

**Post-release assessment of *Aphalara itadori* (Hemiptera:  
Psyllidae) as a classical biological control agent of *Fallopia  
japonica* (Polygonaceae)**

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**A thesis submitted for the degree of  
Doctor of Philosophy of Imperial College London**

**June 2014**

## Declaration

Except for the parts listed below, all work in this thesis is entirely my own and all references to other works are fully acknowledged.

### Chapter 5

- Emily Aldridge collected the data for the rainfall and plant quality experiments as part of her MSc thesis at Imperial College London. These works were carried out under my supervision and all analyses were my own.
- Jennifer Hodgetts (Fera) carried out the molecular assays to detect *A. itadori* DNA using extractions I personally prepared and sent.

### Chapter 6

- Sarah Pierce (Imperial College London) carried out the nitrogen and phosphorous content assessment for the activated carbon samples.

Signed

Gary Clewley

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## Abstract

*Fallopia japonica* is a damaging invasive weed that is difficult to control in the UK. In 2010, *Aphalara itadori* (Hemiptera), an insect herbivore native to Japan, was released in the UK as a biocontrol agent. Classical biocontrol of weeds has a long history across the world but this is an unprecedented type of release for the European Union. This provided the opportunity to investigate the conditions for establishment and the potential impacts of *A. itadori* under semi-natural conditions awaiting the potential establishment of large field populations in the UK.

A meta-analysis, using global data, was carried out to see if classical biocontrol is an effective method of weed management. Field cage trials were then used to assess some life-history traits of *A. itadori*, including fecundity and longevity. Factors influencing establishment, including rainfall, competition and the role of generalist predators were investigated. The ability to cause damage to *F. japonica* is essential for a successful programme and this was tested, both directly and indirectly considering the performance of native plant species grown with *F. japonica* with and without biocontrol. Finally, the integration of biocontrol with existing control methods and preliminarily with another biocontrol agent, *Mycosphaerella polygoni-cuspidati*, a leaf-spot pathogen was investigated.

*Aphalara itadori* showed the potential to be an effective biocontrol agent with the capacity to successfully reproduce outside, with potentially two generations per year in some areas of the UK. There were demonstrable impacts of *A. itadori* herbivory on *F. japonica* within a single growing season. Nonetheless, there was a discrepancy between the predicted performance from this study and what has been observed at the open field release sites. This is possibly a result of the effect of predation on *A. itadori* survival so recommendations, such as the use of protective cages, are suggested to inform the release strategy.

## **Acknowledgements**

Thanks go to my supervisors Prof Denis Wright and Dr Richard Shaw for their guidance and support throughout my PhD.

I would like to thank CABI for hosting much of the research from this thesis and in particular all the staff that have assisted in any way, including Alex Brooks, Kate Jones, Suzy Wood , Kate Pollard, Marion Seier, Ghislaine Cortat and Tony Cross. I would especially like to thank René Eschen for his invaluable advice on all aspects of this work.

Thanks go to the consortium of funders that have supported the biological control programme of Japanese knotweed. These are: CABI, The Department for Environment, Food and Rural Affairs, The Environment Agency, The Welsh Assembly Government, The South West Regional Development Agency, Network Rail, Cornwall Council and The Canals and Rivers Trust.

This thesis was carried out on a studentship funded by the Biotechnology and Biological Sciences Research Council.

Finally, thanks must also go to Prof Julia Koricheva (Royal Holloway University), Prof Simon Leather (Harper Adams University), Dr Nigel Straw (Forest Research), Sarah Pierce (Imperial College London), Emily Aldridge (Imperial College London), Helen Hipperson (Imperial College London), Louise Greenwood (Hart District Council) and Jennifer Hodgetts (FERA) for their advice or support.

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# Chapter 1 - Introduction

## 1.1 Invasion ecology

The redistribution of species around the globe through human activities, intentional or otherwise, is a major concern. Species that are introduced to a non-native range can become invasive if they are able to establish and spread. Biological invasions are currently occurring on an unprecedented scale (Ricciardi 2007) and can often have considerable negative impacts. Mostly the detrimental impacts on their exotic environment can include the alteration of biotic interactions (Carvalho et al. 2010) or ecosystem function (Ehrenfeld 2003), displacement of native species (Vila et al. 2011), loss of genetic diversity through hybridisation (Lei et al. 2010), direct physical damage (Williams et al. 2010) or public health concerns (Derraik 2007). Invasive species have been considered the second largest threat to biodiversity after habitat loss (Vitousek et al. 1997). However, there can also be social impacts, for example, the vigorous growth of *Arundo donax* L. across wetland areas of the southern states of the USA makes policing the country borders more difficult due to reduced visibility (Seawright 2009). Many of the detrimental impacts are attributable to the regular observation that plants can grow larger and become stronger competitors in a non-native range (Hinz & Schwarzlaender 2004). The spread of non-native species into new geographic areas presents a huge challenge to the management and conservation of global natural resources. The economic costs of invasive non-native species are considerable with an estimated loss of US\$1.4 trillion to the global economy annually (Pimentel et al. 2001) and this does not include the cost of less tangible detrimental impacts on the environment and society. A recent estimate puts the economic cost to Great Britain of £1.7 billion annually (Williams et al. 2010).

Not all non-native species have a negative impact in new areas and some may even have benefits (Ewel & Putz 2004). Global agriculture relies on the success of non-native species, such as corn (*Zea mays* L.) or rice (*Oryza sativa* L.) and many other non-native species that establish have limited negative impacts. With the increasing scale of international commerce and the intentional import of non-native species e.g. the demand for ornamental plant species, many species are able to establish, spread, and have negative impacts. It is estimated that Great Britain has the highest

number of naturalised non-native species in Europe at 3254 with 1834 being terrestrial plants (DAISIE 2013). Plant invaders are among the most numerous and prominent in invasion ecology (Callaway & Maron 2006), partly due to long-term viability and difficulty in the detection of propagules. Williamson (1996) suggested the idea of the 'tens rule', that c.10% of introduced species will establish. Of these c.10% will spread and 10% of those will cause damage. Although this clear cut value is contested (Jeschke & Strayer 2005; Jaric & Cvijanovic 2012) it does illustrate the process of filtering that species pass through outside of their native range. Only a small proportion of potential invaders will succeed due to a range of factors including adaptation, climate, founder group size, available resources and competitors. The probability of a particular species invading a new area (or a particular area being invaded) varies from place to place, among species and over time.

With any invasion where there are unwanted impacts, it is important to consider what is causing the undesired effect. A non-native species, invading an area may be actively displacing native species and driving ecosystem change, or alternatively, the presence of the non-native species may be a response to another underlying driver that facilitates the invasion by improving the suitability of the area. This is outlined by the 'driver vs. passenger' models (MacDougall & Turkington 2005). This will greatly affect any management or mitigation decisions as the removal of a 'passenger' species would not change the other underlying factors so there would be no recovery. Whereas, if the removed species was a 'driver', then there is a better chance that management efforts would be beneficial, although some impacts of invasive species can continue beyond their removal, for example, persistent changes to soil chemistry (Ehrenfeld 2003). The management required to see any benefits would then depend on what the threshold is for negative impacts to occur, some invasive species can have a large impact even at low population densities (Thomas & Reid 2007). MacDougall & Turkington (2005) provide support for the 'passenger' model with their work on invasive grasses in an oak savannah. The traits of the dominant invasive species were better suited to a frequently disturbed system and were able to become abundant relative to the native community. Competition alone from the invasive species wasn't structuring the community and 36 out of 79 species studies did not respond to the removal of invasive species. This will vary between

systems and it has been shown that invasive species can directly drive the loss of biodiversity (Hulme & Bremner 2006).

Understanding the mechanisms of invasion and why some species become successful invaders and others don't, has long been a challenge in invasion ecology. Some authors focussed on the traits of the invaders themselves (Duncan & Williams 2002; Sutherland 2004) to predict which species may become invasive. Some traits, such as high growth rate (van Kleunen et al. 2010) or reproductive output (Richardson & Pysek 2006), are more often associated with invasive species. Considering traits alone inevitably lacks enough complexity to be useful for prediction as the introduction of a species with a high potential to become invasive will still be influenced by a wide range of external factors, such as climate or disturbance. Other approaches look at the invasibility of different areas or the variation in biotic resistance (Levine et al. 2004).

There is a whole suite of mechanisms posited to explain invasions. These include the Novel Weapons hypothesis (Callaway & Ridenour 2004) whereby native species in the recipient area are not adapted to the chemical exudates of invading species so are at a competitive disadvantage. Alternatively, the Evolution of Increased Competitive Ability hypothesis (Blossey & Notzgold 1995; Joshi & Vrieling 2005) states that invasive species will be able to reallocate resources away from actions such as defence and into adapting competitive ability in the introduced range. It is seemingly clear that no one mechanism can explain a wide range of invasions, plus many mechanisms are not mutually exclusive and often related (Blumenthal 2005). A more holistic approach is now being considered to explain invasion success incorporating multiple mechanisms (Catford et al. 2009; Gurevitch et al. 2011).

One popular mechanism in the plant invasion literature is the Enemy Release Hypothesis (ERH), which suggests that the increased performance of invasive species is a result of the absence of specialist natural enemies in the introduced range (Keane & Crawley 2002). For this to be true, some assumptions need to be met. Firstly, natural enemies must be able to significantly regulate plant populations, generalist natural enemies would exert a greater impact on native species than invasive species and plants would need to respond to the release by increased

investment in growth or reproduction. Across multiple systems there is evidence both for and against the role of ERH in plant invasions (Colautti et al. 2004).

Evans (2008) takes the idea of enemy release further by considering the role of fungal endophytes, which form asymptomatic associations with plants. The range of these associations is from harmful pathogen to co-evolved beneficial symbiont, which may confer defence against pests and disease. Invasive plants that maintain their beneficial endophytes or acquire new associations in the introduced range could gain an advantage over competitors and also remain resistant to natural enemies. Those species arriving without their endophytes can also gain an advantage through reallocation of resources otherwise consumed but would be more susceptible to attack if the association with natural enemies were re-established. Evans (2008) suggested that the associations with endophytes in the introduced range may explain some of the variation seen in the success of classical biological control (biocontrol).

## **1.2 Classical biological control of weeds**

Once an invasive weed is widely established in a new region, eradication is often prohibitively expensive and unfeasible to carry out. Conventional management practise relies on the frequent application of chemical herbicides and/or physical removal. This approach can often have detrimental environmental impacts, including non-target damage and unintended dispersal of weed propagules. Ongoing management also requires a sustained economic investment, otherwise, problem species can re-emerge or spread in the absence of control efforts. Biocontrol is an alternative to physical or chemical control, often advocated as a preferred option, or can form part of a larger integrated pest management (IPM) programme.

An accepted definition of biocontrol is “the actions of parasites, predators, and pathogens in maintaining another organism’s density at a lower average than would occur in their absence” (DeBach 1964). There are three main approaches;

- Classical – The importation and release of coevolved natural enemies as biocontrol agents for the permanent suppression, not eradication, of a target species with the expectation control will be self-perpetuating and the

biocontrol agents, which may be invertebrates, pathogens or microorganisms will become naturalised.

- Inundative – Regular releases that temporarily increase the population size of biocontrol agents. This can refer to the use of mycoherbicides and invertebrate or vertebrate herbivores.
- Conservation – The protection or manipulation of existing populations of biocontrol agents (native or non-native) to promote an additional control pressure. This is generally not used for weed control.

Since invasive weeds often cover large areas, the introduction of specialist natural enemies from their native range using the classical approach is the most commonly used and there is a long history, dating back to the 19<sup>th</sup> Century, with many introductions worldwide (Julien & Griffiths 1998; MacFadyen 1998). If successful, there are multiple benefits to using classical biocontrol. If established, a classical biocontrol agent population would be self-sustaining and self-dispersing and the chemical input is reduced which is economically and environmentally beneficial.

The use of classical biocontrol does make the assumption that damage from coevolved natural enemies of the weed has the potential to regulate the population density of the target weed. The ability of biocontrol agents to control target weeds does vary depending on the target but there are many examples of classical biocontrol being used successfully against invasive weeds. One of the earliest and most cited examples of successful weed biocontrol is the control of *Opuntia stricta* Haw., a cactus native to the Americas, in Australia from the late 1920's. A moth, *Cactoblastis cactorum* (Berg), was released and effectively cleared millions of hectares of infested land (Dodd 1959). Though, the resources invested in the control programme were atypically high with over 200 workers producing billions of *C. cactorum* eggs for release, contrasting with more recent programmes with releases typically in the thousands (Raghu & Walton 2007). Currently well over 1000 releases have been made worldwide against over 133 target weeds (Julien & Griffiths 1998), many with considerable success (Fowler et al. 2000). However, classical weed biocontrol remains rare in Europe (Sheppard et al. 2006). Although there are cases of classical biocontrol agents present in Europe that have been accidentally introduced. For example, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae),

a biocontrol agent of the North American *Ambrosia artemisiifolia* L. (Asteraceae) in China, has been found in over 130 sites in Switzerland and Italy since 2013 (Müller-Schärer et al. 2014). Damage to *A. artemisiifolia* is up to 100% with complete defoliation at some sites but it is unclear whether the arrival of *O. communis* will have significant impacts on non-target plants as it is possible for *O. communis* to complete development on sunflower, *Helianthus annuus* L., though this is not a preferred host under choice conditions (Zhou et al. 2011)

The processes of carrying out a weed biocontrol programme have been well reviewed (MacFadyen 1998) and outlined (e.g. De Clerck-Floate et al. 2006). There are extensive pre-release studies to ensure the suitability of the target and the host specificity of the candidate agents (Briese 2005). These include the identification of the native range of the target weed as accurately as possible and carry out surveys to collect natural enemies. Typically, specificity to the target is the principle requirement when prioritising candidate biocontrol agents (rather than potential to cause damage). Specificity is determined through various laboratory studies including feeding trials under choice and no choice conditions, oviposition trials and development trials. Test plant lists constructed for host range studies largely use the centrifugal phylogenetic method of assessment (Wapshere 1974; Schaffner 2001) under the assumption that the more closely a plant is related to the target weed, the more likely it is at risk of attack. At which level non-target plant use is acceptable, depends on the particular programme, for example, it may still be suitable to release a biocontrol which oviposited on a non-target species under no choice conditions if the eggs couldn't develop. Although pre-release studies are critical to ensure a greater chance of success and reduce the risks of non-target impacts, relatively little work is carried out post-release (Blossey & Skinner 2000).

Success rates for weed biocontrol inherently vary, for example because many biocontrol agents simply fail to establish (Julien and Griffiths 1998) or a lack of desired impacts on the target or to the benefit of the recipient environment. It has been suggested that success rates may have changed over time with higher establishment rates pre-1980 (Crawley 1990), but it is not clear whether this is the result of a shift in release strategies or that target species more suited to control from biological agents were selected earlier. Moreover, the reporting of success rates is

inconsistent as it varies from system to system and depends on the specific aims of a programme (van Klinken & Raghu 2006). Success is not simply the reduction in the density of the target weed, but may also include a decline in the rate a weed is spreading or an economic saving from reduced investment in conventional management that exceeds the amount invested in the biocontrol programme. The outcome of a programme can take several years to become evident and will be influenced by a wide range of factors including climatic mismatch (Byrne et al. 2004), predator interference (Crider et al. 2011) or release strategy (Grevstad 1999).

There have been concerns over the intentional release of non-native organisms for use in biocontrol. Rare cases where there has been non-target damage, for example, feeding on *Cirsium* thistles native to the USA by the introduced *Rhinocyllus conicus* Froel. (Coleoptera: Curculionidae) have been used to illustrate the risks of biocontrol (Simberloff & Stiling 1996; Louda et al. 2003). In addition, many negative public perceptions of biocontrol have been founded on experiences of the outcomes of poorly regulated releases of inappropriate species, for example the introduction of the cane toad, *Rhinella marina* L., into Australia in 1935 which has gone on to have considerable ecological impacts (Shine 2010). Aside from this, genuine concerns over the risk of non-target impacts need to be addressed. Host specificity is largely considered the most important trait of a biocontrol agent to reduce the risk of non-target damage. On the whole, weed biocontrol has a good safety record, Paynter et al. 2004 surveyed 20 biocontrol agents in New Zealand for evidence of non-target attack. Two species demonstrated rare and minor non-target attack which was predicted from pre-release testing, although two more species also demonstrated some non-target attack but it was highlighted that the pre-release studies were inadequate.

Questions on the indirect effects of biocontrol releases are more difficult to answer. Releases of specific biocontrol agents may still have indirect effects on non-target species. Indirect interactions between species can be mediated via shared predators, a form of apparent competition (Holt & Lawton 1994), particularly when a predator has a positive numeric response to a sudden increase in one species, which then alters the top-down pressure on another species. Willis & Memmott (2005) highlight the potential risk of introducing novel species and the consequential

perturbation of food web structure. Using the release of *Mesoclanis polana* Munro (Diptera: Tephritidae) to control *Chrysanthemoides monilifera* ssp. *rotundata* (DC.) T. Norl (Asteraceae) in Australia as an example, they demonstrated that native parasitoids that were able to attack *M. polana* were more abundant than those parasitoids unable to utilise *M. polana* as a host. The unexpected consequences of biocontrol agent releases can also include conflicts of interest between groups. An interesting example is the control of Tamarisk in the USA. Invasions of *Tamarix* sp. have drastically altered riparian areas in the Western USA. A biocontrol programme was carried out and resulted in the release of *Diorhabda* sp. Weise leaf beetles. Dramatic defoliation of trees was recorded over large areas in the first few years. In some areas where native vegetation had been displaced, an endangered bird, *Empidonax traillii* ssp. *extimus* A.R Phillips (Passeriformes: Tyrannidae), was known to nest in tamarisk. There were concerns over the breeding success of *E. traillii* *extimus* and the biocontrol programme received much negative attention and had the release permits withdrawn. Legal actions were started even though an integrated restoration approach, including biocontrol, would have likely improved the chance of survival of this bird species in the longer term (Dudley & Bean 2012). This example demonstrates the potential scale of conflict and that biocontrol practitioners should consider concerns of other parties, regardless of their validity. Conflicts of interest may also commonly arise through a commercial or amenity value to the target weed or the differing values of bordering nations or regions.

### 1.3 Japanese knotweed

*Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene (hereafter referred to as *F. japonica* or Japanese knotweed) is widely considered to be among the worst invasive species in the UK and is included in the IUCN top 100 World's worst invaders (Lowe et al. 2000). Now found throughout the country, *F. japonica* has been present in the UK for over 150 years and has subsequently invaded many disturbed and riparian areas. There is a relatively large amount of information on this species in the literature and this section outlines the ecology and traditional management of *F. japonica*.

## Taxonomy and nomenclature

*Fallopia* is a member of the Polygonaceae, other genera in the family from the UK include *Polygonum* (knotgrasses), *Rumex* (docks and sorrels), *Rheum* (rhubarb), *Fagopyrum* (buckwheats) and *Persicaria* (smartweeds).

*Fallopia japonica* was first described by Houttuyn in 1777 as *Reynoutria japonica*. This work was published in Dutch and subsequently *F. japonica* was independently described by Siebold & Zuccarini in 1846 as *Polygonum cuspidatum* how it became more widely known (Bailey & Conolly 2000). There are a range of synonyms for *F. japonica* (Table 1.1) and *P. cuspidatum* remains widely used in some literature.

There are several invasive *Fallopia* species and hybrids in the UK, though, *F. japonica* var. *japonica* is the most problematic. A dwarf Japanese knotweed, *F. var. compacta* (Houtt.) Bailey, was also introduced but has not become as widespread. Other knotweeds of concern include the giant knotweed, *F. sachalinensis* (F. Schmidt ex Maxim.) Ronse Decraene, and its hybrid with Japanese knotweed, Bohemian knotweed, *F. x bohemica* (Chrtek & Chrtková) Bailey. *Fallopia sachalinensis* can be readily identified from its larger size (4-5m tall) and the leaves have a distinct heart-shaped base. *Fallopia x bohemica* is usually closer to *F. japonica* in height but otherwise the phenotype and chromosome number are an intermediate of the parents (Child & Wade 2000). Russian vine, *F. baldschuanica* (Regal) Holub., and its rare hybrid with Japanese knotweed, *F. x conollyana* Bailey, are also present in the UK. Black bindweed (*F. convolvulus*) and copse bindweed (*F. dumetorum*) are the only native species of *Fallopia* in the UK.

**Table 1.1** Common synonyms of *F. japonica*. Information taken from CABI Invasive Species Compendium (CABI 2014; [www.cabi.org/isc](http://www.cabi.org/isc)) and Bailey & Conolly (2000).

| Synonym                        | Authority                |
|--------------------------------|--------------------------|
| <i>Fallopia japonica</i>       | (Houtt.) Ronse Decraene  |
| <i>Pleuropterus cuspidatus</i> | (Sieb. & Zucc.) Mildenke |
| <i>Pleuropterus zuccarinii</i> | (Small) Small            |
| <i>Polygonum cuspidatum</i>    | Sieb. & Zucc. 1846       |
| <i>Polygonum reynoutria</i>    | Makino 1901              |
| <i>Polygonum seiboldii</i>     | de Vriese 1846           |
| <i>Polygonum zaccharini</i>    | Small                    |
| <i>Reynoutria japonica</i>     | Houtt. 1777              |

## Biology

*Fallopia japonica* is a rhizomatous perennial that produces annual hollow stems reaching an excess of 3m, forming dense stands and can be concentrated into semi-woody crowns. Stems and leaves are often red at first spring emergence before turning green, but some purple flecking can remain on the stems. New growth in spring can be very rapid (Beerling et al. 1994). The unfurled leaves are approximately 10-18cm long and heart-shaped with a flattened base, growing alternate on the stems. At the base of the petioles extra-floral nectaries are often produced. The rhizomes are dark brown, nodal and fleshy with a bright orange interior. The rhizome network can be very extensive spreading up to 7m laterally from the parent plant and to a depth of 2m (Child & Wade 2000). Cream /white flowers are produced in clusters usually around late summer. Aboveground material dies back in autumn leave standing dead canes which become brittle.

There are no male-fertile *F. japonica* plants in the UK and it has not been shown to have any genetic diversity (Beerling et al. 1994; Hollingsworth & Bailey 2000; Bailey et al. 2007). Other invasive *Fallopia* taxa have been shown to have both male and female-fertile plants in the UK which set viable seed and have higher levels of genetic diversity (Hollingsworth & Bailey 2000), as does *F. japonica* in other introduced ranges (Grimsby et al. 2007; Krebs et al. 2010). In the UK, *F. japonica* disperses and reproduces solely through vegetative means. New material can grow from fragments of rhizome and stem (including internode sections), though regeneration from rhizome is more successful with a fragment as little as 0.7g capable of producing a new plant (Child & Wade 2000).

The description above contrasts with *F. japonica* found in its native range of Asia. In Japan, *F. japonica* typically grows to a maximum of 1.5m and is often a pioneer species on volcanic soils (Beerling et al. 1994). Both sexual and asexual reproduction is observed together in Japan (Tanaka 1966; Maruta 1976). Additionally, monocultures of *F. japonica* on Mt. Fuji naturally show a central die-back where shoot density is reduced in the middle of the stand. This forms part of the natural succession and facilitates other species' establishment in the bare patches (Adachi et al. 1996).

