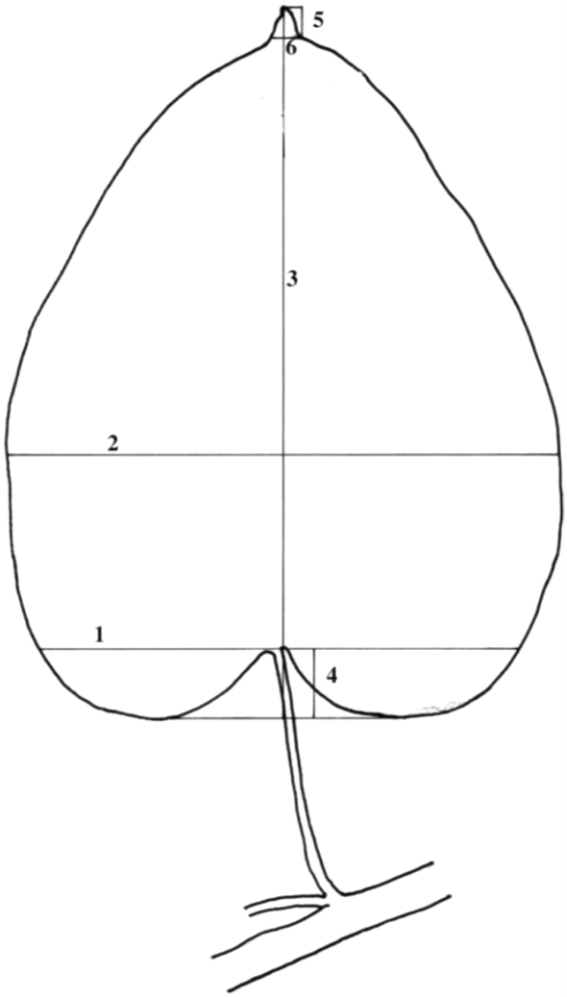


Figure 16a Measurements of leaf morphological characters. (1) Leaf basal width, (2) leaf central width, (3) leaf length, (4) leaf cord length, (5) leaf apex length, and (6) leaf apex width (from Tiebre et al., 2007a).

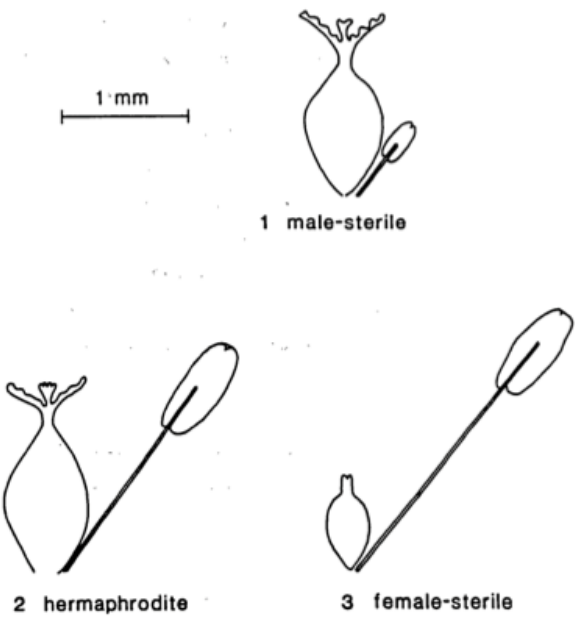


Figure 16b Flower morphologies of the invasive Fallopia taxa. (1) Male-sterile, (2) hermaphrodite and (3) female-sterile (from Bailey, 1989).

2.1.2.1 Morphological characterization

Each stand was assigned to a taxon using the diagnostic morphological characters described by Handeland (1991), Fremstad and Elven (1997), Bailey and Wisskirchen (2004) and Tiebre et al. (2007a). The morphological characters used in Tiebre et al. (2007a) were measured in three to five leaves from each stand (Figure 16a). The thricomes or papillae on the lower side of the leaves were examined using a Leica MZ125 stereomicroscope (up to 100X magnification). When flowers were present, sex was determined according to the descriptions of flower morphology in Bailey (1989) (Figure 16b). A herbarium specimen was made for each sampled stand. Mean measurements of the leaf characters were compared between taxa, and between collection areas in one-way ANOVA analyses with Minitab 16 statistical software. All analyses were followed by Tukey HSD post hoc tests with 95% confidence levels.

2.1.2.1 Molecular characterization

2.1.2.1.1 DNA extraction

DNA was extracted using Qiagen's Plant DNeasy Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturers protocol (Qiagen, 2006, Appendix 1), starting from step 7. For most of the samples, DNA was extracted from frozen plant material. For some samples, DNA was extracted from silica dried plant material. Frozen plant material was thoroughly grinded in liquid nitrogen to a fine powder using a mortar and pestle. A maximum of 100 µg grinded sample was put in 2 ml Eppendorf tubes, which had been
filled with 400 µl buffer AP1 before the sample was grinded. It was important to work quickly to avoid thawing of the sample. When DNA was extracted from silica-dried samples, the plant material was disrupted by placing maximum 20 µg of dry sample in 2 ml Eppendorf tubes together with a 3 mm tungsten carbid bead and mixed for 1 min. at 30 Hz in a mixing mill. The AP1 buffer was then added after the disruption of plant material. After this, the procedure was the same for dried and frozen samples. The procedure is here briefly described:

RNase A was added to digest RNA in the sample. After thorough vortexing, the mixture was incubated for 10 min. in a water bath holding 62°C. This step lysed the cells.

Buffer AP2 was added, followed by vortexing, and incubation on ice for 5 min. In this step, detergent, proteins and polysaccharides were precipitated. The debris was separated from the supernatant by centrifugation, and the supernatant was pipetted into a QIAshredder Mini spin column, which contains a filter that removes most of the precipitates and cell debris. Buffer AP3/E was added to help precipitation of DNA, allowing for isolation of DNA from other compounds in the solution. The mixture was then pipetted into a DNeasy Mini spin column and centrifuged. The spin column contains a special filter (silica-beads) that binds the DNA, while other compounds are washed through. Addition of washing-buffer AW, followed by centrifugation, rinsed the DNA. Buffer AE was pipetted onto the filter, followed by incubation for 5 min. in room temperature. AE is a low salt buffer that dissolves the DNA and washes it off the filter. After a final centrifugation step, the DNA was immediately stored in -20°C.

2.1.2.1.2 Gel electrophoresis

Gel electrophoresis is a fundamental technique in molecular biology that separates molecules by size. Gel electrophoresis was used to verify the quality of the extracted DNA. A 0.8% agarose gel was made as follows: Agarose and 10x TBE (Tris/Borate/EDTA) buffer were mixed in an Erlenmeyer flask. The mixture was boiled in a microwave oven until a clear solution formed. The temperature of the solution was reduced by holding the flask under cold, running water, before ethidium bromide was added as a stain to the solution (625 µg/ml, or 1 drop per 50 ml). Ethidium bromide is a fluorescent dye that binds to DNA, and allows for visualization of the DNA under UV light. The mixture was poured into a mould of appropriate size and a plastic comb was added to form wells in the gel. The solution was cooled in room temperature until the gel solidified. The comb was removed, and the gel was submerged in 10x TBE buffer in an electrophoresis tank. 2 µl DNA isolate from sample was mixed with 1 µl loading-buffer and 3 µl distilled water (sdH_2O) and pipetted into the gel wells. When other amounts of isolate DNA were used (1 µl and 5 µl DNA isolate were used from some samples), the volume of distilled water was adjusted to achieve a total volume of 6 µl. The loading buffer is denser than the TBE buffer and is added to make the DNA sit in the wells. A 1 kb DNA ladder was pipetted into one of the wells. A DNA ladder is a set of fragments of known lengths that can be used for comparison to determine the size of the fragments in the sample (Biology Student Handbook, 2013). Electrophoresis was carried out at approximately 90 V until the fragments were separated. The negatively charged DNA

moves through the gel matrix towards a positively charged pole (anode). The matrix of the gel restricts migration of larger fragments more than it restricts smaller fragments. Bands were visualized and photographed under UV light using a gel documentation system (BIO-RAD Gel Doc™ XR+ System) and analysis software (Quantity One® 1-D, version 4.5.1).

2.1.2.1.3 Simple sequence repeats (SSRs) analysis

Simple sequence repeats (SSRs) (Tautz et al., 1986), also called microsatellites (Litt and Luty, 1989), are short, tandemly repeated sequences 2-6 basepairs long (Chambers and MacAvoy, 2000, cited in Semagn et al., 2006) that are dispersed throughout the genome. SSRs are found in a wide variety of eukaryotes and in the chloroplasts of plants (Jarne and Lagoda, 1996). They are highly polymorphic and informative markers that can be used in studies of closely related species (Kumar et al., 2009). SSR analysis is based on the amplification of DNA by polymerase chain reaction (PCR). The PCR is a fundamental method in molecular biology that is used can amplify DNA sequences that are present in very small amounts, allowing for molecular analyses of those specific sequences (Klug et al., 2007).

115 samples were analysed using the SSR markers KW2 and KW6 (Grimsby et al., 2007). The same PCR reactions and cycling conditions as in Grimsby et al. (2007) were used, except it was used undiluted DNA in the present study. *Taq*-polymerase, forward and reverse primers for each SSR marker, and deoxyribonucleotide triphosphates (dNTPs) were added to the reaction mixture. Polymerases are enzymes that catalyse replication of DNA. Primers are short pieces of single stranded DNA that serves as initiators for polymerase to start DNA replication. dNTPs are the buildingblocks of DNA and contain the four bases adenin (A), thymine (T), guanine (G) and cytosine (C) (Klug et al., 2007). One sample of *F. japonica* of known taxonomic identification, kindly provided by John P. Bailey, University of Leicester, UK, was included in all PCR runs as a positive control. Sterilized distilled water (SdH_2O) was used as a negative control in all PCR runs. The PCR was run in a thermocycler (BIO-RAD T100™Thermocycler) that can raise and lower temperatures to optimal levels for the different steps of the PCR. The DNA denatured into single strands at 94°C, primers annealed to the DNA regions flanking the SSRs at 54°C, and the polymerase extended the primers at 71°C (Figure 17). Primer-sequences, PCR reactions, and thermocycling programs used in the PCR amplification are found in Table 3. Gel electrophoresis was used to verify the presence

or absence of amplified fragments. 10 µl amplification product mixed with 2 µl loading buffer was run on a 1% agarose gel at 90-120 V until bands were properly separated. A 100bp ladder was used for comparison.

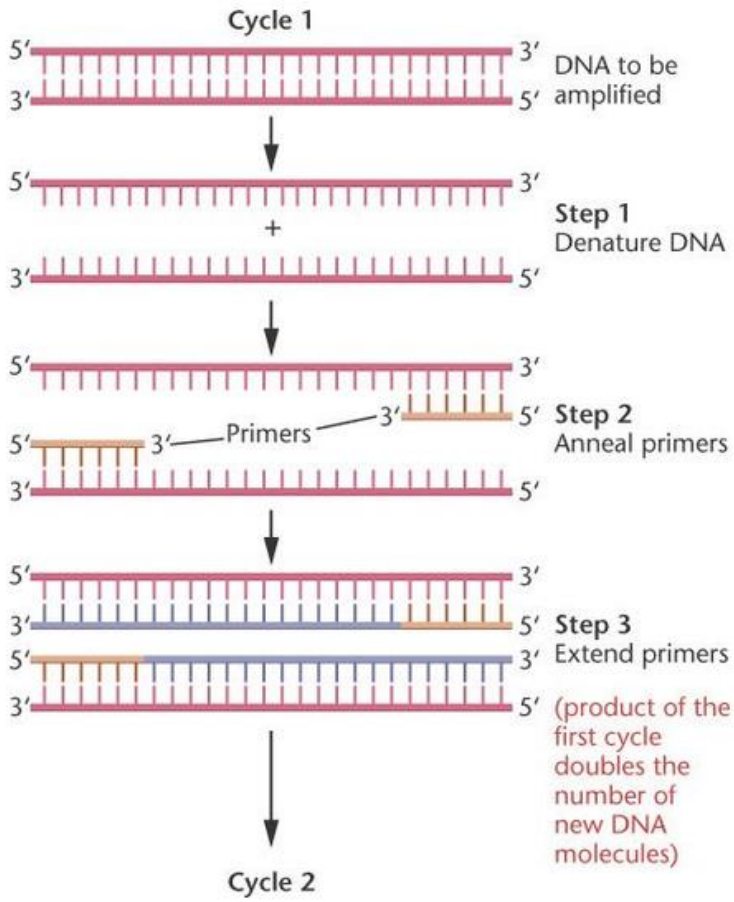


Figure 17 The three steps of polymerase chain reaction (PCR) (From Klug et al., 2007).

2.1.2.1.4 DNA Barcoding

DNA barcoding is a method for identifying species by using short, standardized DNA sequences. The sequences are PCR amplified with specific primers, and are compared to reference sequences found in databases (CBOL, n.d.). The Consortium for the Barcode of Life (CBOL) Plant Working group has approved the chloroplast coding regions maturase K (*matK*) and ribulose-1.5-bisphosphate-carboxylase (*rbcL*) as regions for plant barcodes (CBOL, 2009). The *rbcL* region is found to be easy to PCR amplify, sequence and align, but the discriminatory power of *rbcL* is limited. The *matK* region is a rapid evolving section of

the genome and has a stronger discriminatory power than the *rbcL* region, but is more difficult to PCR amplify. It is therefore recommended that both the *rbcL* region and the *matK* region is used for barcoding of plants (Hollingsworth et al., 2011).

A BLAST search in the nucleotide database GenBank at NCBI (www.ncbi.nlm.nih.gov/) indicated that it is possible to discriminate between *F. sachalinensis* and *F. japonica* by sequencing of the chloroplast DNA regions *matK* (5 informative sites among 833 bp) and *rbcL* (7 informative sites among 797 bp). Chloroplast DNA is maternally inherited (Chase et al., 2005) and cannot be used to differentiate between *F. japonica* and the hybrid *F.* x. *bohemica.* The nuclear ribosomal internal transcribed spacers 1 and 2 (ITS1 and ITS2) were therefore sequenced to check for possible differences between plants morphologically identified as *F. japonica* and plants morphologically identified as *F. x bohemica*. Primers used in the PCR amplification of the DNA sequences are found in Table 4. Polymerases and reaction mixtures are found in Table 5. PCR amplification was carried out in a total volume of 25 µl reaction mixture. The ITS sequence was amplified once with *Taq* DNA polymerase and once with Phusion DNA polymerase. The *matK* sequence and the *rbcL* sequence were only amplified with Phusion DNA polymerase. Phusion DNA polymerase is known to have a lower error-rate than *Taq* DNA polymerase (New England Biolabs, 2014). The PCR was run in a thermocycler (GeneAmp® PCR system 9700) with the cycling conditions given in Table 5. Amplification success was verified by gel electrophoresis, using 2 µl amplification product on a 1% agarose gel, and a 100 bp ladder for comparison. Successfully amplified PCR products were send to GATC Biotech (Germany) for sequencing.

2.1.2.2 Flow cytometry

Flow cytometry analyses were performed by Plant Cytometry Services (Schijndel, Netherlands). 96 samples were analysed. Both fresh and silica-gel dried leaves were analysed for 16 samples. 80 samples were analysed with silica-dried leaves only. The fresh samples were analysed first, with *Vinca major* as internal standard. Because the DNA amount of *F. sachalinensis* was very similar to the DNA amount of *Vinca major*, which could result in difficulties with detecting the *Fallopia* sample, tetraploid *Pachysandra terminalis* was used as internal standard for the remaining analyses of the dried samples.

Table 3 SSR analysis. Primer sequences, reactions, and thermocycling program used in the PCR amplification of the SSR markers KW2 and KW6 (Grimsby et al. 2007).

SSR Marker (size)	**Primer sequence (5' -> 3')**	**PCR reaction mixture (total volume 25 µl)**
KW2 (456 bp)	F: CGATGGAGTAGGTCTTATCTATTTAT R: CCTCTACTCAGTTCTCTAGTGAAGGTC	1.0 µl of undiluted DNA, 1.0 µl of each primer (10 pmol/µl), 2.5 µl of 25 mM $MgCl_2$, 2.5 µl of 100X BSA, 0.2 µl of *Taq* polymerase 17.8 µl sdH_2O
KW6 (338 bp)	F: TGGTTTTGTTTCAAGTTTCTTGTG R: TGTTGATGGTTGGTTGCTTC	
PCR thermocycling program (*Taq*-polymerase):		2 min denaturation at 94°C; 35 cycles of 94°C for 30 s, 54°C for 39 s, 71°C for 30 s; followed by a final extension at 71°C for 5 min, then 4°C for forever.

Table 4 Primers used in the PCR amplification of the ITS, *matK*, and *rbcL* sequences.

DNA region	**Primers**	**Primer sequence (5' -> 3')**	**Reference**
ITS	*ITS1* *ITS4*	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	White et al. (1990)
matK	*matK_X* *matK_5*	TAATTTACGATCAATTCATTC GTTCTAGCACAAGAAAGTCG	Ford et al. (2009)
rbcL - accD	*rbcL-f* *accD-r*	TAGCTGCTGCTTGTGAGGTATGGA AAATACTAGGCCCACTAAAGG	Dong et al. (2012)

Table 5 Polymerases, reactions and thermocycling programs used in the PCR amplification of the different DNA sequences.

DNA sequence	DNA Polymerase	PCR reaction mixture (total volume 25µl)	PCR thermocycling program
ITS1 -ITS4	*Taq*-polymerase	2.5µl buffer (10x), 2µl dNTP(2.5µM), 0.5µl ITS1(50µM), 0.5µl ITS4(50µM), 0.125µl *Taq*-polymerase, 17.375µl mQ H_2O, 2µl template DNA	Initial denaturation at 94°C for 5 min., followed by 35 cycles consisting of 94°C for 30 sec., 55°C for 30 sec. and 72° for 1 min., with a final extension step at 72°C for 7 min and then 4°C for forever.
ITS1 – ITS4	Phusion	5µl buffer HF Phusion (5x), 2µl dNTP (2.5µM), 1.5µl forward primer (10µM), 1.5µl reverse primer (10µM), 0.75µl DMSO, 0.25µl Phusion polymerase, 12.5 µl nuclease-free H_2O, 2µl template DNA	Initial denaturation at 98°C for 30 sec., 35 cycles consisting of 98°C for 10 sec., 52°C for 30 sec and 72°C for 30 sec. were performed, with a final extension step at 72°C for 7 min. and then 4°C for forever.
rbcL-f – accD-r	Phusion		
matK_X – matK_5	Phusion		

2.1.3 Results

2.1.3.1 Morphological characterization

The ANOVA analyses of leaf character measurements revealed significant differences between *F. japonica* and *F.* x *bohemica* in all measured leaf characters ($p<0.05$), except the ratio apex width to apex length (Table 6). *F. sachalinensis* was not included in the analysis because only one of the two stands had full-grown leaves.

Average leaf length in *F. japonica* ranged from 12-20 cm. Leaf bases were mostly straight to slightly cordate, but some leaves in some stands were more distinctly cordate (Appendix 3, Figure 59 - Figure 60). *F. x bohemica* had longer and broader leaves, generally with more cordate leaf bases than *F. japonica*, but shape of leaf base varied between different leaves

(Appendix 3, Figure 61 - Figure 62). Average leaf length in *F.* x *bohemica* ranged from 18.5 – 26.5 cm. *F.* x *bohemica* had a smaller leaf width to leaf length ratio than *F. japonica* (Table 6). *F. sachalinensis* had much larger leaves than the other taxa and distinctly cordate leaf bases (Appendix 3, Figure 63).

The papillae on the lower leaf surface of *F. japonica* varied from smooth and rounded, to more elongated, upright and bluntly pointed (Appendix 3, Figure 64 - Figure 71). *F. x bohemica* had stout, upright trichomes of variable lengths. Some trichomes seemed to be at least 4 cells long (Appendix 3, Figure 72 - Figure 75). *F. sachalinensis* had long, thin, flexious trichomes (Appendix 3, Figure 76 - Figure 77).

Shoot height was not measured in most stands, but measurements of some stands showed that *F. japonica* can reach heights well over 3 m. *F.* x *bohemica* seems to generally be taller than *F. japonica*, but shoot height varied in both taxa. *F.* x *bohemica* seemed to have more upright branches than *F. japonica*, but this character was not measured. Additional extrafloral nectaries on the sides of the stem were found in *F. sachalinensis*, but not in *F.* x *bohemica.*

The ANOVA analysis of variations in leaf characters between different areas gave significant differences in leaf length between *F. japonica* in Bergen and *F. japonica* in Ås and Moss, and in cord length between *F. japonica* in Bergen and *F. japonica* in Drammen ($p<0.05$, Table 7). No significant differences were found in leaf characters between *F. x bohemica* from different areas ($p>0.05$).

2.1.3.1.1 Flower morphology

All flowering *F. japonica* were male-sterile (Appendix 3, Figure 78). The two *F. sachalinensis* were also male-sterile (Appendix 3, Figure 79). *F. x bohemica* had hermaphrodite flowers with variable morphology (Appendix 3, Figure 80 - Figure 82). One stand had flowers that had characters similar to female-sterile flowers, with large, pollen-filled anthers on long filaments, but the pistil seemed to be more developed than in female-sterile flowers (Appendix 3, Figure 82). Both the inflorescences and the leaves of this stand differed morphologically from the other stands of *F. x bohemica*. The leaves were somewhat more oblong (Appendix 3, Figure 61 B.), and the inflorescences were longer and less

compact than the inflorescences observed on the other *F. x bohemica* in this study. No seeds were found in any of the stands during the study.

Table 6 Mean measurements (cm) of leaf characters of *F. japonica*, *F. x bohemica* and *F. sachalinensis.* Some of the largest leaves found on the mid to lower part of the shoots were selected for measurement. Different letters within a row indicates significant differences between taxa ($p<0.05$). FJ = *F. japonica* (n=76), FB = *F. x bohemica* (n=22), FS = *F. sachalinensis* (n=1). Average = Average of all stands. SE = Standard error. Range = range of averages.

Character	**FJ**			**FB**			**FS**
	Average	**SE**	**Range**	**Average**	**SE**	**Range**	
Leaf length	15.7 [a]	1.73	11.9 – 19.7	21.1 [b]	1.81	18.5 – 26.5	38.1
Leaf basal width	13.2 [a]	1.13	9.4 – 15.4	15.8 [b]	0.92	14.0 – 16.9	29.9
Leaf central width	14.1 [a]	2.09	11.5 – 18.5	18.0 [b]	1.12	15.4 – 20.7	30.6
Leaf cord length	0.3 [a]	0.27	0 – 1.3	0.9 [b]	0.47	0.2 – 2.1	6.2
Leaf apex length	1.2 [a]	0.28	0.7 – 2.0	1.4 [b]	0.31	0.9 – 2.1	2.7
Leaf apex width	1.3 [a]	0.28	0.6 – 1.9	1.6 [b]	0.31	1.0 – 2.2	1.3
Cord length to leaf length	0.02 [a]	0.02	0 – 0.07	0.04 [b]	0.02	0.01 – 0.11	0.14
Leaf basal width to leaf length	0.85 [a]	0.08	0.59 – 1	0.75 [b]	0.05	0.64 – 0.85	0.79
Apex width to apex length	1.11 [b]	0.20	0.59 – 1.67	1.13 [b]	0.19	0.86 – 1.57	0.48

Table 7 Mean measurements (cm) of leaf characters of *F. japonica* from different collection areas. Different letters in a row indicate significant differences between localities ($p<0.05$).

Locality	**Leaf length**	**Basal width**	**Central width**	**Cord length**	**Apex length**	**Apex width**	**Cord length to leaf length**	**Basal width to leaf length**	**Apex width to apex length**
Bergen	16.8 [a]	13.3 [a]	14.5 [a]	0.4 [a]	1.3 [a]	1.2 [ab]	0.02 [a]	0.80 [b]	1.07 [a]
Drammen	15.6 [ab]	13.5 [a]	14.4 [a]	0.1 [b]	1.2 [a]	1.3 [ab]	0.01 [b]	0.87 [ab]	1.05 [a]
Moss	15.2 [b]	12.9 [a]	14.0 [a]	0.3 [ab]	1.3 [a]	1.5 [a]	0.02 [ab]	0.85 [ab]	1.22 [a]
Oslo	15.3 [ab]	13.3 [a]	13.6 [a]	0.3 [ab]	1.1 [a]	1.1 [b]	0.02 [ab]	0.88 [a]	1.01 [a]
Ås	14.4 [b]	12.9 [a]	13.6 [a]	0.2 [ab]	1.2 [a]	1.4 [ab]	0.02 [ab]	0.90 [a]	1.16 [a]

2.1.3.2 Molecular characterization

2.1.3.2.1 SSR analysis

The KW6 marker amplified fragments in 24 of the 113 samples, indicating that these were either *F. x bohemica* or *F. sachalinensis*. The KW2 marker amplified fragments in all samples, indicating that DNA could be successfully amplified from all samples (Figure 18).

2.1.3.2.2 DNA Barcoding

Sequences were successfully PCR amplified with the primers ITS1 and ITS4, using Phusion DNA polymerase, and with the primers matK_X and matK_5, using Phusion DNA polymerase. Fragment amplification was not successful in sample no. 8 and 10 (Figure 19). PCR amplification was not successfull with the primers ITS1 and ITS4 using *Taq* DNA polymerase, or with the primers rbcL-f and accD-r using Phusion DNA polymerase.
It was not possible to differentiate between the samples identified as *F. japonica* and *F. x bohemica* by sequencing of the ITS and *matK* regions, since the sequences of the two taxa were identical.

Five *F. japonica* and five *F. sachalinensis* sequences were available for comparison of the ITS region in GenBank (Table 8). The sequence from the present study differed both from the *F. japonica* sequences and from the *F. sachalinensis* sequences found in GenBank. Alignment of the ITS sequence with reference sequences from Genbank showed that the sequence from the present study had more in common with the *F. japonica* sequences than the *F. sachalinensis* sequences, indicating that this region can be used to distinguish *F. sachalinensis* from the other two taxa. The sequence from the present study was also more similar to one of the *F. japonica* sequences in GenBank, than to the other sequences in GenBank. This *F. japonica* was from Japan, Kyoto, Mt. Kurama and was sequenced by Won, H and Park, C. -W., (1999) (GenBank ID: AF189734.1). The sequence from the present study and the sequence from Japan both differed from the other GenBank *F. japonica* sequences in seven positions (see Appendix 4 for alignment of sequences).

One *F. japonica* and one *F. sachalinensis* were available for alignment of the *matK* sequence (Table 8). These sequences were from the UK (Cuénoud et al., 2002), and both differed from the sequence found in the present study. The sequence from the present study differed from

the *F. japonica* sequence from Cuénoud et al. (2002) in six positions. The sequence from the present study and the *F. japonica* sequence were equal to each other, but different from the *F. sachalinensis* sequence, in two positions. The sequence from the present study was equal to *F. sachalinensis*, but different from the other *F. japonica*, in four positions. The sequence from the present study differed from both the other sequences (*F. sachalinensis* and *F. japonica*) in three positions (see Appendix 4 for alignment of sequences).

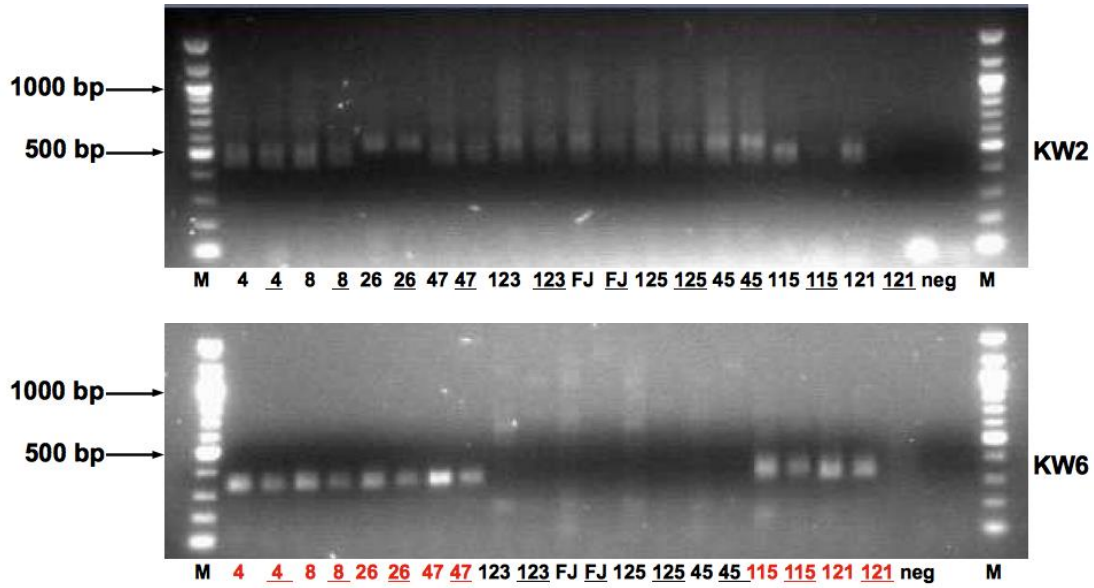
2.1.3.3 Flow cytometry

The flow cytometry analysis revealed that the samples identified as *F. japonica* were octoploid, samples identified as *F. x bohemica* were hexaploid and samples identified as *F. sachalinensis* were tetraploid. Examples of DNA histograms showing flow cytometry results are found in Figure 20.

Table 8 Reference sequences found in GenBank for alignment of the ITS sequence and the *matK* sequence.