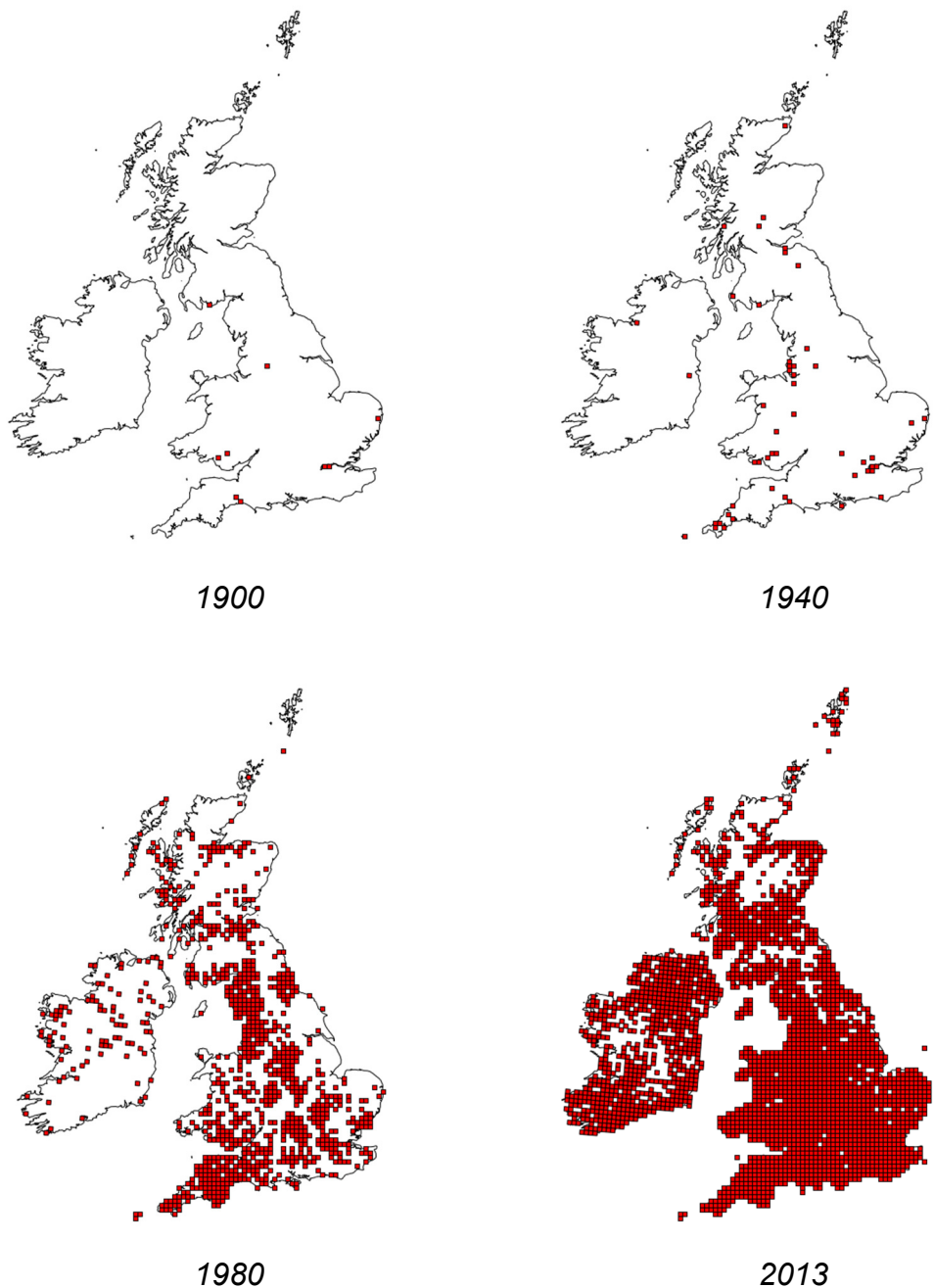
### *Habitat and climate range*

*Fallopia japonica* can be found growing on a large range of habitat types in its introduced range but particularly nutrient rich, riparian habitats and areas of high human disturbance but can also be found growing along woodland edges. The plant can grow on a range of substrates including, clay, alluvial soil, peat, shingle and tolerate a wide pH range from 3.7 to 7.0 (Beerling et al. 1994) and areas of high salt concentrations (Rouified et al. 2012). Generally in the UK *F. japonica* is restricted to lowland areas, rarely found more than 200m ASL, although on the continent it is regularly recorded up to 800m ASL in Switzerland and in its native range the altitudinal limits are around 2400m ASL in Japan and 3800m ASL in Taiwan. In Europe, *F. japonica* seems to prefer areas with an oceanic climate but is nonetheless widespread north-south from Norway to Portugal and into Russia at its eastern limit and can tolerate mean annual temperatures of 5-17 °C and mean annual rainfalls of above 500mm. Its native range includes Japan, Taiwan, Korea and China where it can be found on exposed upland areas and road sides (Beerling et al. 1994).

### *History and spread*

A detailed history of Japanese knotweed in the UK has been published (Bailey & Conolly 2000). The *F. japonica* clone that is now invasive in Europe was first introduced to the UK by Phillipe von Siebold as an ornamental. It was first available commercially in 1848 and was highly prized as a shelter plant with edible young leaves and stems. Japanese knotweed subsequently became very popular and was distributed by a number of nurseries across the country. After a lag phase common to many plant invasions (Crooks 2005), *F. japonica* has rapidly spread throughout the UK (Fig. 1.1). Traits linked with invasiveness often include high reproductive output (Richardson & Pysek 2006). Though, *F. japonica* does not produce any viable seed in the UK, dispersal has primarily been through human movements of contaminated soil and vegetative propagules spread through river systems (Bailey & Conolly 2000). As such, it would have been extremely difficult to predict the spread of *F. japonica* but explains why urban and waste areas are particularly affected. Some legislative measures have been taken to limit the rate of spread and *F. japonica* is listed under Schedule 9 of the 1981 Wildlife and Countryside Act which

makes it illegal to cause it to grow in the wild. There are also official guidance documents produced detailing management options (Environment Agency 2007).



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**Figure 1.1.** The development of the distribution of *F. japonica* in the British Isles over time (NBN Gateway 2013; data providers - [https://data.nbn.org.uk/Taxa/NHMSYS0000458716/Grid\\_Map](https://data.nbn.org.uk/Taxa/NHMSYS0000458716/Grid_Map))

## Impacts

Invasions of *F. japonica* drive ecological change. Native plant communities in direct competition with *F. japonica* are often altered (Aguilera et al. 2010) and the resulting monoculture stands support a depauperate invertebrate fauna (Bimova et al. 2004; Gerber et al. 2008). Although there is a large amount of leaf litter at the end of each season, detritivores didn't perform as well in *F. japonica* stands compared with surrounding vegetation (Gerber et al. 2008). Other evidence suggests that other detritivore groups are not affected and there is a shift towards a detritus-based food web (Kappes et al. 2007). This can have cascade effects on species at higher trophic levels (Maerz et al. 2005). Aside from the direct biotic disruptions, *F. japonica* invasions can alter soil nutrient properties (Dassonville et al. 2007; Aguilera et al. 2010) as well as the soil microbiota (Tanner & Gange 2013).

*Fallopia japonica* is also responsible for causing physical damage to infrastructure accounting for an estimated annual cost of £166m to Great Britain's economy (Williams et al. 2010). New shoots have a rapid growth rate which is able to damage tarmac and paved areas (Child & Wade 2000) and are particularly disruptive to the construction industry. Additionally, where large stands occur in riparian areas, there is an increased risk of flooding in winter as the aboveground vegetation dies back (Child & Wade 2000). More recently, increased public awareness to the impacts of *F. japonica* has affected the property market, reducing house prices where *F. japonica* is present.

## Management

Once established, stands of *F. japonica* are difficult to remove and eradication can take several years at considerable cost (Child & Wade 2000). There is also a growing public perception regarding the difficulty of *F. japonica* control which has received considerable media attention. Conventional methods rely on physical removal, with care not to exacerbate the problem, due to the regenerative capacity of plant fragments (Child & Wade 2000), or chemical control (Environment Agency 2007). Bashtanova et al. (2009) discuss ways of improving chemical control and there are weed barriers than can be effective (Environment Agency 2007) but in the long term, the effectiveness and sustainability of conventional management is unclear (Kabat et al. 2006). It has been estimated in basic terms that a UK wide

eradication of *F. japonica* would cost £1.56bn (Defra 2003). This estimate does not include the indirect costs associated with such a large project or the need for repeat treatments, therefore, eradication is not a viable option.

### *Classical biocontrol of Japanese knotweed*

Research began in 2000 to find a suitable classical biocontrol agent for *F. japonica*. Natural enemy surveys in Japan identified 186 species of arthropod and over 40 species of fungi to be associated (Shaw et al. 2011). After host range testing (Shaw et al. 2009), *Aphalara itadori* Shinji (Hemiptera: Psyllidae) was selected as the first candidate for release (Shaw et al. 2009). A second candidate, a leaf-spot pathogen *Mycosphaerella polygoni-cuspidati* Hara, is also under consideration (see Chapter 7). The licensing and regulation of the biocontrol agent release was complex and required parallel applications to government to allow both the release of an organism from quarantine restrictions and the open release into the environment, which are covered under different legislation. This project carried out a modified European and Mediterranean Plant Protection Organisation Pest Risk Assessment, an application to the Advisory Committee on Releases to the Environment and underwent independent review and public consultation (Shaw et al. 2011). Permission was granted in 2010 for the release of *A. itadori* and psyllids were released at eight sites across England and Wales since. Each release site was paired with a similar control site and a 5-year monitoring plan is being carried out which records the impacts on *F. japonica* and the associated plant and invertebrate communities four times a year.

At the time of writing *A. itadori* had not established self-sustaining populations at any of the release sites. Therefore, questions on the release strategy, efficacy or interactions of *A. itadori* remain unanswered and are difficult or impossible to answer in a quarantine environment and require investigation under more realistic conditions.

## **1.4 Aims and objectives**

The aim of this research was to investigate the potential effectiveness of using *A. itadori* as a classical biocontrol agent for *F. japonica* by considering factors associated with the establishment and impacts of the psyllid. Primarily, field cage

and mesocosm studies were conducted as an interim method in the absence of established field populations.

The specific objectives of this thesis were to:

- 1) Quantitatively review the recent literature on the effectiveness of classical biocontrol of invasive plants and the response of non-target species.
- 2) Investigate generation time and fecundity of *A. itadori* under more natural conditions than pre-release studies.
- 3) Investigate factors that can influence the likelihood of establishment. In particular, the effect of rainfall and plant stress of psyllid reproductive performance and to test the hypothesis that protection from generalist natural enemies will increase psyllid survival.
- 4) Test the hypothesis that *A. itadori* can cause adequate damage to *F. japonica* to reduce plant performance and whether native plants perform better when grown with *F. japonica* under attack from psyllids.
- 5) Investigate the combined impacts of conventional and biological control methods on *F. japonica* to determine any synergistic impacts. To test the hypothesis that two commonly used herbicides may reduce survival of various life stages of *A. itadori* and the effect of herbicide application on *A. itadori* oviposition preference.
- 6) To test the effects of another candidate biocontrol agent for *F. japonica*, the leaf spot pathogen *M. polygoni-cuspidati* on oviposition preference of *A. itadori*.

## Chapter 2 – General materials and methods

### 2.1 Plants

All *F. japonica* plants used were propagated from rhizome fragments collected in Surrey, UK from an established stand with no known history of control. Rhizomes were dug by hand and washed with tap water to remove excess soil which may harbour unwanted arthropods. Fragments were then placed in a plastic tray filled with tap water and covered with either another plastic tray or plastic bag until there was new growth at the nodes. If rhizome fragments were measured prior to planting, dial callipers were used for measuring length and diameter and a digital balance was used for fresh weight.

A controlled environment glasshouse unit maintained at c.25°C at Silwood Park (Ascot, UK) was used for initial propagation. All waste material contaminated with *F. japonica* was disposed of using a licensed waste disposal contractor (Grundon, Wallingford, UK) and taken for incineration.

Unless stated otherwise, the potting media used for experiments detailed in Chapter 4.2.1 and Chapter 6.1.1 was a 2:1 mixture of Westland multipurpose compost (Westland Horticulture Limited, Dungannon) and John Innes no. 2 (John Innes Manufacturers Association, Reading). For all other experiments only Westland multipurpose compost was used. In experiments where potted plants were placed in grow bags, 38L Westland grow bags (Westland Horticulture Limited, Dungannon) were used.

All leaf area measurements (mm<sup>2</sup>) for *F. japonica* were estimated using the following;  $\text{area} = (\text{length} \times \text{width}) \times 0.669 + 44.28$ , where the leaf was measured at the widest part (Dray 2010).

### 2.2 Insects

The *A. itadori* culture was established at CABI from individuals collected in Kumamoto prefecture, Kyushu, Japan in 2004 (Shaw et al. 2009). Psyllids were reared on *F. japonica* plants in 0.4x0.4x0.5m ventilated Perspex cages under a controlled conditions (22±1.5°C, 60-85% RH, 13:11 L:D). Psyllids used in

experiments detailed in Chapter 5.1.2 and Chapter 7 were reared under the same controlled conditions but on plants placed on plastic trays and covered in a mesh sleeve. New cohorts were regularly set up and the plants were watered as required by filling plastic trays lined with capillary matting. Psyllids were transferred from rearing cages to experiments using a pooter and 30ml tubes. Nymphs were transferred using a fine paintbrush.

## 2.3 Study sites

### *CABI garden*

The grounds at CABI (Egham, UK) were used to house all experiments using field cages and some open or sleeved plant experiments. The area used was c.0.15ha of mostly mixed grassland with patches of *Urtica dioica* L. and *Rubus fruticosus* L. and bordered by mixed deciduous trees. The field cages used (unless otherwise mentioned) were 2x2x2m in size with a mesh size of 0.2x0.8mm. Internal frames supporting the cages were constructed from steel tubes. All sleeve material used was nylon with a mesh size of 0.2x0.6mm.

### *Field sites*

The following release sites were used in the predator exclusion experiment in Chapter 5.3.2 (precise locations are not given as the details of release sites are not public knowledge).

- Cornwall – A 1200m<sup>2</sup> stand of *F. japonica* interspersed with *Impatiens glandulifera* Royle located within a 0.5ha paddock with light grazing. Bordered by mature deciduous trees. On the edge of an urban environment.
- Surrey – A 150m<sup>2</sup> stand of *F. japonica* set at the back of a 0.4ha field bordered by mixed deciduous trees in a suburban environment.
- Sussex – A 50m<sup>2</sup> stand of *F. japonica* on the edge of amenity grassland adjacent to a mixed deciduous tree border in a semi-rural area.

- West Glamorgan – A 75m<sup>2</sup> stand of *F. japonica* set amongst parkland and woodland on the edge of an urban area. The stand is immediately surrounded by mixed deciduous trees and large patches of *U. dioica*.
- Norfolk – A 130m<sup>2</sup> stand of *F. japonica* amongst an area of scrubland on the edge of an urban environment.

## 2.4 Statistical analyses

Specific details are outlined in each chapter but following general practise was followed throughout the thesis. Linear models were used on data which conformed to the assumptions of these models, or did so after transformation. Linear regression was used when both the response and explanatory variables were continuous. ANOVA models were used where the explanatory variable was categorical and ANCOVA models with a combination of categorical and continuous explanatory variables. Count data were analysed using generalised linear models (GLM) with a Poisson error distribution. If the model residual deviance was larger than the residual degrees of freedom then this indicates overdispersion. In these cases a quasi-Poisson error distribution was used. Proportion data were analysed as per count data but with a binomial error distribution and a quasi-binomial distribution if the data was overdispersed. If a significant main treatment effect was found for a model with a categorical response variable then differences between means were determined using Tukey's Honestly Significant Difference (HSD) test for linear models and by manual contrasts using the t values for GLMs. If multiple explanatory variables were included in a model then firstly a maximal model was fitted with all interactions followed by a stepwise deletion of non-significant terms until only a minimum adequate model remained (Crawley 2007).

All analyses were carried out using R v. 3.0.2 (R Core Team 2013) and RStudio v. 0.97.551.

## **Chapter 3 – The effectiveness of classical biological control of invasive plants**

*The work presented in this chapter formed the basis of the following paper:*

*Clewley, G. D., Eschen, R., Shaw, R. H., & Wright, D. J. (2012). The effectiveness of classical biological control of invasive plants. Journal of Applied Ecology, 49, 1287-1295.*

### **3.1 Introduction**

One criticism of classical biocontrol has been the lack of quantitative assessment of effectiveness, especially post-release (McEvoy & Coombs 1999). Many classical biocontrol programmes have been concerned with the identification, safety-testing and release of control agents, but less with the control success once the agent was released (McEvoy & Coombs 1999) where often little information is available (Julien & Griffiths 1998). However, without thorough assessment of the effectiveness of control efforts it is difficult to understand why these programmes worked (or not) and how to prioritise potential target species, potential control agents and resources. Detailed monitoring is also required to understand non-target impacts, such as the floral community changes after the creation of free space (Barton et al. 2007). In order to objectively assess the effectiveness of biocontrol, standardised measures of success are needed. However, most studies have used subjective or imprecise descriptions of success, such as classifying the degree of control as 'slight' or 'marked' (Crawley 1989), or focused entirely on impact on the target species (Hoffmann 1995; Fowler et al. 2000).

Ultimately, the measure of success should be whether specific aims, defined at the start of a biocontrol programme, are met (van Klinken & Raghu 2006). Aims and objectives will vary depending on context, such as the impact on the target species and costs and benefits of biocontrol relative to conventional control measures. For example, success in an agricultural setting may depend on the reduction in the cost of conventional control methods or increased crop yields as a result of effective biocontrol of the invasive plant. In a natural environment, by contrast, the invasive

species may not be managed and its economic cost may be unknown. Here, an increase in the abundance of native species after biocontrol of the invasive plant, or the establishment of a community similar to the original community that was displaced by the invasive species may indicate success. If the aims of a control programme are to mitigate or reverse the detrimental environmental impacts of an invasive plant, only monitoring the reduction in abundance of the target species may not be sufficient when the negative impacts include the loss of native species. In this case, the impact on native species and communities and their functioning should also be recorded. However, few control programmes have been quantitatively assessed regarding the community composition and the results of such assessments have rarely been reported. Recently, the importance of studying post-control changes in the plant and invertebrate community have been discussed and components of effective study designs, such as control sites where no biological control agents are released, have been suggested (Blossey & Skinner 2000; Carson et al. 2008; Morin et al. 2009), but no analysis of the combined results has occurred. Furthermore, setting specific goals based on the impacts of the target species prior to release, for example setting an acceptable target density, against which the effectiveness of control can be measured, can be beneficial (Paterson et al. 2011). This will help to distinguish between success and impact since a successful biocontrol project need not necessarily demonstrate changes in all possible measures of impact, for example, if the goal was solely to reduce seed production (Norambuena & Piper 2000).

Meta-analysis provides an excellent tool to combine data from multiple studies to identify broad patterns (Gurevitch & Hedges 1993; Harrison 2011). There have been two previous meta-analyses on the effectiveness of biocontrol agents on invasive plants. Stiling and Cornelissen (2005) considered the impact of weed biocontrol agents as part of a broader analysis of biocontrol. They reported significant declines in plant performance but only impacts on individual plants were considered. However, depending on the target plant biology and ecology, the impact on individual plants may not relate to population scale impacts and therefore the results of such studies may have limited value. Thomas and Reid (2007) addressed this problem in their analysis of biocontrol agents in Australia by including measures of target plant density and indirect non-target plant response as a result of target

removal. Whether or not the non-target species were native or not was not clear. They found significant reductions in plant performance and target density but no firm conclusions could be drawn for the impact on abundance of non-target plants due to a sample size of two studies, highlighting the scarcity of available and detailed post-release monitoring data.

The aim of this chapter was to quantitatively assess the effectiveness of classical biocontrol of invasive plants, not only at the level of individual plants, but also at the level of the plant population and, ultimately, to assess changes in the associated plant community using post release data. In the present study, only results published after 2000 were included to provide a reflection of modern biocontrol practise and to assess the effectiveness of classical biocontrol over relatively short time scales.

## **3.2 Materials and methods**

### **3.2.1 Literature search**

Data were primarily gathered through a literature search using both ISI Web of Knowledge (<http://apps.webofknowledge.com>) and CAB Direct ([www.cabdirect.org](http://www.cabdirect.org)). The search was restricted to publications from the years 2000 to 2011 and to the key words “*weed biological control*” and “*invasive plant biological control*”. The search included sub-searches for “*community impact*” and “*population impact*”. Also, a systematic search through the journals Biological Control (vol. 17–56) and BioControl (vol. 45–56) and through the Proceedings of the XI and XII International Symposia on the Biological Control of Weeds was carried out. Any appropriate studies found in the references of those papers found as above were also included.

Studies were included if they met the following criteria. The biocontrol agent must be non-native to the region where it has been intentionally introduced and the study must be carried out after the release of the biocontrol agent. Pre-release and native range studies which only reflect potential impacts were not included. There had to be an appropriate design with control and treatment groups. Studies with biocontrol agent absence/presence comparisons from control and release sites and pre- and post-release comparisons from the same sites were pooled together only if the nature of response variables measured (magnitude and direction) were not

significantly different from each other using heterogeneity analysis (see below). If significant differences were found then only control and release site comparisons were included in the analysis.

### *3.2.2 Data extraction*

Of the total 151 studies identified, 61 studies were included in the final analyses that covered the period 1992–2008 in which the studies were carried out. These produced 173 independent comparisons which represent 49 different biocontrol agents used against 28 target invasive plants from across the world (Appendix S1 in Supporting Information). Studies were most often rejected due to being carried out in the native range, with unintentionally released biocontrol agents or poor reporting of variance and sample sizes.

If multiple samples were taken over time or various agent densities tested, then only the most recent sample and highest density treatments were used in the analyses. If multiple measurements were taken from the same plants that could be included in the same analysis (e.g. above and belowground biomass), then they were combined to avoid pseudoreplication. Additionally, if multiple studies reported data on a target plant species with the same biocontrol agent, response variable and geographical region they were combined to produce an average effect size for that species.

Classical biocontrol programmes from any country were included and data used was collected predominantly under field conditions (64% of studies: Appendix 1). Field cage and laboratory studies were only included if they demonstrated the efficacy of biocontrol agents already released into the field. These studies were excluded if the effect sizes differed significantly from those under field conditions determined using heterogeneity analysis. The response variables related to the target plants were plant size (height or diameter), plant mass, flower production, seed production (number produced) and target density. Response variables for the associated plant community were the abundance and diversity of non-target plant species. If the data were available, changes in the abundance or diversity of only native non-target plant species were analysed separately.

The means from control and experimental treatments and their respective standard deviations (calculated from the standard error if necessary) and sample sizes were

extracted from Tables and Figures in each study. Data were extracted from graphs using UTHSCSA Image Tool software (University of Texas, USA).

The extracted data were converted to a common effect size, Hedges'  $d$  (Gurevitch & Hedges 1993) for each comparison, which is based on a standardised mean difference between treatments (equation 1). Hedges'  $d$  can be negative or positive indicating the general direction of the effect and when  $d = 0$  there was no difference between treatments.

$$d = \frac{\bar{X}_E - \bar{X}_C}{SD_{Pooled}} J \quad (\text{Eqn. 1})$$

Where  $\bar{X}_E$  is the mean of the experimental treatment (with control agents) and  $\bar{X}_C$  the mean of the control treatment (without control agents). The  $SD_{Pooled}$  indicates the pooled standard deviation of both treatments, which was calculated as per Equation 2. The term  $J$  weights studies based on their sample size (Equation 3).

$$SD_{Pooled} = \sqrt{\frac{(n_E - 1)(SD_E)^2 + (n_C - 1)(SD_C)^2}{n_E + n_C - 2}} \quad (\text{Eqn. 2})$$

Where  $SD_E$  and  $SD_C$  are the standard deviations for the experimental and control groups respectively and  $n_E$  and  $n_C$  are the sample sizes of those groups.

$$J = 1 - \frac{3}{4(n_E + n_C - 2) - 1} \quad (\text{Eqn. 3})$$

Various characteristics of the studies were recorded and used as additional explanatory factors in the analyses. These were the invasive (study) region, the native region (continental scale), growth form (defined using the United States

Department of Agriculture (USDA) growth habit codes: forb, shrub, tree or vine, plus the additional 'aquatic') and longevity (annual, biennial or perennial) of the target weed, the feeding guild (miner or borer, sap-feeder, root-feeder, folivore, gall-former, seed-feeder, flower-feeder, pathogen or a mixture of strategies) and taxonomic group (Order and Family) of the biocontrol agent, and the study duration or period in years between the earliest and latest sampling dates (Appendix 1).

The majority of studies were carried out on plant species invasive in North America (66%), followed by Oceania (18%). Most target plant species were native to Europe (56%) or Central and South America (28%). Arthropods represented the

predominant control agents, used in 93% of studies and 10% using fungal pathogens. Three percent of studies used both arthropods and pathogens. Over half of the studies used coleopteran biocontrol agents (57%), with the Curculionidae (38%) and Chrysomelidae (17%) as the most common families. One third of all studies used folivores (31%), followed by internal aboveground feeders (15%). There has been a strong emphasis to target perennial species (82%) and around half were forbs (54%).

### 3.2.3 Statistical analysis

The change in the annual number of publications available for analysis from 2000–2011 was analysed using linear regression. The influence of the biocontrol agent on the target plants and on the associated plant communities were analysed in separate meta-analyses.

The uniformity of effect size across the studies in the analysis of each response variable was assessed using the  $Q$  test of homogeneity (Gurevitch & Hedges 1993). A significant  $Q_{\text{total}}$  statistic indicates more variation across the effect sizes than expected by sampling error alone, i.e. a significant heterogeneity between the biocontrol agent and the control treatment. This variation can be explained by the magnitude of the effect sizes or the direction, identified by the respective number of studies that showed an increase or decrease in the response.

Analyses were carried out using random-effects models, as the results were from studies that represent a larger population of possible effect sizes of interest. Fixed-effects models are only appropriate for a set of studies with no heterogeneity in effect size and when the inference of results is limited to the observed studies only (Hedges & Vevea 1998). To study the variation between studies, the additional explanatory factors were included as fixed factors in a mixed-effects model. For this the  $Q_{\text{between}}$  statistic was calculated (Gurevitch and Hedges 1993). A sensitivity analysis was carried out to identify any extraneous studies if any significant heterogeneity was found. This approach reiterates the analysis  $n$  times, omitting each study in turn. Excluding studies through the sensitivity analysis did not change any of the observed results, which justified the inclusion of all studies.

Many studies are likely not to be published, especially if they demonstrate non-significant effects or represent failed biocontrol attempts, an issue known as the ‘file-drawer problem’ (Rosenthal 1979). To account for this publication bias, fail-safe numbers were calculated for each analysis. The fail-safe number indicates how many additional, non-significant unpublished studies would have to be included in the analysis to change the result from a significant one to a non-significant one. If the fail-safe number is larger than  $5n + 10$ , where  $n$  is the number of comparisons, then the analysis is considered robust (Rosenberg 2005).

All analyses were carried out using R version 2.12.1 (R Development Core Team 2010) and the ‘metafor’ package (Viechtbauer 2010).

### 3.3 Results

Over the past decade there has been a significant, linear increase in the number of publications reporting quantitative assessments of the impact of biocontrol and which met our criteria for inclusion in the meta-analyses, ( $n = 11$ ,  $R^2 = 0.49$ ,  $P < 0.05$ ; Fig. 3.1). There was significant heterogeneity in the effect sizes within each analysis (Table 3.1), indicating variation in the magnitude, and direction for some analyses, of the responses to biocontrol.

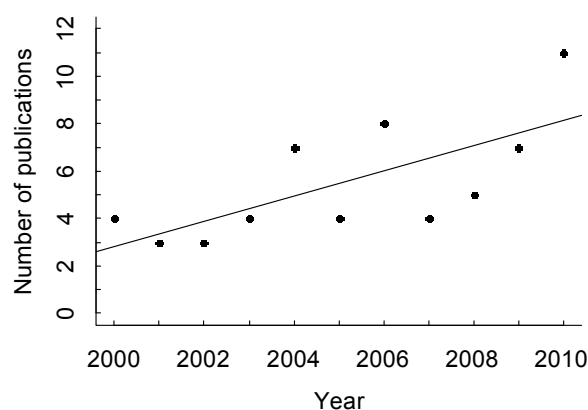


**Figure 3.1.** The annual number of publications over the past eleven years that met the criteria for inclusion in the analysis. The line indicates a significant linear relationship ( $y = 2.82 + 0.53 x$ ,  $P < 0.05$ ).

**Table 3.1.** Heterogeneity ( $Q_{total}$ ) for each variable of observed effect sizes from studies of the impact of biocontrol on invasive plants.  $N_{increase}$  and  $N_{decrease}$  refer to the number of studies with an effect size in a particular direction between the treatment group relative to the control. Native species data was a subset of non-target species.

| Variable                 | $Q_{total}$ | $N_{increase}$ | $N_{decrease}$ | $P$    |
|--------------------------|-------------|----------------|----------------|--------|
| Plant size               | 93.3        | 1              | 31             | <0.001 |
| Plant mass               | 88.3        | 2              | 36             | <0.001 |
| Seed production          | 84.8        | 3              | 23             | <0.001 |
| Flower production        | 104.0       | 4              | 18             | <0.001 |
| Target density           | 169.3       | 2              | 29             | <0.001 |
| Non-target abundance     | 66.1        | 12             | 3              | <0.001 |
| Non-target diversity     | 35.2        | 10             | 0              | <0.001 |
| Native species abundance | 54.9        | 4              | 2              | <0.001 |
| Native species diversity | 5.3         | 4              | 1              | ns     |

Overall, the results revealed the impact of biocontrol on the target species: all measures of individual plant performance decreased significantly (Fig. 3.2). The observed reduction in plant size was  $28 \pm 4\%$  (mean  $\pm$  1 S.E.), plant mass  $37 \pm 4\%$ , flower production  $35 \pm 13\%$  and seed production  $42 \pm 9\%$ . There were also significant reductions in the target plant density with mean declines of  $56 \pm 7\%$ , demonstrating impacts at the population level. All target level analyses were robust, as all the fail-safe numbers were high.

There was an increase in non-target plant species diversity ( $88 \pm 31\%$ ) as a result of the release of classical biocontrol agents (Fig. 3.3). Non-target abundance also increased markedly by  $227 \pm 125\%$ , but the low fail-safe number indicates that this result should be interpreted with care. A subset of the non-target data including only plants native to the study region (using the studies where this information was available) was analysed. There was a significant increase in the diversity of native non target species ( $342 \pm 268\%$ ), but with a high variance and a very low fail-safe number. No significant effect on the abundance of native plant species was found (Fig. 3.3).

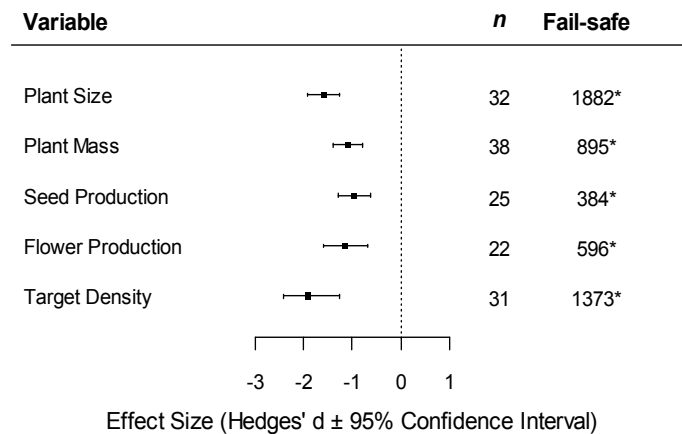


**Figure 3.2.** Mean effect size ( $\pm$  95% Confidence Interval) for the use of biocontrol agents against invasive plants. The response variables are detailed on the left followed by the number of independent comparisons in each analysis (*n*) and fail-safe numbers on the right. The effect is considered significant if the 95% Confidence Interval does not overlap with zero. Analyses are considered robust if the fail-safe numbers are larger than  $5n + 10$ . Significant and robust results are indicated with an asterisk.

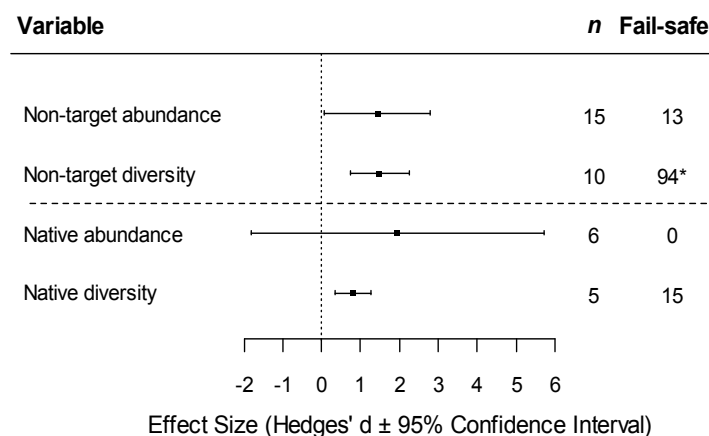


**Figure 3.3.** Mean effect size ( $\pm$  95% Confidence Interval) for the change in non-target plant diversity and abundance following the introduction of biocontrol agents against invasive plants. The number of independent comparisons in each analysis (*n*) and fail-safe numbers are on the right. Fail-safe numbers are considered robust if they are larger than  $5n + 10$ . The effect is considered significant if the 95% Confidence Interval does not overlap with zero. For the analyses of native plant responses a subset of all the non-target data was used. Details on the native status of the non-target plants were not available for all the studies. Significant and robust results are indicated with an asterisk.

Some of the observed heterogeneity within the analyses of the effect of biocontrol was explained by the additional explanatory factors (Table 3.2). Classical biocontrol caused significant declines in the size of invasive plants native to Oceania, Europe or South America ( $-94\%$   $n=1$ ,  $-34 \pm 5\%$   $n=13$  and  $-24 \pm 4\%$   $n=15$  respectively), but not of those native to Asia or Africa (Fig. 3.4). There was also a significant difference in plant size responses as a result of agent families (Fig. 3.5): agents from the Chrysomelidae, Curculionidae, Psyllidae and Tingidae caused significant declines ( $-37 \pm 6\%$ ,  $-24 \pm 4\%$ ,  $-94\%$  and  $-40\%$  respectively. All  $P < 0.05$ , except Curculionidae:  $P = 0.054$ ). There was a significant difference between agent families in their impacts on flower production. An agent from the Agromyzidae caused a significant decrease ( $98\%$ ) in flower number (Hedges'  $d$  (95% CI) =  $-2.96$  ( $-5.24, -0.69$ )  $n = 1$ ). No significant effects of agents belonging to any other family were observed.

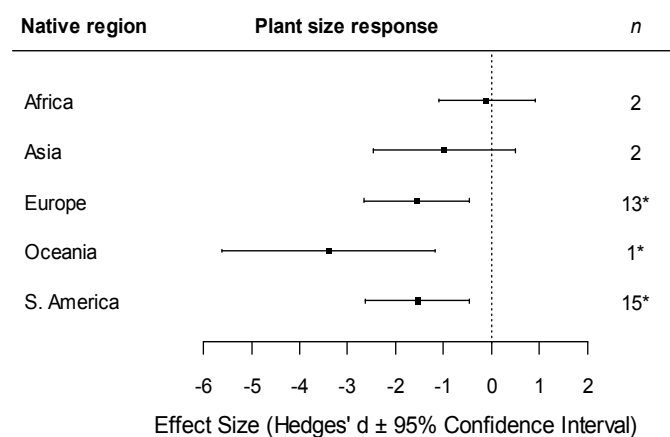


**Figure 3.4.** Mean effect size ( $\pm 95\%$  Confidence Interval) for the change in plant size after the introduction of biocontrol agents against invasive plants. The studies are grouped by the native region of the target plant. The number of independent comparisons in each analysis ( $n$ ) is displayed on the right. The effect is considered significant if the 95% Confidence Interval does not overlap with zero. Significant results are indicated with an asterisk.

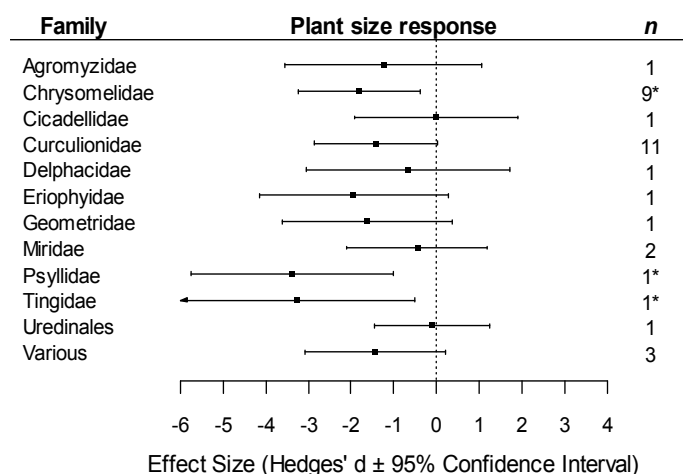


**Figure 3.5.** Mean effect size ( $\pm$  95% Confidence Interval) for the change in plant size following the introduction of biocontrol agents grouped according to taxonomic Family. The number of independent comparisons in each analysis (*n*) is on the right. The effect is considered significant if the 95% Confidence Interval does not overlap with zero. Significant results are indicated with an asterisk.

There were significant effects of study duration on non-target diversity and abundance. The longest studies (seven years) showed increases in non-target diversity, but not the shorter studies (Fig. 3.6). Only one study of intermediate length (five years) showed a significant increase in non-target abundance (Hedges' *d* (95% CI) = 11.64 (7.15,16.14) *n* = 1), but no such effects were observed in longer or shorter studies.

**Table 3.2.** Heterogeneity ( $Q_{\text{between}}$ ) between different study characteristics considered to explain variation in each meta-analysis of the impact of biocontrol of invasive plants. Characteristics are invasive region (continental scale), native region (continental scale), feeding guild or attack mode and taxa of the biocontrol agent and the growth form (defined using the USDA growth habit definitions), longevity (annual, biennial or perennial) of the target plant and the study duration. Significant results are in boldface.

| Variable             | Study characteristic |           |                 |                    |          |                 |                  |      |    |                |          |                  |
|----------------------|----------------------|-----------|-----------------|--------------------|----------|-----------------|------------------|------|----|----------------|----------|------------------|
|                      | Invasive region      |           |                 | Native region      |          |                 | Agent guild      |      |    | Agent order    |          |                  |
|                      | Q                    | d.f.      | P               | Q                  | d.f.     | P               | Q                | d.f. | P  | Q              | d.f.     | P                |
| Plant size           | 1.63                 | 3         | ns              | <b>12.79</b>       | <b>4</b> | <b>&lt;0.05</b> | 8.79             | 7    | ns | 6.17           | 6        | ns               |
| Plant mass           | 5.42                 | 3         | ns              | 4.01               | 4        | ns              | 9.61             | 6    | ns | 7.58           | 9        | ns               |
| Seed production      | 2.44                 | 3         | ns              | 2.83               | 4        | ns              | 8.72             | 8    | ns | 5.02           | 6        | ns               |
| Flower production    | 7.62                 | 3         | ns              | 0.36               | 3        | ns              | 0.88             | 5    | ns | 1.01           | 3        | ns               |
| Target density       | 2.19                 | 2         | ns              | 0.93               | 3        | ns              | 12.33            | 8    | ns | 7.05           | 6        | ns               |
| Non-target abundance | 0.50                 | 1         | ns              | 1.56               | 2        | ns              | 3.30             | 4    | ns | 0.83           | 2        | ns               |
| Non-target diversity | 0.50                 | 1         | ns              | 0.50               | 1        | ns              | 4.75             | 3    | ns | 0.50           | 1        | Ns               |
|                      | Agent family         |           |                 | Target growth form |          |                 | Target longevity |      |    | Study duration |          |                  |
|                      | Q                    | d.f.      | P               | Q                  | d.f.     | P               | Q                | d.f. | P  | Q              | d.f.     | P                |
| Plant size           | <b>27.10</b>         | <b>12</b> | <b>&lt;0.05</b> | 7.75               | 5        | ns              | 0.03             | 1    | ns | 9.34           | 7        | ns               |
| Plant mass           | 6.67                 | 15        | Ns              | 1.41               | 5        | ns              | 5.66             | 2    | ns | 12.19          | 6        | ns               |
| Seed production      | 8.98                 | 9         | Ns              | 3.56               | 4        | ns              | 0.09             | 2    | ns | 2.81           | 4        | ns               |
| Flower production    | <b>24.30</b>         | <b>6</b>  | <b>&lt;0.05</b> | 6.06               | 3        | ns              | 2.17             | 2    | ns | 3.84           | 4        | ns               |
| Target density       | 8.02                 | 9         | ns              | 6.08               | 4        | ns              | 0.80             | 1    | ns | 5.93           | 9        | ns               |
| Non-target abundance | 5.76                 | 3         | ns              | 0.94               | 1        | ns              | 0.41             | 1    | ns | <b>48.28</b>   | <b>7</b> | <b>&lt;0.001</b> |
| Non-target diversity | 4.72                 | 2         | ns              | 0.50               | 1        | ns              | 0.13             | 1    | ns | <b>11.31</b>   | <b>3</b> | <b>&lt;0.05</b>  |

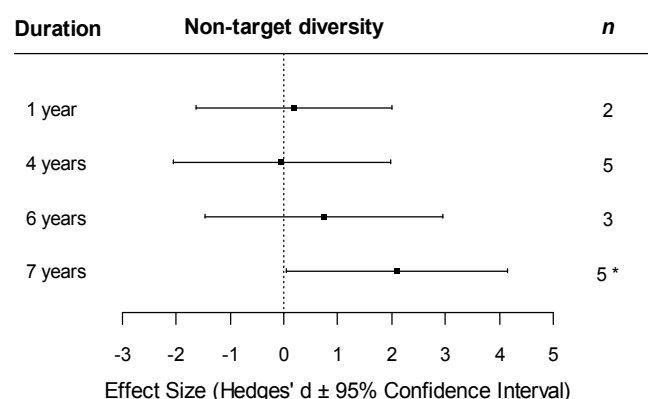


**Figure 3.6.** Mean effect size ( $\pm$  95% Confidence Interval) for the change in non-target plant diversity following the introduction of biocontrol agents against invasive plants. The comparisons are grouped by the duration in years that the studies were carried out. The number of independent comparisons in each analysis (*n*) is on the right. The effect is considered significant if the 95% Confidence Interval does not overlap with zero. Significant results are indicated with an asterisk.

### 3.4 Discussion

This analysis demonstrates the effectiveness of classical biocontrol in invasive plant management. There were significant reductions in all individual plant measures considered, which confirms the results of a previous study (Stiling & Cornelissen 2005). However, the present analysis had more stringent selection criteria and considered broader measures of impact from a larger number and variety of biocontrol programmes. Unlike the previous study, results collected from the native region were excluded here as they do not represent the effectiveness of biocontrol. This analysis also revealed significant declines in target abundance as a result of classical biocontrol, but using data from a larger range of study systems than those included in a previous analysis of biocontrol programmes in Australia (Thomas & Reid 2007).

In 94% of the comparisons that considered the impact on the target plant, the response was a decline in performance or abundance (Table 3.1), which indicates the generality of these results. The overall reduction of performance at the level of individual plants may help mitigate the impact of the target plant, either through reduced growth or seed production. Crawley (1989) stated in a descriptive review of

biocontrol programmes that the Coleoptera were the most widely used and the most successful agents. In the present, quantitative study the Coleoptera were still the most prevalent agents, but there was no clear evidence that they are more effective than agents of other Orders. However, agents of two of the coleopteran families that were considered had a negative impact on plant size and we conclude that Coleoptera are the most effective agents for reducing plant size. The evidence for impact of families in other Orders was not as consistent and robust. For example, in the Hemiptera only significant reductions were found for the Psyllidae and Tingidae due to individual studies. Significant heterogeneity was also found in the effect of agent families on flower production. This was due to the result of a single study, where the leaf miner, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), was released against *Lantana camara* L. in South Africa and the flower number of the target was reduced by 97.5% (Simelane & Phenyne 2005). For all other response variables, only overall impacts of biocontrol were found (Fig. 3.2). Hence, the studies considered in this analysis provide broad and fairly consistent evidence for the effectiveness of classical biocontrol of invasive plants, but the most effective agents at reducing plant size were beetles in the Chrysomelidae and Curculionidae families and we recommend these be prioritised in cases where potential agents of different taxa have been identified.

We calculated the fail-safe N value to assess the robustness of each main response variable (Rosenberg 2005). The analyses of the overall effects of biocontrol on individual plants and non-target plant diversity were robust, but the analyses of characteristics of the studies were less robust. The significant variation between the impacts of different agent families was a result of a small number of studies, studies with large effect sizes, or comparisons of groups with very unbalanced sample sizes and the results of these comparisons should be interpreted with care. For example, there were far more studies to control invasive plants with European or South American than Asian origins and 85% of studies that reported non-target responses were carried out in North America. Similarly, the significant reductions in target plant size by some of the agent families only represent the results of single studies (Agromyzidae, Psyllidae and Tingidae). All the data in the present analyses were from published sources. It is likely that biocontrol programmes that are successful have a better chance of being published (Rosenthal 1979). However, data from more

studies and especially where the target plant was not controlled could provide valuable insights into which factors are associated with effective biocontrol efforts. As such, sources that provide information on both successful and unsuccessful control efforts will continue to be particularly useful (Klein 2011).

A key impact of invasive plants is the loss of local plant diversity (Vilà et al. 2011). The reduction in size of plants of the target species following the release of control agents is likely to reduce the competitive strength of the target species and enable other plants to become established. On a longer timescale, a reduction in seed production may reduce the spread or regeneration of the target species. Prior to this analysis, there has been no quantitative demonstration of the response of the non-target plant community to a broad range of biocontrol efforts. Our analysis revealed that the number of plant species increases following the release of biocontrol agents, but it is unclear whether this was due to an increase in native species, or secondary invasion by other non-native species. The increase in non-target plant abundance is also encouraging, although there were insufficient data for a robust comparison. Many of the studies included were designed before the publication of current post-release monitoring advice (Blossey & Skinner 2000; Carson *et al.* 2008) or may not have been running long enough to for such impacts to occur. The opportunity for large-scale ecological study in biocontrol systems has long been recognised (Crawley 1989) and much of the work is focused on post-release research. It is likely that in the coming years more data, collected over longer periods, will become available regarding the impact of biocontrol which may allow separation of long and short term patterns. Especially long-term patterns could not be evaluated with great certainty in this study as a result of the paucity of long-term studies. However, slower ecosystem responses, such as re-establishment of native vegetation and invertebrates, would be the best measure of biocontrol success.

The number of studies that met our selection criteria with respect to reporting of data increased consistently in recent years (Fig. 3.1). This is probably a result of the increasingly common scientific evaluation of biocontrol programmes, demanded by funding agencies, and of the development of biocontrol as an ecological science through the higher involvement of academic researchers. However, despite recent improvements in the reporting of the effect of biocontrol on the diversity and abundance of non-target species, it was often unclear which species were occupying

the free space created as a result of biocontrol. Only around one third of studies detailed the recovery of native species and in the other studies the identity of the non-target species was either not clear or a combination of native and invasive. If the information was available and only native species were considered, the positive non-target diversity response was still significant, but no longer robust due to the small sample size. The increase in abundance and diversity of non-native non-target species may indicate secondary invasions (Symstad 2004), i.e. that free space created by the removal of one invasive species made possible the establishment of another. This would severely undermine the overall success of a control programme, even one that was 'successful' at removing the target plant, as the original impacts may not be alleviated. Clearly, greater detail regarding the identity of non-target plants is needed in future studies. The control of *Ageratina riparia* (Regel) R. King and H. Robinson in New Zealand (Barton et al. 2007) is a good example, as this study demonstrated a 30% increase in the diversity of non-target vegetation. More importantly, data from paired control and release sites that were monitored over several years were presented and the establishing native and non-native plant species were identified, revealing that secondary invasion did not happen. Nonetheless, classical biocontrol resulted in a reduction in the target plant and increases in diversity in invaded areas, which is likely to impact other trophic levels due to the provision of additional niches (Florens et al. 2010). These impacts occurred in the absence of additional control and restoration efforts, such as seeding. It is possible that the impact of biocontrol on non-target plant species can be enhanced by an integrated approach (Paynter 2003) or by active restoration by land managers (Seabloom et al. 2003). Reid et al. (2009) considered the impact of invasive plant management using a combination of control methods on ecosystem restoration in Australia through a literature review and land manager surveys. The authors identified similar problems with information provided in the scientific literature as the present study: only 20% of the studies they included considered non-target impacts and many reported only qualitative data. However, the land manager surveys provided more detail. In 52% of cases there was an increase in both native and invasive plants and 33% of cases reported increases in native plants only. Although these results are still mostly based on qualitative assessments and confirm the lack of this information in the literature.