DNA region	Taxon	Country	Reference	GenBank ID
ITS	*F. sachalinensis*	Korea	Won,H. and Park,C.-W (1997)	AF040073.1 AF040074.1
ITS	*F. sachalinensis*	Korea	Won,H. and Park,C.-W (1999)	AF189737.1 AF189735.1 AF189736.1
ITS	*F. japonica*	Korea	Won,H. and Park,C.-W (1997)	AF040070.1 AF040071.1
ITS	*F. japonica*	Germany	Kersten,T. and Knoess,W. (2008)	EU808015.1
ITS	*F. japonica*	China	Zhang,P., Meng,X. and Zhang,C. (2010)	HM357906.1
ITS	*F. japonica*	Japan	Won,H. and Park,C.-W. (1999)	AF189734.1
matK	*F. sachalinensis*	UK	Cuénoud et al. (2002)	AY042635.1
matK	*F. japonica*	UK	Cuénoud et al. (2002)	AY042586.1

A.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500 bp
KW2
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1000 bp
500 bp
KW6
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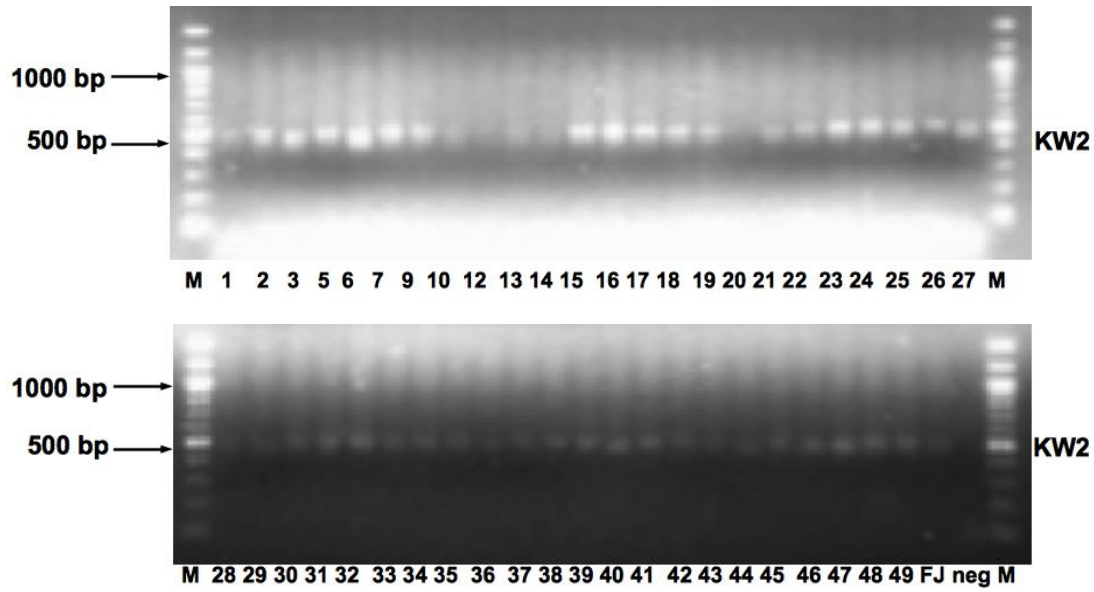
B.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500 bp
KW2
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1000 bp
500 bp
KW2
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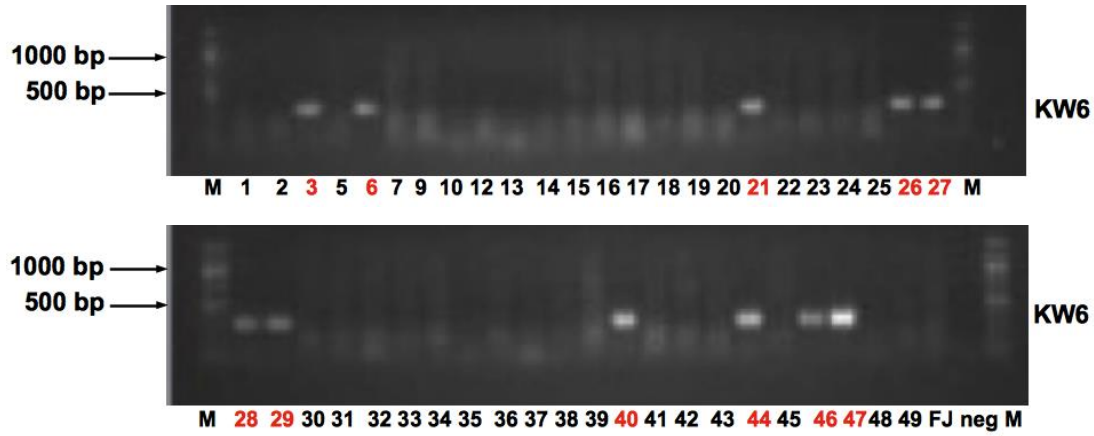
C.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500 bp
KW6
M 1 2 3 5 6 7 9 10 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 M
1000 bp
500 bp
KW6
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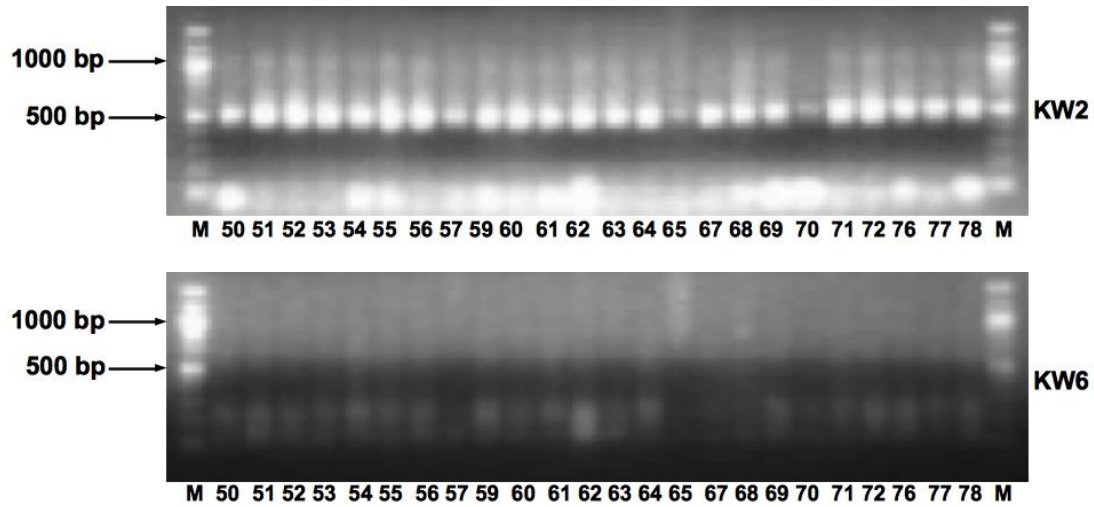
D.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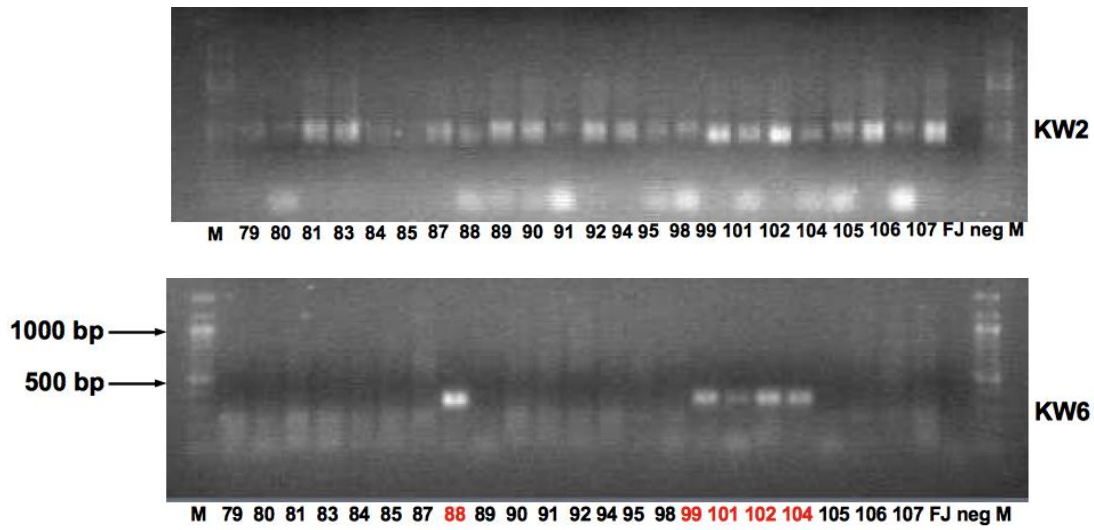
500 bp
KW2
M 50 51 52 53 54 55 56 57 59 60 61 62 63 64 65 67 68 69 70 71 72 76 77 78 M
1000 bp
500 bp
KW6
M 50 51 52 53 54 55 56 57 59 60 61 62 63 64 65 67 68 69 70 71 72 76 77 78 M

E.


KW2
M 79 80 81 83 84 85 87 88 89 90 91 92 94 95 98 99 101 102 104 105 106 107 FJ neg M
1000 bp
500 bp
KW6
M 79 80 81 83 84 85 87 88 89 90 91 92 94 95 98 99 101 102 104 105 106 107 FJ neg M

F.

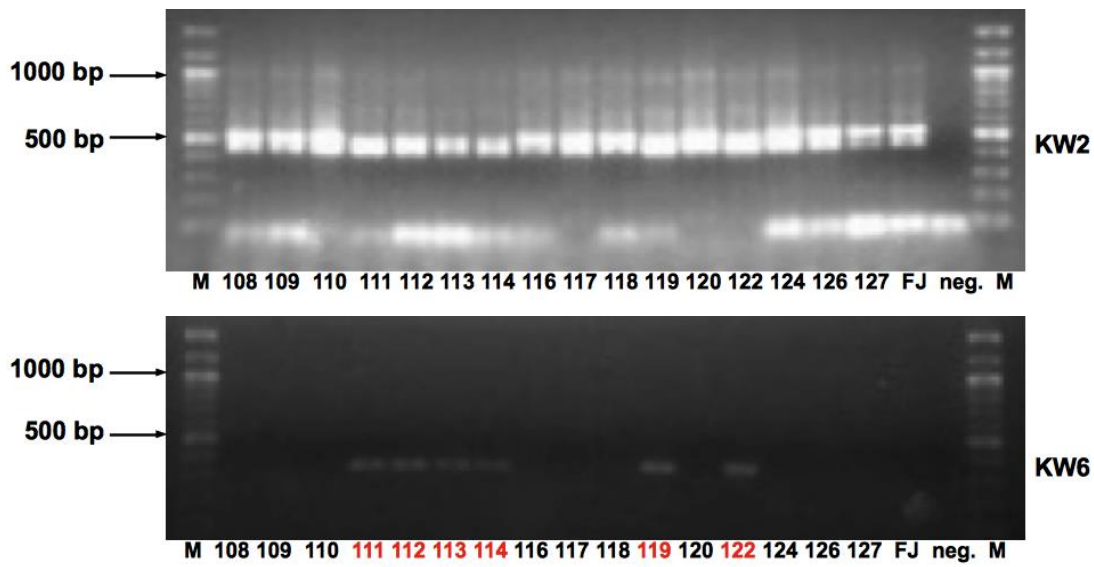

1000 bp
500 bp
KW2
M 108 109 110 111 112 113 114 116 117 118 119 120 122 124 126 127 FJ neg. M
1000 bp
500 bp
KW6
M 108 109 110 111 112 113 114 116 117 118 119 120 122 124 126 127 FJ neg. M

Figure 18 (previous pages) PCR products amplified by SSR markers KW2 and KW6 (Grimsby et al., 2007). Lanes M contain 100 bp ladders. FJ = control sample of *F. japonica* of known taxonomic identity. Neg = negative control (sdH_2O). Samples in which the KW6 fragment was amplified are highlighted with red colour. Amplification of KW6 fragments indicates that a sample is *F. x bohemica* or *F. sachalinensis.* The SSR marker KW2 amplifies fragments in all three taxa (*F. japonica, F. x bohemica and F. sachalinensis*). **A.** PCR nr 1. Test SSR (optimization). Undiluted and diluted (10x) DNA was used. Underlined numbers denotes diluted DNA. **B.** PCR nr 2. SSR marker KW2. **C.** PCR nr 3. SSR marker KW6. **D.** and **E.** PCR nr 4. SSR markers KW2 and KW6. **F.** PCR nr. 5. SSR markers KW2 and KW6.

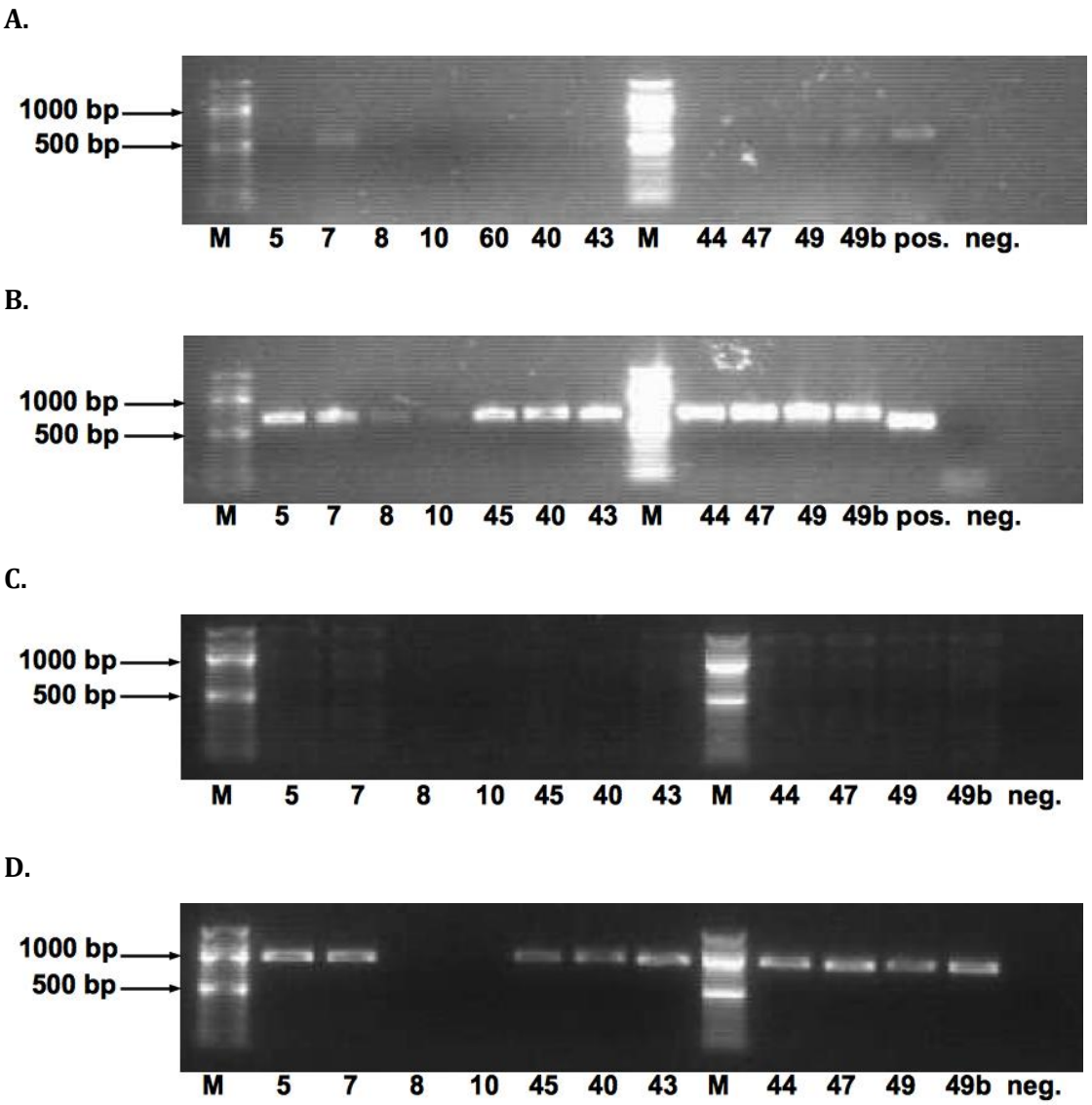
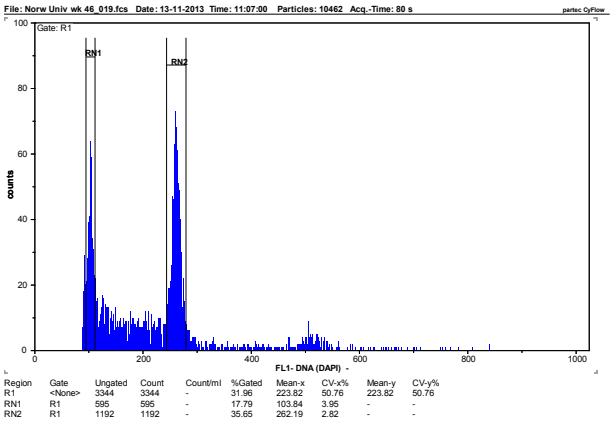
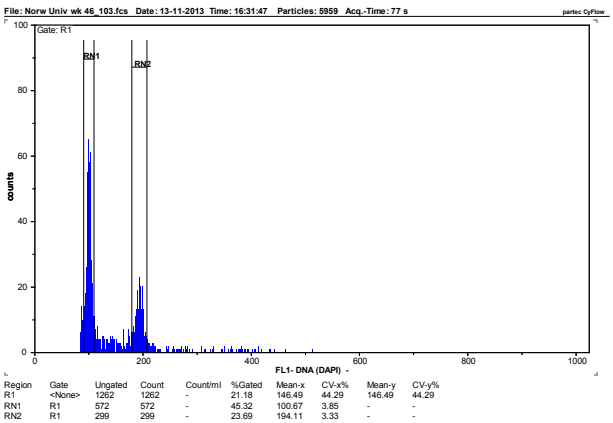


Figure 19 (above) Results of PCR amplification of **A.** ITS region with primers ITS1 + ITS4 and *Taq* DNA polymerase. **B.** ITS region with primers ITS1 + ITS4 and Phusion DNA polymerase. **C.** *rbcL* region with primers rbcL-f and accD-r and Phusion DNA polymerase. **D.** *matK* region with primers matK_X and matK_5 and Phusion DNA polymerase. Lanes M contain 100 bp ladders. Pos. = positive control (fungal DNA). Neg. = negative control (sdH_2O). Other lanes contain DNA from *Fallopia* spp. morphologically identified as *F. japonica* (5, 7, 10, 45, 43, 49 and 49b) or *F. x bohemica* (8, 40, 44 and 47).

A



B



C

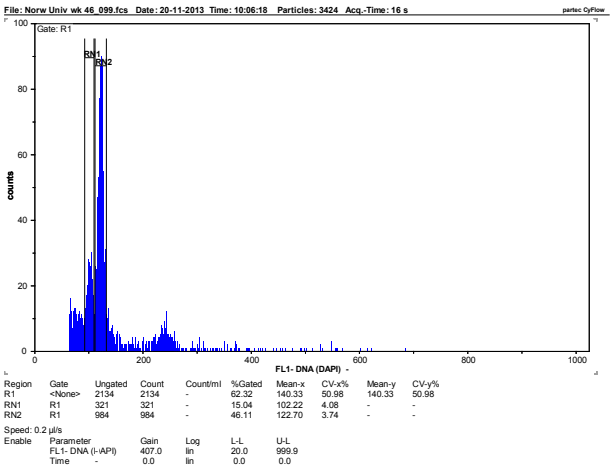


Figure 20 Examples of DNA histograms from flow cytometry analysis of **A.** octoploid (8X) *F. japonica*, **B.** hexaploid (6X) *F. x bohemica*, **C.** tetraploid (4X) *F. sachalinensis.* Internal standard is *Pachysandra terminalis* (4X) (RN1, bar to the left).

2.1.3.4 Summary of results of the analyses

A summary of the results from the morphological identification, SSR and flow cytometry analyses is found in Table 9.

Table 9 Summary of results of morphological identification, SSR analysis and flow cytometry analysis.

Morphological identification (n=121)	**SSR analysis (n=113)**		**Ploidy level (n=96)**
	KW2 fragment amplified	**KW6 fragment amplified**	
F. japonica	+	–	8X Octoploid
F. x bohemica	+	+	6X Hexaploid
F. sachalinensis	+	+	4X Tetraploid

2.1.3.5 Distribution of the taxa

Identification based on morphological characters, SSR analysis and flow cytometry revealed that 92 stands (76.7%) were *F. japonica*, 26 stands (21.7 %) were *F. x bohemica*, and 2 stands (1.7%) were *F. sachalinensis*. The relative proportions of the three taxa differed between the areas (Figure 21). *F. x bohemica* was the most frequent taxon in the examined area in Oslo (mainly along Akerselva river), where it represented 61% of the sampled stands. *F. x bohemica* was found in four of the five areas, but not in the examined area in Bergen. *F. sachalinensis* was rare in the study area, and was only found in Drammen. The single sample collected in Sogndal in western Norway was *F. japonica. F. x bohemica* was more frequent than what was previously recorded in the Species Map Service. Some of the *F.* x *bohemica* had been recorded as *F. japonica*, and a few as *F. sachalinensis.* Some of the *F. sachalinensis* stands recorded in the Species Map Service were searched for in the present study, but not found. Maps showing the locations of the stands sampled in the study area are found in Appendix 2.

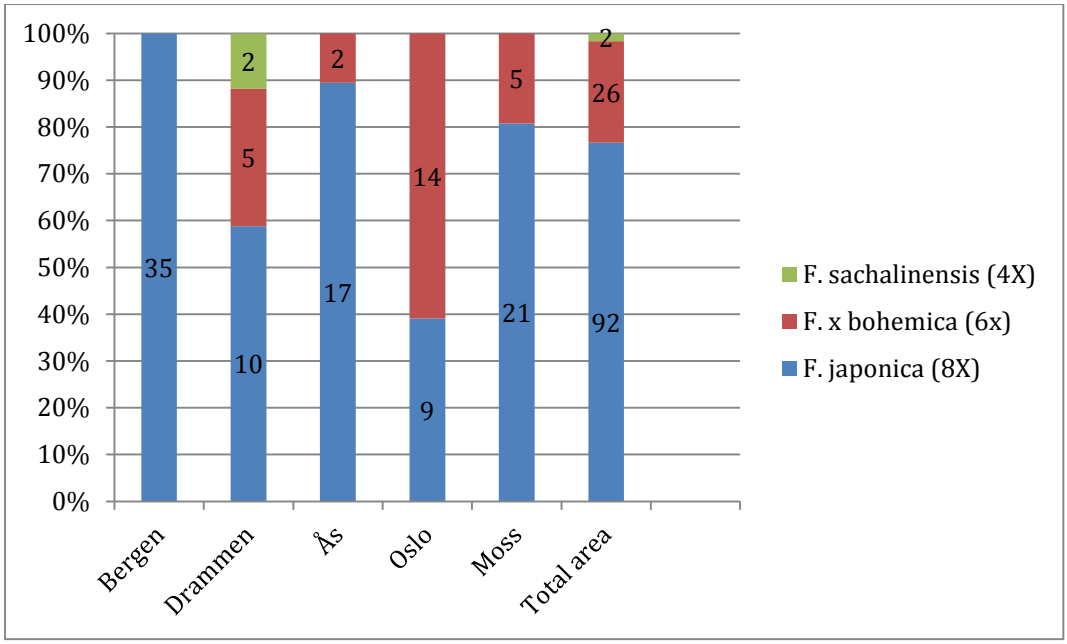


Figure 21 Percentage proportions represented by each taxon in the different areas and the total study area. Values within bars indicate the number of stands recorded (n).

2.1.4 Discussion

F. japonica was the far most frequent taxon in the study area, representing 92% of the sampled stands. *F.* x *bohemica* was more frequent in the study area than what was previously recorded in the distribution map of the Species Map Service. Where *F.* x *bohemica* was present in areas that had been previously surveyed, it had been identified mostly as *F. japonica*, and sometimes as *F. sachalinensis*. The area along the Akerselva river in Oslo, where *F.* x *bohemica* was most frequent, had not been surveyed previously. The results of the present study indicate that the distribution of *F.* x *bohemica* in Norway is underestimated in currently available distribution maps, due to misidentification of *F.* x *bohemica,* and the occurrence of *F.* x *bohemica* in areas that have not yet been examined. *F.* x *bohemica* was not present in the area examined in Bergen, but was present in the four other areas. *F.* x *bohemica* is however recorded previously in Bergen, but in a different area than the area examined in the present study (Handeland, 1991). *F. sachalinensis* was rare, and was only represented by two stands, both of them located in Drammen.

Other European studies have also found that *F. japonica* is most frequent of the three taxa and that *F. sachalinensis* is generally less frequent than *F.* x *bohemica* (Bailey, 2003; Mandak et al., 2004; Tiebre et al., 2008; Krebs et al., 2010), the relative importance of *F.*

japonica and *F*. x *bohemica* can differ between regions (Tiebre et al., 2007a). The lower frequency of *F. sachalinensis* may be connected to the lower regeneration potential of vegetative fragments (Bimova et al., 2003, Bailey et al., 2009). Mandak et al. (2004) found that *F.* x *bohemica* is spreading faster than the parental taxa, which could be due to the increased regeneration potential of *F.* x *bohemica* compared to the parental taxa (Child, 1999; Bimova et al., 2003; Bailey et al., 2009). Tiebre et al. (2008) however found that *F. japonica* and *F.* x *bohemica* had comparable increase in distribution over three years.

The results of the SSR analysis supported the morphological identification, indicating that the taxa can be distinguished by the morphological characters described by Handeland (1991), Fremstad and Elven (1997), Bailey and Wisskirchen (2004) and Tiebre et al. (2007a).
The identification was also supported by a parallel study, where the same DNA used in the present study was analysed by Amplified Fragment Length Polymorphism (AFLP) (Elameen, A., unpublished). The AFLP study showed that all samples that had been assigned to the same taxon, were the same genotype. The results of the AFLP study contrasts with other studies in Europe in that no genetic variation was found within *F. sachalinensis* and *F.* x *bohemica* (Hollingsworth and Bailey 2000a; b; Mandak et al., 2005; Tiébré et al., 2007a; Krebs et al., 2010). The absence of genetic variation within *F. japonica* however corresponds with other European studies, except the recent Polish study where several *F. japonica* genotypes were found through AFLP analysis (Bzd*f*ôga et al., 2012). The British *F. japonica* sample used as control in the present study was also included in the AFLP analysis (Elameen, A., unpublished), and the results showed that the British *F. japonica* and the *F. japonica* found in the Norwegian study area is the same genotype.

The results of the morphological characterization correspond with the results of Tiebre et al. (2007a) in that the measured leaf characters differed significantly between taxa. The measurements from the present study were larger for both *F. japonica* and *F. x bohemica* than what was found by Tiebre et al. (2007a) in Belgium. It may be that the taxa generally produce larger leaves in the Norwegian study area compared to in Belgium, but part of the differences in the results may also be due to differences in the method when leaves were selected for measurements. While Tiebre et al. (2007a) systematically collected three leaves from three shoots in each stand, leaves were not collected systematically in the present study. Instead, the larger leaves were selected, and only 3-5 leaves were measured in each stand. For further studies, it may be suggested that the method and the leaf characters described by

Tiebre et al. (2007a) is used as a standard for morphological characterization of invasive *Fallopia* taxa. This would allow for comparison of the leaf morphology of the taxa between different parts of the distribution range.

In the present study, the size ranges of all measured leaf characters were overlapping between *F. japonica* and *F.* x *bohemica*. Tiebre et al (2007a) also found continuous variation between *F. japonica* and *F.* x *bohemica*, but found that the presence of trichomes on the lower leaf surface and the somewhat cordate leaf bases in *F.* x *bohemica* were sufficient to distinguish between the taxa. This corresponds to the findings of the present study. However, while cordate leaf bases were generally much more pronounced in stands of *F. x bohemica*, cordate leaf bases were also present in some *F. japonica*. This indicates that the shape of the leaf base may not always be a reliable diagnostic character used alone. Tiebre et al. (2007a) found that the characters that best distinguished the taxa were leaf length, leaf basal width and leaf central width. These characters differed significantly between the taxa also in the present study, although the ranges of the two taxa were overlapping. The presence of stout trichomes on the lower side of the leaves seems to be the most reliable diagnostic character for *F.* x *bohemica*. The papillae of *F. japonica* could be elongated, rough and upright, but differed distinctly from the trichomes of *F. x bohemica. F.* x *bohemica* always had trichomes that were clearly more than one cell long, and the trichomes were more pointed than the papillae of *F. japonica*. *F. sachalinensis* could be distinguished from the other taxa by its much larger, oblong leaves, the typical "lyre-shaped" leaf base, and the clearly multi-celled, flexious trichomes on the lower side of the leaves.

Comparison of leaf morphology between different locations gave significant differences in leaf length and cord length between *F. japonica* from Bergen and *F. japonica* in some of the areas in southeast Norway. The results of the statistical analyses should however be accepted with some reservations, due to the non-systematical method for collection of leaves. Handeland (1991) noted that some *F. japonica* in Bergen had particularly large, robust stems, and large leaves up to 18 cm long, and suggested that these plants could be a different variety of *F. japonica* than the common *F. japonica* var. *japonica*. Since the AFLP analysis showed that no genetic variation was present within any of the taxa, (Elameen, A., unpublished), the morphological variations may be explained by phenotypic plasticity and epigenetic effects. Phenotypic plasticity refers to the potential of specific traits of a genotype to respond to

different environments (Richards et al., 2006). Previous studies have found plasticity in several functional traits in the invasive *Fallopia* taxa, e.g. height, leaf area, succulence, and root-to-shoot ratio (Richards et al., 2008; Walls, 2010; Herpigny et al., 2012; Richards et al., 2012). Plasticity in ecologically relevant traits can increase fitness and adaptation in a range of habitats, and may contribute to the success of some invasive species (Richards et al., 2006; Bossdorf et al., 2008a; Richards et al., 2008; Walls, 2010; Richards et al., 2012). The variation in leaf size found in *F. japonica* in the present study may be due to phenotypic plasticity in response to more shaded habitats. Shaded leaves have increased specific leaf area (SLA) compared to leaves that grow in full sunlight (Gratani, 2014, and references therein). *F. japonica* is a light dependent plant that is affected by moderate shadow (Beerling, 1994), and phenotypic plasticity in response to different light conditions would therefore be important for the ability of *F. japonica* to adapt to diverse habitats.

Phenotypic plasticity can be mediated by epigenetic effects (Bossdorf et al., 2008b; Richards et al., 2008; Aubin-Horth and Renn, 2009; Richards et al., 2012). Epigenetic effects are molecular processes that can activate, reduce, or disable the activity of particular genes. Gene expression and function can be altered without changes in DNA sequence, and new phenotypes can be formed within the same genotype (Richards et al., 2006; Bossdorf et al., 2008b). Epigenetic changes can persist through sexual and asexual reproduction (Verhoeven et al., 2010; Richards et al., 2012). High levels of epigenetic variation have been found in invasive *Fallopia* population with low levels of genetic variation (Richards et al., 2012). The invasive *Fallopia* taxa provide a good system for epigenetic studies because of their low genetic diversity and their broad ecological distribution (Bossdorf et al., 2008b; Richards et al., 2012). In Norway, where seed production is not reported, the genetic variation within the taxa can be expected to be particularly low. The conditions in Norway may therefore be well suited for studies of epigenetic effects in these taxa.