In conclusion, the results present here show that classical biocontrol of invasive plants significantly reduces the size and the reproductive output of individual plants and the abundance of target species. Thus, these results illustrate that classical biocontrol of plants is a valuable management tool. They also reveal the enhanced establishment of other, non-target plant species. Many of these impacts were recorded within several years of the initial release of the agents, which is encouraging, but long term monitoring is essential to improve understanding of the effectiveness of classical biocontrol. The outcome of control efforts are not always the same due to the highly variable nature of plant invasions (Shea *et al.* 2010), and reliable predictors of successful programmes are still lacking. However, the results presented here provide indications for selection of the most promising control agents, as some coleopteran agents were more effective at reducing plant size than other taxa. We therefore recommend that Coleoptera be prioritised when selecting potential agents for future biocontrol projects. Studies presenting a lack of control or recovery are still relatively few (e.g. Butler & Wacker 2010). Yet, reporting the outcome of unsuccessful projects, which has long been advocated (Crawley 1989), will be invaluable to inform future control efforts and we recommend that post-release monitoring not be restricted to the biocontrol agent and its target and that they include a range of non-target species (Denslow & D'Antonio 2005; Carson *et al.* 2008). Especially as some key functional groups, such as predators, pollinators and decomposers, may respond quicker to target control than non-target plant species (Fiedler *et al.* 2011).

## Chapter 4 – Life history

*The work on psyllid overwintering presented in this chapter forms the basis of the following paper:*

Clewley, G.D. & Wright, D.J. (2014). Winter hosts of *Aphalara itadori* (Hemiptera: Psyllidae), a classical biological control agent of *Fallopia japonica* (Polygonaceae), in the United Kingdom. *Biocontrol Science and Technology*. DOI: 10.1080/09583157.2014.923378

### 4.1 Introduction

Psyllids (Hemiptera: Psyllidae) are more widely known and studied as plant pests than as beneficial organisms (Sagar & Balikai 2013), as is often the case with herbivorous insects. Worldwide, c.3000 species have been described that occur in a wide range of climatic and vegetation types. There are detailed reviews on the biology and life-histories of psyllids generally (Hodkinson 1974; Hodkinson 2009). At least 77 species have been recorded in the UK including five species in the genus *Aphalara* (Hodkinson & White 1979).

*Aphalara itadori* is a species native to Japan where it can be widely found across the country (Sasaki 1954) and up to 2150m ASL (Shaw et al. 2009). *Aphalara itadori* has a typical psyllid life-cycle as it passes from egg through five nymphal instars to a sexually reproducing adult stage. It is this adult stage that overwinters on shelter plants once the host dies back at the end of autumn. Adults are dark brown in colour with mottled wings and are around 2mm long (Fig. 4.1), see Burckhardt & Lauterer (1997) for further details on the *Aphalara* genus.

As part of the agent selection process, detailed life-cycle and host range data for *A. itadori*, which form the basis of predicting establishment potential (Byrne et al. 2004), were collected under quarantine conditions prior to its release in 2010 (Shaw 2007; Shaw et al. 2009; Grevstad et al. 2013) which are briefly summarised here.

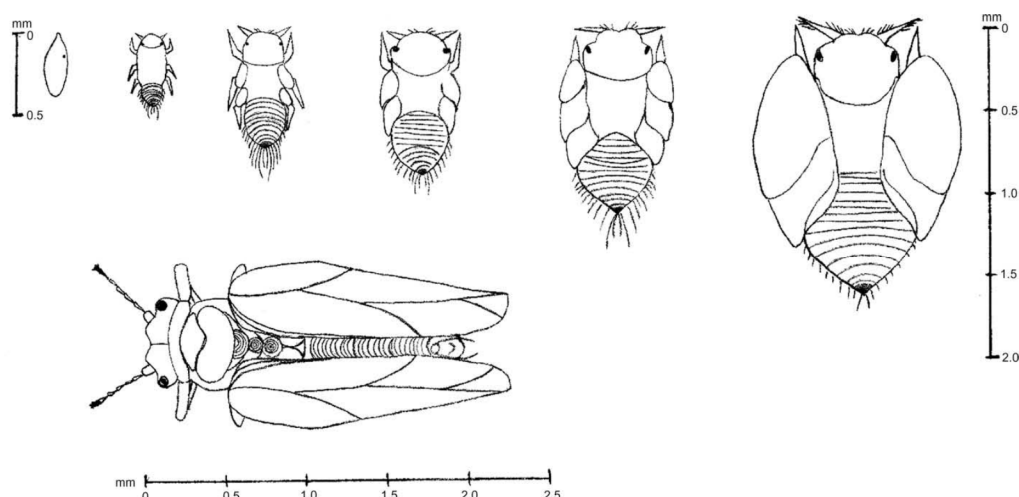


**Figure 4.1.** Diagrams of each life stage (Egg, N1-5 and adult) of *A. itadori*. From Shaw et al. (2009).

*Aphalara itadori* was shown to have a high reproductive potential, from 11 pairs tested, the mean  $\pm$  SE eggs laid per female was  $637 \pm 122$ . Since the nymphs of *A. itadori* do not move between plants that are not touching, adult oviposition preference is a key trait in host selection. Multiple-choice oviposition studies were carried out in which three *F. japonica* plants were placed alongside three plants each of two other species. Thirty adult psyllids were placed on the plants for one week to oviposit. In total 90 plant species were tested this way and the position of 146 000 eggs were recorded. Eight species received eggs that did not belong to the genus *Fallopia*. The egg densities were much lower for this non-target group, ranging from a mean  $\pm$  SE of  $0.1 \pm 0.1$  -  $17.3 \pm 7$  eggs per plant, compared to a mean  $\pm$  SE of  $433.7 \pm 28.2$  eggs per plant for *F. japonica*. Furthermore, none of the nymphs hatched from eggs laid on the non-target group were able to develop beyond the third larval instar. Oviposition was recorded on the native *F. dumetorum* and *F. convolvulus* and the non-native *F. baldshuanica*, both in the presence and absence of *F. japonica*. There was no significant difference in the number of eggs laid on the non-target *Fallopia* whether *F. japonica* was present or not. Nymphs that had hatched on their preferred host were also transferred onto the non-target species that received eggs during the oviposition study. Only one species not in the *Fallopia* genus, *Muehlenbeckia complexa* (A. Cunn.) Meisn., was found to be suitable for development to adulthood but only in four cases out of 60 nymphs. Incidental use of

*M. complexa* as a host is unlikely and considered low risk as it is a non-native species in Europe. Even though adults are mobile and could potentially use a wider range of hosts, adult survival was shown to be considerably reduced when psyllids were restricted to any host which was not *F. japonica*. Based on the evidence it was concluded that *A. itadori* is host specific.

Successful establishment of an insect in a novel area can, among other factors, depend on temperature. There must be periods above a lower temperature threshold for development to occur (van Lenteren et al. 2006) as well as a certain number of accumulated degree days (dd) (Pilkington & Hoddle 2006). *Aphalara itadori* development was recorded under laboratory conditions at various constant temperatures (Table 4.1) for both an imported population in the UK and a wild collected population in Japan (Birkin 2008; Shaw et al. 2009; Myint et al. 2012). Data from Birkin (2008) and Myint et al. (2012) both report that the predicted lower development threshold for *A. itadori* is 5.8°C although development to adult was not observed below 12°C (Table 3.1). The predicted degree day requirements for development from egg to adult from these two studies were 575dd and 607.4dd respectively, both higher than previously predicted 462.5dd (CABI 2007).

**Table 4.1** Mean  $\pm$  SE duration (days) development for different life stages of *A. itadori* at various constant temperatures in °C ( - indicates no development occurred). Values presented from Myint et al. (2012) are for females only. N1-5 refers to nymphal instars.

| Temp. | Egg            | N1             | N2            | N3             | N4                  | N5             | Total          | Reference           |
|-------|----------------|----------------|---------------|----------------|---------------------|----------------|----------------|---------------------|
| 8     | -              | -              | -             | -              | -                   | -              | -              | Birkin (2008)       |
| 10    | 37 $\pm$ 1     | -              | -             | -              | -                   | -              | -              | Birkin (2008)       |
| 12    | 22.0 $\pm$ 0.8 | 13.2 $\pm$ 0.5 | 9.3 $\pm$ 0.3 | 15.0 $\pm$ 1.3 | 14.3 $\pm$ 1.3      | 13.0 $\pm$ 2.7 | 86.8 $\pm$ 3.5 | Birkin (2008)       |
| 15    | 15.8 $\pm$ 0.7 | 6.8 $\pm$ 0.8  | 7.6 $\pm$ 1.5 | 7.8 $\pm$ 0.6  | 7.6 $\pm$ 1.4       | 16.4 $\pm$ 1.5 | 62.0 $\pm$ 4.3 | Birkin (2008)       |
| 15    | 18.6 $\pm$ 0.2 | 8.8 $\pm$ 0.2  | 6.6 $\pm$ 0.2 | 7.6 $\pm$ 0.2  | 9.6 $\pm$ 0.2       | 16.3 $\pm$ 0.3 | 67.5 $\pm$ 0.5 | Myint et al. (2012) |
| 20    | 11.5 $\pm$ 0.1 | 5.5 $\pm$ 0.1  | 4.7 $\pm$ 0.2 | 5.1 $\pm$ 0.1  | 5.9 $\pm$ $\pm$ 0.2 | 10.2 $\pm$ 0.2 | 42.8 $\pm$ 0.3 | Myint et al. (2012) |
| 22    | 9.2 $\pm$ 0.1  | 4.8 $\pm$ 0.2  | 3.3 $\pm$ 0.2 | 3.9 $\pm$ 0.3  | 4.5 $\pm$ 0.1       | 7.1 $\pm$ 0.3  | 32.9 $\pm$ 0.8 | Shaw et al. (2009)  |
| 25    | 7.9 $\pm$ 0.2  | 3.8 $\pm$ 0.1  | 3.7 $\pm$ 0.1 | 3.3 $\pm$ 0.1  | 5.2 $\pm$ 0.1       | 8.8 $\pm$ 0.2  | 32.7 $\pm$ 0.3 | Myint et al. (2012) |
| 30    | 8.1 $\pm$ 0.3  | 8.8 $\pm$ 0.6  | 5.7 $\pm$ 0.5 | 5.1 $\pm$ 0.5  | 5.9 $\pm$ 0.4       | 14.2 $\pm$ 0.9 | 47.8 $\pm$ 1.5 | Myint et al. (2012) |

Insects in temperate regions must be adapted to survive low winter temperatures in order to establish and persist. Like other insect groups (Musolin & Saulich 1999), psyllids have evolved a wide range of physiological mechanisms to improve winter survival (Hodkinson 2009). Generally, these mechanisms allow insects to avoid freezing through the accumulation of compounds such as glycerol and the reduction of water content in their cells. Other mechanisms may allow freeze tolerance by controlling the process to prevent lethal damage (Bale 2002). Poor adaptation to winter conditions in the introduced range may preclude establishment altogether, or reduce the effectiveness of an agent. For example, Diaz et al. (2011) reports that *Gratiana boliviana* (Coleoptera: Chrysomelidae) was not as successful in Northern Florida in controlling Tropical soda apple (*Solanum viarum* Dunal) despite success further south (Medal & Cuda 2010). Poor overwintering survival was considered to be the limiting factor.

For *A. itadori* to overwinter successfully in the UK the climatic conditions must fall within the fundamental tolerances of the species and there must be suitable overwintering sites available. There will be a natural variation in the cold-hardiness of any species depending on the conditions of the native region (Zhou et al. 2010). This can influence the selection of a particular population, not just the species identity, of a classical biocontrol agent to ensure the best match between the native and introduced regions. The *A. itadori* population released in the UK was originally collected from Kyushu, Japan. Climate comparisons between single sites in the native and introduced regions (Myint et al. 2012) do indicate a discrepancy with a wider range of temperatures experienced in the native range. Nonetheless, Myint et al. (2012) reported that temperatures in Southeast England can be above the lower development threshold for *A. itadori* throughout the year and generally the summer months would be suitable for full development.

Different population biotypes may be required if releases are planned over a wider climatic region, as has often been necessary in North America (e.g. Grevstad et al. 2013). Birkin (2008) reports that adult psyllids had a high tolerance for low temperatures, taking 4h for mortality to exceed 50% at -10°C. It was considered unlikely that the lowest extremes of temperature experienced in the UK will be a limiting factor to establishment. As their host plants dieback in late autumn, many psyllids overwinter in temperate regions as adults on shelter plants, often conifers

(Hodkinson 2009). The question whether there is a lack of suitable overwintering sites for *A. itadori* remains unanswered. It is reported that *Pinus densiflora* Sieb. & Zucc. and *Cryptomeria japonica* (Thun. ex L. f.) D. Don are reported to be the principle winter hosts of *A. itadori* in their native range (Baba & Miyatake 1982; Miyatake 1973; Miyatake 2001). Both these species have a sparse, low density distribution in the UK, but it is possible that close relatives, such as Scot's pine, *Pinus sylvestris* L., which is common, may serve as suitable overwintering hosts.

Information from the literature and previous quarantine studies provide a baseline of what can be expected from the insect once released, but there is often a discrepancy between performance between laboratory and field conditions (Koricheva et al. 1998). The aim of this study was to gain a more accurate understanding of development requirements, fecundity and longevity of *A. itadori* under more natural conditions than the quarantine conditions of the previous studies with the population that was released in the UK, to refine expectations of performance at the release sites and to assess overwintering potential on an analogue to its native winter host as well as non-conifer hosts. The hypotheses tested were 1) conditions are suitable outside in Southeast England for *A. itadori* to complete its development; 2) fecundity and longevity differ under caged conditions to that in quarantine; 3) *A. itadori* will be able to overwinter on commonly found shelter plants, such as *P. sylvestris*.

## **4.2 Materials and methods**

See General methods section (Chapter 2) for origins and culturing of *A. itadori* and plant material. All statistical analysis was carried out using R v3.0.2 (R Core Team 2013).

### *4.2.1 Development and thermal requirements*

Rhizomes were collected in March 2011 and planted in 6L pots. Plants were grown in a glasshouse for three weeks before being transferred to 12L pots and placed outside.

Seven field cages (1.5x1.5x2m tall with 0.2x0.8mm mesh) were set up and three similarly sized *F. japonica* plants were placed in each. Potted plants were placed in grow bags and the floor of the cage up to a few inches below the rim of the pots was covered in a layer of sawdust to reduce water loss. Plants were left for one week to

acclimatise and were watered as necessary. A single data logger (DS1921G-F5 thermochron, HomeChip) was placed in each cage and recorded temperature at three hour intervals for the duration of the study. Plants were individually sleeved and either 20, 100 or 200 adult psyllids were introduced to plants at random in each cage. Psyllids were allowed to oviposit for one week and then both the adults and the sleeves were removed. Removing the sleeve removed its effect on the microclimate (i.e. the data loggers recorded the temperature experienced by the psyllids) but in the nymph stage psyllids do not move between plants. The number of eggs on each plant was counted using a stereomicroscope. At weekly intervals, the numbers of nymphs were counted in situ using a 10x magnifying hand lens and the life stages present at each sampling was recorded. Once a fifth instar nymph was identified on a plant it was re-sleeved. Newly emerged adults were collected and removed from the plant to quantify survival to adulthood. Degree days were calculated (Eqn 4.1) as the accumulated average of the daily maximum and minimum temperature ( $T_{\max}$  and  $T_{\min}$ ) above a lower developmental threshold of 10°C ( $T_{\text{base}}$ ).

$$\frac{T_{\max} + T_{\min}}{2} - T_{\text{base}} \quad (\text{Eqn. 4.1})$$

#### 4.2.2 Fecundity and longevity

In order to estimate fecundity under semi-natural conditions, mated pairs were removed from culture on, or just, after the day of emergence prior to any oviposition. Mated pairs were transferred to *F. japonica* plants between 10-20cm tall in 3L pots. Plants were individually sleeved before being placed outside. The number of eggs laid was counted weekly and fresh plants were provided for each pair, if the female was still alive. Oviposition was recorded for 27 pairs of *A. itadori* and plants were kept outside until both adults had died. This took place in Egham, UK, between May and October 2012 and temperature was recorded inside the sleeves using data loggers (DS1921G-F5 thermochron, HomeChip).

### 4.2.3 Overwintering

#### *No choice*

This study was carried out between October 2012 and March 2013 in Egham, UK. Prior to use, adult psyllids were taken from culture and placed on fresh *F. japonica* plants outside in a field cage during September 2012. Any psyllids that began to show a darkening in colour, indicating the transition to an overwintering morph (Hodkinson 2009), were used. Four potential winter hosts were tested; *F. japonica* plants (c. 40cm tall in 3L pots), trunk sections of *Quercus robur* L. and *P. sylvestris* (c. 30cm tall and 20cm diam.) and two year old living *P. sylvestris* saplings (c. 150cm tall in 7.5L pots; watered as necessary). Each host was sleeved to prevent dispersal and 100 psyllids were placed in each sleeve. There were 10 replicates for each potential host housed in field cages for protection. Temperatures inside the sleeves were recorded every three hours using data loggers (DS1921G-F5 thermochron, HomeChip) placed in two sleeves from each treatment.

#### *Choice*

Choice arenas were set up in field cages to assess the overwintering capability of *A. itadori* on two different stages of *P. sylvestris*. 100 adult psyllids were placed on a single *F. japonica* plant in the centre of the cage with a potted pine (7.5L, 24cm diameter pot) between 100-150cm tall and a section of Scots pine trunk (30-40cm tall, 25-30cm diameter) positioned randomly in two opposing corners. There were twelve replicates. The psyllids used were taken from culture and placed outside on large *F. japonica* plants for 4-6 weeks at the start of September 2011 to acclimatise to outdoor conditions. When transferred to the choice cages all adults used exhibited black colouration indicative of an overwintering morph (Hodkinson 2009). The plants were watered if necessary but otherwise the cages were not disturbed until March 2012 when the two pine overwintering sites were sleeved to capture emerging psyllids. The dead stems from the central *F. Japonica* plant and the cage substrate were also placed in mesh bags to check for any emerging psyllids.

## 4.3 Results

### 4.3.1 Development and thermal requirements

The total generation time from egg to adult ranged from 63 – 98 days. There was overlap in the instar stages recorded at each sampling period (Fig. 4.2) with up to four different stages recorded in a single week. A minimum of 439dd was required for complete development from egg to adult and the mean  $\pm$  SE across all plants was  $503 \pm 12$  dd. A minimum of 153dd was required for egg development before the first nymph was observed. The mean temperature in the cages across this development period was 16.2°C.

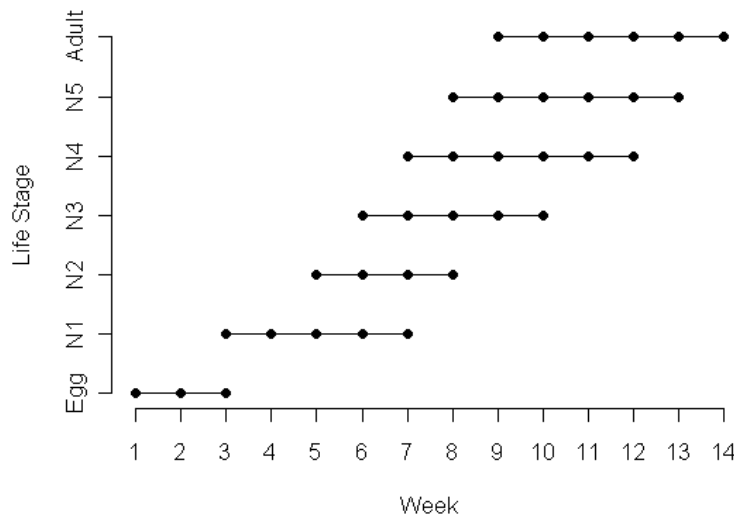


**Figure 4.2.** Range of *A. itadori* life stages present (egg to adult through five nymphal instars) in each week. The experiment began 26<sup>th</sup> April 2011 and a mean  $\pm$  SE of  $503 \pm 12$  degree days above a base of 10°C were accumulated before first adult emergence.

### 4.3.2 Fecundity and longevity

There was considerable variation in the total fecundity and longevity of *A. itadori* (Table 4.2) with a difference of 400 eggs per female between the highest and lowest fecundity recorded. There was no significant difference in longevity between males and females ( $F_{1,52} = 0.49$ ,  $P = 0.49$ , ANOVA). The number of eggs laid in each week

was significantly positively correlated with the mean temperature over the previous 7 days (Fig. 4.3).

**Table 4.2.** Fecundity and longevity of *A. itadori* placed outside between May and October 2012 (n=26). Mated pairs were placed on individually sleeved *F. Japonica* plants. Eggs were counted and plants were replaced weekly.

|              | Eggs (per pair) $\pm$ 1SE |                | Longevity (days) $\pm$ 1SE |                |
|--------------|---------------------------|----------------|----------------------------|----------------|
|              | Total                     | Per week       | Male                       | Female         |
| <b>Mean</b>  | 156.2 $\pm$ 22.2          | 29.6 $\pm$ 2.5 | 34.6 $\pm$ 4.9             | 39.4 $\pm$ 4.6 |
| <b>Range</b> | 18-418                    | 9-60           | 10-111                     | 10-96          |

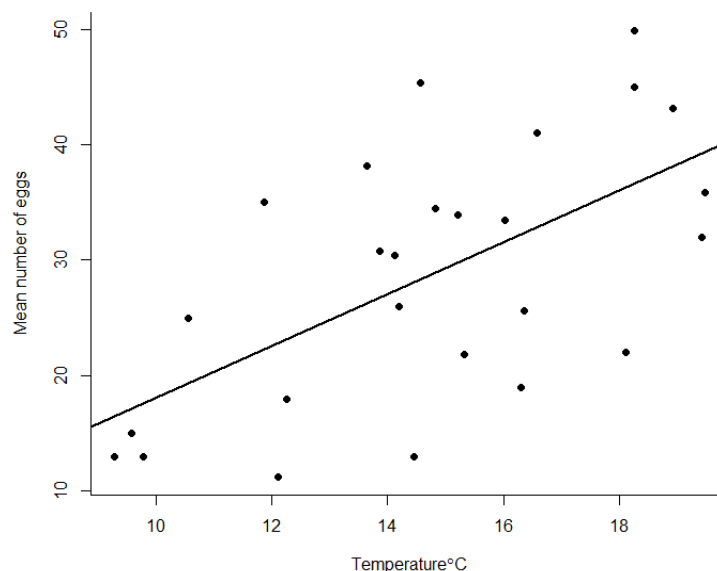


**Figure 4.3.** Relationship between the mean number of eggs laid and the mean temperature over a seven day period. Data was averaged across all *A. itadori* females (N = 26 in total) active during each period.  $y = -4.29 + 2.25x$ ,  $r^2 = 0.34$ ,  $P < 0.01$ ).

#### 4.3.3 Overwintering

##### *No choice*

The number of live adults found in spring (Fig. 4.4) did differ significantly between hosts ( $F_{3,36} = 8.77$ ,  $P < 0.001$ , GLM quasibinomial) with *A. itadori* found to have successfully overwintered on all potential hosts except the *P. sylvestris* saplings. The

temperatures inside the sleeves (Fig. 4.5) were comparable to ambient conditions but the experimental set up would have modified the microclimate to some extent. In this case this was not an issue as the aim of this investigation was not to quantify survival rates or physiological tolerances.

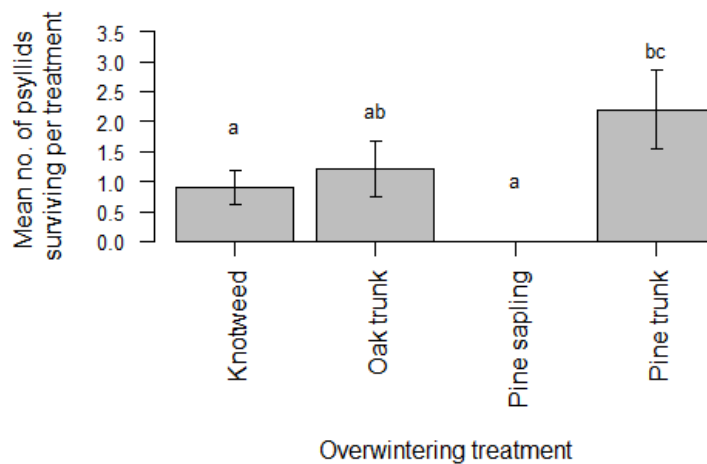


**Figure 4.4.** Mean number of adult psyllids found successfully overwintered in spring (March 2013) on four potential hosts. N=10 for each host and each was placed in a sleeve to prevent psyllids escaping. 100 psyllids were added to each sleeve in Oct 2012. Error bars are  $\pm 1$  SE and bars with different letters above are significantly different from each other.

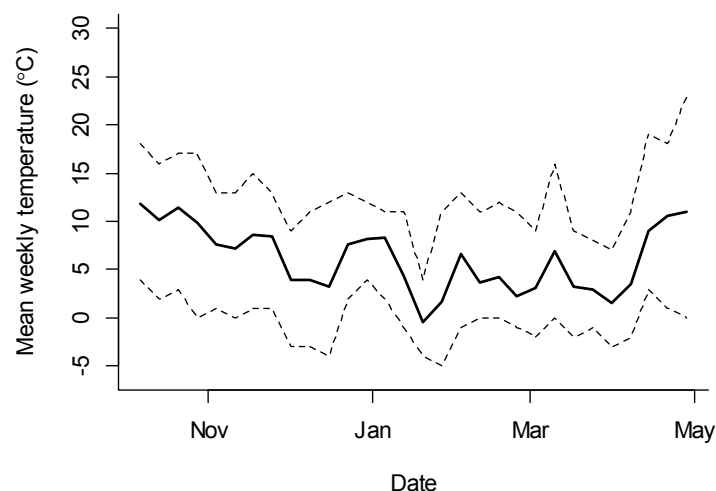


**Figure 4.5.** Mean weekly temperatures recorded inside the overwintering sleeves using eight dataloggers (DS1921G-F5 thermochron, HomeChip). Recordings were taken every 3 hours. Upper and lower dashed lines represent the maximum and minimum temperature recorded in that weekly period.

## Choice

Psyllids were only found in spring on sections of pine logs with a mean  $\pm$  1 S.E. of  $1.58 \pm 0.31$  and zero found on the saplings.

## 4.4 Discussion

Calculating insect development rates can be complicated by non-linear responses to temperature and the difference in response to a constant or fluctuating temperature (Beck 1983; Garcia-Ruiz et al. 2011). In this present study, individual development rates were not recorded, as no individuals were followed but rather a cohort at weekly intervals. Nonetheless, these data serve to corroborate some of the previous laboratory based development work. Birkin (2008) tested the closest temperature to the overall mean recorded during this study at 15°C. The mean duration from egg to adult at this temperature was 62 days (Table 4.1), this is very close to the minimum duration of 63 days recorded from *A. itadori* reared outside. Psyllids reared outside demonstrated a much wider range in development times. The consequence of this is that the offspring of some individuals may be sexually mature with sufficient time in the season to produce another generation whereas as other individuals may not. It is likely that there will be overlapping generations under natural conditions and possibly variation in voltinism.

Development rates will vary temporally and spatially, so accumulated dd is a useful tool for predicting insect development (Pilkington & Hoddle 2006; Mangan & Baars 2013). The results here are closer to the lower predictions of dd requirements (CABI 2007) rather than the higher predictions of Birkin (2008) and Myint et al. (2012). Despite this, all studies agree that thermal requirements should not be a barrier to the development of *A. itadori* in the south of the UK. Though, other areas of the UK, where fewer dd accumulate across the year, would be unlikely to see multiple generations of the psyllid. Additionally, multiple complete generations may not be realised in every year. For example, dd data for recent years (<http://www.degreedays.net/>, accessed May 2014) indicate that only 840dd above a base of 10°C were accumulated between March and September 2012 in Ascot, southeast England. Since the minimum expected is 880dd for a complete second

generation only a partial second generation may have developed in that year. Although this would inevitably affect population growth, the impacts on *F. japonica* would not necessarily be considerably affected as it is the nymphs which cause damage to the plant and immature psyllids would still be present even if unable to complete development.

Other traits are also likely to be effected by fluctuating temperatures. The psyllids in this study were living approximately 25 fewer days than those at a constant temperature with a comparable mean from the work by Myint et al. (2012), though caution should be taken when comparing these groups as different populations of *A. itadori* were used. Female fecundity was also lower outside, with the total eggs per female around 25% of that reported in Shaw et al. (2009). It was expected that there would be a discrepancy in performance between artificial and semi-natural conditions. Similarly, a small discrepancy is expected between the caged performance and open field performance because of the slightly raised temperature in the cages. This study carried out under semi-natural conditions does serve to estimate the expectations of psyllid performance at the open release sites. There will be a quarter of the eggs taking twice as long to develop compared with insects reared at a constant 22°C, which will revise the upper limit of potential expected density.

If *A. itadori* was restricted to *P. densiflora* and *C. japonica* for overwintering, then this would be a considerable barrier to successful establishment. Understanding the fundamental range of *A. itadori* winter hosts is vital in predicting its overwintering success, especially as it appears to be physiologically suited to cope with winter conditions in the UK (Birkin 2008). Hatherly et al. (2005) used the lethal time needed to kill 50% of a population at 5°C as a reliable predictor of winter survival, this remains something to be tested for *A. itadori*.

Most live psyllids were recovered from trunk sections of *P. sylvestris* or *Q. robur* (Fig. 4.4) suggesting that winter hosts need not be living. The saplings had suitable overwintering sites among the needles but the bark was relatively smooth. Although mature bark seems favourable, some psyllids overwintered when left on *F. japonica*. Since they had access to dead canes, leaf litter and soil it is unclear precisely where they overwintered, but nonetheless suitable sites are not limited to tree trunks.

Forced overwintering on alternative sites, such as leaf litter, has been reported for other psyllid species (Hodkinson 2009). However, based on the (non-significantly) higher number of live adults recovered from tree trunks, we hypothesise that the microhabitat of mature tree trunks, with cracks and crevices, was an important factor of a suitable overwintering site.

That *A. itadori* is able to successfully overwinter on non-living hosts may also have wider implications since one concern over the release of *A. itadori* was the chance of creating a new pathway of disease transmission to a winter host. Some psyllids, such as *Diaphorina citri* Kuwayama, are known to act as vectors in their respective systems (Hall et al. 2013). If *A. itadori* can overwinter successfully on non-living hosts then this suggests that winter host feeding is not essential and further supports that the risk of disease vectoring from this release is very low (Shaw et al. 2009).

This study shows that *A. itadori* will not be restricted to just a few rare conifers in the UK and has a larger overwintering host range. Although further research is required on the extent of this host range and winter survival, this can be achieved fairly easily in the field (Hodkinson 2009) and could reveal the order of preference for winter hosts too (Kristoffersen & Anderbrant 2007). For such studies to be carried out in the field, it would require much larger natural populations than currently occur.

Establishment rates for weed biocontrol agents vary considerably (Julien & Griffiths 1998) and failures are often accredited to poor life-history adaptations in the new range (McClay 1996). Field cage trials using the introduced population can provide a quick and cheap means of assessing the suitability of biocontrol agents without having to wait for field populations to establish, if similar work has not already been carried out prior to release. These results demonstrate that *A. itadori* is able to develop through all life stages in the outdoor environment of the UK where it has been released and some predicted life-history traits have been revised. Furthermore, winter survival is not likely to be a limiting factor in terms of thermal tolerance or available sites. This latter result is supported by the observation that *A. itadori* has successfully overwintered at some of the release sites in the UK, albeit in low numbers (Eschen & Brook, Unpublished report to Fera).

## Chapter 5 – Establishment

### 5.1 Introduction

It is difficult to predict whether a biocontrol agent will establish a self-sustaining population (Simberloff 1989). As discussed in Chapter 4, one of the key factors involved in establishment are the climatic tolerances of the species and incompatibility has been reported as the reason for failure in a number of biocontrol introductions (McEvoy & Coombs 2001). Apart from this, there are a whole suite of other factors that can determine establishment success, including other abiotic factors, biotic interference, regulatory restraints, biocontrol agent genetic variation and release strategy (Fowler et al. 2010). Reported establishment rates for weed biocontrol agents vary greatly (Julien et al. 1984; MacFadyen 1998; Syrett et al. 2000; McEvoy & Coombs 2001; Denoth et al. 2002). This will also change over time, in New Zealand overall establishment rates were reported at a low of 44% (Cameron et al. 1993) but this increases to 95% if only recent programmes are included (Hayes 2000). It is worth remembering that the chance of establishment of an agent is not synonymous to success rates (MacFadyen 2000). Success often means that the release of biocontrol agents partially or wholly negates the need for other control measures, but may also refer to the meeting of any specific aims of a programme. Control programmes could have variable establishment rates for particular biocontrol agents themselves but ultimately still achieve a high rate of target control. For example, Fowler et al. (2000) report 83% of programmes to have at least partial success. In the early stages of a release programme, biocontrol practitioners are essentially managing small populations of a rare species and face many of the same challenges as conservationists (Fauvergue et al. 2012), such as stochastic events at release sites (Norris et al 2002) and Allee effects, the reduced population growth at low densities (Dennis 1989), which have been suggested as a factor in biocontrol before (Hopper & Roush 1993). It is prudent to ensure that the selected agent(s) have the best chance of success and the release efforts are optimised during the early stage of a biocontrol programme. In this chapter, several factors that could influence the way in which *A. itadori* is released are studied through a series of separate experiments in order to inform the strategy for future releases of *A. itadori*.

### 5.1.1 Release conditions

#### Release size

The resources of a biocontrol programme are finite so it is desirable to use what is available in the most effective way. Releasing a certain number of biocontrol agents could be divided into a large number of individuals at few sites or fewer individuals at many sites. Grevstad (1999) used a simulation approach to consider this issue. It was concluded that demographic stochasticity alone was not likely to influence the chance of establishment but environmental stochasticity and the Allee effect were crucial and could affect optimal release strategy. The Allee effect is the reduction in growth rate while the population is at low densities brought about by difficulty in mate finding for example (Dennis 1989). In a population likely to experience the Allee effect, being released into a relatively stable environment, fewer larger releases are preferred, whereas, many small releases would improve the chance of establishment in a variable environment. The effect of environmental stochastic events were apparent in the release of *A. itadori* in 2010, a late frost killed off early *F. japonica* growth and prevented any insect establishment in that year (R. Shaw, personal communication). In a more recent review of the release and establishment of 74 species in the USA, it was found that release size did not affect the establishment rate but in general, biocontrol programmes were making too few releases to optimise the chance of establishment (Grevstad et al. 2011). Experimental releases at different densities in the field are ideal for working out the release strategy for a particular biocontrol agent. Memmott et al. (2005) did this for *Arytainilla spartiophila* Förster, a psyllid agent released to control *Cytisus scoparius* (L.) Link in New Zealand. Releases of 2-270 individuals were made and followed for 6 years. There was some evidence of a positive correlation between release size and establishment likelihood, but only in the first year. If the psyllid survived the first year and an initial lag phase then the chance of establishment increased to 96%. Interestingly, even some of the smallest releases possible with a chance of establishment, two individuals, were present five years later. Even though large scale manipulative field releases are not possible for *A. itadori* in the UK as the number of release sites are limited it would be beneficial to know whether release density influences the chance of establishment of *A. itadori*. The hypothesis that larger releases of *A. itadori* will be more likely to produce more offspring and increase the likelihood of a growing

population was tested with an experiment to study the reproductive success of *A. itadori* released at three different densities in a mesocosm environment.

### *Rainfall*

It is not only the number of releases made but the timing that must be considered. Although rarely quantified, the direct effect of adverse weather soon after a release of a biocontrol agent is thought to reduce the chance of establishment (Freckleton 2000). Norris et al. (2002) demonstrated that simulated rainfall can significantly increase post-release mortality in *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), a species shown to have a reduced chance of establishment at lower densities (Memmott et al. 1998). *Aphalara itadori* nymphs are dorsoventrally flattened which may improve adhesion to the plant during periods of rainfall, nonetheless, the numbers of another flattened phloem feeder, *Dactylopius opuntiae* Cockerell, remaining on the host plant was negatively correlated with the duration of rainfall (Moran & Hoffman 1987). Unlike *A. itadori* however, *D. opuntiae* is native to arid regions of the Americas so may well not be adapted to avoid dislodgement in periods of heavy rain. If a direct effect of rainfall is identified for a release then it is important to take it into account, relatively little effort may be needed to delay or carry out a sequential release yet the probability of establishment could increase. An experiment was carried out to assess the impacts of simulated rainfall on the adhesion of *A. itadori* eggs and nymphs to their host plant to test the hypothesis that extended period of rainfall will decrease the number of eggs and nymphs present on the host plant.

### *Plant stress*

There is a large body of literature on how host plant quality and water-stress can affect the performance of herbivorous insects (e.g. Awmack & Leather 2002; Coley et al. 2006) with support for both the Plant Stress (White 1969) and Plant Vigour (Price 1991) hypotheses. Respectively, these hypotheses suggest that herbivores may perform better on either stressed plants, which may have higher levels of accessible nutrients, or on unstressed plants. Huberty & Denno (2004) demonstrate that different feeding guilds differ in their response to water-stressed plants. Under continuous stress, phloem-feeders had, despite the increased levels of nitrogen,

reduced performance due to reduced accessibility. The responses of chewing insects were more varied. Contrasting with observations of phloem-feeders performing better on stressed plants this is explained by Huberty & Denno (2004) with the Pulsed Stressed Hypothesis. Plants that undergo prolonged water-stress but with short periods of hydration may provide sufficient turgor to facilitate feeding. Since the nymphs of *A. itadori* do not move large distances, it is up to adults to assess plant quality and choose to oviposit. There may then be a fitness cost if adults are released at sites which are likely to experience periods of water-stress. The aim of this experiment was to assess oviposition preference on *F. japonica* plants experiencing different levels of water-stress under semi-natural conditions, thereby testing the hypothesis that adults will prefer unstressed plants.

### 5.1.2 Species interactions

Since *A. itadori* is not being released into a closed system and the aim is for permanent establishment, there will inevitably be interactions with the existing fauna. It is possible that interspecific competition or top-down pressure from predators could preclude establishment or ultimately reduce the impact on the target. Through mesocosm studies, and even more so with quarantine trials, it is extremely difficult to predict the likelihood of that certain interactions will occur in the field but the outcome of manipulated systems can be informative.

#### *Competition*

*Fallopia japonica* supports a poor invertebrate assemblage (Gerber et al. 2008) so it would be unlikely that *A. itadori* would encounter strong competition from other herbivores once established on a patch. Nevertheless, some sporadic herbivory does occur on *F. japonica* from a small range of insects so there is a potential for localised competition. This is more of a problem for programmes that release multiple insect control agents as the more species utilising a host plant. In particular if they directly share resources, the higher the likelihood of antagonistic interactions (McEvoy & Coombs 1999) as in the case of *Lythrum salicaria* L. control in North America (Denoth et al. 2002). Even in the case of *F. japonica* where only a single insect agent is being released, understanding its competitive ability can be useful if it

is possible that other herbivores sharing the same resource will be encountered. In this study, the performance of *A. itadori* was studied in the presence of an interspecific competitor with similar feeding requirements, *Myzus persicae* Sulzer (Hemiptera: Aphidae), to test the hypothesis that psyllid performance will be lower in a competitive environment.

### *Predator exclusion*

There have been concerns over the release of non-native insect herbivores for the control of weeds (Pearson & Callaway 2003; EPPO 2010; Seymour & Veldtman 2010), because there is the potential for food web disruption and indirect non-target impacts mediated through shared natural enemies, i.e. apparent competition, even with the release of host specific biocontrol agents (Carvalho et al. 2008; Pearson & Callaway 2008). In order for these impacts to occur, the biocontrol agent must be palatable to the predator in question and should be preferred compared with native prey. The predator population should be able to increase in response to additional resources and regulate alternative prey populations. A specific set of circumstances are required for adverse impacts through apparent competition. Tipping et al. (2013) detailed the release of *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) where it has become an abundant biocontrol agent of *Melaleuca quinquenervia* (Cav.) Blake in Florida, USA. There has been no evidence that the introduction of a novel herbivore, with an increasing population, led to any indirect effects or predator spill over despite an estimated predation rate of *O. vitiosa* larvae of 8.4%. *Aphalara itadori* is a relatively unprotected species and psyllids are often being preyed upon by a wide variety of generalist predators, often members of the aphidophagous guild (Hodek & Honek 2009; Sigsgaard 2010). In laboratory trials, *A. itadori* has been shown to be palatable to three coccinellid species, but of a lower nutritional quality compared with an aphid alternative, resulting in a longer development period and lower body mass (Broom 2009). This is unsurprising as many coccinellids can have a wide ranging diet (Lucas et al. 2007) and their foraging strategy tends to use tactile cues and be opportunistic (Ferran & Dixon 1993). Hence they are unlikely to show strong preferences for single species. There are also fitness costs to feeding on sub-optimal prey as body weight is correlated with fecundity (Kajita & Evans 2010).

The key concern about predation for a biocontrol practitioner is that predation could cause local extinction of the biocontrol agent while it is still present at low densities at the early stages of release or reduce the effectiveness of the biocontrol agent (Crider 2011). Paynter et al. (2010) demonstrated that higher parasitoid attack rates on biocontrol agents are linked with failure to control the target weed. The likelihood of such failure to establish not only depends on the severity of the top-down pressure but also the release strategy used. Protecting the biocontrol agents from predators, either physically or temporally, could increase the chance of establishment. Often, a simple mesh cage enclosing the biocontrol agents can increase survival rates (Manrique et al. 2011).

It was hypothesised that *A. itadori* will have a higher survival rate when predator density is lower, aiding establishment likelihood. To test this hypothesis an experiment was carried out to assess the effectiveness of partial predator exclusion to the establishment of *A. itadori* nymphs as they are the most vulnerable stage.

#### *Molecular detection of predation*

Detecting predation of biocontrol agents in the field can be difficult, requiring long periods of observation and complicated by the nocturnal feeding habits or short periods of activity of many generalist predators (King et al. 2008). Quantifying the predation pressure without manipulative experiments needs even more investment of resources. A common approach in agricultural systems and food web studies is the molecular analysis of predator gut contents to detect consumed prey. This method is well reviewed (Symondson 2002; King et al. 2008) and allows the identification of a prey species or group of interest by the amplification of small residual amounts of DNA using specific primers and polymerase chain reaction (PCR). In its simplest application, a presence/absence of a prey species is determined for a sample of predators with only a fairly crude quantification (Hoogendoorn & Heimpel 2001) but using real-time PCR, information on the amount of DNA present can be collected (Pompanon et al. 2012). Considerations must be made with this method, as detection rates of DNA will differ across different predators and feeding methods as will the degradation rate of the prey DNA (Greenstone et al. 2010). Care must also be taken when collecting predator samples that they are not contaminated with contact with the prey of interest or regurgitation

onto other predators (King et al. 2008). Although work is underway at Landcare Research in New Zealand, there are no published examples of predator gut content trails being used in weed biocontrol but would be a useful tool to help quantify if generalist arthropod predators are having a considerable impact on population size of biocontrol agent releases. The Food & Environment Research Agency (Fera) have been developing a primer to detect *A. itadori* and distinguish it from other psyllid species in the UK (N. Boonham, personal communication). The available primer was specific to the *Aphalara* genus but due to a lack of *Aphalara* specimens other than *A. itadori* it is unknown whether it is effective at species-specific identification. This problem is currently being addressed (J. Hodgetts, personal communication.). The aim of this preliminary work was to identify if the existing primer available would be suitable for use in gut content analysis and whether it was a tool worth pursuing for this system in order to better assess the relative importance of predation of *A. itadori* in the field.

## **5.2 Materials and methods**

See General methods section (Chapter 2) for origins and culturing of *A. itadori* and plant material. All statistical analysis was carried out using R v3.0.2 (R Core Team 2013).

### *5.2.1 Release conditions*

#### *Release size*

See Chapter 4.2.1

#### *Rainfall*

A rainfall simulator was used to evaluate the effect of rainfall duration on psyllid numbers and constructed around a 2x2x2m steel field cage frame. Plastic sheeting covered all vertical sides of the frame and was attached to additional upright wooden supports to a height of 4m. A hollow cone nozzle (Raindrop Hollow Cone raindrop, 1/4", Delavan, Widnes, UK) with a 1 bar constant flow valve (Birchmeier, Stetton, Switzerland) was fitted at a height of 3m in the centre of the cage. The simulator was supplied and powered by an outside tap. The output of a hollow cone nozzle is not uniform across the spray area, with the least amount of flow directly below the

nozzle. Plastic pots (13cm) were placed at multiple spots within the spray area and water ran for 5 minutes to calibrate the quantity of water received. The eight most similar areas in terms of volume collected were marked and subsequently used to place experimental plants. See Aldridge (2012) for additional details.

Experimental plants of 6-10 weeks old were caged with 60 adult *A. itadori* each and were allowed to oviposit for 10 days before being removed. Plants were exposed to 30, 60 or 90min of continuous simulated rainfall on dry still days with control plants for each duration (n=8 for each). The number of eggs were counted before and after (allowing 1h for plants to dry off) using a binocular microscope on three leaves selected before the treatment was applied and marked with cotton tied to the base of the leaves. The recorded response was the proportion of eggs or nymphs displaced from the marked leaves after the trial.

### *Plant stress*

Rhizome fragments were planted in pots (13cm diam.) filled with J. Arthur Bower's Multi-Purpose Compost with added John Innes (Lincoln, UK). Four different plant stress conditions were established using different watering regimes and defined by the volumetric soil moisture content. These were 1) Soaked (>45%); 2) Medium (25-40%); 3) Pulse stressed (rapid change from <20% to >45%) and 4) Drought (<20%). Moisture levels were regularly recorded using a soil probe (ThetaProbe Soil Moisture Sensor ML2x, Delta-T Devices, Cambridge, UK). All pots were covered in plastic bags to help maintain the correct moisture range, holes were placed in the bags for the saturated plants. Choice arenas (n=15) were set up by placing a single plant from each treatment in a randomised position on a plastic tray and covered with a large sleeve. Plants were selected to be as similar as possible in size and quality. Thirty adult psyllids were released in the centre of the tray and allowed to oviposit for 16 days, after which the number eggs laid were counted under a dissection microscope.

### *5.2.2 Species interactions*

#### *Competition*

*Myzus persicae* were taken from existing cultures reared on Chinese cabbage (*Brassica rapa* L. ssp. *chinensis*) at Silwood Park (Ascot, UK) and placed on 10-

25cm *F. japonica* plants in wooden framed Perspex and gauze cages (54x29x44cm). Insects were left to reproduce on *F. japonica* for a minimum of two months before use in a controlled environment room (20°C, 70% RH, 16:8 light:dark regime). Fresh plant material was added and watering carried out as required. *Myzus persicae* was selected to be the competitor as it is one of a very few insects found transiently feeding on *F. japonica* in a similar way to *A. itadori* in the field (R. Eschen, personal communication).

To assess the effect of interspecific competition on *A. itadori* performance an experiment was carried out in a 3x3x2m tall cage between April and August 2010 in Egham, Surrey. There were twelve replicates (36 plants) in the cage, each sleeved individually and placed in grow bags and covered to halfway up the pot in sawdust to reduce water loss. Plants were watered as necessary. Each plant was randomly assigned one herbivore treatment; Psyllid (50 *A. itadori* adults), Aphid (15 apterous *M. persicae* adults) or both (50 psyllids and 15 aphids). Insect numbers were recorded every two weeks in a separate inspection cage using a 10x magnification hand lens. Psyllid adults were recorded at every sampling, nymphal instars present were recorded but number only for the final sampling and eggs only at the first sampling. This was to minimise disturbance to the plant and insects escaping. Adult aphids (apterous) and alates were counted precisely and nymphs were estimated. After 14 weeks all plants were destructively sampled and insect numbers counted.