F. x *bohemica* and *F. sachalinensis* were successfully distinguished from *F. japonica* by use of the SSR marker KW6. Expensive fluorescent labels were not needed, and the PCR products could be run on regular agarose-gel. SSR analysis with KW6 provides therefore a useful and achievable tool for taxonomic identification in situations where taxonomic identity is uncertain and an absolute identification is needed. KW6 can however not distinguish between *F.* x *bohemica* and *F. sachalinensis.*

Sequencing of the ITS region appears to be more useful than the *matK* region in distinguishing *F. sachalinensis* from the two other taxa. The limited suitability of *matK* in distinguishing between *F. japonica* and *F. sachalinensis* may be due to the low number of reference sequences available for comparison in Genbank (one *F. japonica* and one *F. sachalinensis*). None of the regions sequenced in the present study could distinguish between *F. japonica* and *F.* x *bohemica*. The identical *matK* sequences of *F. japonica* and *F.* x *bohemica* can be explained by maternal inheriting of chloroplast DNA (Chase et al., 2005). The identical ITS sequences may be explained by the close relationship between the hybrid and its parent, and the short time since hybridization. The ITS sequence from the present study had more in common with a *F. japonica* sequence from Japan than with the *other F. japonica* ITS sequences in Genbank. This similarity could be due to the Japanese origin of the European *F. japonica* genotype (Bailey and Conolly, 2000). Finding robust markers that can effectively distinguish between species is a challenge in barcoding of land plants (Hollingsworth, 2011, Chase et al., 2005). Barcoding has limitations in identification at the species level in complex groups, but in some situations, a less accurate identification can be tolerated, depending on the aim of the analysis (Chase et al., 2005). If barcoding can be used to identify invasive *Fallopia* on a genus level, barcoding could be a useful tool in situations where it is sufficient to know that one of the taxa is present, e.g. identification of rhizomes in soil masses where aboveground parts are not available.

Most reference sequences for invasive *Fallopia* taxa were from the native range. Five of the six reference sequences used for alignment of the ITS sequence were from Japan, China or Korea. One of the ITS sequences was from a German study (Kersten,T. and Knoess,W., 2008), but the sample seems to be from an Asian herbal product. The two reference sequences from Genbank used for alignment of the *matK* sequence were from the UK, one *F. japonica* and one *F. sachalinensis* (Cuénoud et al., 2002). Both sequences differed from the sequence obtained in the present study. No genetic variation is found within *F. japonica* in the UK (Hollingsworth and Bailey, 2000a), and it is shown through AFLP analysis that the *F. japonica* genotype in the study area is the same genotype that is found in the UK (Elameen, A., unpublished). The nearest explanation for the variations found between the sequences is therefore somatic mutations. Somatic mutations can be caused by environmental factors, or by errors during DNA replication in somatic cells. Somatic mutations can accumulate in plants with time, and are particularly frequent in long-lived plants and in plants with vegetative regeneration (Klekowski and Godfrey, 1989, cited in Elameen, 2009). The *F.*

japonica sequence from Cuénoud et al. (2002) and the sequence from the present study differed from each other in 6 out of 833 base pairs. Variations between individuals in single base pairs are called single nucleotide polymorphisms, or SNPs (Klug et al., 2007). SNPs can occur with or without affect on the phenotype, depending on the location of the SNP in the gene sequence. SNPs may remain undetected when multi-locus methods such as RAPD or AFLP are used to examine genetic diversity. RAPD and AFLP are based on the comparison of fragment lengths, and similar sized fragments are not necessarily homologous (Kumar et al., 2009).

By use of flow cytometry analysis, ploidy levels were easily determined to an affordable price. Samples dried in silica gel were usable, but it is not the ideal material for flow cytometry, as it can be difficult to get enough nuclei from dried material. The DNA histograms produced may be less clear than histograms produced from fresh samples, and it is not possible to detect aneuploid individuals (Gerard Geenen., Plant Cytometry Services, personal communication). One advantage of using dry samples is that samples can be collected and stored until analysis. Flow cytometry is a relevant method in the study of invasive species, since polyploidy is found to play an important role in the evolution of invasiveness in some species (Booth et al., 2011; te Beest et al., 2011). In the present study, flow cytometry revealed that the ploidy levels of the invasive *Fallopia* taxa found in the study area are the same as the most common ploidy levels for these taxa in other parts of Europe (8X *F. japonica*, 6X *F.* x *bohemica* and 4X *F. sachalinensis*) (Bailey et al., 2009). Three types of hexaploid *F. x bohemica* with different origins are detected in Europe. The most common is formed by hybridization between octoploid male-sterile *F. japonica* and tetraploid male-fertile *F. sachalinensis* (Bailey and Wisskirchen, 2004). A rare hexaploid *F. x bohemica* is formed by pollination of octoploid *F. japonica* by tetraploid *F. japonica var. compacta.* (Bailey et al., 2007). A third hexaploid, which is produced through the pollination of 8X *F.* x *bohemica* by male-fertile *F. sachalinensis*, has also been detected. The *F.* x *bohemica* found in the present study has most likely been introduced to the country, since the taxa do not reproduce by seeds in Norway. Considering the rarity of the two latter hexaploid hybrids compared to the one most common, it is most likely that the hybrid found in the present study is a cross between *F. japonica* and *F. sachalinensis*.
Fremstad and Elven (1997) reported that most findings of *F. sachalinensis* in Norway are of male-sterile plants, and Handeland (1991) had only observed male-sterile *F. sachalinensis*. The two *F. sachalinensis* sampled in the present study were also male-sterile. *F. japonica* is

only known as male-sterile. The most common hexaploid hybrid has irregular meiosis and reduced fertility, but can produce viable aneuploid or unreduced gametes (Bailey and Wisskirchen, 2004). If future climatic conditions support seed production in the invasive *Fallopia* taxa in Norway, *F.* x *bohemica* could be a potential pollen source for *F. japonica* and *F. sachalinensis*. Hexaploid hermaphrodite *F.* x *bohemica* can also produce seeds, but seed production is found to be much lower than in male-sterile *F. japonica* or *F. sachalinensis* (Bailey, 1994). No seeds were observed in any of the taxa during the present study. The parallel AFLP study (Elameen, A., unpublished) found no genetic diversity within any of the taxa, which supports the theory that seeds are not produced in the study area (Handeland, 1991; Fremstad and Elven, 1997).

The presence of *F.* x *bohemica* in the study area is concerning. If seed production in the invasive *Fallopia* taxa becomes possible in Norway under future climatic conditions, the hybrid can be determining for the consequences. *F.* x *bohemica* is also found to have more effective vegetative regeneration than the parental taxa (Child, 1999,; Bimova et al., 2003), a greater negative impact on native species (Parepa et al., 2013) and spreads faster in some areas (Mandak et al., 2004). Some studies also indicate that the hybrid is less susceptible to control efforts (Bimova et al., 2001, Rouifed et al., 2011). Spread of *F.* x *bohemica* could increase the problems already associated with the invasion that is currently dominated by *F. japonica.* Considering the increased threat posed by *F.* x *bohemica* in the long term, it may be suggested that eradication of this taxon is prioritized.

The high frequency of both *F. japonica* and *F.* x *bohemica* along the river Akerselva underlines the importance of preventing spread of the taxa into riparian habitats, and of controlling established stands. Established stands of *F. japonica* and *F. x bohemica* were also found by Drammenselva in Drammen, and by lakes in Moss, Ås and Bergen. Vegetative fragments can spread with the water, and with time, the habitats could become seriously invaded by the taxa. Fremstad and Elven (1997) reported that the invasive *Fallopia* taxa were not commonly found along watercourses in Norway. The taxa may have become more common in riparian habitats since then. Distribution surveys and rapid response to new establishments are necessary and effective means to prevent *Fallopia* invasions along watercourses (Colleran and Goodall, 2013, Nickelson, 2013).

2.2 Experiment 2:

Growth and allocation pattern in *F. japonica* and *F.* x *bohemica*

2.2.1 Introduction

The pattern of assimilate distribution between different plant organs is fundamental for finding the most effective timing for both mechanical and chemical control efforts against perennial weeds (Price et al., 2001; Bashtanova et al., 2009; Mangerud and Brandsæter, 2009). The success of mechanical control strategies in reducing rhizome biomass in *F. japonica* depends on the biomass allocation in the plants at the current time (Seiger and Merchant, 1997). Phloem-transported herbicides will be most effectively delivered to the rhizomes at a time when assimilate allocation is directed to belowground parts (Bashtanova et al., 2009).

Growth pattern and biomass allocation in *F. japonica* has previously been studied in detail by Price et al. (2001), who used ^{14}C to trace the distribution of assimilates throughout the growing season. They found that more than 80% of the given ^{14}C was retained in the shoots in May and June, and that increasing amounts of ^{14}C was transported to the rhizomes after this. This corresponds to the observations of Dauer and Jongejans (2013), that the production of new rhizomes started in June – July. Price et al. (2001) found significant transport of assimilates from shoots to rhizomes in late autumn, at a time that corresponds with senescence (October). Only 15% of the carbon fixed in May was recovered from the rhizomes in September, while almost 90% of carbon fixed in May was recovered from the rhizomes in April the following spring. This shows that the rhizome is the most effective sink in late autumn. Assimilate recycling was tight in *F. japonica*, and only a small, not significant amount of the fixed ^{14}C was not recovered. Stored resources were remobilized to new shoots in the spring.

Less is known about the growth and biomass allocation in *F.* x *bohemica*, the hybrid between *F. japonica* and *F. sachalinensis*. Herpigny et al. (2012) found that *F. japonica*, *F. sachalinensis* and *F.* x *bohemica* had a similar growth pattern, with rapid height growth during the first two months, until a plateau was reached in June. In one of two years, *F. japonica* reached the plateau one month earlier than *F. sachalinensis*, and *F.* x *bohemica* was intermediate. Shoot height differed significantly between the taxa at some sites, but no

significant differences were found in the number of leaves. In contrast, Rouifed et al. (2011) found that *F.* x *bohemica* produced more leaves than *F. japonica*.

F. japonica and *F.* x *bohemica* are found to differ in their regeneration success from rhizome fragments. *F.* x *bohemica* has shown higher regeneration rates and more rapid shoot growth and leaf production than *F. japonica* (Child, 1999; Bímová et al., 2003). Parepa et al. (2013) did not find a difference in regeneration rates between the two taxa, but found that *F.* x *bohemica* had a final biomass almost three times greater than *F. japonica*. Some studies also indicate that *F.* x *bohemica* is more tolerant to some control treatments than *F. japonica*, and that the taxa may differ in growth and allocation in response to disturbance (Bimova et al., 2001; Rouifed et al., 2011).

In the present study, growth and biomass allocation of *F. japonica* and *F.* x *bohemica* is examined through a growth experiment involving destructive harvests of aboveground and belowground biomass at different times of the growing season. The hypotheses were:

1) Biomass allocation to aboveground growth increases from spring to June. After this, aboveground growth decreases and biomass allocation to belowground parts increases.

2) *F. japonica* and *F.* x *bohemica* show more or less the same growth pattern as described, but *F.* x *bohemic*a both has a stronger below- and aboveground growth during the growing season.

2.2.2 Material and methods

Young, white rhizomes were collected from two sites, one stand of *F. japonica* in Frogn and one stand of *F. x bohemica.* in Ås, (59°N), south-east in Norway, June 27, 2011. Taxonomic identities of the stands were determined in Experiment 1 (sample no. 8 and 9, Table 16, Appendix 2). After excavation of the rhizomes, they were immediately stored in closed, insulated boxes together with moistened paper to prevent desiccation during the work.

The day after, intact rhizomes with full length from base to tip were chosen. For each taxon, 40 rhizome fragments with three nodes were individually planted in pots (10 L) filled with garden soil (L.O.G. 'Gartnerjord', Mixture 840 g/kg $^{-1}$ sphagnum peat, 100 g/kg $^{-1}$ fine sand, 60 g/kg $^{-1}$ clay, 5.5 kg/m^{-3} dolomite lime , 1.2 kg fertilizer (NPK 15-4-12), 0.2 kg F.T.E. no 36, pH 5.5 – 6.5, and density 270 kg/m^{-3}). The pots were placed in greenhouse (about 16/8 h

day/night, 22 ˚C and 15 ˚C respectively, and natural light during day) and watered when needed. No extra fertilization was given.

Before planting of the rhizome fragments, fresh weight and length of each fragment were measured. Fresh and dry weight were measured for five extra fragments. These weights were used to estimate initial dry weight for the other rhizome fragments.

At August 9, 2011, 42 days after planting of the rhizomes, 16 out of 20 plants from each taxon were transplanted to the experimental garden. Immersed cement pipes (diameter 1 m, depth 2 m) were used as plant containers to prevent unwanted spreading through rhizome growth. The growth medium was pre-used peat-soil (as described above). Plants were sorted by height into four blocks to reduce unexplained variation from differences in initial plant height and plant position in the garden (Figure 22).

Aboveground monitoring

Aboveground growth was monitored from shoot emergence to harvest. Height was measured as the distance from soil level to the highest node. Leaves (folded and unfolded) were counted, and width of the broadest leaf was measured.

Harvest of biomass

The same day as the 16 plants were transplanted outdoor, the remaining 4 plants of each taxon were harvested. Five harvests were performed during the experiment: August 9, 2011; October 14, 2011; May 24, 2012; June 15, 2012 and July 7, 2012. Four plants of each taxon were harvested each time.

At harvest, height was measured as the distance from the soil surface to the highest node. The number of leaves was counted and width of the broadest leaf was measured. The plants were washed and divided in component parts: old rhizome, new rhizomes (white rhizomes), roots and aboveground parts. Plant material was oven dried at 60 °C for at least 1 week and then weighed.

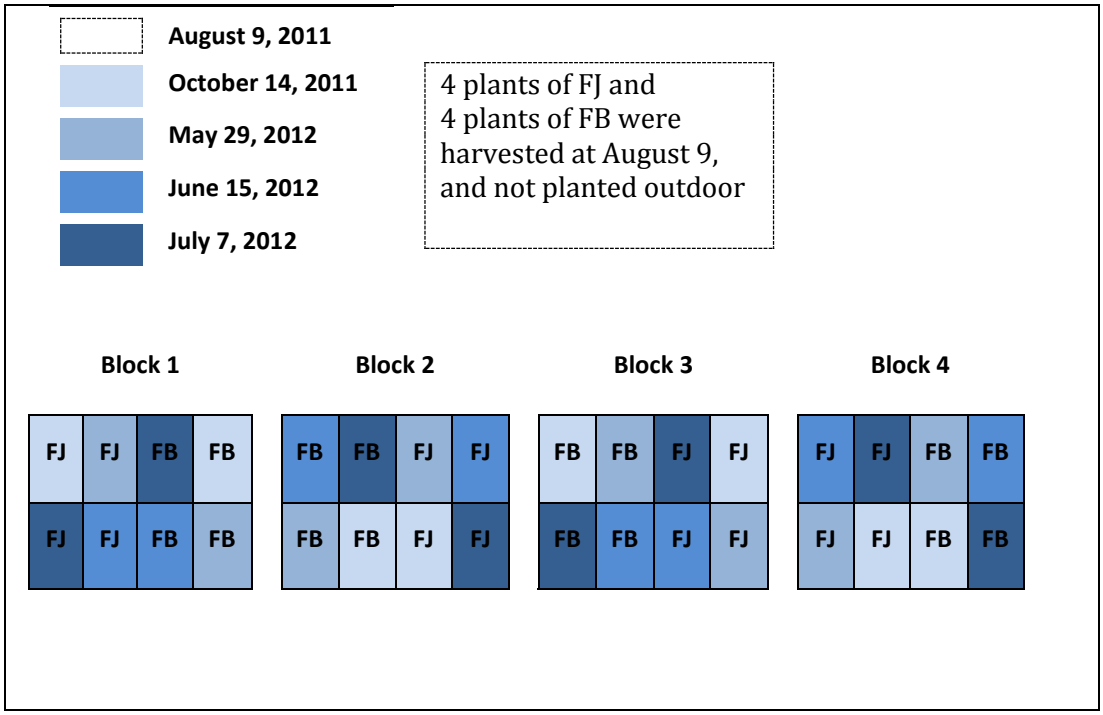


Figure 22 Overview of experiment. The plants were sorted by plant height at August 9, 2011, and arranged in four blocks with eight plants per block. FJ = *F. japonica*. FB = *F.* x *bohemica*. Fill colours denotes time of harvest. Four plants from each taxon were harvested at August 9, the same day as the16 other plants were transplanted outdoor (stippled box).

Statistical analyses

1) Differences in biomass of different plant parts in the two taxa were analysed in a three-way ANCOVA with taxon and harvest date as fixed factors, block as random factor and initial rhizome mass as covariate. The analyses were followed by Tukey HSD post hoc test with 95% confidence intervals.

2) Height, number of leaves and width of widest leaf were analysed in repeated measures ANOVA (Mixed procedure) with SAS 9.1, and with classes = plant, time, taxon and block; repeated = time; subject = plant, random = block. Height and number of leaves were analysed from August 9. Width of widest leaf was analysed from August 30. The analyses were followed by Tukey HSD post hoc tests with 95% confidence intervals.

A confidence level of 95% was used in all analyses. The logarithms of the response variables were used to meet the assumptions of normal distribution. All analyses were followed by multiple comparisons using Tukey HSD post hoc test with 95% confidence level.

2.2.3 Results

Biomass

The ANCOVA analyses of biomass of different plant parts showed an effect of harvestdate, taxon and block on biomass of all plant parts except new rhizomes. Initial rhizome mass had no effect. The interaction harvestdate*taxon had effect on the biomass of aboveground parts and roots (Table 10).

Table 10 Summary of statistical output (P-values) for ANCOVA analyses of the effect of harvestdate, taxon, initial rhizome mass, and block, on the biomass of different plant parts.

Source	Df	Aboveground	Roots	Old rhizomes	New rhizomes
Harvestdate	4	0.000	0.000	0.000	0.782
Taxon	1	0.000	0.000	0.000	0.611
Initial rhizome mass	1	0.580	0.581	0.433	0.379
Block	3	0.013	0.002	0.020	0.127
Harvestdate*Taxon	4	0.034	0.000	0.071	0.119

August 9, 2011 - October14, 2011

Aboveground biomass differed significantly between *F. japonica* and *F.* x *bohemica* at harvest in August (Figure 23). No significant differences between the taxa were found in any other plant parts at this time (Figure 24 - Figure 26). None of the taxa had produced new rhizomes in August (Figure 26). From August to October, biomass increased significantly in all plant parts, except new rhizomes, in both taxa ($p<0.05$) (Figure 23 - Figure 26). The period from August to October was the only time during the experiment when there was a significant increase in old rhizome biomass in any of the taxa (Figure 25). There was no significant difference in old rhizome biomass between the taxa in August, but old rhizome biomass was significantly greater in *F.* x *bohemica* than in *F. japonica* in October (Figure 25). All four harvested *F.* x *bohemica* plants had produced new rhizomes in October, while the harvested *F. japonica* plants had produced none (Figure 26). The length of the new rhizomes in *F.* x *bohemica* ranged from 2 cm – 23.5 cm, n=8.

October14, 2011 – May 24, 2012

Aboveground biomass was significantly greater in *F.* x *bohemica* than in *F. japonica* at May 24 (Figure 23). Root biomass decreased significantly in *F.* x *bohemica* with 43% from October 2011 to May 2012, but not in *F. japonica* (Figure 24).

Two out of four harvested *F. japonica* plants had produced new rhizomes in May. Length of new rhizomes ranged from 2 cm - 10.5 cm, n=13. 10 out of 13 new rhizomes had produced aboveground shoots in *F. japonica*. Three out of four harvested *F.* x *bohemica* plants had produced new rhizomes. Length of the new rhizomes in *F.* x *bohemica* ranged from 2 cm - 21 cm, n=15. 13 out of 15 new rhizomes had produced aboveground shoots in *F.* x *bohemica.*

May 24, 2012 – June 15, 2012)

Aboveground biomass increased significantly in both taxa from May to June (Figure 23). A significant increase in root biomass during this time was found for *F.* x *bohemica*, but not for *F. japonica* (Figure 24). Four out of four harvested *F. japonica* plants had produced new rhizomes in June. Length of new rhizomes in *F. japonica* ranged from 9.1 cm – 33.5 cm, n=8. One out of four harvested *F.* x *bohemica* plants had produced one new rhizome. Length of the new rhizome in *F.* x *bohemica* is missing, but the rhizome was small, with a dry weight of 0.03 g (Figure 26).

June 15, 2012 – July 7, 2012

Aboveground biomass increased, but not significantly, from June to July in both taxa (Figure 23). Root biomass increased significantly in *F.* x *bohemica* during this period, and the increase during this time was greater than from May to June (Figure 24). Root biomass was doubled in *F. japonica* from June to July, but the increase was not significant (Figure 24). All harvested *F. japonica* plants had produced new rhizomes in July. Length of new rhizomes ranged from 1.8 cm – 55.5 cm, n=11. Three out of four harvested *F.* x *bohemica* plants had produced new rhizomes. Length of new rhizomes ranged from 3 cm – 14.5 cm, n=3. Young rhizome biomass did not differ significantly from June to July in any of the taxa, and did not differ significantly between taxa in July or at any other times of harvest (Figure 26).

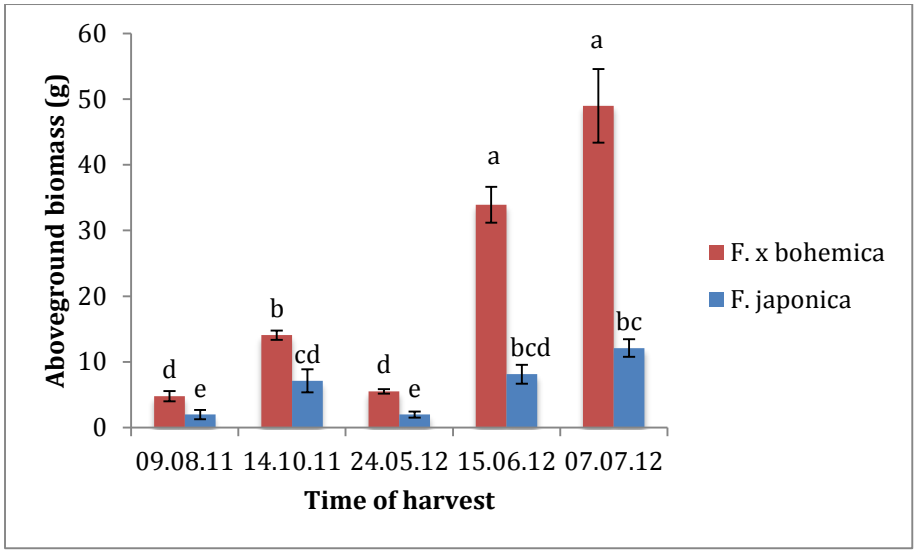


Figure 23 Biomass of aboveground parts in *F. japonica* and *F.* x *bohemica* at different times of harvest. Different letters indicate statistical significant difference ($p<0.05$). Error bars represent standard error.

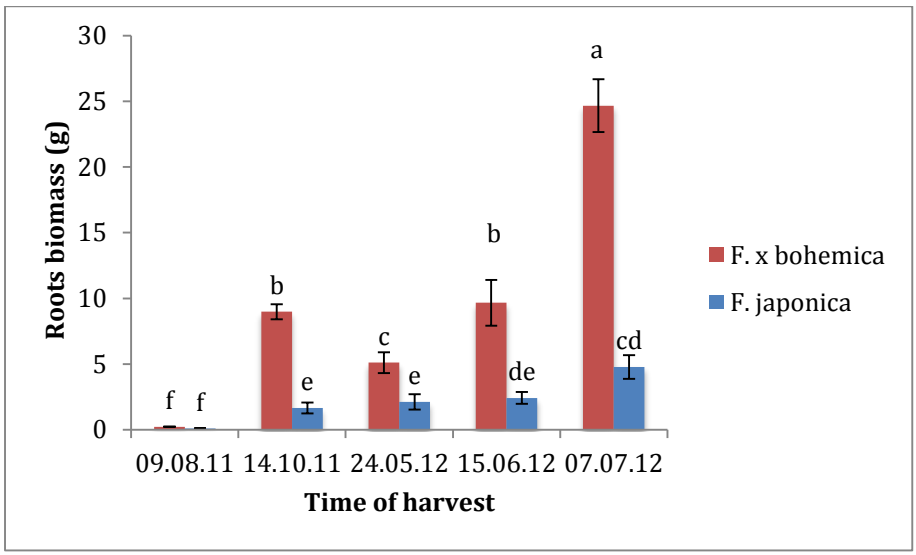


Figure 24 Biomass of roots in *F. japonica* and *F.* x *bohemica* at different times of harvest. Different letters indicate statistical significant difference ($p<0.05$). Error bars represent standard error.

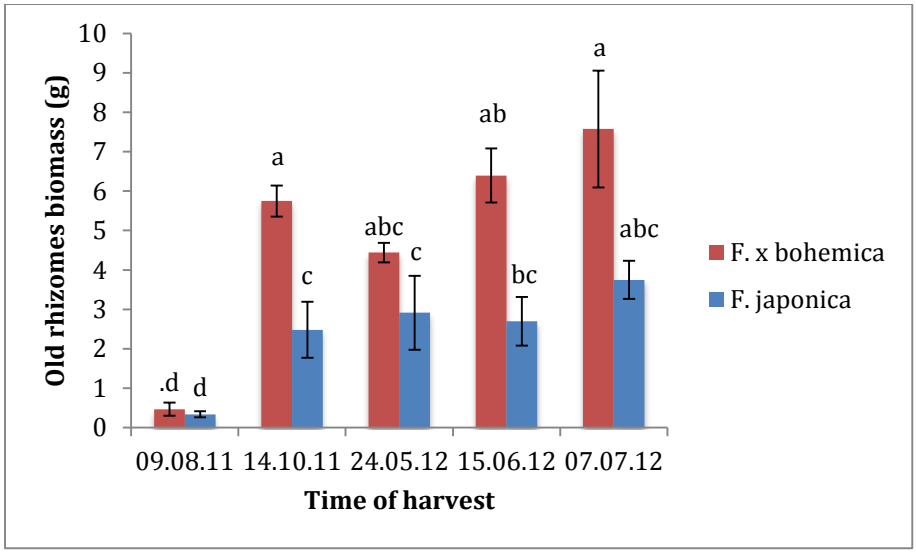


Figure 25 Biomass of old rhizomes in *F. japonica* and *F.* x *bohemica* at different times of harvest. Different letters indicate statistical significant difference ($p<0.05$). Error bars represent standard error.

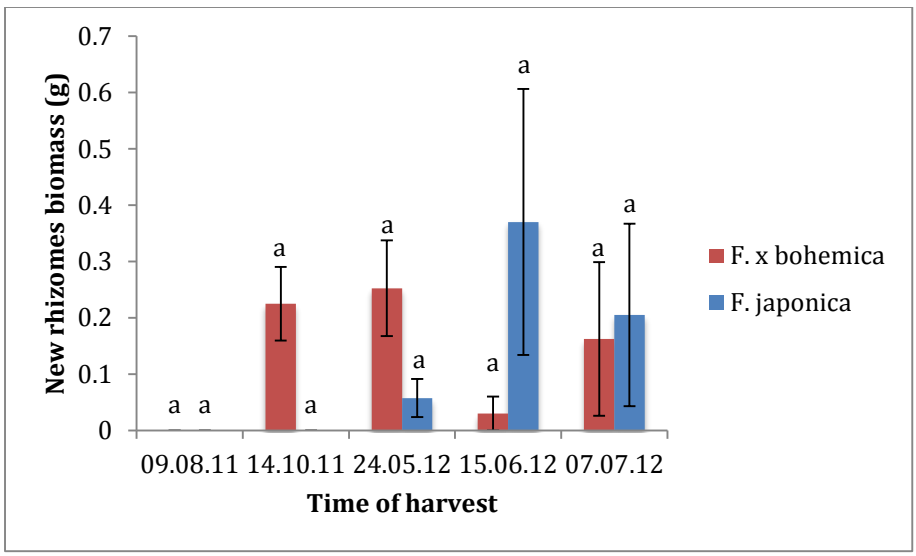


Figure 26 Biomass of new rhizomes in *F. japonica* and *F.* x *bohemica* at different times of harvest. Different letters indicate statistical significant difference ($p<0.05$). Error bars represent standard error.

Height growth

Time, initial rhizome mass and the interaction time*taxon had significant effect on height ($p<0.05$) (Table 11). Taxon and block did not have significant effect on height ($p>0.05$) (Table 11). Both taxa had significant height growth from August 9 to August 30 ($p<0.0001$). After this, height growth was not significantly different from date to date the rest of the season. The following season, height growth increased significantly between the time intervals May 8 - May 15 - May 24 in both taxa. Height growth was significant from May 24 to May 30 in *F.* x *bohemica*, but not in *F. japonica*. Height growth increased significantly from May 24 to June 9 in both taxa. Both taxa continued height growth after this, but height growth was not significant. Height growth reached a plateau in mid June in both taxa (Figure 27).

Number of leaves

Time, initial rhizome mass and the interaction time*block had significant effect on number of leaves ($p<0.05$) (Table 11). Taxon and the interaction time*taxon did not have significant effect ($p>0.05$) (Table 11). Number of leaves increased significantly in both taxa from August 9 to August 30. After this, there was no significant increase for the rest of the season (Figure 27). The following season, number of leaves increased significantly between each time-interval from April 29 - May 8 – May 15 – May 24. After this, leaf production continued to increase significantly towards July 7, but longer time intervals were needed to reach statistical significance.

Leaf width

Time had significant effect on width of broadest leaf ($p<0.05$) (Table 11). Block, 'initial rhizome mass', taxon and time*taxon did not have significant effect ($p>0.05$) (Table 11). Width of the broadest leaf was statistically analysed from August 30. Leaf width did not increase significantly from August 30 to the end of the season. Leaf width increased in both taxa from April 29 to May 8 following season (Figure 27). This was the only time when there was a significant increase in leaf width.

Table 11 Summary of output for repeated measures ANOVA analyses of height, number of leaves and width of broadest leaf.