### *Predator exclusion*

Two concurrent predator exclusion trials were carried out between May and July 2013, one using potted *F. japonica* plants carried out in the CABI garden, Egham and the other using sleeved natural stems at five of the psyllid release sites (Cornwall, Surrey, Norfolk, Sussex and West Glamorgan). The plants used for the garden study were planted from rhizome fragments (15-20g) originally in spring 2012 so had already developed a small rhizome network in 12L pots facilitating growth of multiple stems 90-160cm tall in 2013. Plants were placed on plastic trays to act as a barrier to the ground and arranged inside 2x10m wire fence (50mm mesh). The experiment was set up adjacent to a natural stand on *U. dioica* (c. 4x5m) to act as a source of predators. Four treatments were used (n=10 for each treatment); 1) Unsleeved – plants and insects exposed to open conditions throughout; 2) Sleeved –

170cm tall sleeve placed over plant to exclude the majority of predators and pots covered in a trapping adhesive (Oecotak A5, Oecos, Kimpton, UK); 3) Temporarily sleeved – sleeve placed over plants as before to prevent psyllid dispersal for 21d then removed; 4) Predator addition – as per sleeved treatment but with the addition of two field caught individuals of *Anthocoris nemorum* L. (Hemiptera: Anthocoridae) after 21d. *Anthocoris nemorum* was selected as it is a common and widespread generalist predator that can be found on a variety of vegetation, occasionally including *F. japonica*, and has been observed predating on *A. itadori* (Personal observation). Each plant was randomly assigned a treatment and 100 adult psyllids taken from culture were introduced to each plant. After 21 days the number eggs laid were counted and after 7 weeks the number of nymphs were counted. Any evidence of predation was noted during watering and sampling visits.

The same treatments as the garden study were set up at the field release sites with the exception that no Oecotak was used. Twelve stems were selected (n=3 for each treatment) at each site that were on the periphery of the stand and concealed as possible from the public. Stems with a diameter of 1-1.5cm were used so the full grown plant could be comfortably sleeved (secured with string and insulating tape to main stem c. 15cm aboveground level). Each stem was randomly assigned a treatment and 100 adult psyllids were introduced. After 19d, sleeves were removed and predators were added to the respective plants and eggs were counted for all stems. After 8 weeks, all nymphs were counted *in situ*. Four data loggers were placed in shaded locations at each site and recorded temperature every four hours.

#### *Molecular detection of predation*

Specimens of *A. nemorum* were collected from Silwood Park (Ascot, UK) using a beating tray and were immediately placed into individual 1.5ml tubes. The predators were starved for 24h prior to use. Predators were placed in 3cm Petri dishes separately with two fifth instar *A. itadori* nymphs and allowed to feed for 30min. Any individual which was not observed feeding was not used and excluded from the study. Once fed, predators were placed in 1.5ml tubes and killed by freezing at -18°C within 1h. Control predators were also starved for 24h but were killed without any contact with *A. itadori*. DNA was extracted from predators according to the manufacturer's instructions for two extraction kits; DNeasy Blood & Tissue Kit

(Qiagen) and Purelink Genomic DNA Kit (Invitrogen). Extractions were then sent to Fera (York, UK) to screen for *A. itadori* DNA using real-time PCR with TaqMan and minor-groove binding (MGB) probes (Applied Biosystems, Paisley, UK). The response of real-time PCR is the Ct (cycle threshold) value. This is the number of cycles required for the amplifications to cross a background level, as such the value is inversely proportional to the amount of target DNA. Assays run for 40 cycles and a Ct value of <38 indicates positive detection. Two replicates of individual predators with and without *A. itadori* present in their gut were sent for each extraction method as well as one replicate containing four individuals per sample extracted using the Qiagen kit.

### 5.2.3 Predicting establishment rates

Grevstad et al. (2011) states that the overall probability ( $P_{all}$ ) of a biocontrol agent establishing is the same as it not failing at all release sites. This is dependent on an underlying establishment rate ( $P_1$ ) for the biocontrol agent and the number of releases made ( $N$ );  $P_{all} = 1 - (1 - P_1)^N$ . Using this equation and the results from field releases (hard release) or manipulated releases into sleeves or cages, predicted establishment rates for *A. itadori* can be calculated. A release was considered successful if the number of individuals in the F1 generation was the same or higher than the founder number

## 5.3 Results

### 5.3.1 Release conditions

#### *Release size*

The high release density yielded significantly more eggs than the medium ( $t = 2.87$ , d.f. = 2, 18,  $P < 0.05$ ) or low ( $t_{2,18} = -6.21$ ,  $P < 0.01$ ) releases (Fig. 5.1). The absolute total number of adult psyllids in the low release treatment was significantly lower than both medium ( $t_{2,18} = 2.51$ ,  $P < 0.05$ ) and high ( $t_{2,18} = -2.71$ ,  $P < 0.05$ ) density releases but the difference between the medium and large was not significant (Fig. 5.1). The proportion of the eggs laid that survived to adulthood did not differ significantly between treatments (low = 0.15, medium = 0.18, high = 0.14,  $F_{2,18}$

=0.01,  $P = 0.98$ ). Only in one release in the low and high treatments and three for the medium did the number of adults produced in the F1 generation exceed the number of adults initially introduced. The chance of an increased population did not differ significantly between treatments (Fisher's Exact test,  $P = 0.55$ ).

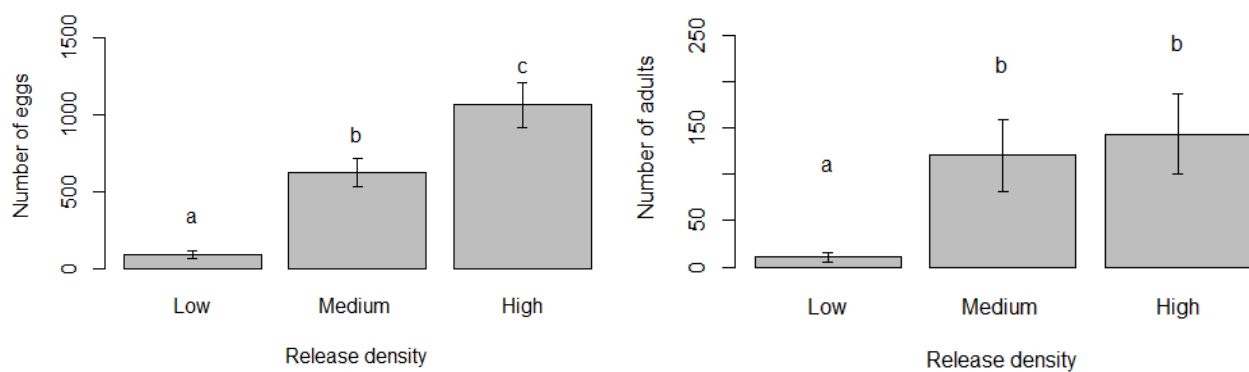


**Figure 5.1.** Total number of eggs laid after seven days at three release densities (low – 20; medium – 100; high – 200) of adult *A. itadori* (left) and the total number of adults emerged from each release density (right). Bars with a different letter above are significantly different.  $N = 7$ .

### Rainfall

Significantly more eggs were displaced from the leaf when exposed to simulated rain compared with controls ( $t_{2,45} = 2.86$ ,  $P < 0.05$ ; Fig. 5.2) but the duration of the rainfall had no effect ( $t_{2,45} = 1.7$ ,  $P = 0.09$ ).

There were no significant effects of the addition of simulated rainfall ( $t_{2,42} = 1.29$ ,  $P=0.2$ ) or the duration ( $t_{2,42} = 0.16$ ,  $P=0.86$ ) on numbers of first instar nymphs (Fig. 5.2).

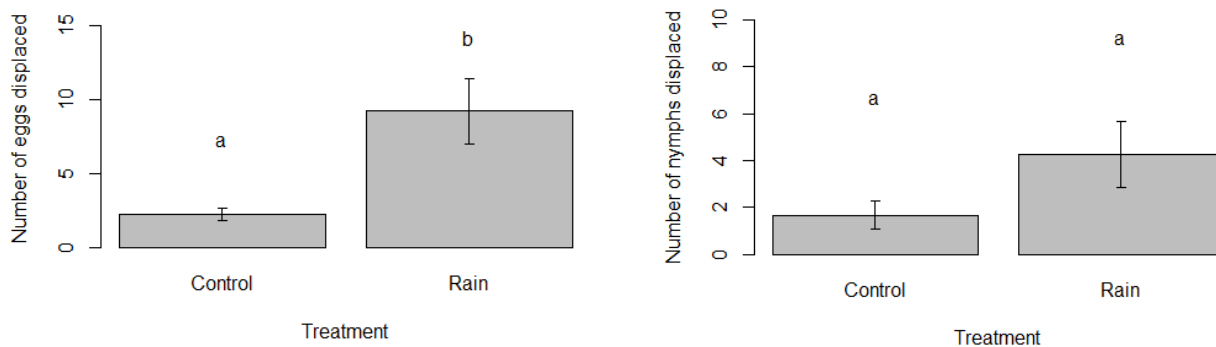


**Figure 5.2.** Total number of eggs (left) and nymphs (right) displaced from marked leaves after the addition of simulated rainfall (combined data from exposure of 30,60 and 90min). Bars with a different letter above are significantly different.  $N = 24$ .

### *Plant stress*

*Aphalara itadori* showed a significant oviposition preference between plant treatments (Fig. 5.3). Soaked plants were preferred over pulse stressed ( $t_{3,56} = 3.4$ ,  $P<0.01$ ) or medium ( $t_{3,56} = 3.18$ ,  $P<0.01$ ) as well as for drought stressed plants over pulse stressed ( $t_{3,56} = 4.21$ ,  $P<0.01$ ) and medium ( $t_{3,56} = 3.42$ ,  $P<0.01$ ). There were no significant differences between soaked and drought stressed ( $t_{3,56} = 0.28$ ,  $P=0.78$ ) or pulse stressed and medium ( $t_{3,56} = 1.1$ ,  $P=0.27$ ). The total number of eggs laid in each trial ranged from 22-369.

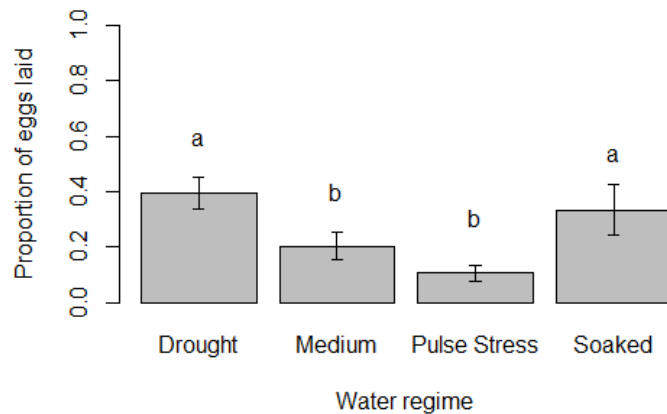


**Figure 5.3.** Proportion of eggs laid by 30 *A. itadori* adults in a choice arena outside in field cages. The watering regime maintained soil moisture content; Soaked (>45%); Medium (25-40%); Pulse stressed (rapid change from <20% to >45%) and Drought (<20%). Bars with a different letter above are significantly different. N = 15.

### 5.3.2 Species interactions

#### Competition

Under direct interspecific competition (psyllid + aphid treatment), the number of adults and nymphs of *A. itadori* present at the end of the study were reduced significantly compared with the psyllid only treatment (adult;  $t_{1,22} = 42.9$ ,  $P < 0.001$ . Nymph;  $t_{1,22} = 2.4$ ,  $P < 0.05$ , GLM, quasi-Poisson; Fig. 5.4). The competitor *M. persicae* was unable to persist in a single replicate.

#### Predator exclusion

In the garden study, two plants were damaged and excluded from the analysis. The number of eggs laid after 21d were significantly lower in the unsleeved treatment compared with the others which were not significantly different from each other ( $t_{3,33} = 10.91$ ,  $P < 0.001$ , GLM quasi-Poisson; Fig. 5.5). At the final counts, there were significantly more nymphs present in the sleeved treatment compared with the other treatments ( $t_{3,33} = 2.31$ ,  $P < 0.05$ , GLM quasi-Poisson; Fig. 5.5). A variety of

generalist predators were commonly seen on the plants throughout the study with some evidence of predation and reproduction (Table 5.1).

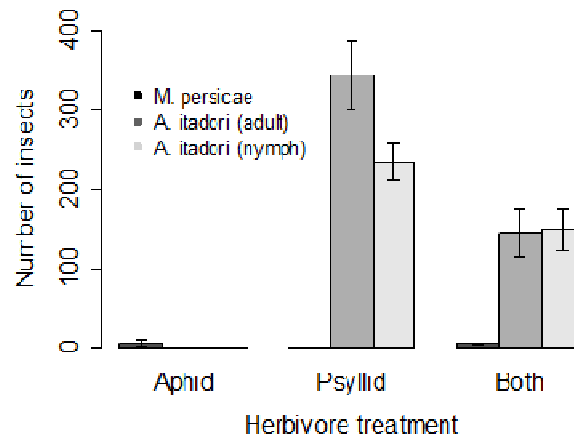


**Figure 5.4.** Mean number of insects collected from 36 *F. japonica* plants (12 per treatment) after 14 weeks in field cages. *M. persicae* numbers are a combination of adults, alates and nymphs whereas *A. itadori* adults and nymphs were counted separately.

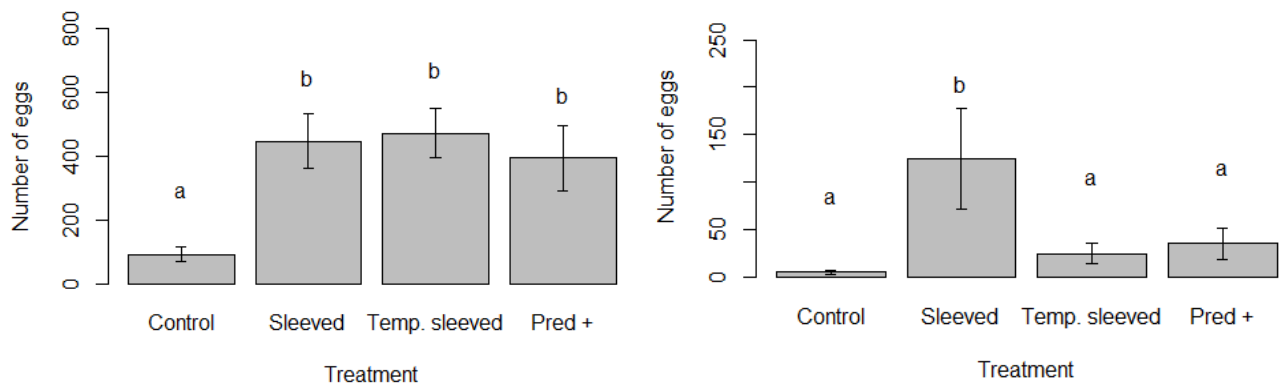


**Figure 5.5.** Total number of eggs laid after three weeks (left) and surviving nymphs after eight weeks (right) of *A. itadori* under four predator manipulation treatments carried out outside in a garden environment. 100 adults were released on unprotected (control) plants, protected for the entire study (sleeved), protected only oviposition period (temp. sleeved) or protected for the entire study with the addition of two *A. nemorum* predators (pred +). Bars with a different letter above are significantly different. N = 10 except for control and sleeved treatments where 1 plant was excluded from each.

**Table 5.1.** Observations of generalist predators occurring on *F. japonica* plants with *A. itadori* under the unprotected control treatment outside in a garden environment between May and July 2013.

| Species                              | Taxon         | Life stage present       | Direct predation of <i>A. itadori</i> observed |
|--------------------------------------|---------------|--------------------------|------------------------------------------------|
| <i>Anthocoris nemorum</i>            | Anthocoridae  | Egg, Nymph, Adult        | Yes                                            |
| <i>Harmonia axyridis</i>             | Coccinellidae | Egg, Larvae, Pupa, Adult | Yes                                            |
| <i>Propylea quatuordecimpunctata</i> | Coccinellidae | Larvae, Adult            | Yes                                            |
| Predatory mite sp.                   | Acari         | Adult                    | Yes                                            |
| <i>Chrysoperla carnea</i>            | Coccinellidae | Egg, Adult               | No                                             |
| <i>Coccinella septumpunctata</i>     | Coccinellidae | Adult                    | No                                             |
| <i>Helophilus pendulus</i>           | Syrphidae     | Egg, Larvae, Pupa, Adult | No                                             |
| <i>Lasius niger</i>                  | Formicidae    | Adult                    | No                                             |
| Various spiders                      | Araneae       | Adult                    | No                                             |

There were problems with data collection for the field experiment. Half of all stems had died back at the time of final sampling and 13 more showed signs of heavy damage. This was particularly a problem at the West Glamorgan and Cornwall sites where eight and nine stems out of twelve were excluded respectively. Counts of eggs laid after 19d was possible for all plants and showed that the exposed control plants had significantly fewer eggs than the other treatments ( $F_{3,22} = 9.96$ ,  $P < 0.001$ , LME; Fig. 5.6). Of the living stems at the end of the trial, there were no significant differences between treatments in the number of nymphs found ( $F_{3,22} = 1.11$ ,  $P = 0.37$ , LME; Fig. 5.6). Likely establishment was only recorded for one stem; one of the sleeved plants at the Norfolk site had a count of 324 nymphs and the majority were in the fourth or fifth instar. The average, minimum and maximum temperatures recorded over the whole period were similar across sites (Table 5.2).

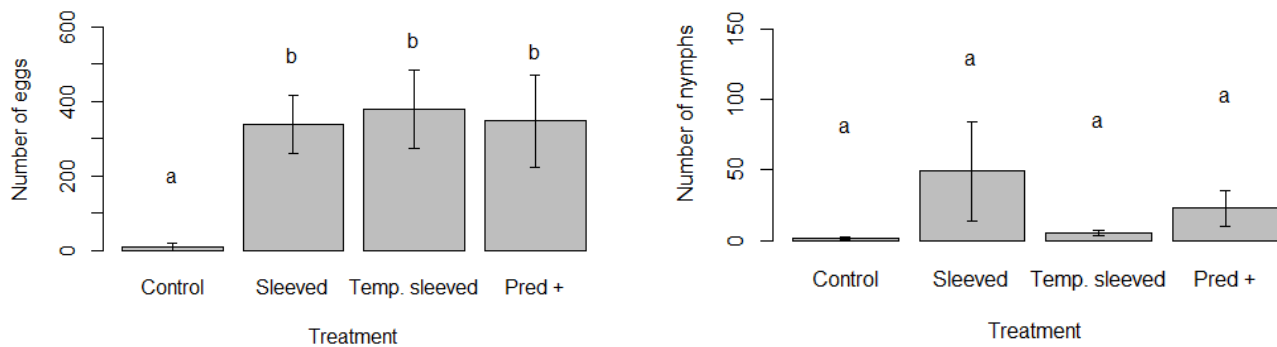


**Figure 5.6.** Total number of eggs laid after 19 days (left) and surviving nymphs after eight weeks (right) of *A. itadori* under four predator manipulation treatments carried out at five open release sites across the UK. 100 adults were released on unprotected (control) plants, protected for the entire study (sleeved), protected only oviposition period (temp. sleeved) or protected for the entire study with the addition of two *A. nemorum* predators (pred +). Bars with a different letter above are significantly different. Many plants died and were excluded from the analysis, N = 7, 9, 8, 6 respectively.

**Table 5.2.** Mean, minimum and maximum temperatures (°C) recorded using four DS1921G-F5 thermochron dataloggers at each of the release sites over an 8 week period between May and July 2013.

| Temp (°C)   | Site        |             |            |                |            |
|-------------|-------------|-------------|------------|----------------|------------|
|             | Cornwall    | Surrey      | Sussex     | West Glamorgan | Norfolk    |
| <b>Mean</b> | 14.1 ± 0.11 | 15.4 ± 0.13 | 14.3 ± 0.1 | 13.5 ± 0.1     | 14.3 ± 0.1 |
| <b>Min</b>  | 5.5         | 8           | 8          | 3              | 2.1        |
| <b>Max.</b> | 26          | 29          | 26.5       | 25             | 35.1       |

#### *Molecular detection of predation*

All predator samples that had fed on *A. itadori* were positive for the presence of target DNA whereas control specimens were not (Table 5.3). This was the case for both extraction methods and for groups and individual predators.

**Table 5.3.** C<sub>T</sub> values (mean of two runs from each sample) from preliminary assays to detect *A. itadori* DNA from the contents of a generalist predator, *A. nemorum*, either fed or not fed psyllid under control conditions. Two extraction methods, PCR probes and predator number were trialled. A cut-off of C<sub>T</sub> < 38 was marked positive and are indicated in bold.

| Treatment                 | No. of predators | Extraction | TaqMan probe | MGB probe   |
|---------------------------|------------------|------------|--------------|-------------|
| <i>A. itadori</i> present | 1                | Qiagen     | <b>33.9</b>  | <b>33.3</b> |
| <i>A. itadori</i> present | 1                | Qiagen     | NA           | <b>32.9</b> |
| Control                   | 1                | Qiagen     | 40           | 40          |
| Control                   | 1                | Qiagen     | 40           | 40          |
| <i>A. itadori</i> present | 1                | Invitrogen | <b>34.3</b>  | <b>33.3</b> |
| <i>A. itadori</i> present | 1                | Invitrogen | <b>33.2</b>  | <b>33.4</b> |
| Control                   | 1                | Invitrogen | 40           | 40          |
| Control                   | 1                | Invitrogen | 40           | 40          |
| <i>A. itadori</i> present | 4                | Qiagen     | <b>31.9</b>  | <b>31.3</b> |
| Control                   | 4                | Qiagen     | 39.6         | 40          |

### 5.3.3 Predicting establishment rates

Only once out of 24 releases (8 sites from 2011-13) has *A. itadori* shown good signs of establishment, producing large numbers of eggs and nymphs, but ultimately still hasn't established in the field (R. Shaw, personal communication). The protected releases used for the insect development study (Chapter 4) were considered successful for four out of 21 plants. Finally, there was one successful release out of the 23 surviving plants in the field predator exclusion study (combining all treatments that used protective sleeves at some point). Using these data as a baseline, the expected probability of establishment and the relationship with the number of releases is shown in Fig. 5.7.

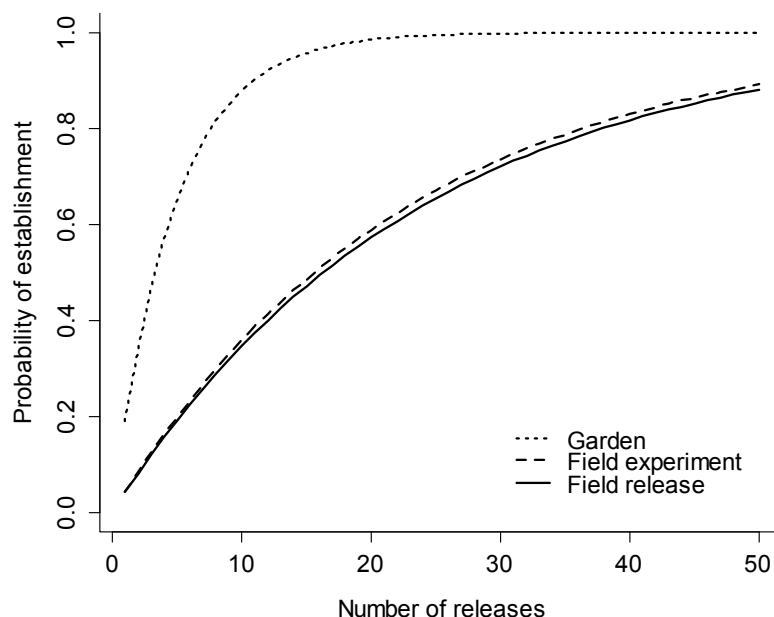


**Figure 5.7.** The predicted probability that at least one release of *A. itadori* will establish as a function of the total number of independent releases. Different curves were calculated using data from the garden and field predator exclusion study (Chapter 5.3.2) and from the CABI programme of open field releases.

## 5.4 Discussion

### 5.4.1 Release conditions

#### *Release size*

Insects will likely need a minimum density of founder individuals to establish successfully (Lockwood et al. 2005), but above this threshold, additional individuals may not proportionately increase the chance of success. In the present study, there was no difference in adult numbers produced between the larger two release sizes (Fig. 5.1) though the scale of this study is coarse with only three release densities. Given that practitioners are unlikely to know the effect of release density or the likelihood of establishment for a particular biocontrol agent, it is advised that a mixed release in terms of size and number is carried out in the early stages in order to inform future efforts (Shea & Possingham 2000). In the example of *A. spartiophila* releases in New Zealand (Memmott et al. 2005), the insect had a very high chance of establishment with some populations which had just two founding individuals

surviving. Though, in the Memmott et al. (2005) study, a total of 55 releases were made and after six years, 22 had psyllids. Localised extinction occurred at over half the sites. Yet, although release size may have an effect on establishment success, the effect of the number of releases appears to be stronger (Grevstad et al. 2011). The *A. itadori* releases in the UK have been limited to a maximum of eight sites around the country, albeit with large numbers of individuals released (c. 1000-10000 in a given year), with more than 24 releases made in total. It is possible that this shortage of release sites has been a major limiting factor in the establishment of this biocontrol agent. However, it may also be a case that *A. itadori* is not as effective at colonising new environments compared to other psyllid biocontrol agents in which case, additional efforts may be required to encourage population growth in the UK.

### *Rainfall*

The favourability of the environment at the time of release is expected to influence establishment (Fowler et al. 2009). A small, but statistically significant, effect was observed in this study with the addition of rainfall. The number of eggs found on the plant decreased from 98% in the control to 92%. Although a small reduction, it is not one to be ignored and would likely change under natural conditions. Artificial experiments such as this provide a quick assessment but are no substitute for field data. Norris et al. (2002) discusses how field data is underutilised and the benefit of recording climatic data immediately after release alongside establishment in post-release studies. Through the routine collection of climatic data, paired with the establishment success of a biocontrol agent, then gaps may be filled in, helping to understand the causes of failed establishment attempts. This could help inform future releases and potentially the different susceptibilities of different life-stages or taxa. Further experimental work would be useful to determine the benefit of sheltering releases if they must be made during periods of poor weather, but would unlikely be as beneficial as protecting from predators although these need not be mutually exclusive.

### *Plant stress*

Insect herbivory can be affected by plant quality and is often mediated through climatic conditions (Shure et al. 1998). Releasing onto suitable stands of *F. japonica* could then be important for *A. itadori* establishment since unfavourable plant quality

could result in adult dispersal or poor development of immature stages. In the present study, *A. itadori* preferred to oviposit on the extremes of plant quality presented (Fig. 5.3), possibly as a response to the accessibility of plant nutrients. Riparian areas are often favoured by *F. japonica* (Beerling et al. 1994) and could provide an abundance of preferable hosts less likely to suffer from pulse stress.. However, due to conditions laid down by the regulator for the release of *A. itadori*, no sites near any watercourse were selected in case the use of pesticides was required. Nonetheless, psyllids also demonstrated a preference for drought stressed plants, therefore it is unlikely that reduced oviposition will likely lower performance at sites where *F. japonica* may have a poor water supply.

#### 5.4.2 Species interactions

##### *Competition*

Although psyllid numbers were significantly lower in presence of strong competition from *M. persicae* (Fig. 5.4) it seems clear that *A. itadori* is the stronger competitor on *F. japonica* and able to persist. This study did show the extremely unlikely event of a no choice interaction as *F. japonica* is a sub-optimal host for *M. persicae* under natural conditions and will often move off the plant after an initial period of growth, as suggested here with the production of alates and it is likely that the two species would spatially segregate. Denoth et al. (2002) reports that the establishment rate of biocontrol control agents is not influenced by the number of agents released, but there is a higher chance of successful control with additional agents. There are no planned releases of an additional insect agent for the control of *F. japonica* in the UK, therefore, competition between *A. itadori* and another specialist will not be a limiting factor to its establishment. Short term interference from transient herbivores, such as *M. persicae*, may cause localised limitation of population size in the worst case but would not preclude the establishment of *A. itadori*. Not tested directly here, but there is also the potential for indirect competition between herbivores mediated through changes in plant quality induced by herbivore attack (Denno et al 2000). The key recommendation from this would be to ensure psyllid releases do not occur directly with other herbivores present but there is no need to drastically alter release strategy as any other herbivore present are unlikely to persist for long.

## *Predation*

It is likely that a top-down pressure is having a considerable impact on *A. itadori* as a variety of predators (Table 5.1) were found to utilise *A. itadori* as a resource in a short space of time, although they occurred at much lower densities than on surrounding vegetation (G.D.C personal observation). The increase in nymph survival when the majority of predators were excluded is not likely an effect of sleeving the plants as nymph populations were also reduced in the predator addition treatment. The results of this study indicate that protecting the early, vulnerable life-stages of *A. itadori* may improve the chances of survival. Once the psyllids reach a larger population size, then the effects of predation may become less important. So long as *A. itadori* does not become a principal prey item for a particular species, it is unlikely that there would be a numerical response from the predator (Symondson 2002). Food web interactions are complex and it is not only prey switching that need be considered. Intraguild predation is extremely common among generalist predators and increases at higher predator densities which may establish a shift in predator assemblage rather than increase in population size (Lucas 2005)

Protecting the psyllids with a physical barrier after release also has the benefit that it prevents the adults from dispersing. In the open field releases and the unsleeved treatment in this study, adult numbers quickly declined and either through host preference or release methods the cue to oviposit on the release plants was missing (G.D.C, personal observation). If the adults are physically contained then there was no problem with oviposition, this then ensures a population of immature individuals on the plant. It is still unclear in the field whether declining adult numbers are a result of dispersal or mortality and this question would be best answered through manipulative field experiments.

## *Molecular detection of predation*

The work carried out here is preliminary but nonetheless shows proof of concept that the molecular detection of predation can be used in this system with existing resources. More work is needed to get this method functional. Prey DNA degrades over time once ingested (King et al 2008), the rate at which the DNA degrades will vary according to predator type and conditions predation occurred (Greenstone et al.

2007). Laboratory feeding assays to determine detection curves are essential in order to calibrate samples collected in the field (Greenstone et al. 2010). One downside of this approach is the care required when field collecting predators to ensure there is no cross-contamination (Greenstone et al. 2011), this usually involves sampling each predator individually. This can make it difficult to either standardise sampling methods, or collect sufficient predators. There has been some progress recently with steps to optimise the PCR process (Sint et al. 2011) and methods to allow the removal of contaminating DNA and open up the use of vacuum or mass sampling (Chapman et al. 2010; Greenstone et al. 2012). This approach is the ideal way to combine biocontrol and ecological research as quantifying the predation pressure on biocontrol agents will not only inform control efforts of the target but could reveal shifts in food webs and provide systems for predator-prey interaction studies (Sheppard & Harwood 2005).

It is unsurprising that a combination of factors discussed here and in Chapter 4 all contribute to the establishment of *A. itadori*. These factors will interact with one another (Masters et al. 1998; Gurevitch et al. 2000) and the relative importance of each can vary from site to site and over time. None of the investigated establishment factors suggested a complete crash of the psyllid population but each had some detrimental effect. It would be worth carrying out changes to release strategy that are relatively simple, such as timing, to reduce the cumulative result of limiting factors. For those factors that are more difficult to manipulate over larger areas, such as predator interference, it would be prudent to monitor releases with and without protected or exclusion areas to look for difference in performance. Alternatively, releasing multiple life stages of the biocontrol agent may be beneficial as the introduction of immature stages removes adult choice as a variable in whether the target host is accepted, for example, Center et al. (2006) report successful establishment of *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae) on an invasive tree, *Melaleuca quinquenervia* (Cav.) Blake, in Florida at a site where only nymphs were released. Many of the limiting factors could be negated by the use of more releases (Grevstad et al. 2011) and this may ultimately have the largest impact on establishment. Using the current release strategy at 30 separate sites, to spread the risk of local extinction, it is predicted that there would be a 72% chance of at least one population establishing (compared with the 30% at eight sites). However,

when the number of release sites are limited, then release strategy can be modified to improve the likelihood of establishment. Multiple factors that could influence establishment were considered in this chapter and their relative average effect will differ (Table 5.4). From the field cage data, it is recommended that the release strategy for *A. itadori* should aim to release as many adults as possible in the absence of competition, but also provide some level of predator exclusion to improve survival rate. Providing protection for the biocontrol agent from predators has been effective in Australia in the control of *Asparagus asparagoides* (L.) W.Wight. Reilly et al. (2004) detailed the results of a field experiment where the number of larvae and pupae of *Crioceris* sp. (Coleoptera: Chrysomelidae), a biocontrol agent released against *A. asparagoides*, were significantly higher when adults were released into exclusion cages compared to plots without cages. Predicting establishment from artificial releases however, is likely to overestimate the probability of success, as there was a difference of 53% between using field and cage data. This overestimation is most probably a result of the over simplification of artificial releases and under field conditions factors such as predator density, climatic conditions and dispersal can all vary and have cumulative negative effect on a small population of a biocontrol agent. The likelihood of success of a biocontrol programme is proportionate to the amount of resources invested (Fowler et al. 2000). In order for the current and future biocontrol programmes in the UK to be effective, it is essential for regulators and funding bodies to be aware of what investment is required for a reasonable chance of success. This does include the availability of more release sites but also the research required to optimise release strategy so that biocontrol efforts are not simply a lottery of releasing as many agents as possible in as many places as possible.

**Table 5.4.** Summary of the percentage difference of the number of *A. itadori* between some of the different conditions of the establishment factors investigated.

| Factor        | Condition                  |   | Numerical increase |       |       |
|---------------|----------------------------|---|--------------------|-------|-------|
|               |                            |   | Egg                | Nymph | Adult |
| Predators     | Excluded<br>Control        | ↑ | 80%                | 96%   | -     |
| Release size  | 200 adults<br>100 adults   | ↑ | 70%                | -     | 19%   |
| Competitors   | Absent<br>Present          | ↑ | -                  | 36%   | 58%   |
| Plant quality | Preferred<br>Not preferred | ↑ | 21%                | -     | -     |
| Rainfall      | Absent<br>Present          | ↑ | 5%                 | 18%   | -     |

## Chapter 6 – Impacts

### 6.1 Introduction

#### 6.1.1 Direct target impacts

Classical biocontrol of invasive weeds can be an effective alternative to conventional management (Culliney 2005) and some of the traits of *F. japonica*, such as asexual reproduction, make it a suitable target for biocontrol (Paynter et al. 2012).

Additionally, the presence of only a single genetic clone present in the UK (Hollingsworth & Bailey 2000) should reduce the variation in susceptibility to herbivory. Finding the most suitable biocontrol agent is essential and regulatory concerns are typically focussed on the host specificity of an agent, to minimise the risk of non-target impacts (EPPO 2010, Pearson & Callaway 2003). Although understanding the host range is crucial, agent efficacy requires more attention as there is little benefit to releasing ineffective agents. Raghu et al (2006) argue that efficacy testing should be considered with higher priority than host range testing when candidate biocontrol agents are being selected and that only agents with demonstrable desired impacts on the target weed should be considered for host range testing. This is in agreement with the 'Code of Best Practices for Classical Biological Control of Weeds' (Balciunas 2000). Despite this, there is yet to be an emphasis on efficacy testing as a requirement in applications for release of biocontrol agents, in Europe at least, and other requirements vary hugely between countries (Bale 2011).

Psyllids have not been commonly used as biocontrol agents but there are some successful examples (Memmott et al. 2005; Center et al. 2011). Furthermore, phloem feeding insects have the potential to reduce plant performance more than defoliators for some plant groups (Zvereva et al. 2010). Grevstad et al. (2013) describe a laboratory efficacy assessment for *A. itadori* as part of a pre-release assessment in North America. Individuals from the same source population of *A. itadori* that have been released in the UK, as well as psyllids from another population collected from Hokkaido in northern Japan were used. It was demonstrated that *A. itadori* can significantly reduce the growth of *F. x bohemica* and *F. sachalinensis* over a period of 50 days. There were also some differences in performance between

the two populations of *A. itadori* depending on the *Fallopia* host. Psyllids originally collected from Kyushu (now in the UK) produced significantly more adults in the F1 generation while on *F. x bohemica* compared with those collected from Hokkaido. Since *A. itadori* is already licensed for open field releases in the UK, it is possible to test the impacts of the psyllid on *F. japonica* at a larger scale and under more natural conditions, using field cages, than previously under quarantine conditions. An early post-release impact assessment can bridge the gap between small scale impacts observed in quarantine (White 2007) and those in the field which may take years to become apparent. To test the hypothesis that *A. itadori* can cause damage to *F. japonica*, an experiment was carried out to quantify the impacts of this species on large potted plants in field cages.

#### 6.1.2 Indirect impacts

A minority of plant species in their non-native range can become invasive (Williamson 1996), disrupting ecosystems, displacing native species and causing considerable economic damage (Pimentel et al. 2005), although, there is a wide range of potential mechanisms that facilitate these invasions (Catford et al. 2009).

The Novel Weapons Hypothesis (Callaway & Ridenour 2004) outlines the role of allelopathy in conferring a competitive advantage to the invader. Allelopathy is the chemically mediated interaction between plant species whereby secondary compounds produced by one species can negatively affect the performance of other species (Inderjit & Callaway 2003). These interactions can be important in structuring communities and have previously been implied in plant invasions (Callaway & Aschehoug 2000).

All of the invasive knotweeds are known to contain secondary compounds (Inoue et al. 1992, Vrchotova et al. 2007, Fan et al. 2010). Some of these have been shown to have inhibitory effects on other plants in growth assays (Vrchotova & Sera 2008, Moravcova et al. 2011), which may explain part of *Fallopia* dominance. Several, more ecologically realistic, studies have been carried out using *F. x bohemica* to investigate the importance of allelopathic in knotweed invasions. Murrell et al. (2008) identified a significant role of allelopathy in the inhibition of the growth of native

species using potted communities. Although not identified as the primary mechanism of dominance, allelopathy has also been indicated as an additional factor affecting native species in a *F. x bohemica* stand under manipulated field conditions (Siemens & Blossey 2007). These types of impacts can also be indirect or persistent. Parepa et al. (2012) demonstrated that the allelopathic effects of *F. x bohemica* does not necessarily require the presence of live knotweed as some effects were detected just from the presence of leaf litter or trained soil.

Allelopathic interactions between species are complex and can be modified by biotic (Glinwood et al. 2011) or abiotic factors (Chen et al. 2012). The strength of allelopathic effects can be affected by insect herbivory but there is a wide range in plant responses. Thelen et al. (2005) demonstrated an increase in the negative allelopathic effect of the invasive *Centaurea maculosa* Lam. against native plant species as a result of herbivory. Other studies show insect herbivory to have either no effect (Norton et al. 2008) or a decrease (Kong et al. 2002) in the production of allelochemicals and their impact on other plant species. Furthermore, plant responses can vary depending on the type of herbivory, for example, feeding by either chewing or sap-feeding insects can activate different chemical signal pathways and illicit different plant responses (Walling 2000). Such variations may alter the outcome of control efforts using specialist insect biocontrol agents unless the importance of allelopathy and its response to herbivory is taken into account within management programmes. If one of the aims of a biocontrol programme is to reduce the competitive ability of the target species to facilitate recovery from native species, but if this does not occur, despite a biocontrol agent successfully damaging the target, the ultimate success of the effort could be questioned. Ideally, native plant species would benefit indirectly from the presence of *A. itadori* as a result of any damage this species causes to *F. japonica*.

The aims of this study were i) to demonstrate whether an allelopathic mechanism is involved in the negative impacts on native European plant species by *F. japonica* and ii) determine whether these impacts are modified by the presence of a specialist herbivore and biocontrol agent of *F. japonica*.

## 6.2 Materials and methods

### 6.2.1 Direct target impacts

See Chapter 2 for general methods

*Fallopia japonica* was grown in a glasshouse in 3L pots for three weeks before being placed outside and transferred to 10L pots and allowed to settle for a further week before use. It was expected that plants would have the space to be able to grow unrestricted over the period of the study. Before planting rhizomes were measured (length and diameter) and weighed.

The experiment was carried out in a 3x3x2m tall cage between April and August 2011. There were twelve replicates (24 plants), each individually sleeved and placed in grow bags and covered to halfway up the pot in sawdust to reduce water loss. Plants were watered as necessary. The presence or absence of *A. itadori* (at a density of 50 adults per plant) was randomly assigned. Before introducing the insects, initial measures of plant performance were recorded. These were: stem height, stem diameter (measured 1cm above soil), leaf number and total leaf area. Plant height and adult psyllid numbers were recorded every two weeks. The number of eggs laid was recorded only once, 14 days after the adults were introduced, and the number of nymphs on each plant was only recorded at the final sampling date. This was to minimise disturbance to the plant and the chance of insects escaping. After 14 weeks all insects were removed and final measures of plant performance were recorded. In addition to the initial measures, all leaves were scored using a devised damage rating, scoring leaves as: 1) Healthy; no visible damage 2) Slight damage; some minor discolouration 3) Moderate damage; heavily discoloured, leaf curling 4) Heavy damage; extensive discolouration, browning, leaf curled 5) Dead (Fig. 6.1). The aboveground and rhizome dry weights were also recorded. Plants were destructively sampled and placed in a drying oven (75-80°C for 72h) then weighed. Only rhizome material was weighed for the belowground measure, all roots were removed to allow for removal of all soil.



**Figure 6.1.** Illustration of the damage rating scores 1-5 for *F. japonica* plants exposed to *A. itadori* for 14 weeks in field cages outside. From left to right; 1) Healthy; no visible damage 2) Slight damage; some minor discolouration 3) Moderate damage; heavily discoloured, leaf curling 4) Heavy damage; extensive discolouration, browning, leaf curled 5) Dead.

The total changes in the stem diameter and total leaf area were analysed using linear models. Above and belowground dry weights were also analysed using linear models with initial rhizome weight used as a covariable in the belowground analysis. Response variables were log-transformed to normalise the data and meet the assumptions of linear models. Change in leaf number and the proportion of damaged leaves were analysed with generalised linear models, using quasi-Poisson and quasibinomial error distributions respectively. Plant height, measured repeatedly over the study, was analysed using a mixed-effects model specifying week of measurement and individual plant as random factors. All analyses were carried out using R v3.0.2 (R Core Team 2013).

### *Sleeve impact*

To assess the impact of sleeves on the growth of *F. japonica*, an additional cage was set up as above with 24 plants randomly assigned a sleeve or no-sleeve treatment. Plants were allowed to grow unrestricted for the duration of the above experiment and the same measures of plant performance were recorded as above.

### *6.2.2 Indirect impacts*

Four herbaceous plants (*Geranium robertianum* L., *Lamium album* L., *Silene dioica* (L.) Clairv. & *Urtica dioica* L.) and one grass (*Poa trivialis* L.) were selected to represent a native community. All seeds were obtained from the following seed

suppliers in the UK; Emorsgate Seeds (King's Lynn), Naturescape (Langar) and Nicky's Nursery (Broadstairs). All of these species regularly co-occur with *F. japonica* in the UK and share some habitat requirements (Beerling et al 1994; *personal observation*).

To test for any inhibitory allelopathic effects of *F. japonica* and the impact of the psyllid on these inhibitory effects, if present, four main treatments were set up. These were: *F. japonica* only, native species only, *F. japonica* + natives and *F. japonica* + natives + psyllids. Each of these treatments were tested with and without the addition of activated carbon (activated charcoal puriss. p.a. powder, Sigma-Aldrich) at a concentration of 20ml/L mixed through the substrate. Activated carbon is a commonly used material in allelopathy studies that can absorb chemical compounds in the soil (Callaway & Aschehoug 2000). There were 15 replicates for each combination totalling 120 pots. The total nitrogen (1100 mg/Kg) and phosphorous (100 mg/Kg) content of the activated carbon was determined using a modified Kjeldahl method (Kjeldahl 1883), modified using a selenium catalyst (Ashton 1936).

Depending on the treatment, a single rhizome fragment (containing one node and weighed before planting) was buried at a depth of 5cm and 25 seeds of each native species were sown just below the surface in 7.5L plastic pots. The pots were placed in grow bags within field cages in a random order and individually sleeved to prevent psyllids from escaping and to maintain a comparable environment for all plants. Psyllids were placed on the *F. japonica* at a density of 50 adults when the plants were c. 10cm tall. All plants were watered equally as required. After 14 weeks all plants were harvested for determination of dry aboveground biomass for *F. japonica*, herbs and grass separately. Plant material was dried at 70-80°C for 72h prior to weighing. Any *F. japonica* rhizome that did not sprout was excluded from the analysis.

ANOVA models were used to analyse the native species aboveground biomass response with the presence of *F. japonica*, *A. itadori* and activated carbon each fitted as factors with two levels along with their interactions. The growth of *F. japonica* itself was analysed separately with the presence of native plants, *A. itadori* and activated carbon included as factors. All data included in these models were log-transformed prior to analysis. The relationship between total biomass of the natives

and *F. japonica*, plus *F. japonica* and the initial rhizome biomass were analysed using linear regression models. All analyses were carried out using R v.3.0.2 (R Core Team 2013).

## 6.3 Results

### 6.3.1 Direct target impacts

The introduction of *A. itadori* significantly reduced the growth of *F. japonica* compared to control plants (Table. 6.1; Fig. 6.2), with a 50% reduction in plant height gain, from a mean  $\pm$  SE start height of  $52.4 \pm 1.01$ cm and  $49.7 \pm 0.96$ cm respectively. The final rhizome dry weight was also reduced by c.50% and a 40% decrease in total leaf area gain by psyllid herbivory. Plant height for both groups increased quite rapidly for the first six weeks, by which time there was a significant difference between groups ( $F_{1,22}=10.6$ ,  $P<0.01$ , ANOVA).

Although there was no change in the number of leaves produced, the amount of visible leaf damage was significantly higher in the psyllid treatment (Table. 6.1). Only 0.5% of the leaves in the control group were given a damage score of 3 or higher compared with 41% from the psyllid group (Fig. 6.2). This damage was typically a yellowing or browning of the leaf along with curling at the edges.

**Table 6.1.** Table summarising the analyses of plant growth measurements of *F. japonica* exposed to an initial density of 50 adult *A. itadori* per plant. Insects were left to develop unhindered for 14 weeks before sampling. All plants were housed outside in field cages under near-normal environmental conditions.

| Plant measure                | Test statistic                | d.f. | P      |
|------------------------------|-------------------------------|------|--------|
| Plant height                 | F= 22.29 (LME)                | 1,22 | <0.001 |
| Change in diameter           | F= 1.13 (ANOVA)               | 1,22 | 0.299  |
| Change in leaf area          | F= 42.13 (ANOVA)              | 1,22 | <0.001 |
| Change in leaf number        | t= -0.62 (GLM, quasi-Poisson) | 1,22 | 0.539  |
| Aboveground dry weight       | F= 0.67 (ANOVA)               | 1,22 | 0.421  |
| Rhizome dry weight           | F= 28.17 (ANCOVA)             | 1,21 | <0.001 |
| Proportion on leaves damaged | t= 4.89 (GLM, quasibinomial)  | 1,22 | <0.001 |

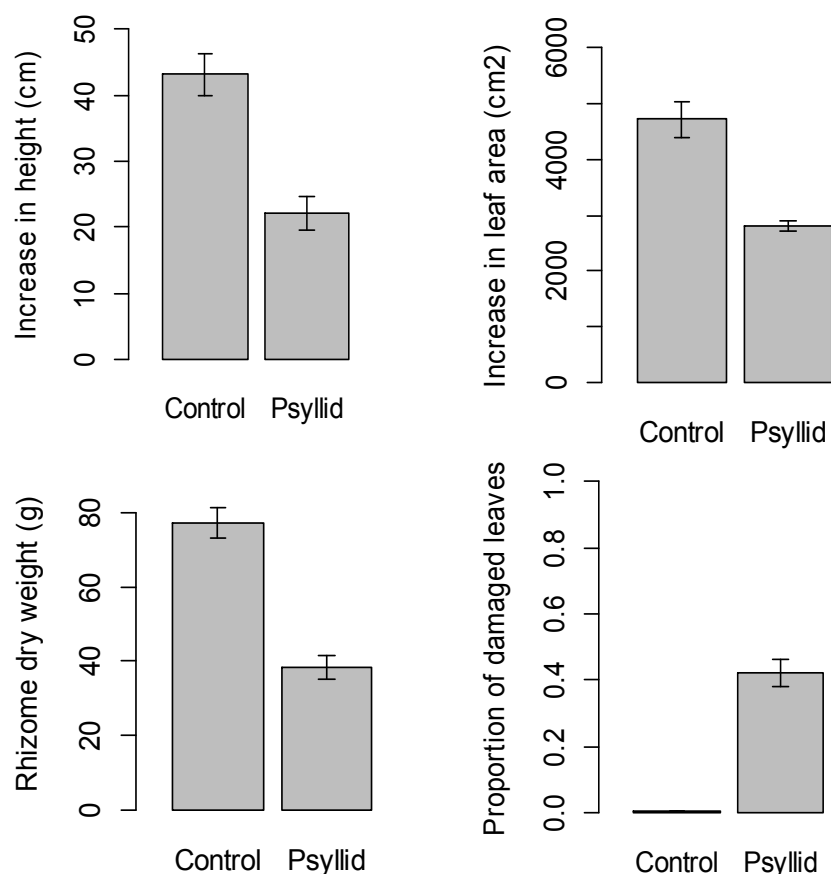


**Figure 6.2.** Plant growth measures of *F. japonica* exposed to *A. itadori* (50 adults) in field cages for 14 weeks. Error bars are  $\pm 1$  S.E. N = 12.