Response variable	Effect	Df	P
Height			
	Block	3	0.15
	Initial rhizome mass	1	0.0006
	Time	12	<0.0001
	Taxon	1	0.45
	Time*Taxon	12	0.0007
Number of leaves			
	Block	3	0.29
	Initial rhizome mass	1	0.04
	Time	12	<0.0001
	Taxon	1	0.07
	Time*Taxon	12	0.39
	Time*Block	36	0.005
Width of widest leaf			
	Block	3	0.26
	Initial rhizome mass	1	0.48
	Time	10	<0.0001
	Taxon	1	0.25
	Time*Taxon	10	0.45

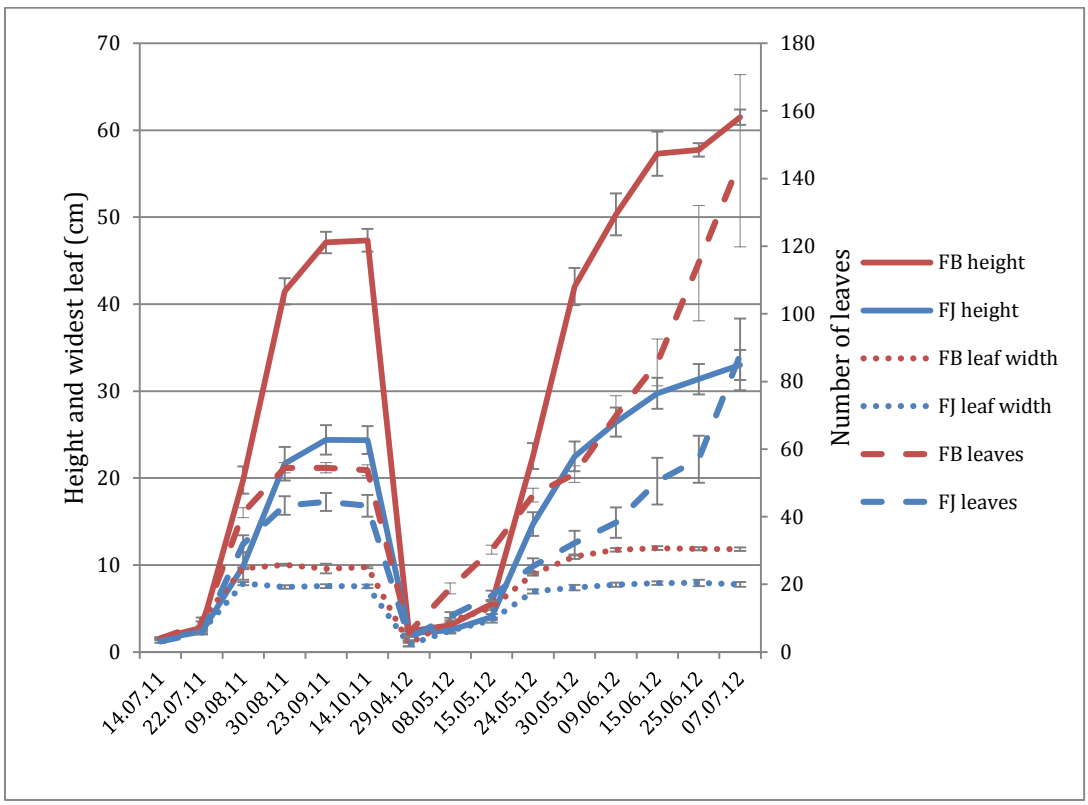


Figure 27 Mean of height, number of leaves and width of widest leaf in plants of *F. japonica* and *F.* x *bohemica* during the experimental period. FB = *F. x bohemica* and FJ = *F. japonica*.

2.2.4 Discussion

Roots and rhizome biomass increased significantly in both taxa from August to October the first season. Following season, aboveground biomass increased significantly from May to June, but not from June to July. Monitoring of height growth showed significant allocation to aboveground growth until mid-June. After this, height growth continued, but was not statistically significant. Root biomass increased significantly from May to June and from June to July in *F.* x *bohemica*, but not in *F. japonica*. Root biomass was however doubled in *F. japonica* from June to July, which is a greater increase compared to from May to June. The results indicate a shift in the direction of resource allocation during June, when allocation to aboveground growth decreased and allocation to belowground growth increased. The results correspond with previous studies, who also found that allocation to belowground parts increased from June onwards in *F. japonica* (Price et al., 2001) and that height growth reached a plateau in June in both *F. japonica* and *F.* x *bohemica* (Herpigny et al., 2012).

F. japonica and *F.* x *bohemica* showed a similar allocation pattern throughout the growing season, but some differences between the taxa were found. Only four plants of each taxon were harvested each time, and starting material was collected from only one stand of each taxon. Due to possible variations within taxon and between the environments, the results should be interpreted with caution.

F. x *bohemica* had a much greater aboveground and belowground biomass than *F.* x *bohemica*. Total plant biomass was more than twice as high in *F.* x *bohemica* as in *F. japonica* already in August the first season, and almost four times greater in July, the following season. This result correspond well with the results of Parepa et al. (2013), who found that *F.* x *bohemica* on average produced a biomass three times greater than *F. japonica*. Other studies have also found that *F.* x *bohemica* produce greater shoots than *F. japonica* from rhizome fragments 30-40 days after planting (Child, 1999; Bimova et al., 2003). The present study showed that the biomass of *F.* x *bohemica* continued to increase compared to *F. japonica* between each harvest date until the experiment ended one year after planting of rhizome fragments. In particular, *F.* x *bohemica* had a greater allocation to roots. While Price et al. (2001) found that biomass allocation to belowground parts was predominantly to rhizome growth in *F. japonica*, in the present study, root and rhizome biomass were quite similar in in this taxon. In contrast, *F.* x *bohemica* had a much greater biomass allocation to roots than to rhizomes. Root biomass in *F.* x *bohemica* increased

significantly already from May to June, while no increase was found in belowground parts in *F. japonica* during this time. This result may indicate that allocation to belowground parts starts at an earlier time in *F.* x *bohemica* than in *F. japonica*.

All four *F.* x *bohemica* plants harvested in October the first season had produced new rhizomes, while *F. japonica* had produced none. This could be a result of coincidence due to the low number of plants harvested. It may however be hypothesized that *F.* x *bohemica* was able to start new rhizome development at a shorter time after planting than *F. japonica*. This could be due to that greater roots and a greater aboveground shoots with more leaves provided *F.* x *bohemica* with sufficient nutrients and energy to start new rhizome production.

The following season, a sharp increase in height growth occurred in mid-May, and significant height growth continued until mid-June. Herpigny et al. (2012) found that height growth in *F. japonica*, *F.* x *bohemica* and *F. sachalinensis* was rapid the first two months of the growing season, and that height reached a plateau in June. This growth pattern was similar for the three taxa, but a difference between the taxa was found in one of two years, when *F. japonica* reached the plateau one month earlier than *F. sachalinensis* and *F.* x *bohemica* was intermediate (Herpigny et al., 2012). In the present study, height growth increased more rapidly in *F.* x *bohemica* than in *F. japonica*, and the rapid increase seemed to continue somewhat longer in *F.* x *bohemica* than in *F. japonica*. While *F.* x *bohemica* had significant height growth during the last week in May, height growth in *F. japonica* during this short time interval was not sufficient to reach statistical significance. This could reflect what was found by Herpigny et al. (2012), that growth slowed down at an earlier time in *F. japonica* than in *F.* x *bohemica.*

Herpigny et al. (2012) recognized two growth phases, with rapid height growth the first two months, followed by increased ramification as height growth decreased. In the present study, leaf production increased rapidly in the beginning of the season, but from the end of May, longer time-intervals were needed for the increase to be significant. Increased variation combined with a decreasing number of plants due to destructive harvests, and not an actual decline in leaf production, might have been the reason for this result. From looking at the curve representing number of leaves in Figure 27, leaf production seems to increase more sharply in both taxa from mid-June onwards. Ramification was not examined in the current study, but the increase in leaf production could be due to increased ramification, as found by

Herpigny et al. (2012). Callaghan et al. (1981) also found that the leaf area index (leaf m^2 /ground m^2) increased during June-August.

Both *F.* x *bohemica* and *F. japonica* established and grew rapidly during the current study. Belowground biomass increased greatly within short time, and new rhizomes up to 55 cm long (*F. japonica*) were produced in less than one year. This underlines the importance of a rapid response to new establishments of invasive *Fallopia*.

Based on the results of the currents study, mechanical control-methods such as fragmentation and cutting, will be most effective from an early time of season (May) until mid June. Stimulation of shoot production through fragmentation (Child et al. 1998), and frequent removal of aboveground biomass through cutting during this time can deplete rhizomes of stored resources and prevent replenishment of carbohydrates to the rhizomes.

Mechanical control methods are most effective when performed at the time when the dry-weight of the plant is at its minimum, called the compensation point. Generally, the production of new regenerative structures in perennial plants starts after the compensation point of the plant is reached (Mangerud and Brandsæter, 2009). The present study could not provide clear information about what time of season new rhizome production is initated in these taxa, nor about when the dry-weight is at its minimum. Dauer and Jongejans (2013) observed that production of new rhizomes started in June-July. In the present study, new rhizomes were observed already in October the first season in *F.* x *bohemica* (possibly between August-October), but no new rhizomes were found in *F. japonica* at the same time. In late May the following season, new rhizomes were found in both taxa, and although some of the new rhizomes in *F. japonica* may have been present in October the first season, the results could indicate that new rhizome production occurred early in the season, before May 24. Some of the plants harvested in June and July had not produced new rhizomes, indicating that new rhizome production depended on other factors than time of season. July was however the time when most plants had produced new rhizomes (4/4 *F. japonica* and 3/4 *F.* x *bohemica*).

A significant reduction in root biomass of 43% was found from October to May in *F.* x *bohemica*. A similar reduction was not found in *F. japonica*. Price et al. (2001) found that root biomass decreased significantly by 60% in *F. japonica* from September to April. These results may indicate that a dry-weight minimum occurs during April/early May.

Eradication of invasive *Fallopia* taxa through herbicide treatments relies on the transport of herbicide to rhizomes and the poisoning of the rhizome buds (Bashtanova et al., 2009). The results of the current study supports previous studies in that allocation to belowground parts increases from June onwards (Price et al., 2001), and that application of herbicides later than June increase transport of herbicide to the rhizomes (Price et al, 2001; Bashtanova et al., 2009). Trials of chemical control methods also indicate that application in July is more effective than application in May (Kabat et al., 2006). An increase in number of leaves during June-August (Callaghan et al., 1981) may also improve herbicide capturing by the plant during the later part of the season compared to earlier in the season.

It is difficult to draw conclusions about the pattern of new rhizome production, or about the timing of a dry-weight minimum, from the results of the present study. A larger experiment with more experimental plants and more frequent harvests, especially during the early part of the growing season, could possibly reveal a clearer pattern. It may be that young plants have a different growth and allocation pattern than mature, well-established stands. Intact stands in the field should therefore be examined parallel to studies of experimental plants.

Several studies indicate that some *F.* x *bohemica* genotypes have a greater invasive potential than the parental taxa, *F. japonica* and *F. sachalinensis*. Based on the results of the present study, it may be hypothesized that both a greater aboveground biomass, and a greater root production, play a role in the increased invasiveness in *F. x bohemica.* More effective root growth could increase establishment success of rhizome fragments. Combined with a greater aboveground biomass production, this may increase competitiveness and survival of newly established *F.* x *bohemica* compared to *F. japonica.* Rouifed et al. (2011) found that *F. japonica* and *F.* x *bohemica* differed in their response to cutting of aboveground shoots. Cutting reduced belowground biomass more in *F. japonica* than in *F.* x *bohemica,* and *F.* x *bohemica* seemed to be able to restore its growth rate without changing its biomass allocation. While cutting resulted in modification of root distribution in *F. japonica,* with fewer roots in the deepest layer, root distribution in *F.* x *bohemica* was not affected by the treatment. Most studies of invasive *Fallopia* taxa focus on the growth and development of rhizomes, which is natural due to the important role of the rhizomes in the regeneration and spread of these taxa. The roots however seem to be sparsely studied. The potential role of allocation to root growth in increasing invasiveness in *F.* x *bohemica* should be further

Rouifed et al. (2011) found that *F.* x *bohemica* produced more leaves than *F. japonica*, and suggest that this could make *F.* x *bohemica* more competitive for light than *F. japonica*. Herpigny et al. (2012) did not find a significant effect of taxon on the number of leaves, but found a significant effect of site. The present study found no significant effect of taxon on the number of leaves, but the number of leaves and leaf width was consistently higher in *F.* x *bohemica* than in *F. japonica*.

Generally, *F. japonica* and *F.* x *bohemica* seem to have similar allocation patterns. The results indicate however, that allocation to belowground parts in *F.* x *bohemica* may be more rapid from an earlier time of season than in *F. japonica*, which could affect the optimal timing of control methods. Limitations of the experiment in terms of number of experimental plants and harvest frequency however make generalization difficult, and the experiment should be viewed as a pre-study. Further studies should involve more experimental plants from different starting material, more frequent biomass harvests, and should include the male parent of the hybrid, *F. sachalinensis.* This could allow for a more representative comparison of the three taxa.

2.3 Experiment 3: Shoot regrowth potential of invasive *Fallopia* taxa throughout the growing season and after covering

2.3.1 Introduction

Eradication of *Fallopia japonica* and related taxa requires a control method that targets and eventually kills the belowground rhizomes (Child and Wade, 2000). Cutting of aboveground shoots reduces belowground biomass in *F. japonica and F.* x *bohemica* (Seiger and Merchant, 1997; Rouifed et al., 2011), and small stands may be eradicated by monthly cuttings over three or more growing seasons (Soll, 2004). Covering restricts light availability and prevents aboveground shoot production. Five to six years of covering is reported to successfully eradicate small stands (Nickelson, 2013), while larger stands may require covering for more than eight years (Sally Nickelson, Wildlife biologist/Watershed ecologist, Cedar River Watershed, Washington, personal communication).

Mechanical control methods are considered most suitable for small, isolated, and easily accessible stands, or where herbicide use is restricted (Seiger and Merchant, 1997). Control of larger stands, or control on a landscape level, will in most cases require a strategy that includes herbicide treatments (Child and Wade, 2000; McHugh, 2006; Nickelson, 2013). Integrating mechanical and chemical methods may increase treatment efficiency and reduce the use of herbicides (Child et al., 1998).

2.3.1.1 Effect of season on the shoot regrowth potential

Knowledge about the shoot regrowth potential of rhizomes throughout the growing season is important to succeed with control methods that aim to deplete the rhizomes of energy reserves. Depletion of rhizomes through repeated cutting is most effective when regrowth after cutting is vigorous. Cutting of aboveground shoots may also be used to provide regrowth of reduced height for a more effective and practical herbicide treatment later in the season. The effect of the cutting treatment will depend on the shoot regrowth potential at the time (Callaghan et al., 1981; De Waal, 1995; Gover et al., 2005).

Shoot production in *Fallopia japonica* is not uniform throughout the growing season (Callaghan et al., 1981; De Waal, 1995; Adachi et al., 1996; Gover et al., 2005). Studies from the UK and Japan report that uncut plants of *F. japonica* produce new shoots until the end of

July, and that shoot density decreases after this, due to die-back of late-emerged shoots (Callaghan et al., 1981; Adachi et al., 1996). Regrowth after a single cutting also decreases as the time of cutting becomes later in the growing season (Callaghan et al., 1981; De Waal, 1995; Gover et al., 2005). Gover et al. (2005) recommend cutting plants around June 1 if a herbicide treatment is carried out later in the same season, as this will result in vigorous regrowth of reduced height. de Waal (1995) reported that little to no regrowth occurred after cutting in mid-August. Seiger and Merchant (1997) found that a single cutting between June 5 and August 28 significantly reduced belowground biomass at the end of the growing season, but found no significant differences between cuttings at different dates within this period. Cutting on September 21 had no significant effect on belowground biomass, indicating that aboveground growth had ceased at this time.

2.3.1.2 Effect of covering on shoot regrowth potential

When covering or deep burial are used to prevent shoot production and eradicate invasive *Fallopia*, it is relevant to ask how long the rhizomes are able to retain their shoot regrowth potential when aboveground shoot production is prevented. Knowledge about how long the rhizomes can survive without shoot production is also relevant when planning the monitoring and use of sites where invasive *Fallopia* taxa has been controlled or buried.

How long the vegetative reproductive organs in perennial weeds can live depends on many factors, e.g. taxon, growth conditions, soil management methods and the level of fragmentation (Mangerud and Brandsæter, 2009). Fykse (1983), cited in Mangerud and Brandsæter (2009), showed that the vegetative reproductive organs in some important perennial weeds could remain alive for at least two growing seasons when they were allowed to produce aboveground shoots. When shoot production was prevented through deep burial, most of the species did not survive longer than 12 months. According to Adachi et al. (1996), intact *F. japonica* rhizomes can remain alive for decades. It is also reported that rhizomes of *F. japonica* can become dormant if they are buried deeply, and unconfirmed observations suggest that rhizomes can stay dormant for more than 20 years (Environment Agency, 2013). An apparent dormancy has been observed following herbicide treatments of invasive *Fallopia*. Plants can appear dead with no aboveground growth for three years, and then continue shoot production (Soll et al., 2008; Nickelson, 2013). Trials from Denmark have found that the rhizome system of *F. japonica* was still alive after three years of covering

(Buttenschøn, 2013). Trials from Washington, USA, found that 5-6 years of covering with geotextile could eradicate small stands (<100 m^2) of *F. x bohemica,* but a longer time of covering was needed to eradicate larger stands. A large stand of *F.* x *bohemica* produced numerous new shoots after eight years of covering (Nickelson, 2013; Sally Nickelson, Wildlife biologist/Watershed ecologist, Cedar River Watershed, Washington, Personal Communication).

2.3.1.3 Aims of the study

Experiment 3.1 To examine the shoot regrowth potential in *F.* x *bohemica* throughout the growing season in Norway, an experiment was carried out that involved single cuttings at different times of the growing season and measuring of regrowth. It was hypothesized that regrowth after cutting would decrease during the growing season, and finally cease in September.

Experiment 3.2 To examine how long the rhizomes of invasive *Fallopia* live when shoot production is prevented, an experiment was initiated that involved covering *F. japonica* and *F.* x *bohemica* with thick, woven plastic. The plastic was removed after different time-lengths and at two different times of the growing season, which made it possible to study both the effect of cover duration, and the effect of season, on the shoot regrowth potential.
It was hypothesized that 1) Three years of covering will reduce plant vigour and the number of shoots, but this period will be too short to completely eradicate the shoot regrowth potential. 2) Removing the plastic in spring will result in stronger regrowth than when removing the plastic in autumn, due to a decrease in the shoot regrowth potential during the growing season.

2.3.2 Material and methods

2.3.2.1 Experiment 3.1: Seasonal changes in shoot regrowth potential of F. x bohemica

The experiment was carried out during 2012 and 2013 on a large, well-established stand of *F. x bohemica,* located in Ås (59°N), southeast in Norway. Taxonomic identity was determined in Experiment 1 (sample no. 8, Table 16, Appendix 2). There were some methodological differences between the two years (Table 12):

2012

Single cutting events were performed at five different dates during the period May 26 – September 15. Two randomly chosen plots, each with an area of 1 m^2, were cut each time. Each plot was only cut once. All plots were located randomly, ca. 0.5-1 m within the outer periphery of the stand.

Shoots were counted within each plot before they were cut at ground level with a lopper. The cut shoots were removed from the plots and piled at the site. New shoots that emerged within the plots were counted 2.5 and 5 weeks after cutting. Aboveground biomass within the plots was harvested 5 weeks after cutting, oven-dried at 60 °C for at least 1 week and weighed. Shoots in plots cut June 25 were mistakenly harvested one week too early (4 weeks after cutting).

2013

Single cutting events were performed at six different dates within the period May 31 to August 15. Four plots were cut each time, except at May 31, when only two plots were cut (Table 12). Because of the great variation between the two plots cut at this time, it was decided to increase the number of replicates, and four plots were cut each time for the rest of the experiment. Each plot was cut only once. The plots were located pairwise, and the paired plots were located randomly in the periphery of the stand. Plots cut in 2012 were avoided.

Shoots within each plot were counted before they were cut at ground level with a lopper. The cut shoots were removed from the plots and piled at the site. New shoots that emerged within the plots were counted 2 and 4 weeks after cutting. The heights of 6 of the tallest shoots in each plot were measured each time. When less than 6 shoots had emerged, heights of the available shoots were measured. Aboveground biomass was harvested 4 weeks after cutting, oven-dried at 60 °C for at least 1 week and weighed.

Table 12 Overview of method used in the two years in Experiment 3.1. Notes: * Two plots were cut at May 31. ** Final counting of shoots and biomass harvest were performed 4 weeks after cutting at June 25.

	Year 2012	**Year 2013**
Number of plots ($1m^2$) cut each time:	2	4*
Dates for counting of shoots and cutting shoots at ground level:	May 26; June 25; July 18; Aug 17; Sept 19	May 31; June 17; July 1; July 16; Aug 3; Aug 15
Time of first counting of new shoots:	2.5 weeks after cutting	2 weeks after cutting
Time of final counting and harvest of new shoots:	5 weeks after cutting **	4 weeks after cutting

Statistical analyses

Each year was analysed separately. The data was analysed in ANCOVA with Minitab16. When analysing number of shoots produced after cutting, the logarithm of the ratio "mean number of shoots within a plot at harvest / mean initial number of shoots within a plot" was used as response variable and "time of cutting" was fixed factor. When analysing aboveground biomass produced after cutting, the logarithm of "harvested biomass" was used as response variable and "time of cutting" was fixed factor. The mean initial number of shoots within a plot was used as a covariate and a confidence level of 95% was used in both analyses.

Shoot height before cutting was analysed in one-way ANOVA with the logarithm of "mean shoot height" as response variable and "time of cutting" as fixed factor. Shoot height 4 weeks after cutting was analysed with the square root of "mean shoot height" as response variable and "time of cutting" as fixed factor.

All analyses were followed by Tukey HSD post hoc tests with 95% confidence levels.

2.3.2.2 Experiment 3.2: The effect of covering on the shoot regrowth potential of Fallopia japonica and F. x bohemica

Establishment of plots and installation of cover

Eight plots, each consisting of two square meters, were established in four stands of *F. japonica* and four stands of *F*. x *bohemica* July 19 2011, in Ås and Moss municipality (59°N) (sample no. 3, 4, 5, 7, 8, Table 16, Appendix 2). Some of the stands were small and isolated, while other stands were larger. Only one plot was established in each of the smaller stands, while two plots were established within the larger stands (Table 13). The plots located within larger stands were separated with a distance that allowed for uncovering of one plot, while leaving the other plots covered (>2m).

Shoots within the plots were counted before shoots were cut at stubble height (ca. 0 cm), using a brushcutter (Husquarna 235 FR). The cut area was covered with thick, woven plastic ("NORGRO Black woven plastic", quality 100g (=100 g m^{-2})). The photon flux transmitted by the plastic was approximately 0.3%, based on the averages of eight light measurements under the plastic (1.03 µmol s^{-1} m^{-2}) and above the plastic (312.3 µmol s^{-1} m^{-2}) using a LI-COR Quantum/Radiometer/Photometer LI-189.

When plots were located in smaller stands, the whole stand was cut and covered. When plots were located within larger stands, only a part of the stand was cut and covered. Cut plant material was removed from the plots and piled at the site. The plastic was weighed down with stones, logs and other objects.

Maintenance

Plots were monitored approximately once per month during the time they were covered, to remove shoots that had sprouted along the margins of the plastic and to ensure that the plastic was in place and undamaged. Some plots (plot 2, 4 and 7) were monitored more frequently because of the more accessible locations of these plots.

Uncovering

Plots were uncovered after five different time-periods, at two different times of season (May 30, 2012; August 21, 2012; May 30, 2013; August 21, 2013; June 9, 2014) (Table 14). Two plots were uncovered each time: one plot within a stand of *F. japonica* and one plot within a stand of *F. x bohemica.* Shoots that had emerged under the plastic by the time of uncovering were counted and then removed with a hand-scissor.

Harvest

New shoots that had emerged 4 weeks after uncovering were counted, harvested, oven-dried at 60°C for at least one week and then weighed. When aboveground biomass within a plot had been harvested, the plastic was again placed over the plot to allow for continuation of the experiment and future recordings.

Statistical analyses

Since only one plot of each taxon was uncovered at each time, the data from *F. japonica* and *F. x bohemica* were pooled for statistical analyses of regrowth. The data was analysed in non-linear regression with Minitab 16. An exponential model was used to estimate the effect of cover duration on regrowth potential.

Table 13 Overview of *Fallopia* stands and plots used in Experiment 3.2. Sample no. refers to Table 16, Appendix 2 (taxonomic identification and locations).

Plot	Taxon	Comment	Sample no.
1	*F. japonica*	Small "island", close to larger stand	7
2	*F.* x *bohemica*	Small, isolated stand	4
3	*F. japonica*	Large stand	7
4	*F. x bohemica*	Small "island", close to larger stand	8
5	*F. japonica*	Large stand	7
6	*F.* x *bohemica*	Large stand	3
7	*F. japonica*	Small, isolated stand	5
8	*F.* x *bohemica*	Large stand	3

Table 14: Overview of the method used in Experiment 3.2

Method	Date		Plot no.
Covering Initial number of shoots was counted.	2011	July 19	1, 2, 3, 4, 5, 6, 7, 8
Uncovering The plastic was removed from the plots. Shoots that had sprouted under the plastic were counted and removed from plots.	2012	May 30	1, 2
		Aug 21	3, 4
	2013	May 30	5, 6
		Aug 21	7, 8
	2014	June 9	1, 2
Harvest New shoots were counted. Aboveground biomass was harvested, dried and weighed.	4 weeks after uncovering		

2.3.3 Results

2.3.3.1 Experiment 3.1: Seasonal changes in shoot regrowth potential of Fallopia x bohemica

Time had a significant effect on the number of shoots, shoot height and aboveground biomass produced after cutting (Table 15). The initial number of shoots had a significant effect only on shoot production in 2013 (Table 15).

2012

Shoot regrowth after cutting decreased during the growing season (Figure 28, Figure 30, Figure 32). Cutting June 25 significantly decreased the number of shoots, compared to cutting May 26 (Figure 30). Note that shoots were mistakenly counted 4 weeks after cutting in June, while 5 weeks after cutting in May. Lower biomass was produced after cutting June 25 than after cutting May 26, but the difference was not significant (Figure 32). No significant decrease in the number of shoots produced after cutting was found during the rest of the growing season (Figure 30), but a significant difference in biomass produced after

cutting was found between cutting May 26 and cutting July 18 (Figure 32). From July 18, biomass production after cutting remained low until it decreased significantly in September (Figure 32).

2013

Shoot regrowth after cutting decreased during the growing season (Figure 29, Figure 31, Figure 33). Number of shoots produced after cutting was lower when cutting June 17 than when cutting May 31, but the decrease was not significant. Number of shoots produced after cutting decreased significantly from May 31 to July 1, and from July 1 to July 16 (Figure 31). Biomass and number of shoots produced after cutting did not change significantly from July 16 to August 15 (Figure 31, Figure 33).

There was significant increase in height growth of uncut shoots from May 31 to June 16, and from June 16 to July 1. Height growth was not significant after July 1 (Figure 34). There was a significant decrease in height growth of new shoots produced after cutting from July 1 to July 16 (Figure 34).

Table 15: Results from ANCOVA and ANOVA analyses of shoot regrowth after cutting.

			ANCOVA		**ANOVA**	
			Number of shoots 4 weeks after cutting (harvest/start)	**Aboveground Biomass 4 weeks after cutting**	**Initial shoot height before cutting**	**Shoot height of regrowth 4 weeks after cutting**
Year	**Source**	**Df**	**p**	**p**	**p**	**p**
2012	Time of cutting	4	0.003	0.004		
	Initial number shoots	1	0.350	0.582		
2013	Time of cutting	5	0.000	0.000		
	Initial number of shoots	1	0.152	0.036		
2013	Time of cutting	5			0.000	0.000

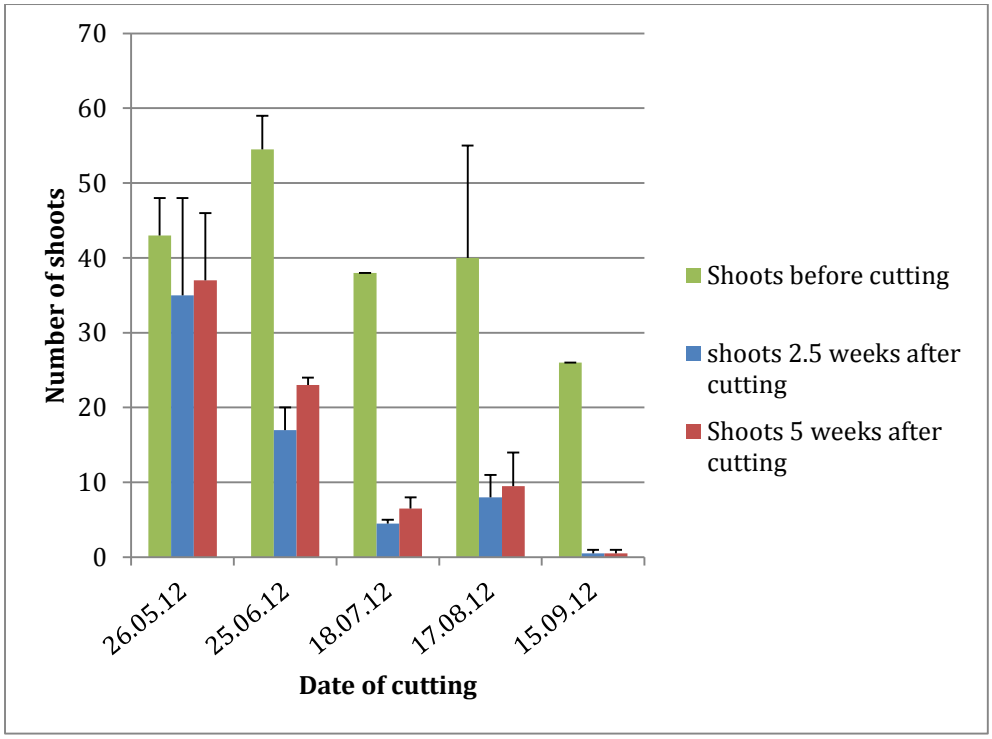


Figure 28 Number of shoots before cutting (green), 2.5 weeks after cutting (blue) and 5 weeks after cutting (red), year 2012. Error bars represent SE.

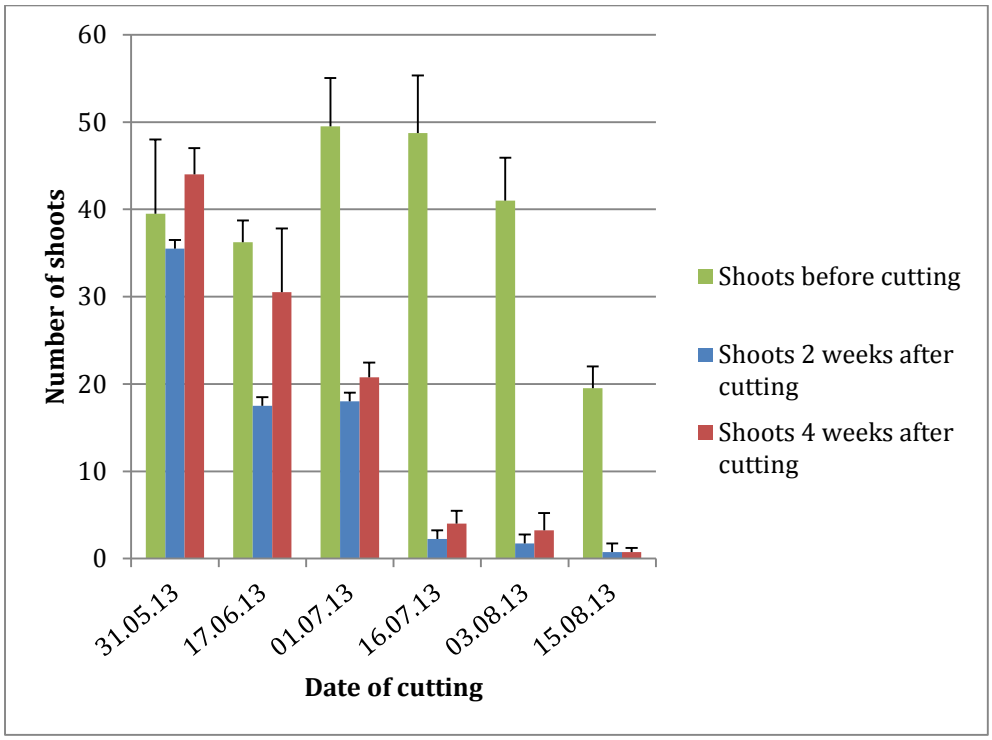


Figure 29 Number of shoots before cutting (green), 2 weeks after cutting (blue) and 4 weeks after cutting (red), year 2013. Error bars represent SE.

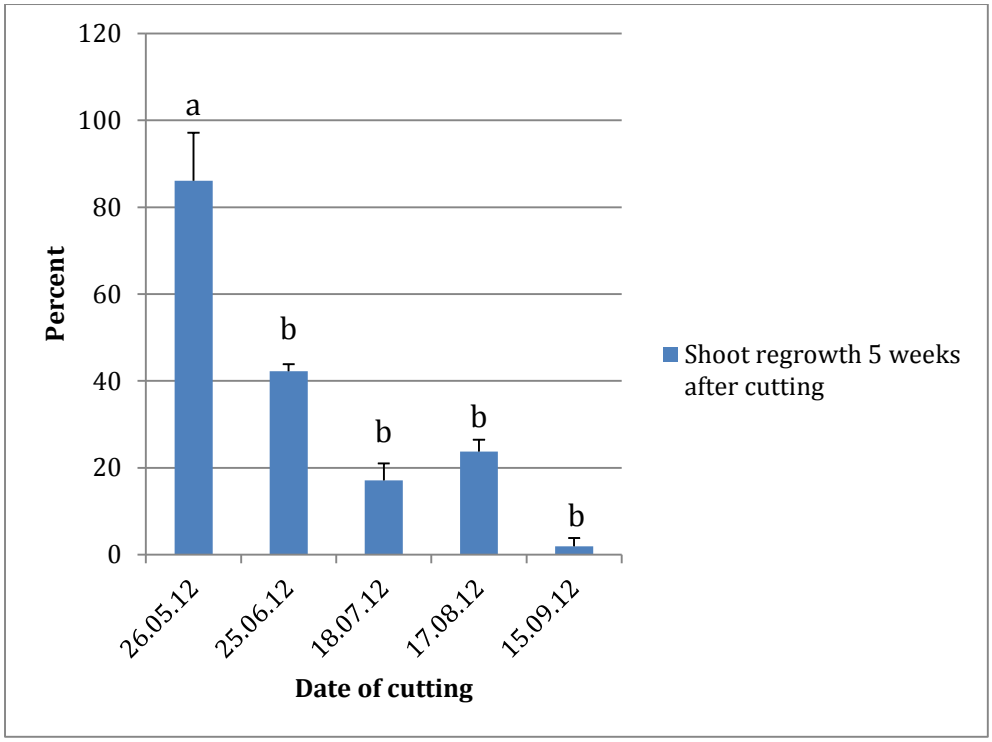


Figure 30 Number of shoots 5 weeks after cutting, as percentage of the initial number of shoots, year 2012. Error bars represent SE. Different letters indicate statistical significance ($p<0.05$)

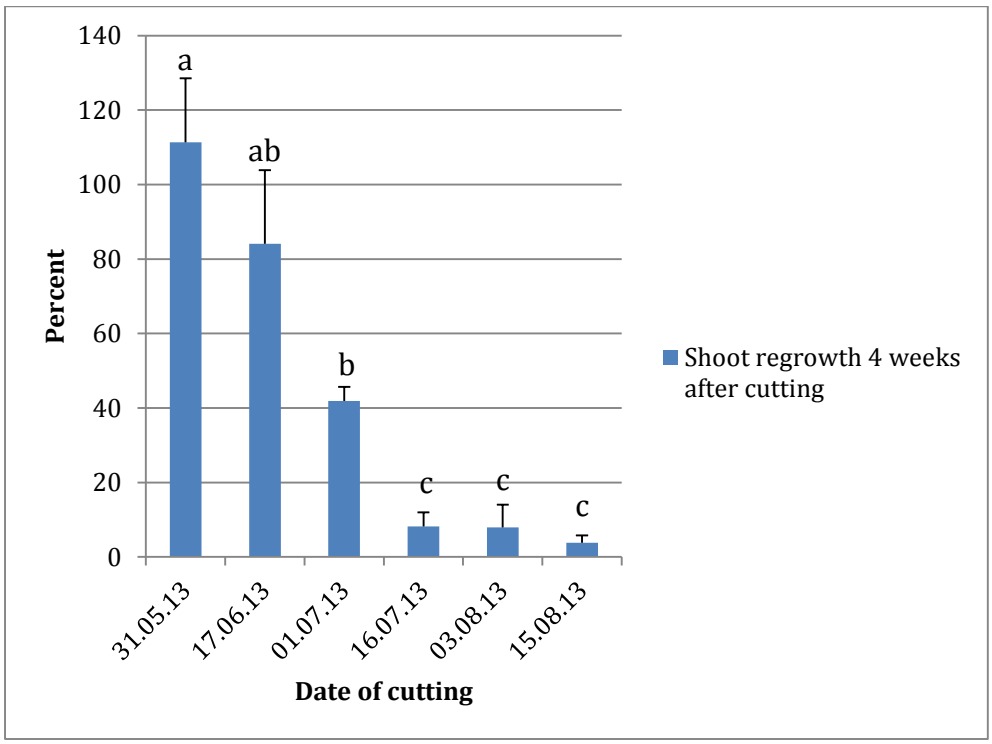


Figure 31 Number of shoots 4 weeks after cutting, as percentage of the initial number of shoots, year 2013. Error bars represent SE. Different letters indicate statistical significance ($p<0.05$)

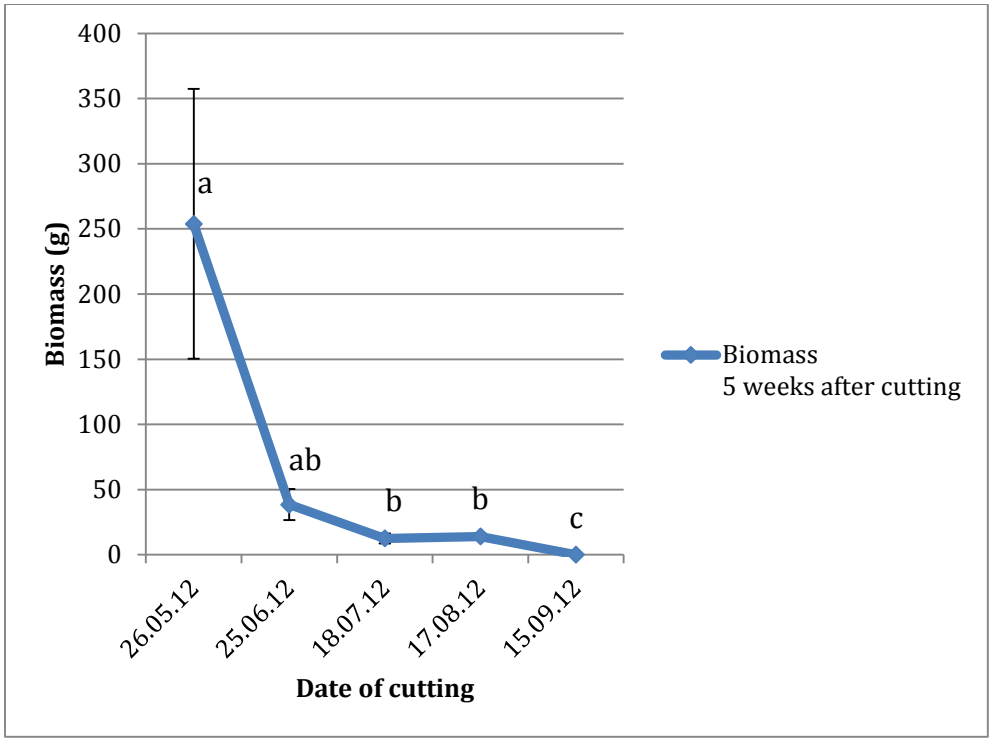


Figure 32 Aboveground biomass harvested 5 weeks after cutting at different times during the growing season, year 2012. Error bars represent SE. Different letters indicate statistical significance ($p<0.05$)

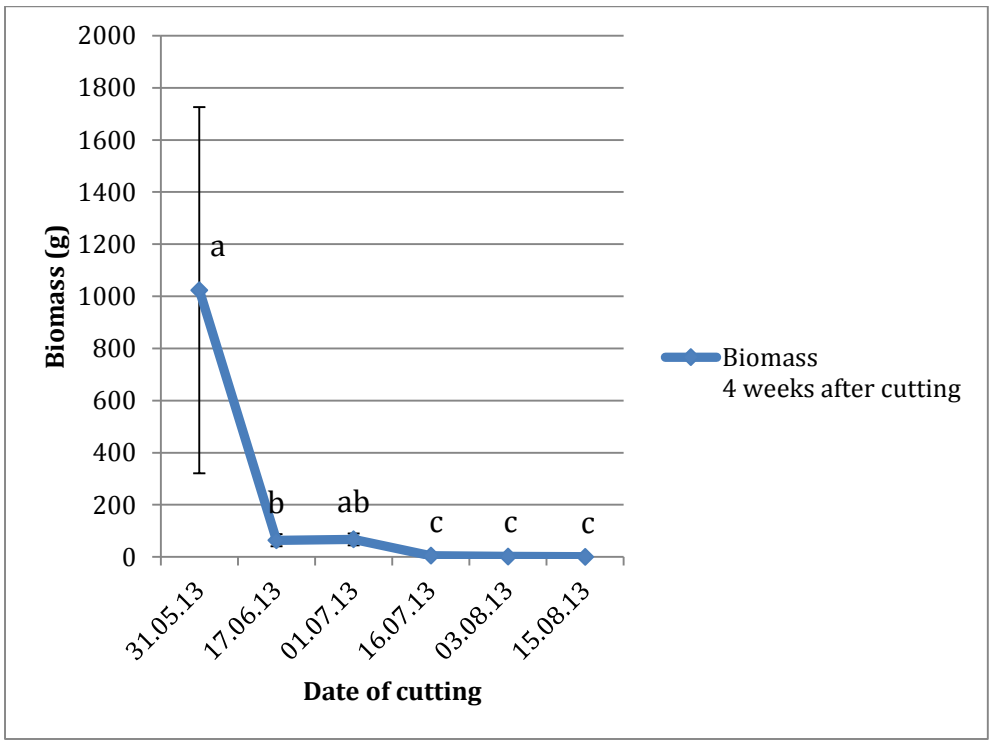


Figure 33 Aboveground biomass harvested 4 weeks after cutting at different dates during the growing season, year 2013. Error bars represent SE. Different letters indicate statistical significance ($p<0.05$).

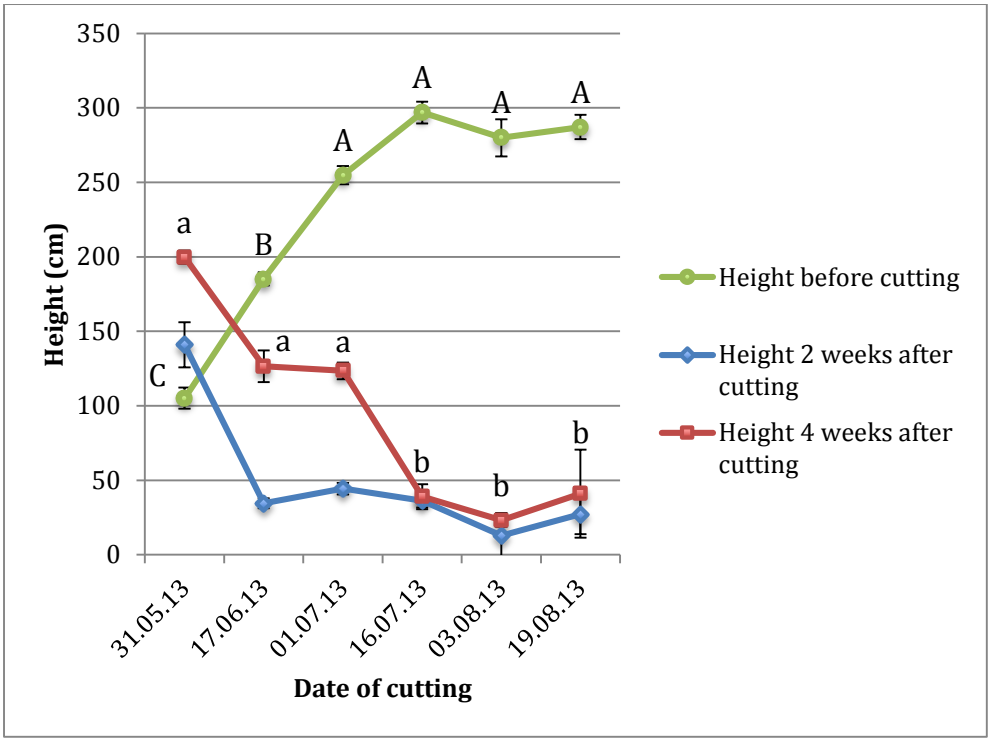


Figure 34 Height measured before cutting, 2 weeks after cutting and 4 weeks after cutting, year 2013. Error bars represent SE. Different lowercase letters indicate statistical significance ($p<0.05$), and different uppercase letters indicate statistical significance ($p<0.05$).

2.3.3.2 Experiment 3.2: The effect of covering on the shoot regrowth potential of F. japonica and F. x bohemica

At the first time of uncovering, in late May 2012, the number of shoots in both plots had increased compared to before covering (Figure 37). Harvested biomass varied greatly between the two plots uncovered at this time, reflected by the large standard error (Figure 38). Uncovering in late August 2012 resulted in a decrease in the number of shoots by 40% in one plot, but an increase in the number of shoots by 511% in the other plot, 4 weeks after uncovering (Figure 37). Mean biomass harvested in August 2012 was lower than mean biomass harvested in May 2012 (Figure 38). Etiolated, twirling shoots with small leaves sprouted under the plastic during the first and the second season (Figure 35).

Four weeks after the uncovering of plots in late May 2013, the number of shoots was reduced by 95% in one plot and increased by 133% in the other plot, compared to the initial number of shoots (Figure 37). Mean biomass harvested at this time was lower than the mean biomass

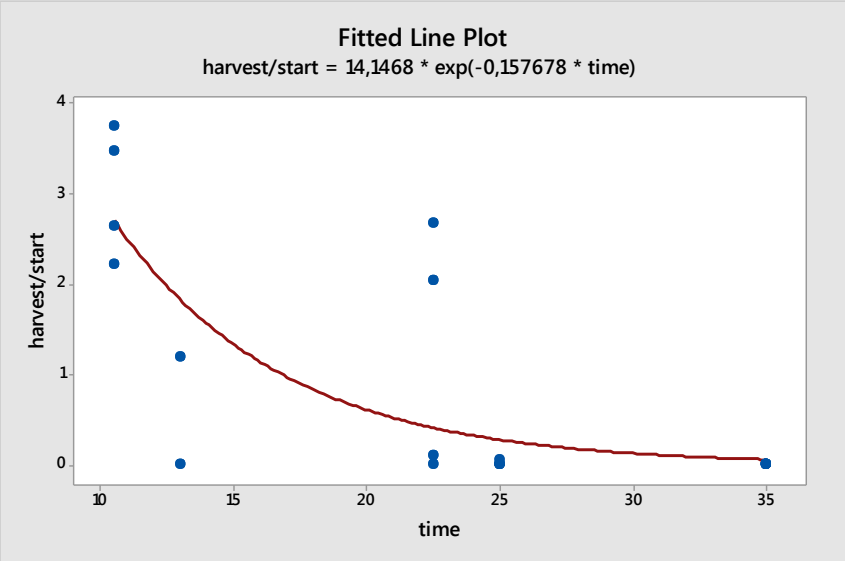
Figure 35 Etiolated shoots sprouted under the plastic. The picture was taken in October 2011 in Moss (plot 6). The plastic was placed back over the plot immediately after the picture was taken.

harvested one year earlier, in May 2012, but higher than the mean biomass harvested in August 2012 (Figure 38). Four weeks after uncovering in late August 2013, the number of shoots was reduced by 98% in one plot and 100% in the other plot, compared to the initial number of shoots. Mean biomass harvested this time was lower than at earlier times of uncovering (Figure 38). Shoot sprouting along the margins of the plastic lessened noticeable in 2013 compared to in 2012.

When plots were uncovered in early June 2014, almost three years after the plots were covered, the number of shoots was reduced by 100% in both plots. No new shoots had sprouted under the plastic, and no new shoots had sprouted four weeks after uncovering (Figure 37, Figure 39). Four weeks after uncovering, a small part of plot 1 had accidently been covered with gravel due to work at the site. At the end of the growing season, no new shoots had sprouted in plot 2. At this time, plot 1 had been completely covered with gravel, and it was not possible to assess whether new shoots were present under the gravel.

During the experiment, only a few shoots grew through the plastic. There was only one occasion where shoot growth lifted the plastic out of place. This happened because the plastic was not properly secured, and this plot was excluded from the experiment.

A scatterplot with time (duration of cover) plotted against the ratio harvest/start (mean number of shoots at harvest / mean initial number of shoots), suggested that the dataset was best fitted with an exponential curve (Figure 36). The function $y = 14.15 * e^{-0.158 * t}$ was used to model the relationship between time (t) and harvest / start (y_0) and estimate approximately how long a stand needs to be covered (t) to achieve a certain reduction in regrowth (harvest / start = y_0). The curve falls exponentially towards y = 0, but can theoretically never reach zero until infinity time. The model estimates that 3 - 5 years of covering is needed to achieve a shoot reduction of 90% -100%.



Model: $y = 14.15 * e^{-0.158 * t}$

y_0	Shoot reduction	t
0,5	50 %	21.2 months = 1.8 years
0,1	90 %	31.4 months = 2.6 years
0,01	99 %	46.0 months = 3.8 years
0,001	99,9 %	60.6 months = 5.0 years

Figure 36 Cover duration (horizontal axis, time = months) plotted against regrowth (vertical axis, harvest/start = 'number of shoots one month after uncovering / number of shoots before covering'). The curve visualizes the model $y = 14.15 * e^{-0.158 * t}$ and estimates the effect of different cover durations (t) on shoot regrowth (y_0).

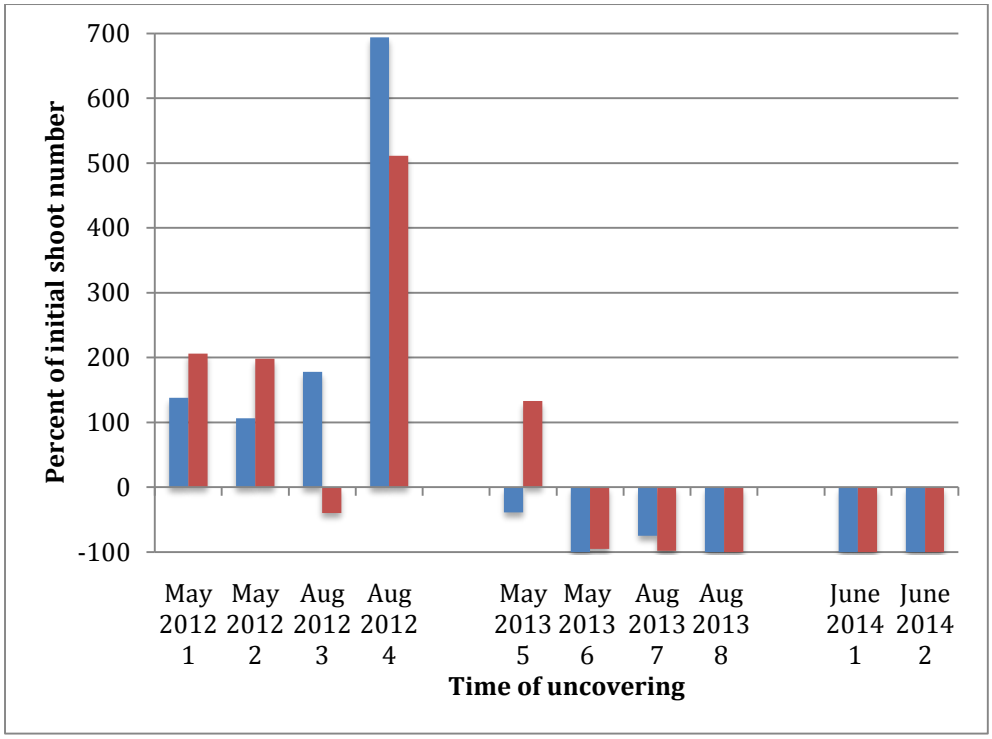


Figure 37 Number of shoots emerged under the plastic at the time of uncovering (blue bars) and 4 weeks after uncovering (red bars), as percentage of the initial number of shoots, in plots uncovered at different times (from left: plot 1, 2, 3, etc.).

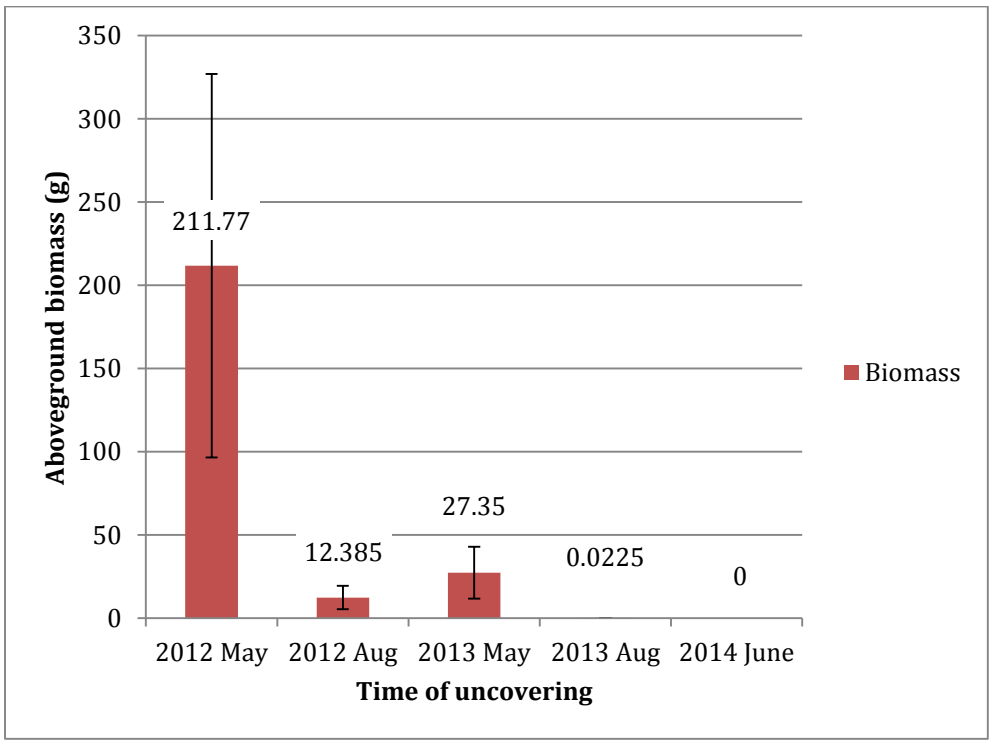


Figure 38 Mean aboveground biomass harvested 4 weeks after uncovering. Aboveground biomass was harvested in two plots each time, and each plot consisted of 2 x 1 m^2. Error bars represent SE.

A

B

Figure 39 No new shoots sprouted after three years of covering. Pictures are taken one month after uncovering in June, 2014. **A.** *F. japonica* (plot 1), **B.** *F.* x *bohemica* (plot 2). Shoots that had remained outside the plastic were alive (upper right corner in picture A).

2.3.4 Discussion

2.3.4.1 Effect of season on shoot regrowth potential

Previous studies have found that the ability of *F. japonica* to produce new shoots after a cutting decreases during the growing season (Callaghan et al., 1981; De Waal, 1995; Gover et al., 2005). The present study has shown that a similar decline in sprouting also occurs in *F. x bohemica*. In Experiment 3.1, biomass production and shoot production after cutting was reduced in mid-June compared to in the end of May, although the reduction was not always significant. The ability to sprout after cutting was significantly reduced from late May to mid-July, when shoot density and biomass production decreased significantly in both years. In 2013, the number of shoots, biomass, and height of regrowth, all decreased significantly from July 1 to July 16, indicating a pronounced change in the regrowth potential during the first part of July. Sprouting ability after cutting remained low during July and August, until September, when biomass production after cutting was significantly reduced in 2012. This corresponds with the findings of Seiger and Merchant (1997), who found that shoot production ceased in September.

A seasonal decline in the shoot regrowth potential was also found in Experiment 3.2. Removing the plastic in August resulted in a lower biomass production than removing the plastic in May. When the plastic was removed in May 2012, the number of shoots produced 4 weeks after uncovering was higher than the number of shoots that had sprouted under the plastic at the time of uncovering. The opposite was found in plots uncovered in August 2012, when the number of shoots that had sprouted under the plastic at the time of uncovering was higher than the number of shoots produced 4 weeks after uncovering.

The seasonal decline in sprouting of *F. japonica* and *F.* x *bohemica* may indicate that the taxa develop sprouting dormancy in the late part of the season. Lang et al. (1987), cited in Liew (2013), mentioned three types of sprouting dormancy (i) eco-dormancy, that is induced by unfavourable environmental factors, (ii) endo-dormancy, defined as “a state in which growth or normal growth cannot be resumed, whatever the external conditions may be”, and (iii) paradormancy, that is induced by physiological factors synthesized and transported from other structures than those affected, e.g. apical dominance. Brandsæter et al. (2010) showed that the sprouting activity of fragmented roots of *Cirsium arvense* and fragmented rhizomes

of *Elymus repens* was relatively uniform during late summer and autumn, and bud dormancy was not apparent. In contrast, *Sonchus arvensis* developed endodormancy in root buds during the late part of the growing season. The same study also found that shoot development and the onset of dormancy could vary between ecotypes. No similar studies of the dormancy pattern in the invasive *Fallopia* taxa are found. In the present study, the decline in sprouting in *F.* x *bohemica* seemed to have started already in June, and became increasingly pronounced during the late part of the growing season. The environmental conditions at the onset of the decline were not unfavourable to growth, and apical dominance was removed through cutting of aboveground shoots, indicating that endodormancy may be the reason for the decline in sprouting (Lang et al., 1987, cited in Liew, 2013).

The ability to sprout throughout the year can differ between intact and fragmented vegetative reproductive organs. This was shown by Liew (2013), who found that the sprouting ability of *C. arvense* was impaired during a period in the autumn, but only in intact roots and not in root fragments. In contrast, a pronounced endodormancy was found in both intact and fragmented roots of *S. arvensis* during a period in the autumn. Locandro (1973), cited in Child (1999), reported that there was no significant seasonal changes in the viability of *F. japonica* rhizomes between May, June and September. Different studies have also shown that rhizome fragments are able to sprout at different times of the year: in late May (Bímová et al., 2003; Pyšek et al., 2003); August (Parepa et al., 2013); August/September (Child, 1999); October (Brock and Wade, 1992) and late November (Sásik and Pavol, 2006). Thus, the possible dormancy observed in intact rhizome systems of *F.* x *bohemica* in the present study, and in *F. japonica* in other studies (Callaghan et al., 1981; De Waal, 1995), may be absent in rhizome fragments.

Signals regulating dormancy in vegetative buds are species specific, and may include day length, temperature decrease or increase, hormonal signals, and gene expression (Chao et al., 2007; Liew, 2013). Dormancy induction and release in temperate regions seem to be primarily regulated by short days and cold temperatures (Chao et al., 2007; Liew, 2013). Liew (2013) found that short days induced endodormancy in root buds in *S. arvensis*, and that high temperatures enhanced the dormancy effect. What factors regulate dormancy induction and release in the invasive *Fallopia* taxa are not known.

Bashtanova et al. (2009) hypothesized that rhizome buds have low activity during the autumn because the plant is preparing for dormancy, and point to the need for more knowledge about rhizome development and bud activity to be able to adjust the timing of herbicide treatments. Glyphosate is a systemic herbicide that is often used to control invasive *Fallopia*. Glyphosate targets plants protein synthesis and may have reduced effect if protein synthesis in the rhizomes is low (Bashtanove et al., 2009). The results of the current study may indicate that a sprouting dormancy develops in the rhizome buds during the late part of the growing season. Whether this dormancy affects the vulnerability of rhizome buds to herbicides is however not answered by the present study.

2.3.4.2 Effect of covering on shoot regrowth potential

The results of the present study show that the persistence of the rhizome system of *F. japonica* and *F. x bohemica* is higher than in some other creeping perennials like *C. arvense* and *E. repens*, where root or rhizome fragments is found to not survive longer than 1-2 year (Fykse, 1983, cited in Mangerud and Brandsæter, 2009). Although two years of covering reduced the number of shoots in *F. japonica* and *F.* x *bohemica* compared to before covering, two years was too short to completely eradicate the shoot regrowth potential. Three years of covering resulted in no new shoot production four weeks after uncovering. It remains however to see whether new shoots will emerge when the plastic has been off for a longer time. It is reported that stands of invasive *Fallopia* can appear dead for three years following herbicide treatments and then continue aboveground shoot production, indicating that the taxa can survive for minimum three years with no aboveground shoot production (Soll et al., 2008; Nickelson, 2013). Experiences with covering used in treatment programs also suggest that the rhizomes of invasive *Fallopia* taxa can survive much longer periods of covering, and that large stands are more difficult to eradicate than smaller stands (Nickelson, 2013). The stands that were uncovered in 2014 in the present study were very small, isolated patches (< 20 m^2).

2.3.4.3 Implications for control

Cutting

Gover et al. (2005) recommend cutting *F. japonica* around June 1 to reduce height prior to a herbicide treatment. In the current study, regrowth after cutting around June 1 reached a height of 2 m in four weeks, which is an unpractical height for herbicide application through foliar spraying. Cutting in mid-June resulted in regrowth with a shoot height of about 125 cm four weeks after cutting, and cutting July 1 reduced shoot density by 60%. If shoot density is greatly reduced, this could reduce the effect of a herbicide treatment. Based on the results of the present study, it may be suggested to cut *F.* x *bohemica* around mid-June to reduce the height of the regrowth and avoid the great reduction of shoot density.

Cutting early in the growing season when shoots have started elongating may increase shoot density (Fløistad, 2010). Scott et al. (1988), cited in Beerling (1990), found that cutting in late May increased shoot density from 40 to 80 shoots pr. m^2 two weeks after cutting. In the present study, shoot density was slightly increased by cutting in late May in 2013, but not in 2012. Cutting later than May reduced shoot density in both years. Bimova et al. (2001) found that cutting in May reduced shoot density at the end of the growing season in *F. japonica* and *F. sachalinensis*, but increased shoot density in *F.* x *bohemica*. In contrast, Rouifed et al. (2011) found no effect of cutting on shoot height, shoot density, or number of leaves in any of the taxa.

When repeated cuttings are used to deplete rhizomes of energy reserves, the frequency of cutting may be higher during May – June, and lower later in the growing season, when regrowth slows down. Soll (2004) recommends cutting at least every 2-3 weeks, or as often as possible, from the shoots emerge in the spring until August. He suggests that the frequency of cutting could be reduced after August.

Covering

The results of the present study suggest that covering could be a relevant method for control of invasive *Fallopia*, but the method requires that the plastic is properly secured, and the site is monitored at least once per month during the first year. The sprouting of shoots along the edges lessened after this, which is also the experience from other trials (McHugh, 2006),

indicating that monitoring may be carried out less frequently the following years. The plastic should be laid loosely over the cut shoots to prevent shoots from pushing through the plastic (McHugh, 2006; King County Noxious Weed Program, 2008). In the current study, shoots growing through the plastic constituted a smaller problem than expected, and shoots seemed to rather grow sideways and twirling under the plastic. There were however a few occasions where shoots grew through the plastic. It was also expected more problems with strong shoot growth lifting the plastic out of place, but this was only a problem where the plastic had not been properly weighed down along the edges. The time of season when the cover is installed probably affects the need for maintenance. In the current study, the plastic was installed in July, after the onset of the decline in shoot regrowth potential (Experiment 3.1, this thesis). Strong shoot growth could probably have caused more problems if the cover had been installed early in the season. It may be suggested to install the cover around mid June – early July. From this time and onwards, the shoot regrowth potential decreases (Experiment 3.1, this thesis), and the allocation of assimilates from aboveground shoots to rhizomes increases (Price et al., 2001; Experiment 1, this thesis). Installation of the cover around this time could therefore reduce the need for maintenance compared to earlier installation, and reduce replenishment of carbohydrates to belowground storage organs compared to later installation.

Covering is considered most suitable for control of small, isolated, and easily accessible stands. This is because of the discouraging results from covering larger stands, and the high costs associated with covering material and installation and maintenance of the cover (McHugh, 2006; Nickelson, 2013). Covering may be used as part of an integrated control method, for example to control regrowth after herbicide treatments (Nickelson, 2013). A suggestion could be to combine covering with digging, repeated cutting and chemical control. Fragmentation of the rhizomes in early spring increases shoot density, depletes rhizomes of energy reserves and provide more shoots for herbicide application (Child et al., 1998). Fragmentation may also reduce the lifespan of the rhizomes and the time of covering needed for eradication (Fykse, 1983, cited in Mangerud and Brandsæter, 2009). Cutting during May – early June when regrowth is strong depletes rhizomes of energy and reduces plant vigour (Seiger and Merchant, 1997). Covering during the later part of the season controls new shoot growth and prevents replenishment of rhizomes. If a chemical treatment with a systemic herbicide is carried out in July or later in the season, the cover could be installed early the following season to control regrowth.

2.3.4.4 Further studies

The effect of timing and number of cutting treatments on shoot regrowth throughout the growing season needs to be studied in more detail to find the most effective way of integrating cutting in a control strategy. Potential differences in the shoot regrowth pattern between the three invasive *Fallopia* taxa (*F. japonica*, *F. sachalinensis* and *F.* x *bohemica*) should also be examined. The shoot regrowth potential throughout the growing season may vary between different genotypes and different geographical regions. Ecotypic variations in the onset of sprouting dormancy have been found for some other perennial weeds (Brandsæter et al., 2010; Liew, 2013). It may therefore be suggested that the experiment is carried out in different geographical regions.

Experiment 3.2 should be continued to examine the effect of covering in the long term. The plots should be monitored for at least four years after aboveground shoot production has ceased. Viability tests of rhizomes from different parts and different depths of the stand could be used to examine if the rhizomes are alive, and if the shoot regrowth potential vary between different parts of the rhizome system.

It is of interest to examine how different cover materials may affect the shoot regrowth potential of rhizomes differently. The woven plastic used in the present study let some light filter through (ca. 0.3%), and etiolated shoots with small leaves sprouted under the plastic during the first years of covering. It could be hypothesized that this shoot production is disadvantageous for the plant. If the plant uses more energy than it gains by producing these shoots, this could be utilized in a control strategy. A cover material that allows for shoot production, but prevents efficient photosynthesis and assimilate allocation to rhizomes, could reduce the time of covering needed for eradication. It is also interesting to examine how different light spectral qualities affect carbohydrate allocation and plant development in invasive *Fallopia*. Bashtanova et al. (2009) discusses light as a mean to manipulate source-sink relationships in invasive *Fallopia*, and refers to the study of Drozdova et al. (2001). Drozdova et al. (2001) found that blue light increased allocation to development of belowground storage organs, while red light increased allocation to aboveground growth in radish plants (*Raphanus sativus L*). Bashtanova et al. (2009) suggest that photo-selective covers that let through blue and far-red light could increase the sink strength of the rhizomes and increase herbicide allocation to the rhizomes. While chemical control with systemic herbicides depends on allocation towards the belowground parts of the plant, mechanical methods that aim to deplete rhizomes depend on allocation to shoot production. Photo

selective covers that let through red light and increase shoot production could perhaps be used in combination with digging and cutting to increase rhizome depletion. Detailed examination of the effect of different light qualities and quantities on the allocation in invasive *Fallopia* could improve covering as a control method.

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Appendices

Appendix 1. DNA extraction protocol. DNeasy Plant Handbook 07/2006

Plant Tissue (Mini Protocol)

Protocol: Purification of Total DNA from Plant Tissue (Mini Protocol)

Important points before starting

- If using the DNeasy Plant Mini Kit for the first time, read "Important Notes" (page 16).
- Ensure that you are familiar with operating the TissueRuptor or the TissueLyser. See "Disruption and homogenization using the TissueRuptor", page 17, or "Disruption and homogenization using the TissueLyser System", page 17. Refer to the *TissueRuptor User Manual* or the *TissueLyser Handbook* for operating instructions.
- Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.

Things to do before starting

- Buffer AP1 and Buffer AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added.
- Buffer AW and Buffer AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.

Procedure

1. **For disruption using the TissueRuptor, follow step 2; for disruption using the TissueLyser, follow steps 3–6.**

   Alternatively, plant or fungal tissue can be ground to a fine powder under liquid nitrogen using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Proceed immediately to step 7.

2. **TissueRuptor procedure: Place the sample material (≤100 mg wet weight or ≤20 mg lyophilized tissue) into a 2 ml microcentrifuge tube. Add liquid nitrogen to the tube, and freeze the sample for 30 s. Keep the sample submerged in liquid nitrogen, and disrupt for approximately 30 s at full speed. Allow the liquid nitrogen to evaporate, and proceed immediately to step 7.**

   Alternatively, fresh or lyophilized material can be directly disrupted in lysis buffer (after step 7) without using liquid nitrogen, but this may cause shearing of high-molecular-weight DNA. We do not recommend disrupting frozen material in lysis buffer as this can result in low yields and degraded DNA.

3. **TissueLyser procedure: Place the sample material (≤100 mg wet weight or ≤20 mg lyophilized tissue) into a 2 ml safe-lock microcentrifuge tube, together with a 3 mm tungsten carbide bead. Freeze the tubes in liquid nitrogen for 30 s.**

   When using lyophilized tissue, the tubes do not need to be frozen in liquid nitrogen.

4. **Place the tubes into the TissueLyser Adapter Set 2 x 24, and fix into the clamps of the TissueLyser. Immediately grind the samples for 1 min at 30 Hz.**

5. **Disassemble the adaptor set, remove the tubes, and refreeze in liquid nitrogen for 30 s.**

   When using lyophilized tissue, the tubes do not need to be frozen in liquid nitrogen.

6. **Repeat step 4, reversing the position of the tubes within the adaptor set. Proceed immediately to step 7.**

   To prevent variation in sample homogenization, the adaptor sets should be removed from the TissueLyser and disassembled after the first disruption step. For the second disruption step, the adaptor sets should be reassembled so that the tube order is reversed. Rotating the racks of tubes in this way ensures that all samples are thoroughly and equally disrupted.

   Note: The majority of plant tissue is ground to a fine powder after 2 disruption steps, however, for some materials one disruption step may be sufficient. Other tissues, such as seeds and roots, may require disruption steps. Optimization of the disruption procedure may be required for some plant material.

7. **Add 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml) to a maximum of 100 mg (wet weight) or 20 mg (dried) disrupted plant or fungal tissue and vortex vigorously.**

   No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of DNA. In rare cases, where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used.

   Note: Do not mix Buffer AP1 and RNase A before use.

8. **Incubate the mixture for 10 min at 65°C. Mix 2 or 3 times during incubation by inverting tube.**

   This step lyses the cells.

9. **Add 130 µl Buffer AP2 to the lysate, mix, and incubate for 5 min on ice.**

   This step precipitates detergent, proteins, and polysaccharides.

10. **Recommended: Centrifuge the lysate for 5 min at 20,000 x *g* (14,000 rpm).**

    Some plant materials can generate very viscous lysates and large amounts of precipitates during this step. This can result in shearing of the DNA in the next step (see "Lysate filtration with QIAshredder", page 21). In this case, optimal results are obtained if the majority of these precipitates are removed by centrifugation for 5 min at 20,000 x *g* (14,000 rpm). After centrifugation, apply supernatant to QIAshredder Mini spin column and continue with step 11.

11. **Pipet the lysate into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at 20,000 x *g* (14,000 rpm).**

    It may be necessary to cut the end off the pipet tip to apply the lysate to the QIAshredder Mini spin column. The QIAshredder Mini spin column removes most precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in step 12.

12. **Transfer the flow-through fraction from step 11 into a new tube (not supplied) without disturbing the cell-debris pellet.**

    Typically 450 µl of lysate is recovered. For some plant species less lysate is recovered. In this case, determine the volume for the next step.

13. **Add 1.5 volumes of Buffer AP3/E to the cleared lysate, and mix by pipetting.**

    For example, to 450 µl lysate, add 675 µl Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AP3/E, but this will not affect the DNeasy procedure.

    Note: Ensure that ethanol has been added to Buffer AP3/E. See "Things to do before starting", page 24.

    Note: It is important to pipet Buffer AP3/E directly onto the cleared lysate and to mix immediately.

14. **Pipet 650 µl of the mixture from step 13, including any precipitate that may have formed, into the DNeasy Mini spin column placed in a 2 ml collection tube (supplied). Centrifuge for 1 min at ≥6000 x *g* (corresponds to ≥8000 rpm for most microcentrifuges), and discard the flow-through.* Reuse the collection tube in step 15.**

15. **Repeat step 14 with remaining sample. Discard flow-through* and collection tube.**

16. **Place the DNeasy Mini spin column into a new 2 ml collection tube (supplied), add 500 µl Buffer AW, and centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm). Discard the flow-through and reuse the collection tube in step 17.**

    Note: Ensure that ethanol is added to Buffer AW. See "Things to do before starting", page 24.

17. **Add 500 µl Buffer AW to the DNeasy Mini spin column, and centrifuge for 2 min at 20,000 x *g* (14,000 rpm) to dry the membrane.**

    It is important to dry the membrane of the DNeasy Mini spin column since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during elution. Discard flow-through and collection tube.

    After washing with Buffer AW, the DNeasy Mini spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to "Darkly colored membrane or green/yellow eluate after washing with Buffer AW" in the Troubleshooting Guide on page 45.

    Note: Following the centrifugation, remove the DNeasy Mini spin column from the collection tube carefully so the column does not come into contact with the flow-through, as this will result in carryover of ethanol.

18. **Transfer the DNeasy Mini spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate for 5 min at room temperature (15–25°C), and then centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm) to elute.**

    Elution with 50 µl (instead of 100 µl) increases the final DNA concentration in the eluate significantly, but also reduces overall DNA yield. If larger amounts of DNA (>20 µg) are loaded, eluting with 200 µl (instead of 100 µl) increases yield. See "Elution", page 21.

19. **Repeat step 18 once.**

    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates. See "Elution", page 21.

    Note: More than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

Plant Tissue (Mini Protocol)

Appendix 2. Locations, taxonomic identities and ploidy levels of *Fallopia* stands used in the study

Table 16 List of Fallopia stands used in the study. Sample ID numbers, taxonomic identities, ploidy levels and GPS coordinates. FJ = F. japonica, FB = F. x bohemica and FS = F. sachalinensis.

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
1	Moss	*F. japonica*	FJ	FJ	8X	N59 28.379 E10 41.233
2	Moss	*F. japonica*	FJ	FJ	8X	N59 28.250 E10 41.354
3	Moss	*F. x bohemica*	FB	FB/FS	-	N59 28.092 E10 41.269
4	Moss	*F. x bohemica*	FB	FB/FS	6X	N59 28.069 E10 41.249
5	Moss	*F. japonica*	FJ	FJ	8X	N59 28.000 E10 41.149
6	Moss	*F. x bohemica*	FB	FB/FS	6X	N59 27.184 E10 41.791
7	Moss	*F. japonica*	FJ	FJ	8X	N59 26.677 E10 42.346
8	Ås	*F. x bohemica*	FB	FB/FS	6X	N59 40.813 E10 46.140
9	Ås (Frogn)	*F. japonica*	FJ	FJ	8X	N59 38.172 E10 39.919
10	Ås (Frogn)	*F. japonica*	FJ	FJ	8X	N59 38.152 E10 39.919
12	Ås	*F. japonica*	FJ	FJ	-	N59 41.210 E10 49.500
13	Ås	*F. japonica*	FJ	FJ	-	-
14	Ås	*F. japonica*	FJ	FJ	8X	N59 39.920 E10 46.978
15	Moss	*F. japonica*	FJ	FJ	-	N59 26.889 E10 42.243
16	Moss	*F. japonica*	FJ	FJ	8X	N59 26.626 E10 40.949
17	Moss	*F. japonica*	FJ	FJ	8X	N59 25.850 E10 40.217
18	Moss	*F. japonica*	FJ	FJ	8X	N59 25.739 E10 40.273
19	Moss	*F. japonica*	FJ	FJ	8X	N59 25.700 E10 40.280
20	Moss (Rygge)	*F. japonica*	FJ	FJ	8X	N59 24.966 E10 40.700
21	Moss	*F. x bohemica*	FB	FB/FS	6X	N59 27.859 E10 41.638

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
22	Ås	*F. japonica*	FJ	FJ	8X	N59 40.897 E10 45.354
23	Ås	*F. japonica*	FJ	FJ	8X	N59 41.538 E10 44.047
24	Ås	*F. japonica*	FJ	FJ	-	N59 41.387 E10 46.531
25	Ås	*F. japonica*	FJ	FJ	8X	N59 39.284 E10 47.217
26	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.518 E10 44.426
27	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 56.056 E10 45.425
28	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.806 E10 45.404
29	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.655 E10 45.236
30	Ås	*F. japonica*	FJ	FJ	8X	N59 39.264 E10 42.275
31	Ås	*F. japonica*	FJ	FJ	-	N59 39.495 E10 49.335
32	Ås	*F. japonica*	FJ	FJ	-	N59 39.980 E10 48.160
33	Moss	*F. japonica*	FJ	FJ	8X	N59 26.385 E10 41.263
34	Moss	*F. japonica*	FJ	FJ	8X	N59 26.362 E10 41.606
35	Moss	*F. japonica*	FJ	FJ	8X	N59 26.311 E10 41.667
36	Moss	*F. japonica*	FJ	FJ	8X	N59 25.961 E10 40.909
37	Moss	*F. japonica*	FJ	FJ	8X	N59 25.989 E10 40.425
38	Moss	*F. japonica*	FJ	FJ	-	N59 25.860 E10 38.478
39	Moss	*F. japonica*	FJ	FJ	-	-
40	Oslo (Lørenskog)	*F. x bohemica*	FB	FB/FS	6X	N59 56.310 E10 58.053
41	Oslo (Lørenskog)	*F. japonica*	FJ	FJ	-	
42	Ås	*F. japonica*	FJ	FJ	8X	N59 40.146 E10 46.384
43	Moss	*F. japonica*	FJ	FJ	-	N59 26.522 E10 42.348
44	Ås	*F. x bohemica*	FB	FB/FS	6X	N59 40.520 E10 48.544

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
45	Ås (Ski)	*F. japonica*	FJ	FJ	8X	N59 42.325 E10 49.872
46	Oslo	*F.* x *bohemica*	FB	FB/FS	6X	N59 55.675 E10 45.300
47	Moss	*F.* x *bohemica*	FB	FB/FS	6X	N59 26.024 E10 40.425
48	Moss	*F. japonica*	FJ	FJ	-	N59 26.758 E10 41.184
49	Moss	*F. japonica*	FJ	FJ	-	N59 26.441 E10 42.376
50	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.506 E5 19.925
51	Bergen (Meland)	*F. japonica*	FJ	FJ	8X	N60 31.200 E5 13.980
52	Bergen (Meland)	*F. japonica*	FJ	FJ	8X	N60 31.940 E5 11.770
53	Bergen	*F. japonica*	FJ	FJ	8X	≈ N60 30.761 E5 16.688
54	Bergen	*F. japonica*	FJ	FJ	8X	≈ N60 30.565 E5 16.506
55	Bergen	*F. japonica*	FJ	FJ	8X	N60 30.380 E5 16.090
56	Bergen	*F. japonica*	FJ	FJ	8X	N60 28.780 E5 15.500
57	Bergen	*F. japonica*	FJ	FJ	8X	N60 27.390 E5 18.190
58	Bergen	*F. japonica*	FJ	FJ	-	-
59	Bergen	*F. japonica*	FJ	FJ	8X	N60 25.120 E5 18.590
60	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.924 E5 19.846
61	Bergen	*F. japonica*	FJ	FJ	-	N60 23.930 E5 19.846
62	Bergen	*F. japonica*	FJ	FJ	8X	N60 24.085 E5 19.297
63	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.432 E5 18.927
64	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.364 E5 18.842
65	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.910 E5 18.401
66	Bergen	*F. japonica*	FJ	-	-	N60 23.921 E5 18.307

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
67	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.799 E5 19.983
68	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.726 E5 20.220
69	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.736 E5 20.086
70	Bergen	*F. japonica*	FJ	FJ	-	N60 23.748 E5 20.088
71	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.530 E5 20.266
72	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.304 E5 20.940
73	Bergen	*F. japonica*	FJ	-	-	N60 23.264 E5 20.995
74	Bergen	*F. japonica*	FJ	-	-	N60 23.253 E5 21.016
76	Bergen	*F. japonica*	FJ	FJ	8X	N60 22.932 E5 21.145
77	Bergen	*F. japonica*	FJ	FJ	-	N60 22.924 E5 21.140
78	Bergen	*F. japonica*	FJ	FJ	-	N60 22.909 E5 21.134
79	Bergen	*F. japonica*	FJ	FJ	8X	N60 22.805 E5 20.867
80	Bergen	*F. japonica*	FJ	FJ	8X	N60 22.803 E5 20.880
81	Bergen	*F. japonica*	FJ	FJ	8X	N60 23.609 E5 19.949
83	Bergen	*F. japonica*	FJ	FJ	8X	N60 24.203 E5 19.859
84	Bergen	*F. japonica*	FJ	FJ	8X	N60 24.262 E5 19.931
85	Bergen	*F. japonica*	FJ	FJ	8X	N60 24.314 E5 19.821
86	Bergen	*F. japonica*	FJ	-	-	N60 24.276 E5 19.883
87	Oslo	*F. japonica*	FJ	FJ	8X	N59 54.960 E10 45.647
88	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 58.022 E10 46.461
89	Oslo	*F. japonica*	FJ	FJ	8X	N59 57.556 E10 46.066
90	Oslo	*F. japonica*	FJ	FJ	8X	N59 57.487 E10 45.995

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
91	Oslo	*F. japonica*	FJ	FJ	8X	N59 57.376 E10 45.980
92	Oslo	*F. japonica*	FJ	FJ	8X	N59 57.334 E10 45.986
94	Oslo	*F. japonica*	FJ	FJ	8X	N59 56.704 E10 45.936
95	Oslo	*F. japonica*	FJ	FJ	8X	N59 56.694 E10 45.931
98	Oslo	*F. japonica*	FJ	FJ	8X	N59 56.231 E10 45.832
99	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.281 E10 45.197
100	Oslo	*F. x bohemica*	FB	-	6X	N59 55.262 E10 45.218
101	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.256 E10 45.258
102	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.210 E10 45.202
103	Oslo	*F. x bohemica*	FB	-	6X	N59 55.193 E10 45.171
104	Oslo	*F. x bohemica*	FB	FB/FS	6X	N59 55.172 E10 45.181
105	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.674 E10 11.063
106	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.671 E10 10.991
107	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.617 E10 10.942
108	Drammen	*F. japonica*	FJ	FJ	-	N59 44.589 E10 10.931
109	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.651 E10 09.072
110	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.974 E10 09.320
111	Drammen	*F. x bohemica*	FB	FB/FS	6X	N59 45.057 E10 09.069
112	Drammen	*F. x bohemica*	FB	FB/FS	6X	N59 45.044 E10 08.670
113	Drammen	*F. x bohemica*	FB	FB/FS	6X	N59 45.073 E10 08.647
114	Drammen	*F. x bohemica*	FB	FB/FS	6X	N59 45.031 E10 08.545
115	Drammen	*F. sachalinensis*	FS	FB/FS	4X	N59 44.587 E10 12.760
116	Drammen	*F. japonica*	FJ	FJ	8X	N59 44.868 E10 13.003

Sample no.	Location	Taxonomic identity	Morphological identification	Molecular identification (SSR)	Ploidy level	GPS coordinates
117	Drammen	*F. japonica*	FJ	FJ	8X	N59 43.495 E10 08.908
118	Drammen	*F. japonica*	FJ	FJ	8X	N59 43.773 E10 09.356
119	Drammen	*F.* x *bohemica*	FB	FB/FS	6X	N59 43.832 E10 08.579
120	Drammen	*F. japonica*	FJ	FJ	8X	N59 43.995 E10 11.147
121	Drammen	*F. sachalinensis*	FS	FB/FS	4X	N59 44.316 E10 11.771
122	Oslo (Asker)	*F.* x *bohemica*	FB	FB/FS	6X	N59 50.027 E10 25.544
123	Ås (Ski)	*F. japonica*	FJ	FJ	8X	N59 42.801 E10 48.338
124	Ås (Ski)	*F. japonica*	FJ	FJ	8X	N59 42.560 E10 49.644
125	Ås	*F. japonica*	FJ	FJ	8X	N59 39.902 E10 46.087
126	Sogndal	*F. japonica*	FJ	FJ	-	N61 15.156 E7 10.124
127	Moss	*F. japonica*	FJ	FJ	8X	N59 28.943 E10 41.946

Maps of locations of the *Fallopia* stands used in the study

The maps show only the locations of stands that are mapped or sampled in the present study, and not the total distribution of the taxa in the areas. Each stand on the map is marked with a sample ID number that refers to Table 16, Appendix 2.

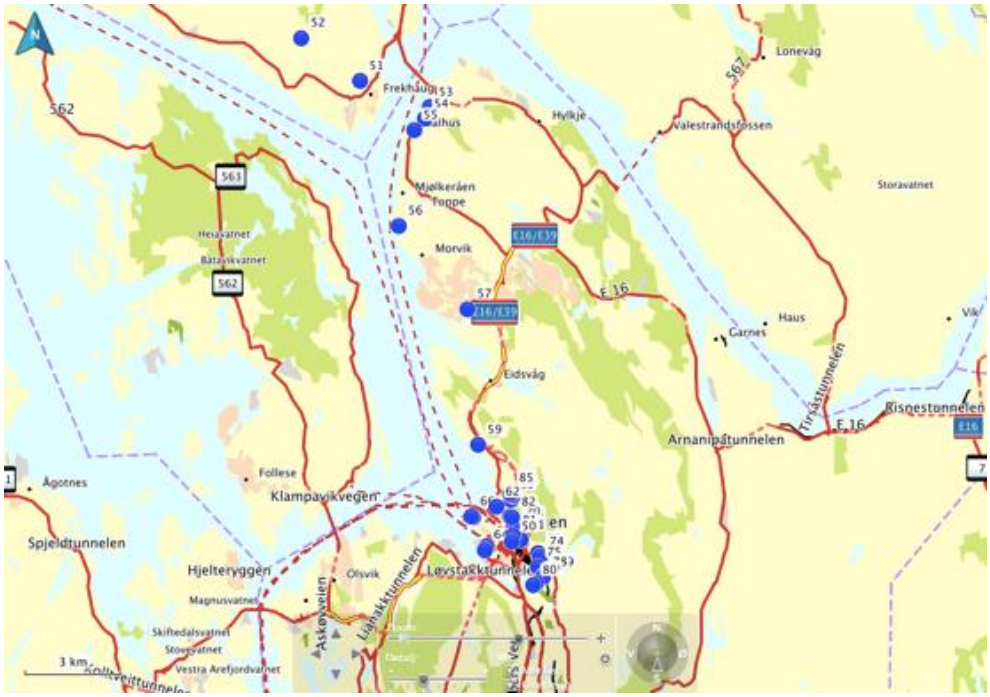


Figure 40 Overview of stands of *Fallopia* spp. in the examined Bergen area. Blue circles = *F. japonica*.

Figure 41 Closer view of the locations of *Fallopia* stands in the Bergen area. Bergen and Meland municipalities. Blue circles = *F. japonica*.

Figure 42 Closer view of the locations of *Fallopia* stands in the Bergen area (Åsane, Bergen municipality). Blue circles = *F. japonica.*

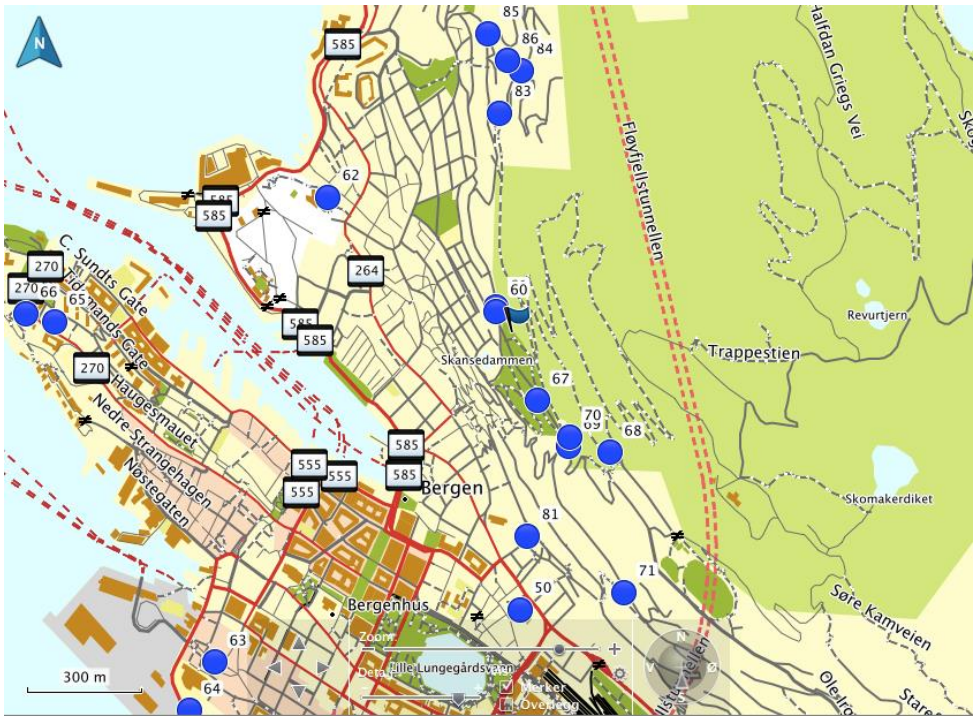


Figure 43 Closer view of the locations of some of the *Fallopia* stands in central Bergen. Blue circles = *F. japonica.* Blue flag = observation on distance only.

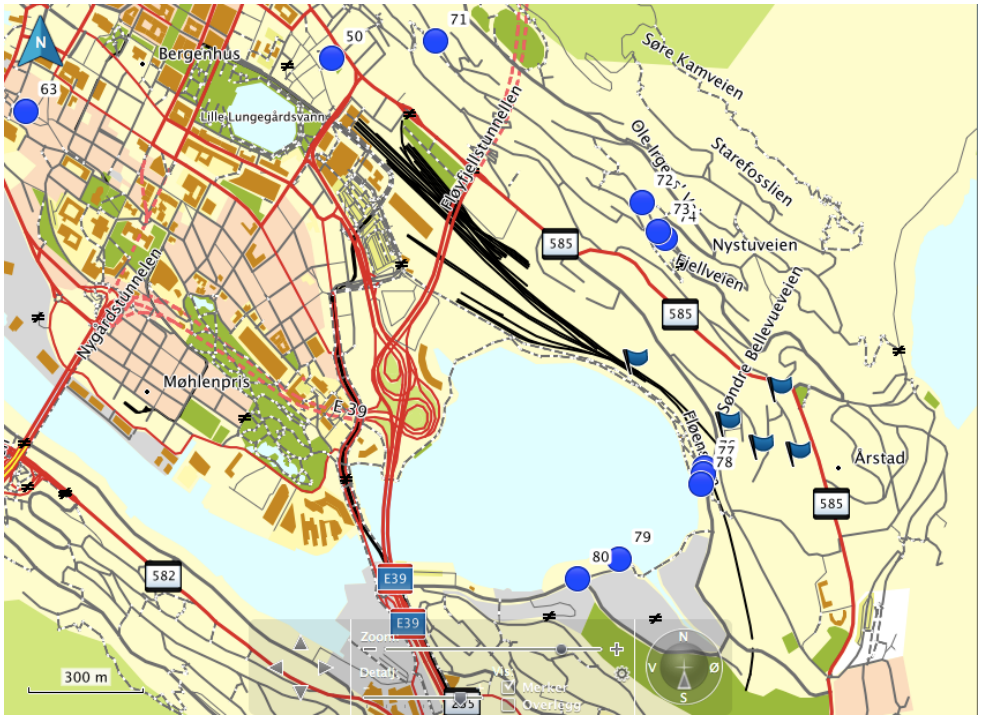


Figure 44 Closer view of the locations of some of the *Fallopia* stands in the Bergen area. Central Bergen. Blue circles = *F. japonica*. Blue flags = observations on distance only.

Figure 45 Locations of *Fallopia* stands in Drammen. Blue circles = *F. japonica*, red circles = *F. x bohemica*, orange circles = *F. sachalinensis*.

Figure 46 The location of the one stand sampled at Barsnes, Sogndal municipality (sample no. 126). Blue circle = *F. japonica.*

Figure 47 The location of the one stand sampled in Asker municipality (sample no. 122).

Figure 48 Overview of *Fallopia* stands along Akerselva river (except stand no. 26, which is located in St. Hanshaugen Park) in central Oslo. Blue circles = *F. japonica*, red circles = *F. x bohemica*.

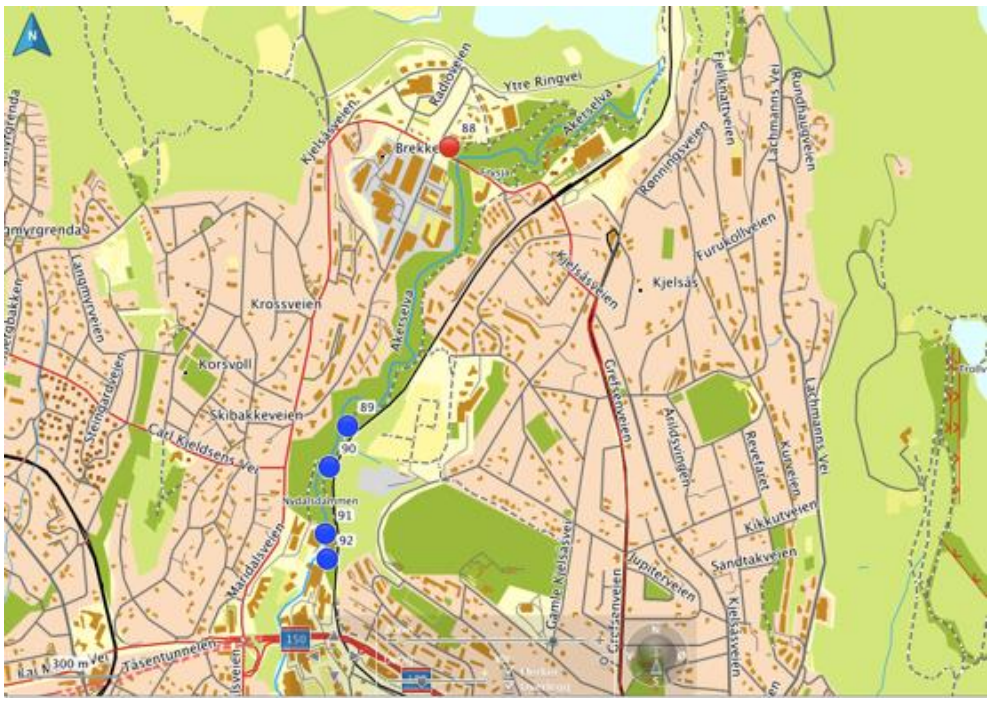


Figure 49 Closer view of the locations of some of the *Fallopia* stands in central Oslo, by Akerselva river. Blue circles = *F. japonica*, red circles = *F. x bohemica*.

Figure 50 Closer view of the locations of some of the *Fallopia* stands in central Oslo, by Akerselva river. Blue circles = *F. japonica.*

Figure 51 Closer view of the locations of some of the *Fallopia* stands in central Oslo, by Akerselva river. Blue circles = *F. japonica*, red circles = *F. x bohemica.*

Figure 52 Closer view of the locations of some of the *Fallopia* stands in central Oslo, by Akerselva river. Blue circles = *F. japonica*, red circles = *F. x bohemica.*

Figure 53 The locations of the two *Fallopia* stands sampled in Lørenskog municipality. Blue circle = *F. japonica*, red circle = *F. x bohemica*.

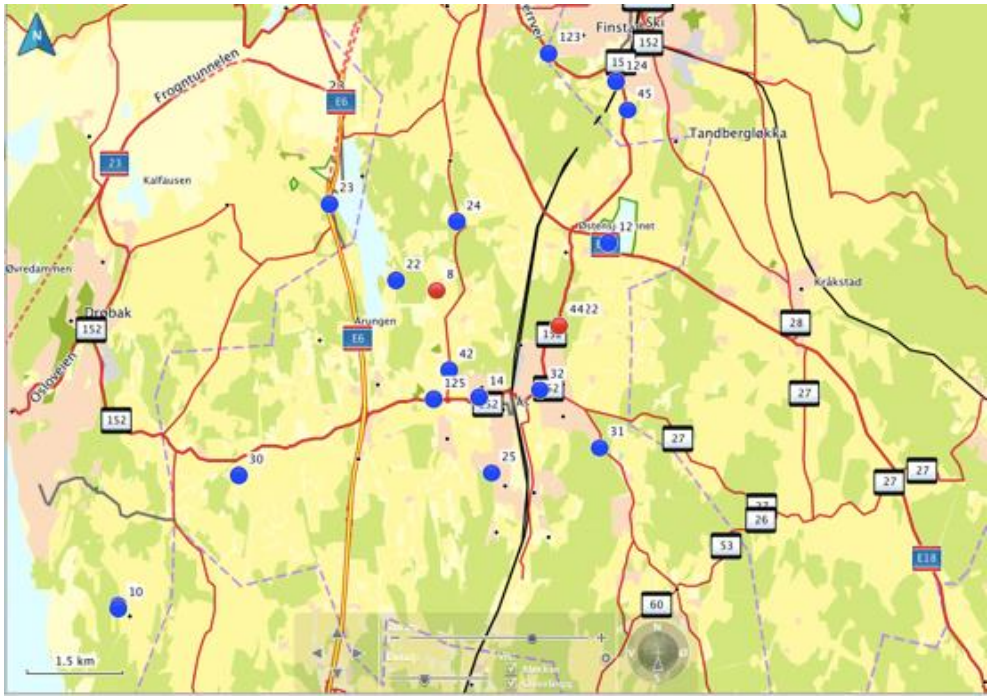


Figure 54 Overview of *Fallopia* stands in the Ås area (Frogn, Ås and Ski municipalities). Blue circles = *F. japonica*, red circles = *F.* x *bohemica*.

Figure 55 Overview of the *Fallopia* stands in the Moss area. Blue circles = *F. japonica*, red circles = *F. x bohemica*.

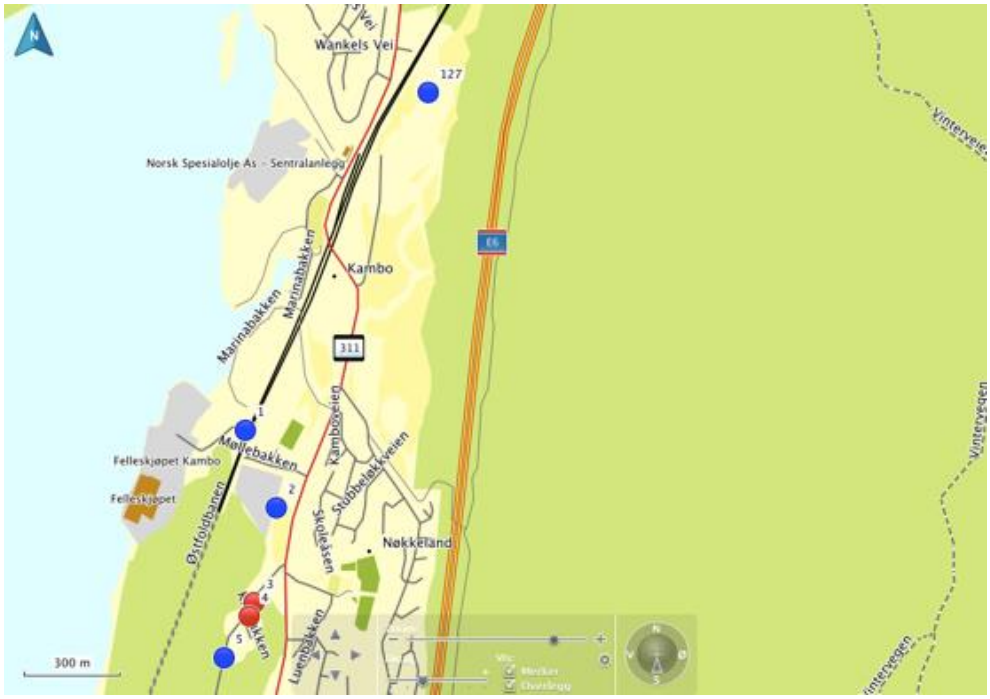


Figure 56 Closer view of the locations of some of the *Fallopia* stands in Moss (Kambo). Blue circles = *F. japonica*, red circles = *F. x bohemica*.

Figure 57 Closer view of the locations of some of the *Fallopia* stands in Moss. Blue circles = *F. japonica*, red circles = *F. x bohemica*.

Figure 58 Closer view of the locations of some of the *Fallopia* stands in central Moss. Blue circles = *F. japonica*, red circles = *F. x bohemica*.

Appendix 3. Morphology

Sample numbers in figure texts refer to Table 16, Appendix 2.

Leaf morphology

A

B

C

Figure 59 *F. japonica* leaves. **A.** sample no. 15, **B.** sample no. 30, **C.** sample no. 10.

A

B

C

Figure 60 *F. japonica* leaves. **A.** sample no. 72, **B.** sample no. 76, **C.** sample no. 71.

A

B

C

Figure 61 *F.* x *bohemica* leaves. **A.** sample no. 29, **B.** sample no. 26, **C.** sample no. 113.

A

B

C

Figure 62 *F.* x *bohemica* leaves. **A.** sample no. 6, **B.** sample no. 11, **C.** sample no. 112.

A

B

C

Figure 63 *F. sachalinensis* leaves. **A.** large, mature leaf. **B.** smaller, younger leaf, **C.** young, unfolded leaves on new shoots. All leaves have deeply cordate leaf bases. Sample no. 115.

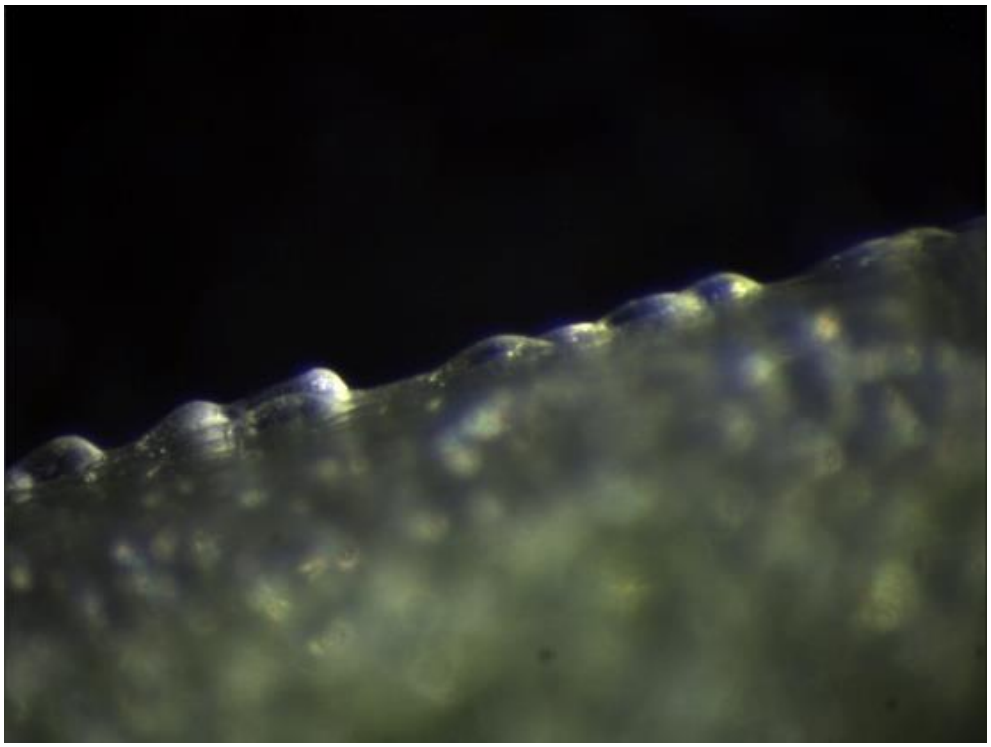


Figure 64 Papillae found on sample no. 87, *F. japonica*.

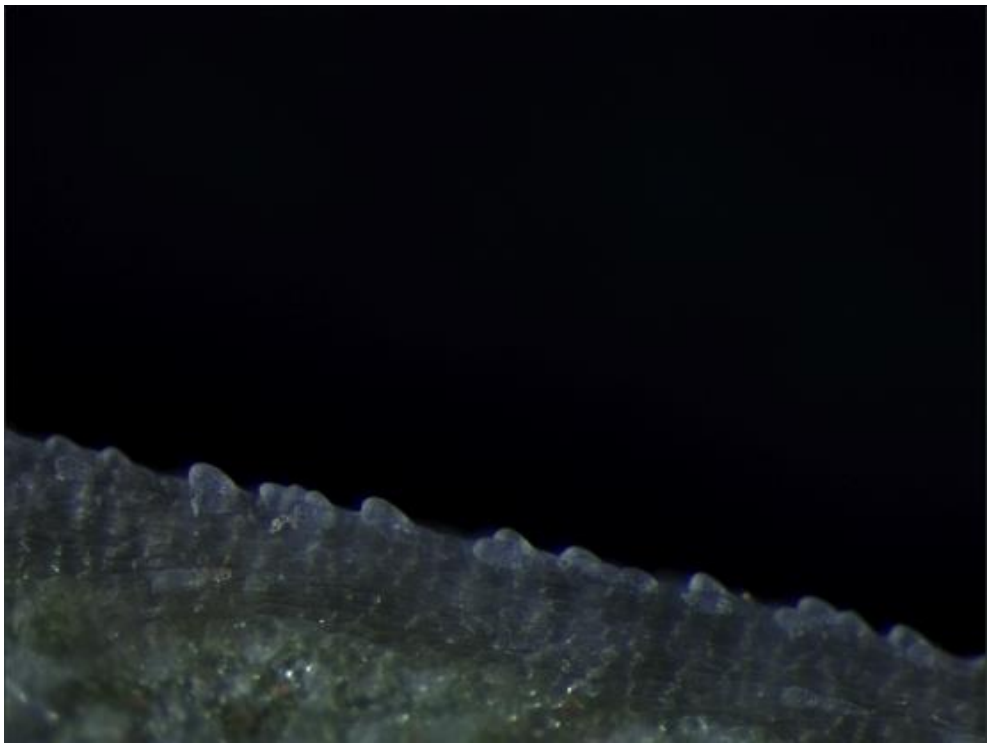


Figure 65 Papillae found on sample no. 123, *F. japonica*.

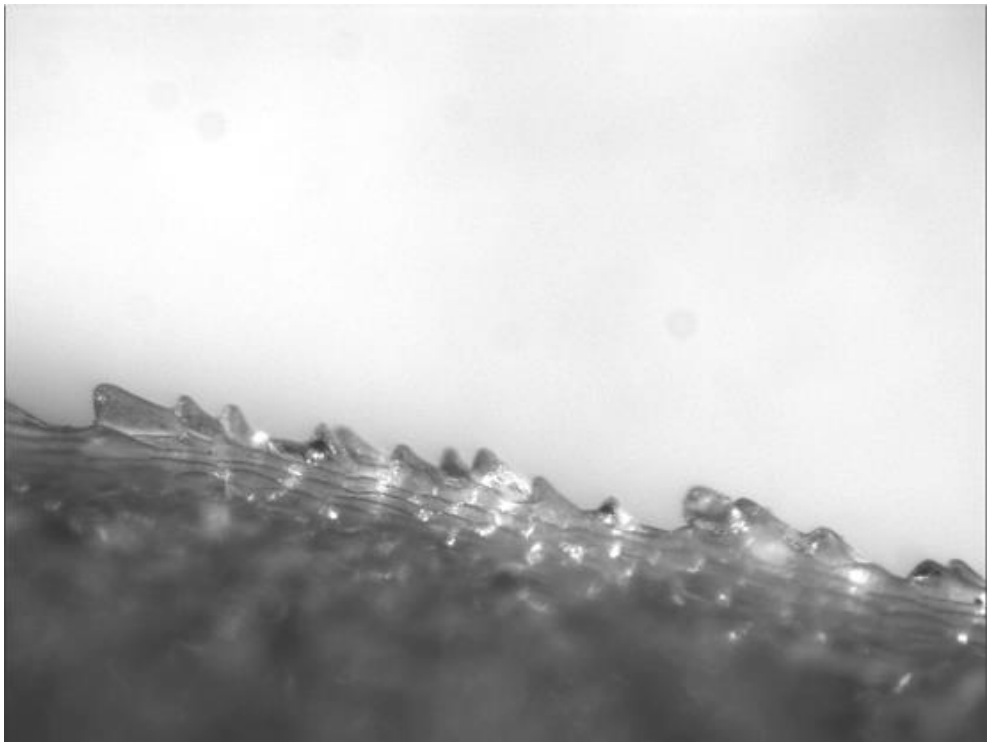


Figure 66 Papillae found on sample no. 107, *F. japonica*.

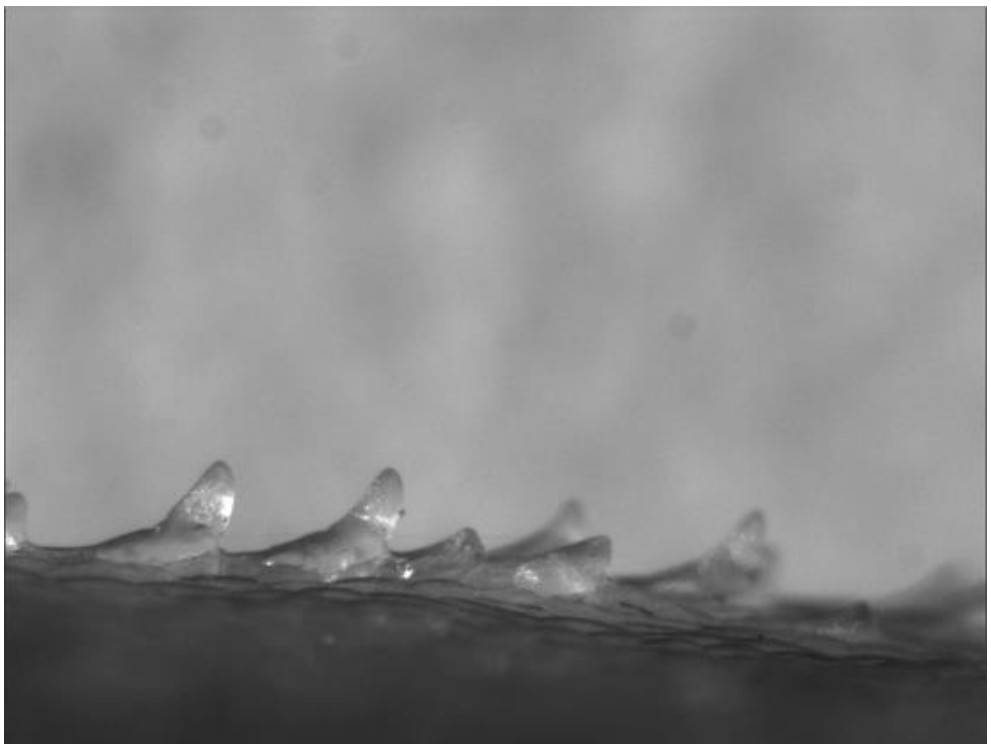


Figure 67 Papillae found on sample no. 10, *F. japonica*.

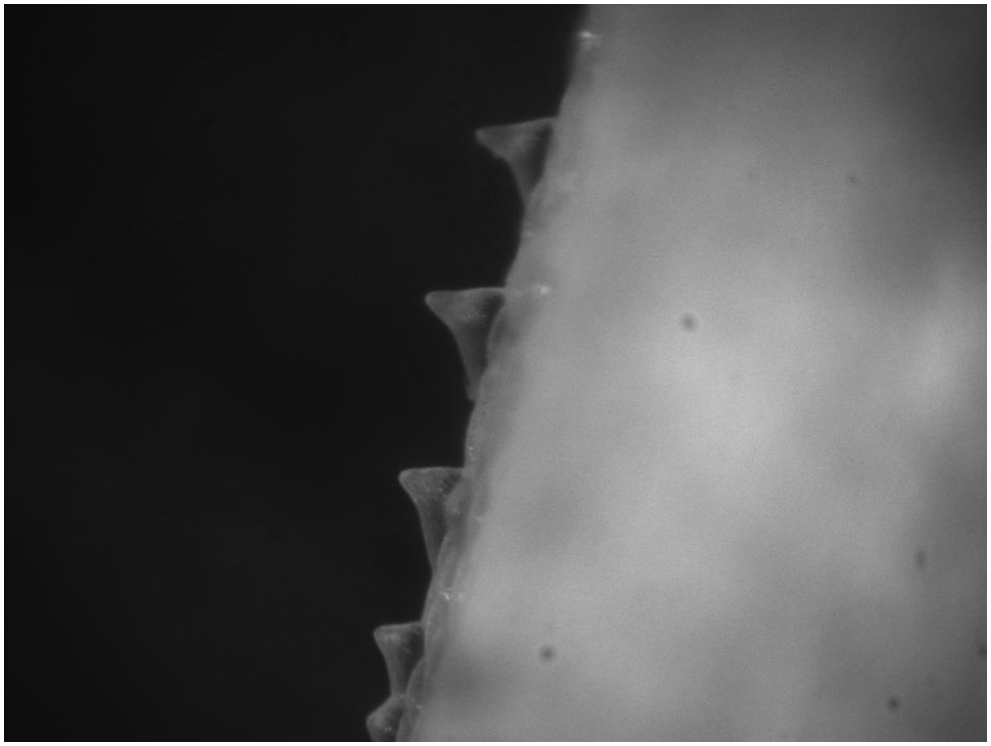


Figure 68 Papillae found on sample no. 118, *F. japonica*.

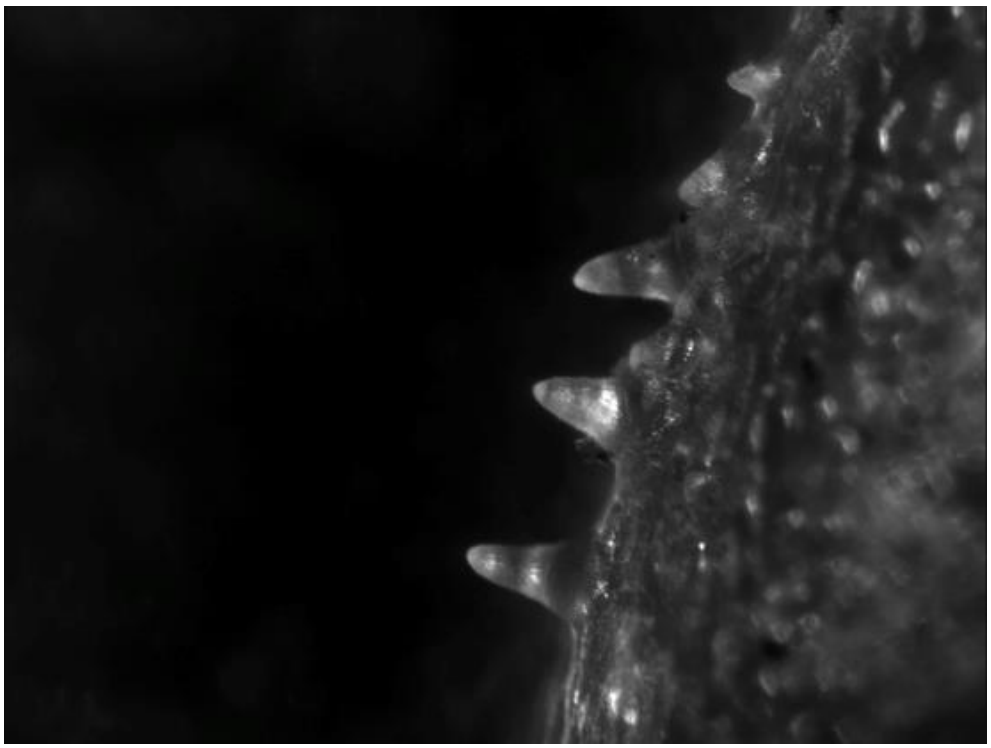


Figure 69 Papillae found on sample no. 124, *F. japonica*.

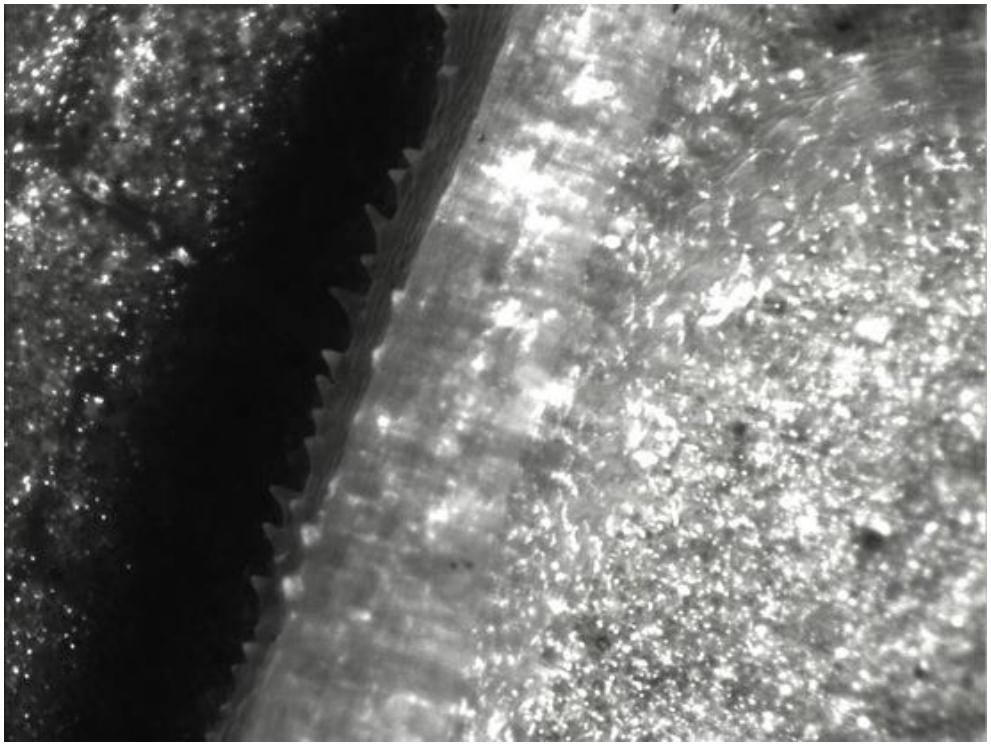


Figure 70 Papillae found on sample no. 117, *F. japonica*.

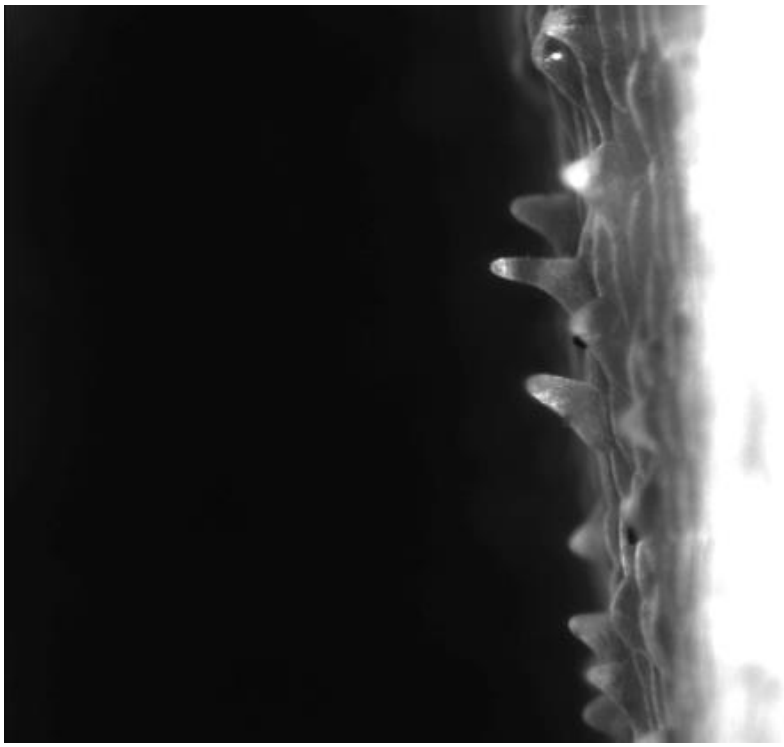


Figure 71 Papillae found on sample no. 7, *F. japonica*.

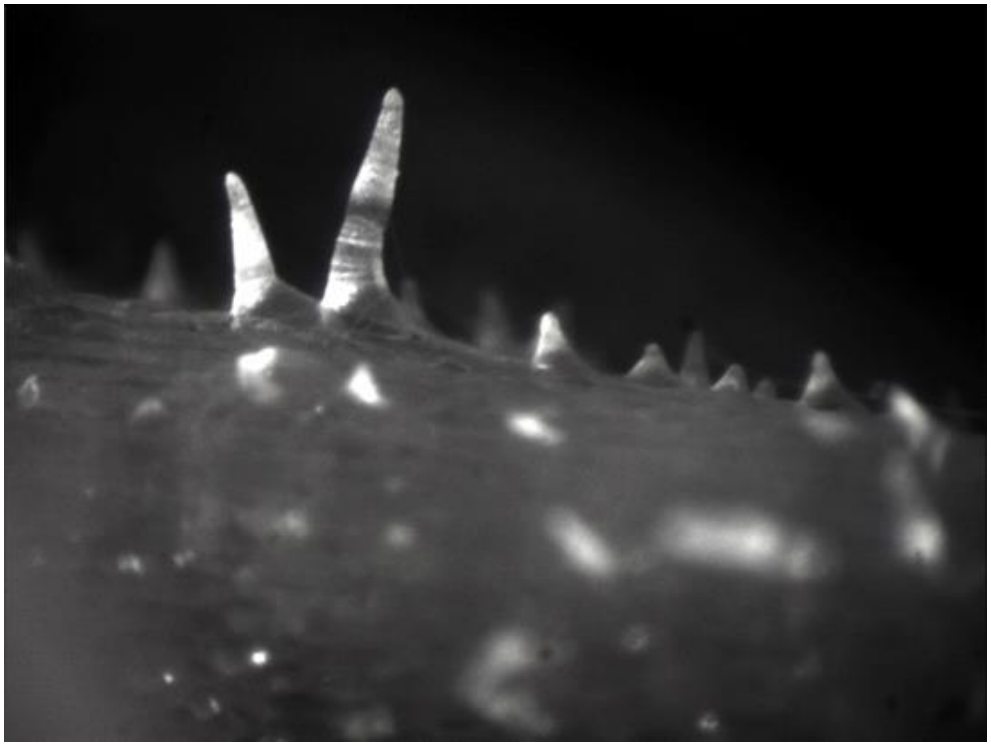


Figure 72 Trichomes found on sample no. 28, *F. x bohemica*.

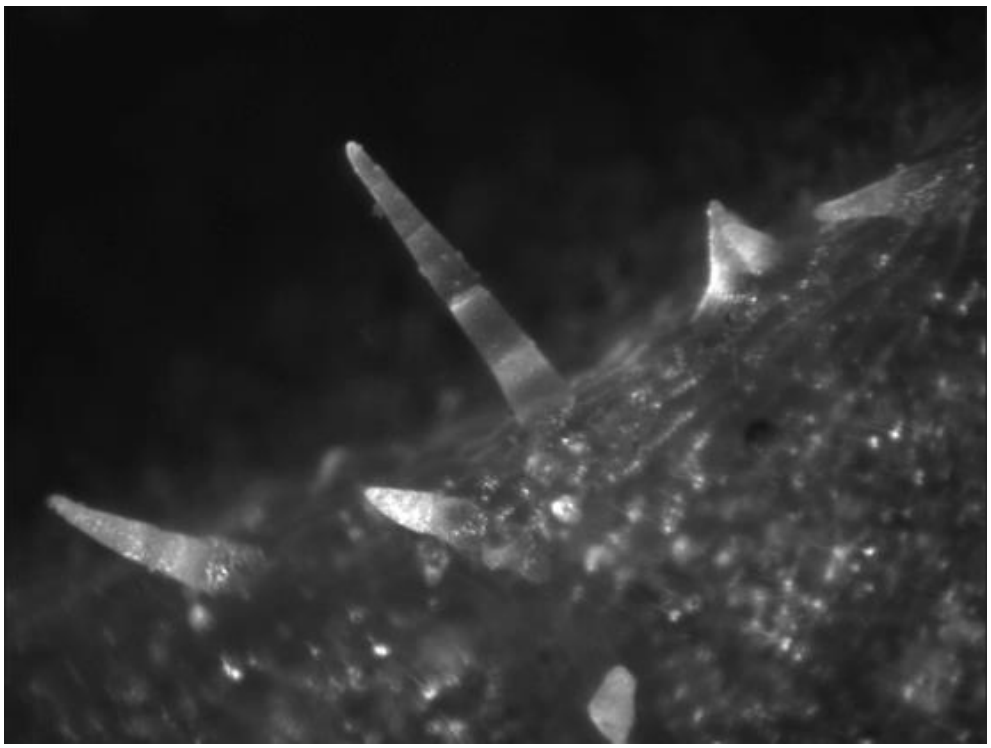


Figure 73 Trichomes found on sample no. 99, F. x bohemica.

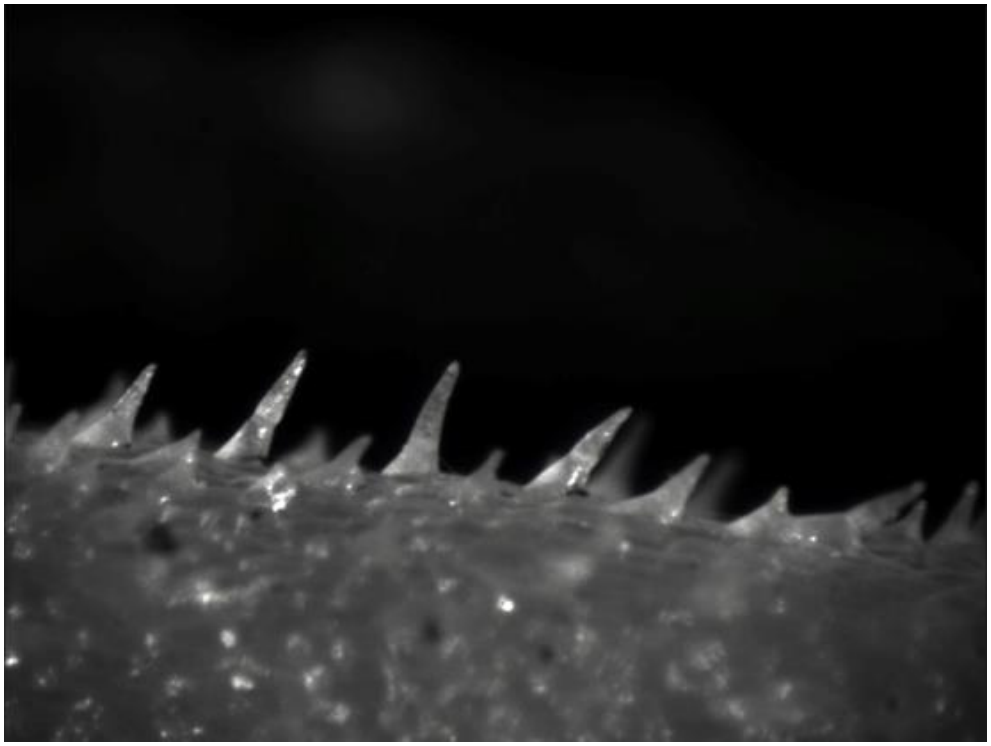


Figure 74 Trichomes found on sample no. 88, *F. x bohemica*.

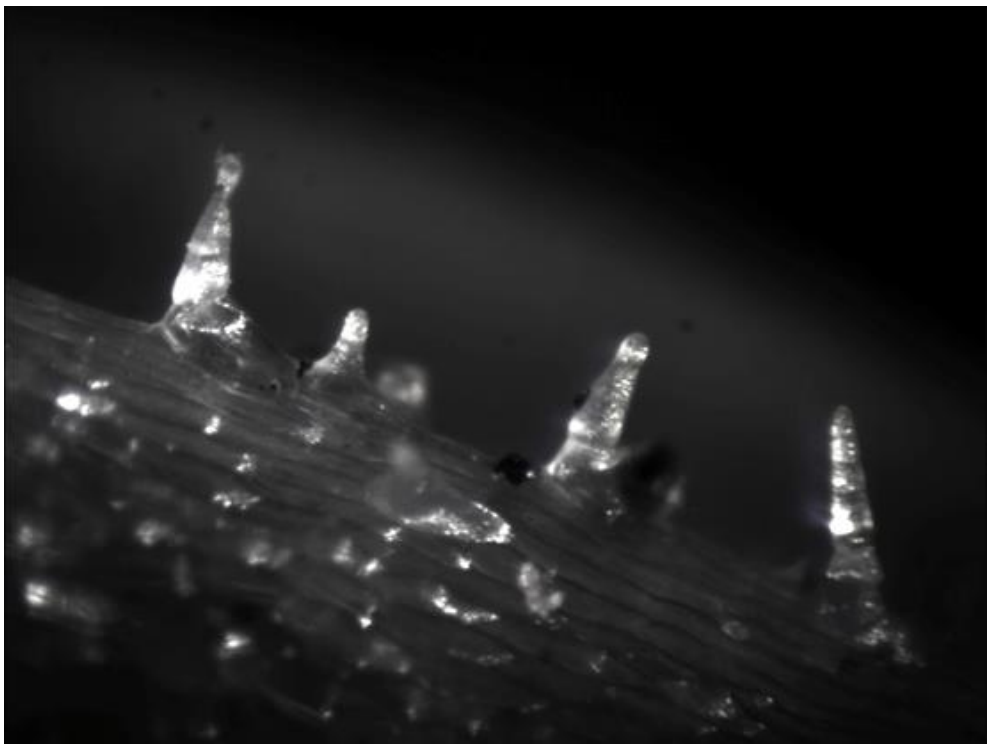


Figure 75 Trichomes found on sample no. 28, *F. x bohemica*.

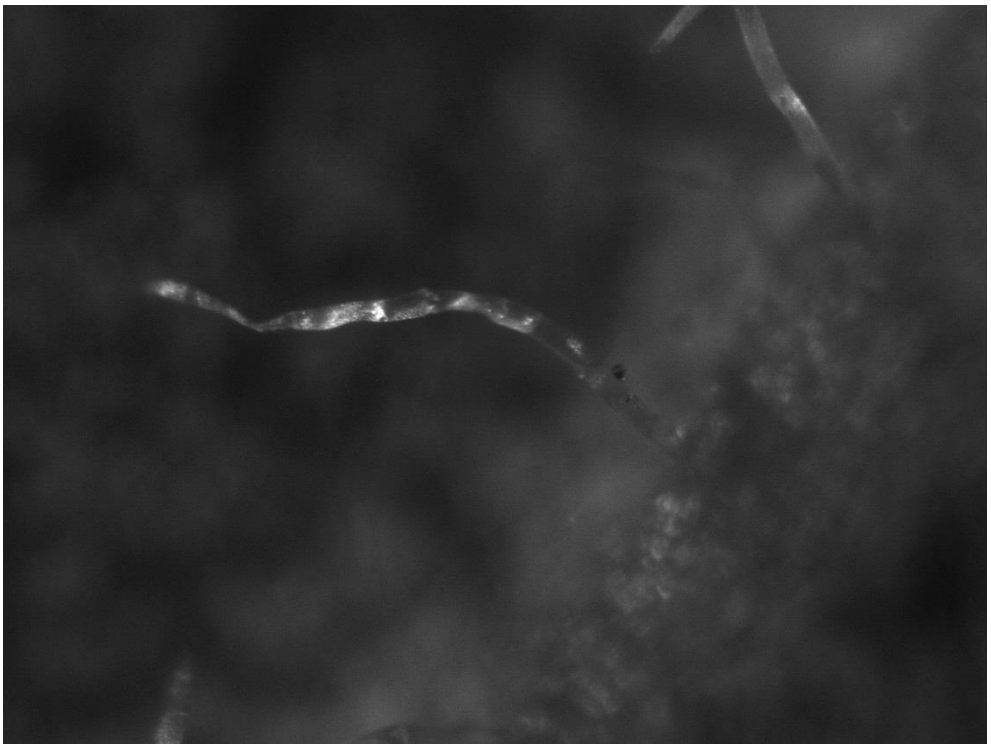


Figure 76 Trichomes on sample no. 115, *F.sachalinensis*.

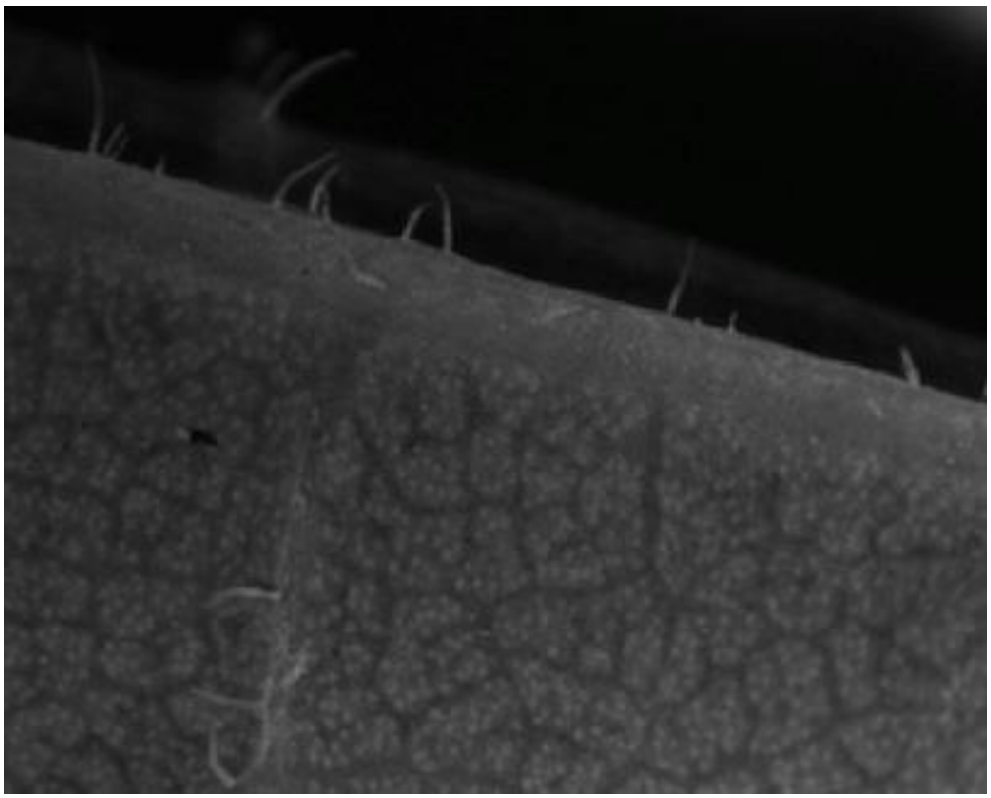


Figure 77 Trichomes on sample no. 115, *F. sachalinensis*.

Figure 78 Male-sterile flowers of *F. japonica* (from sample no. 87).

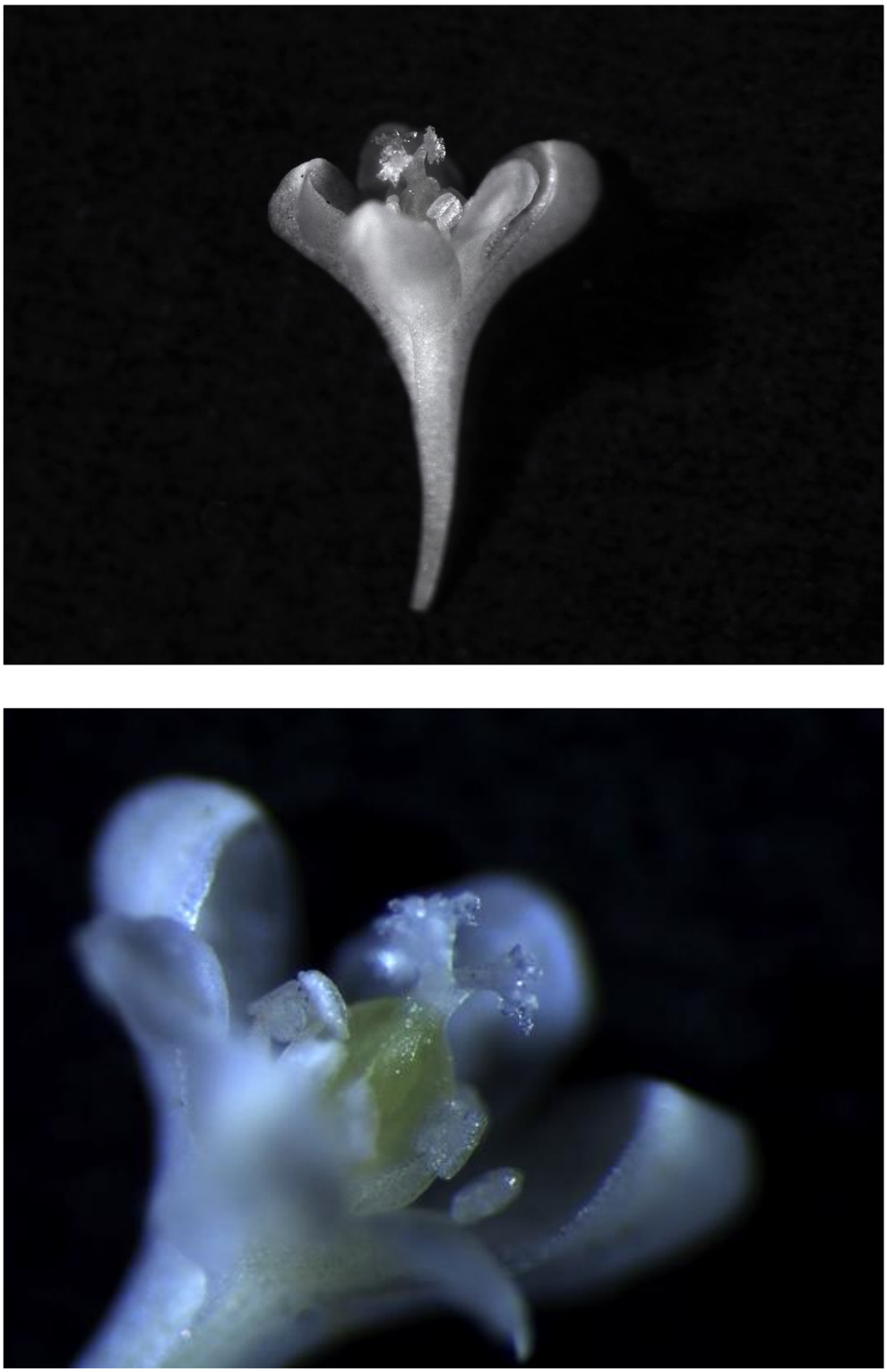


Figure 79 Male-sterile flower of *F. sachalinensis* (from sample no. 115).

Figure 80 Hermaphrodite flower of *F.* x *bohemica* (sample no. 111, upper picture), and variable hermaphrodite flowers of *F.* x *bohemica* (sample no. 113, lower picture).

Figure 81 Morphological variation in two flowers on the same panicle of *F.* x *bohemica* (sample no. 119). Pink colour in the upper picture is due to the colour of the paper underneath the flower.

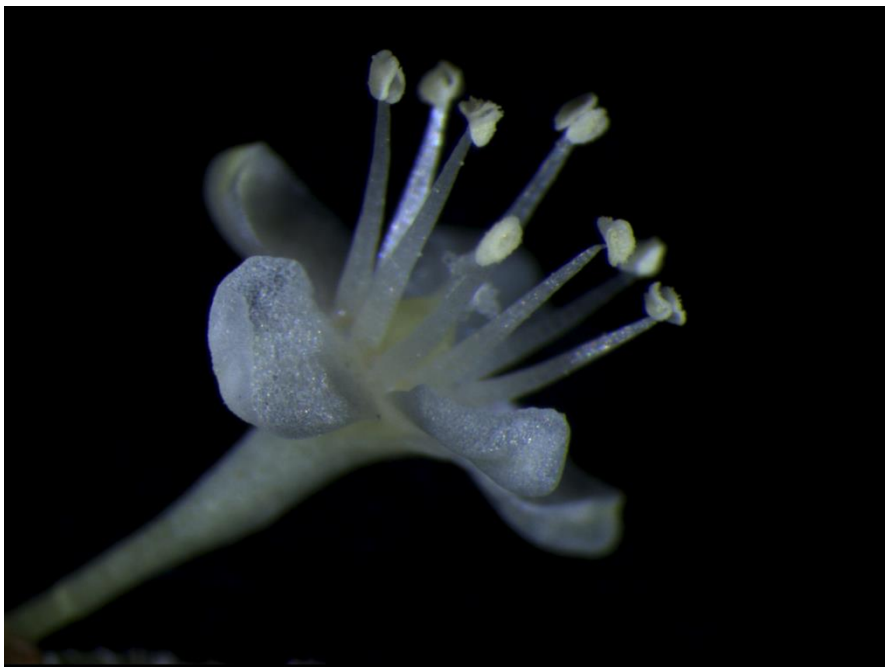
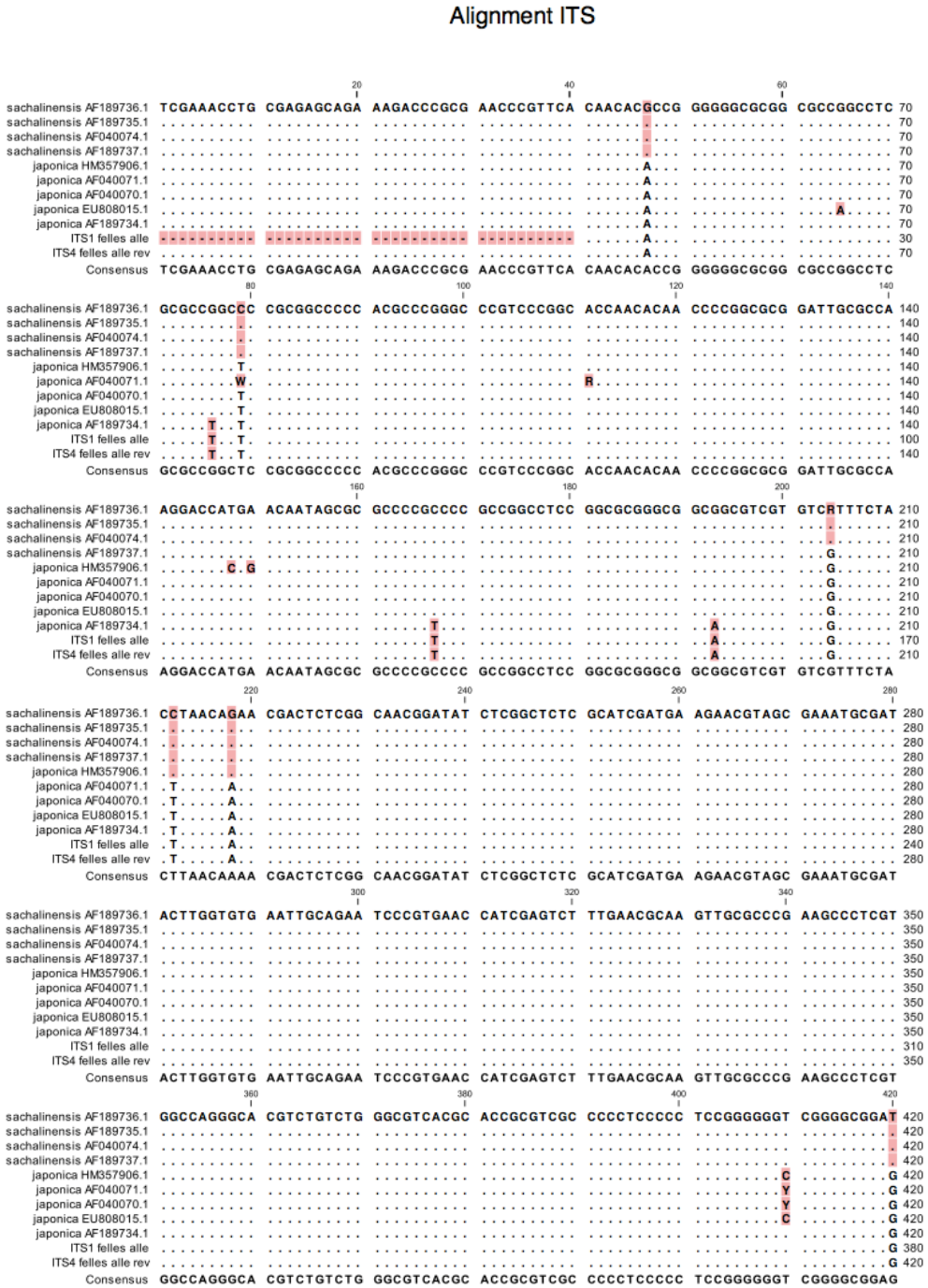
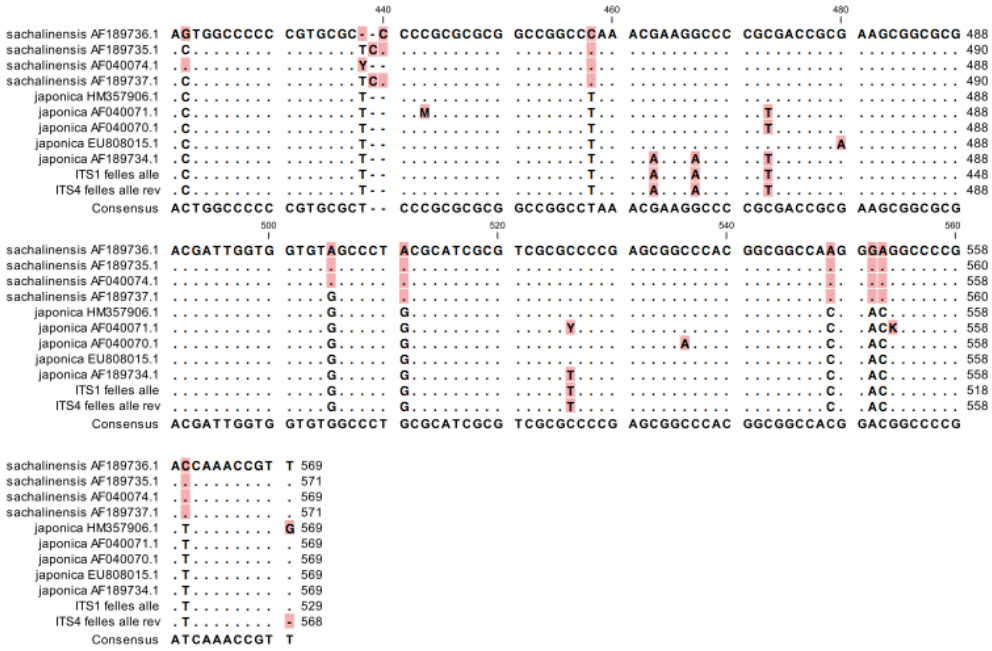


Figure 82 *F. x bohemica* flowers with long filaments and anthers with pollen. Upper picture taken in October 5, 2012 on full-grown stand. Lower picture is from September 12, 2014, from a small shoot. The stand seemed to have been treated with herbicides (sample no. 26).

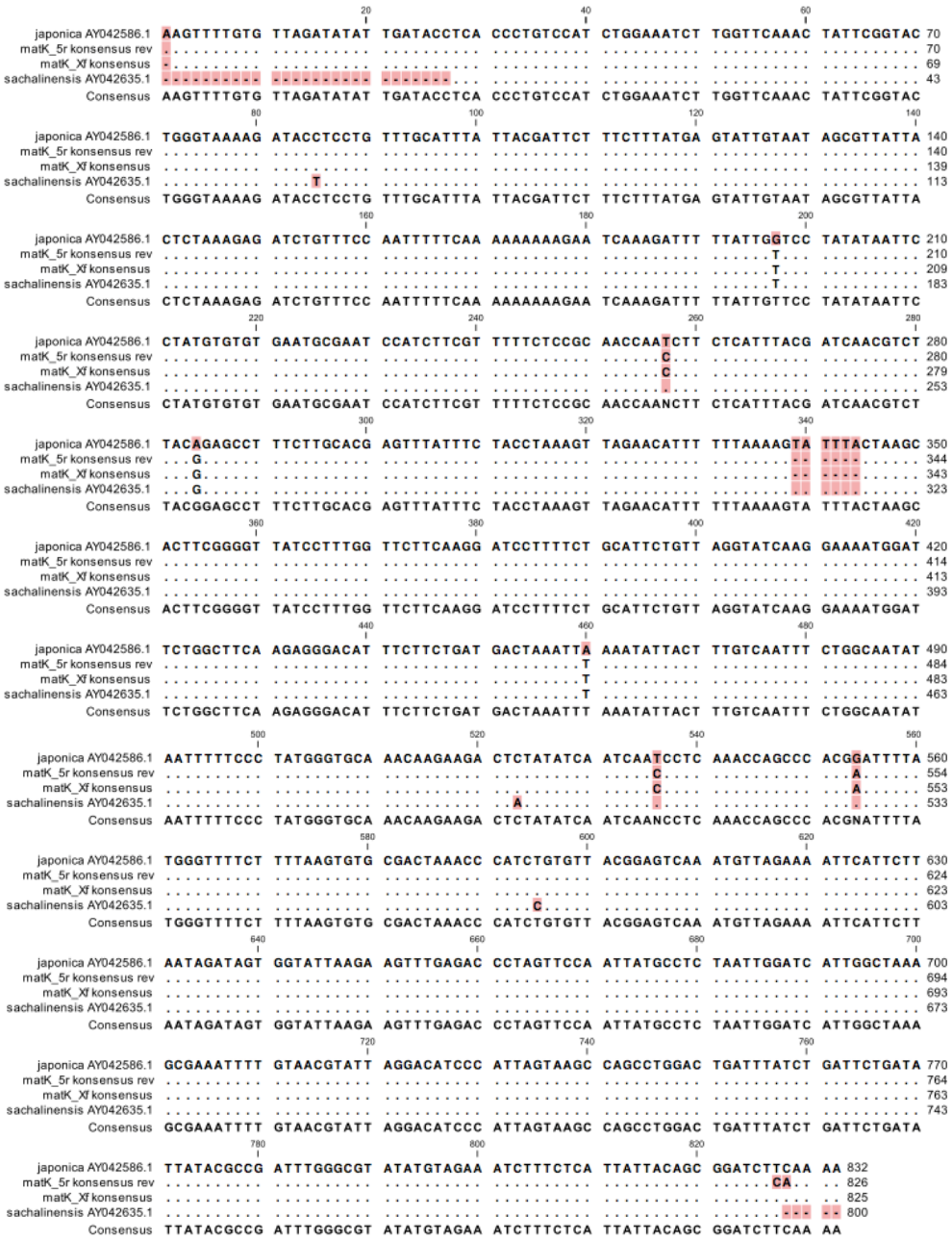
Appendix 4. Alignment of ITS and *matK* sequences.

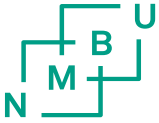


Alignment ITS



Alignment matK





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