### Sleeve impact

Except for change in plant height, all other measures of plant growth were significantly reduced as a result of being sleeved (Fig 6.3. and Table 6.2.).

**Table 6.2.** Impact of sleeves on performance of *F. japonica* grown in a field cage for 14 weeks.

| Measure                | Test statistic                 | d.f.  | P      |
|------------------------|--------------------------------|-------|--------|
| Change in height       | F = 1.67 (ANOVA)               | 1, 22 | NS     |
| Change in diameter     | F = 12.42 (ANOVA)              | 1, 22 | <0.05  |
| Change in leaf area    | F = 11.81 (ANOVA)              | 1, 22 | <0.01  |
| Change in leaf number  | t = 53.21 (GLM, quasi-Poisson) | 1, 22 | <0.01  |
| Aboveground dry weight | F = 11.58 (ANOVA)              | 1, 22 | <0.01  |
| Rhizome dry weight     | F = 22.4 (ANCOVA)              | 1, 21 | <0.001 |

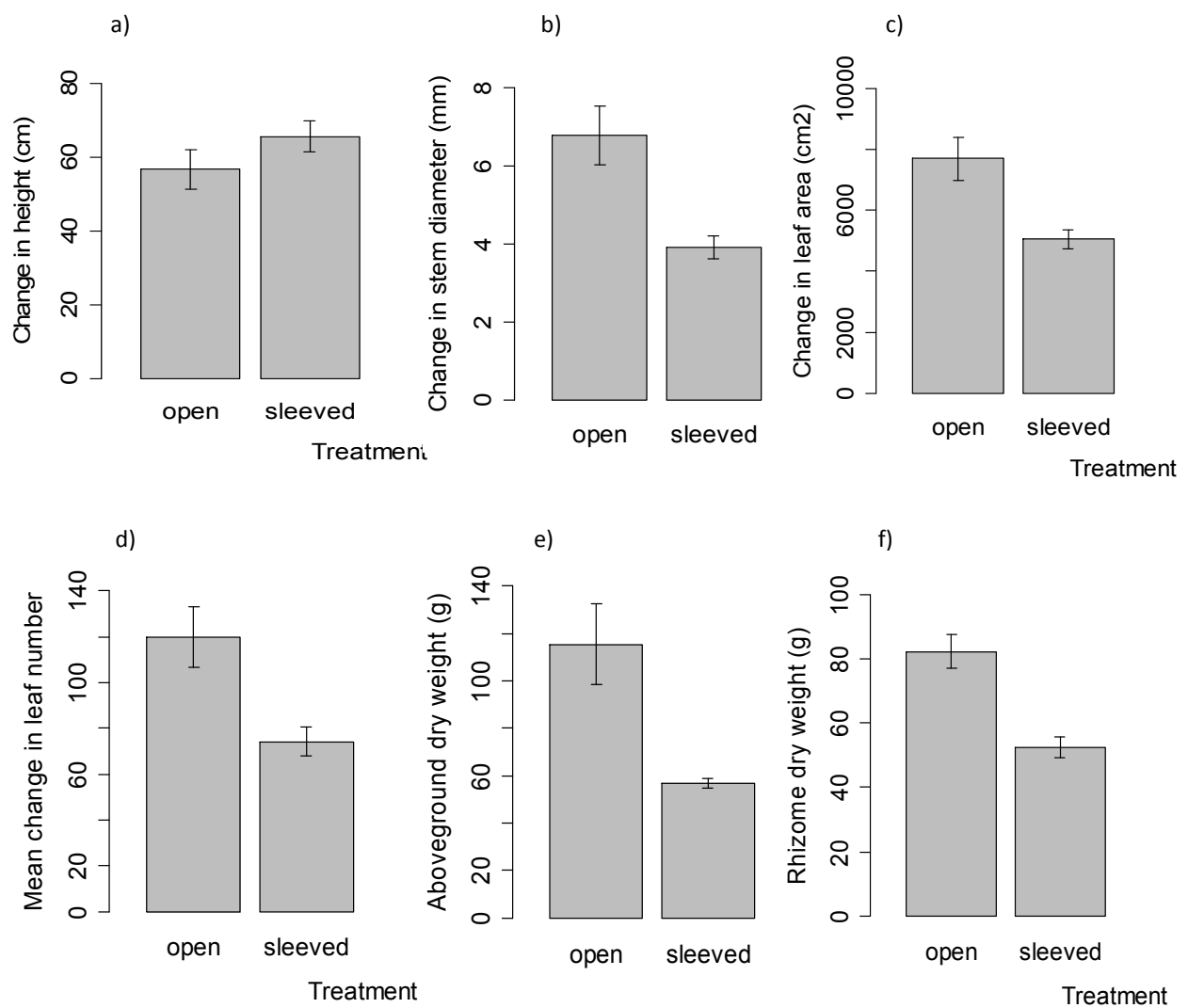


**Figure 6.3.** Impact of sleeves on growth of *F. japonica* a-f) in the absence of herbivory. Plants were grown concurrently with the herbivory impact experiment and destructively sampled at the same time.

### 6.3.2 Indirect impacts

The growth of *F. japonica* was not significantly different in the presence of the native competitors or the addition of activated carbon compared with the controls, but biomass was significantly reduced by 20% as a result of *A. itadori* herbivory ( $F_{5,60} = 2.94$ ,  $P < 0.05$ ).

The final biomass of the native plant species was significantly reduced by 40% when grown in the presence of *F. japonica* compared with controls (Fig. 6.4). This effect was not significantly altered by either the addition of activated carbon or *A. itadori* herbivory and was found for both herb and grass species and their combined total biomass (Table 6.3). There was a significant negative relationship between the biomass of the native species and the biomass of *F. japonica* ( $y = 23.58 + -0.23x$ ,  $r^2 = 0.18$ ,  $n=45$ ,  $P<0.05$ ; Fig. 6.5). The initial size of the *F. japonica* rhizomes did not significantly differ between treatments ( $F_{2,63} = 0.11$ ,  $P = 0.90$ ).

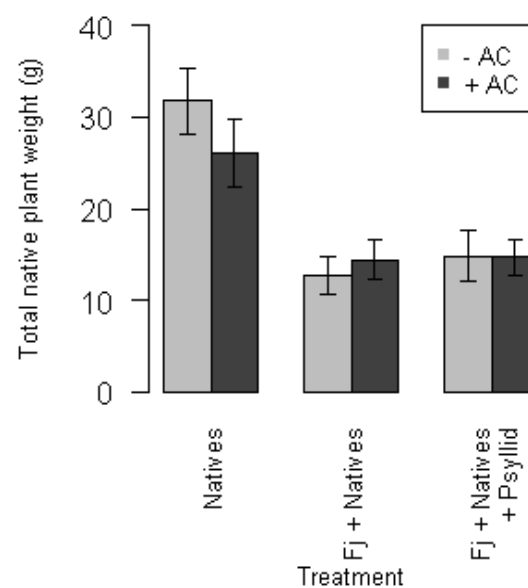


**Figure 6.4.** The dry biomass of aboveground material of artificial native communities (*G. robertianum*, *L. album*, *P. trivialis*, *S. dioica* & *U. dioica*) grown in the presence or absence of *F. japonica* (both with and without *A. itadori* herbivory) and with and without the addition of activated carbon (AC). Experimental pots were grown outside in field cages for 14 weeks before harvest. Error bars represent  $\pm 1$  S.E.

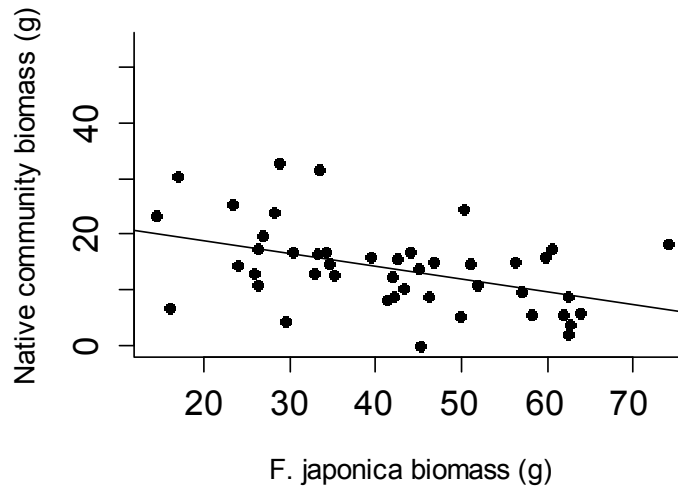


**Figure 6.5.** Relationship between total dry biomass of aboveground material of artificial native communities (*G. robertianum*, *L. album*, *P. trivialis*, *S. dioica* & *U. dioica*) grown in the presence of *F. japonica* and the total dry biomass of *F. japonica*. The line indicates a significant negative relationship;  $y = 23.58 + -0.23x$ ,  $r^2 = 0.18$ ,  $n = 45$ ,  $P < 0.05$ ).

## 6.4 Discussion

### 6.4.1 Direct target impacts

*Aphalara itadori* not only satisfies the host specificity requirements (Shaw et al. 2009) but has the capacity to significantly reduce the growth of *F. japonica* under semi-field conditions and in relatively short time periods. These impacts do correspond to the desired impacts to alleviate some of the problems caused by *F. japonica*. In particular, the reduction in rhizome growth as the belowground rhizome network is important in facilitating extensive regrowth (Beerling et al. 1994). These results build on previously reported impacts of *A. itadori* on *Fallopia* species (Grevstad et al. 2013), where similar effect sizes were found.

In the literature on control agent selection, it is now well established that ineffective control agents should not be released over concerns of indirect non-target impacts on arthropods that can be mediated through shared natural enemies (Holt & Lawton 1994) and food web disruptions (McClay & Balciunas 2005, Pearson & Callaway 2005, Raghu et al. 2006). Though, since species differ in their defences and palatability, not all potential control agents would influence food webs equally. Poorly defended species that are more likely to act as a prey subsidy for generalist predators (Tipping et al. 2011) and if they are also ineffective then could pose the

highest risk of indirect effects. As such, demonstrating the efficacy of relatively poorly defended species (such as non-lerp forming psyllids) is very relevant. Despite the potential risks in classical biocontrol indirect non-target impacts (Seymour & Veldtman 2010), it is important to consider that the presence of a new, abundant, prey species may not necessarily influence the population levels of a generalist predator and these interactions will likely be further complicated through intraguild predation (Tipping et al. 2011).

Broader control agent selection criteria, including the reporting of impact studies will likely improve the chance of releasing successful control agents. Groenteman et al. (2011) discuss the potentially missed opportunities of previously released agents that would not have been released under the current assessment structure despite proving successful at controlling the target weed without any adverse non-target effects. This highlights the potential disparity between the fundamental host range identified pre-release and the realised host range in the field and how specificity shouldn't be the only consideration in agent selection.

These results will also help manage the expectations of any stakeholders regarding field impacts. Here, demonstrable impacts were observed within just one growing season, principally as a reduction in the growing potential and size of the rhizome. These impacts could affect regrowth, and gradually weaken the plant over several years if the herbivory pressure is sufficient. It is likely then, that in the field, biocontrol would limit the spread of *F. japonica* at a local scale if *A. itadori* becomes established and abundant.

Given the habitat range of the target and the impact potential of the control agent it is likely that biocontrol will form a component of a larger integrated approach. This is particularly relevant in urban areas or development sites where complete target removal is often the aim and there are rigid timescales. Integrative weed management can give a better chance of combating the impacts of the target weed (Ainsworth 2003) if the different control methods can be suitably combined (e.g. Moran & Hoffman 2012).

**Table 6.3.** Summary of the ANOVA model results of the native plant species aboveground biomass response to planting of *F. japonica* (Fj) with and without herbivory by *A. itadori* (psyllid) and the addition of activated carbon (AC). The presence of *A. itadori* is not included as a main effect as psyllids were not placed with native plants without *F. japonica*. Total biomass is the herb and grass data pooled together.

|                   | Total biomass |      |      |        | Herb biomass |      |        | Grass biomass |     |       |
|-------------------|---------------|------|------|--------|--------------|------|--------|---------------|-----|-------|
|                   | d.f           | MS   | F    | P      | MS           | F    | P      | MS            | F   | P     |
| Fj                | 1             | 9.65 | 16.6 | <0.001 | 10.41        | 14.5 | <0.001 | 5.97          | 6.1 | <0.05 |
| AC                | 1             | 0.18 | 0.3  | 0.583  | 0.37         | 0.5  | 0.477  | 0.13          | 0.1 | 0.717 |
| Fj x psyllid      | 1             | 0.02 | 0.0  | 0.951  | 0.04         | 0.1  | 0.820  | 0.55          | 0.6 | 0.460 |
| Fj x AC           | 1             | 0.91 | 1.6  | 0.215  | 1.12         | 1.6  | 0.208  | 0.41          | 0.4 | 0.521 |
| Fj x psyllid x AC | 1             | 0.04 | 0.1  | 0.799  | 0.06         | 0.1  | 0.777  | 0.19          | 0.2 | 0.666 |
| Residuals         | 59            | 0.58 |      |        | 0.72         |      |        | 0.99          |     |       |

When considering the level of impacts on a target weed from any control method, the magnitude of the impacts of that target to the environment must also be considered. Under a cost:benefit framework, even a small reduction of a highly damaging weed can give greater returns of investment than greatly reducing a relatively less damaging weed (Page & Lacey 2006).

This study supports that *A. itadori* is a suitable classical biocontrol agent and demonstrates that it has the potential to be effective and damaging to *F. japonica*. If *A. itadori* does become established in sufficient density then it may be expected that the psyllid will limit the new growth of *F. japonica* significantly.

#### 6.4.2 Indirect impacts

There is no evidence for allelopathy in this study, despite the significantly reduced growth of the native plants when grown with *F. japonica* (Fig. 6.5). In this case, it is likely that other mechanisms, such as direct resource competition or shading effects, contributed to the strong competitive ability of *F. japonica*. Furthermore, the growth response of the native species was not altered by the presence of *A. itadori* even though the psyllid did significantly reduce the biomass of *F. japonica*. It is possible that these results are a product of the spatial and temporal scale of the experiment. These constructed communities were only together for a relatively short time and since *F. japonica* was grown from small rhizome fragments the duration of the study may not have been sufficient for any allelochemicals to have an impact. Since this study is comparable in scale to that carried out using *F. x bohemica* (Murrell et al. 2008), then this explanation would only be valid if *F. japonica* demonstrates weaker allelopathy than *F. x bohemica*.

As for the lack of response of the native species in the presence of *A. itadori* observed in the present study, this is likely to be down to the time frame. It is desirable for a biocontrol agent to have indirect beneficial impacts on native plants as simply the reduction of a target weed is rarely sufficient to meet the aims of a control programme. It is the late instar nymphs of *A. itadori* that principally damage the plant (R. Eschen; unpublished data). Late instar nymphs had only developed for

a short time when the study was ended, so communities would need to be followed for longer to see if *A. itadori* herbivory alleviates the negative impacts of *F. japonica* to native plants species, by whichever mechanism this takes place. This was not done in the present study as the principal aim was to identify the role of *F. japonica* allelopathy.

Though widely used, there are potential complications in detecting allelopathy using activated carbon. Through the addition of activated carbon, it is possible that plant performance can be influenced positively, by the addition of nutrients (Lau et al. 2008), or negatively through disruption of signalling with belowground symbionts (Wurst et al. 2010). As such, studies using activated carbon only go so far in explaining how allelopathy can structure plant communities and would ideally be carried out alongside other methods (Inderjit & Callaway 2003, Lau et al. 2008).

It is not necessarily expected that congeneric species will have equal allelopathic effects. For example, *C. maculosa*, an invasive species in North America, is known for inhibiting native species through exuding the allelochemical (-)-catechin (Bais et al. 2003). Though, *C. solstitialis*, a closely related congener, appears not to inhibit the performance of other plants through allelopathy (Qin et al. 2007). There is evidence that *F. japonica* has a lower inhibitory effect on germination of other plant species under laboratory conditions (Moravcova et al. 2011), though allelopathy can act differently on the processes of germination and plant growth (Parepa et al. 2012). In the present study, *F. japonica* allelopathy was not an important factor in inhibiting the growth of nearby plants.

Plant invasions can have complex and lasting effects on the invaded area. Successful invaders can not only demonstrate increased allelopathic impacts in invaded ranges compared with native ranges (Inderjit et al. 2008) but can also disrupt plant-soil feedbacks. Tanner & Gange (2013) discuss the knock-on effects of *F. japonica* invasions. As a species weakly dependent on arbuscular mycorrhizal fungi, sustained monocultures of *F. japonica* could lead to a depletion of the fungal network in the soil. This then has implications for the recovery and establishment of any native species which may be heavily dependent on arbuscular mycorrhizal fungi. Tanner & Gange (2013) also suggest that disturbance of the soil at invaded sites may lead to further disruption of the soil fungi community, whereas Murrell et al.

(2008) recommend frequent cutting of *F. x bohemica* to alleviate the allelopathic impacts. This highlights the need for management that is tailored to the particular target and environment as it is not safe to assume that closely related species will share traits and respond to management in the same way.

It is likely that multiple mechanisms are involved in the competitive advantages of invasive plants (Urgenson et al. 2012). *Fallopia japonica* is able to negatively affect the growth of native plants both directly, as shown in this study, and indirectly by Tanner & Gange (2013). However, there is no evidence that allelopathy plays a considerable role in this, unlike its congener *F. x bohemica*. To further understand the mechanisms involved in knotweed invasions, ecologically realistic studies should be carried out not only using *F. japonica* and *F. x bohemica* but *F. sachalinensis* as well.

## Chapter 7 – Integration of management methods

### 7.1 Introduction

#### 7.1.1 Conventional management

Depending on the particular aims of a control programme (van Klinken & Raghu 2006), a single method of management may be insufficient to have the desired results. This is particularly the case with an invasive species that occupies a variety of habitats, such as *F. japonica*, where considerations about which method to use can vary in each instance (Lym 2005). For example, *F. japonica* occupying land to be developed often needs to be removed according to a particular deadline, often using mechanical methods. Physically removing *F. japonica* fragments the plant material and there is an increased risk of unintentionally spreading the plant, so mechanical removal may not be suitable along river banks. Classical biocontrol certainly is not always successful (Julien & Griffiths 1998) and conventional methods including physical removal, herbicide application or burning have limitations and are often only effective at a local scale at considerable expense (Kettenring & Adams 2011). An integrated weed management approach has the potential to deliver greater control over the target species than the use of a single method alone (Messersmith & Adkins 1995). This has been demonstrated in multiple systems (Lym 2005; Esler et al. 2010; Herrera-Reddy et al. 2012) despite some expectations that biological and chemical control would be incompatible (Harris 1991). Classical biocontrol often forms an essential component of an integrated programme, Moran & Hoffman (2012) reviewed the use of biocontrol for the management of invasive tree species in the fynbos areas of South Africa. Ten invasive tree species were targeted, often with the use of seed feeding insects, in the region with several of them showing considerable declines (Wood & Morris 2007; Impson et al 2011).

A variety of methods are available for the conventional management of *F. japonica*. On sites where *F. japonica* needs to be eradicated over relatively short time periods, then stands can be physically dug out with the contaminated soil either buried on site with use of a weed membrane (to a minimum depth of 2m) or disposed offsite at a licensed landfill site (Environment Agency 2007). Most often, particularly in more environmentally sensitive areas, herbicides and manual cutting are used. Four herbicides are permitted for use against *F. japonica* in the UK (glyphosate, 2, 4-D

amine, Triclopyr & Picloram). Only glyphosate and 2, 4-D amine are approved for use in or near water with the former being most commonly used. Although herbicides and cutting are very destructive to the aboveground *F. japonica* material, due to the persistency and regeneration of the rhizome it can take at least 2-5 years to control a stand (Child & Wade 2000). The preferred timing of glyphosate application is mid to late in the growing season when the rhizome is acting as a sink for nutrients and when the herbicide can disrupt protein synthesis (Bashtanova et al. 2009).

Given the prevalence of conventional control of *F. japonica* and if *A. itadori* achieves widespread establishment, there will be cases where control methods are likely to be combined, whether intentionally or not. Understanding how a biocontrol agent is affected by conventional methods is important in determining the compatibility of methods as well as the combined impacts on the target plant. In some cases, the biocontrol agent performance is not directly affected by the application of herbicides (Lindgren et al. 1999; Lym 2005) but nonetheless, herbicides can reduce performance of biocontrol agents by direct toxicity or through loss of resources or altered feeding and oviposition preferences (Ainsworth 1999). The effectiveness of biocontrol can also be reduced following the use of alternative methods. For example, Larson et al. (2007) reported that populations of a biocontrol agent for *Euphorbia esula* L. in the USA were lower in plots previously treated with herbicides compared with those which were untreated. This disruption was only short term and in the space of three years *E. esula* density had recovered, as had the insect numbers.

It is also possible that a sub-lethal application of herbicides can have a positive effect on herbivore performance. The biocontrol agents *Neochetina* sp. (Coleoptera: Curculionidae), were present at lower densities in areas invaded by Water hyacinth, *Eichhornia crassipes* (Mart.) Solms, in the USA which were treated regularly with herbicides compared to those sites which were not. Despite this, Center et al. (1999) report that treated plants were of a higher nutritional value and as result the biocontrol agents had higher reproductive performance. Though, Katembo et al. (2013) investigated whether herbicide application improved the nutritional quality of the plant and increased the insect feeding rate, again with water hyacinth and *Neochetina* sp., in South Africa, but concluded there were no significant effects.

Much like the use of herbicides, physical control can affect biocontrol agent performance (Hatcher & Melander 2003). Aside from the direct removal of resources, regrowth plant material is often of a different nutritional quality and depending on the herbivore or pathogen present, this change in quality could be to their benefit or detriment. For example, *O. vitiosa* (Coleoptera: Curculionidae), a biocontrol agent of *M. quinquenervia*, is limited to feeding on regrowth plant material which has a higher nitrogen content, so cutting may increase the amount of suitable resources (Wheeler 2001). Conversely, regrowth foliage may be less palatable for herbivores due to a reduction of available nutrients (Mattson 1980). There may also be a higher resistance to pathogens in regrowth material (Green & Bailey 2000). Hatcher & Melander (2003) distinguished between plant regrowth and plant wounding and their effects on biocontrol agents. Wounded foliage is unlikely to yield nutritional benefits for herbivores but may increase infection by a pathogen. If best practise is followed during physical control efforts of *F. japonica* then wounded plant material should not be encountered but regrowth will be very common. It is important to consider the consequences of combining multiple control measures, particularly in terms of potential detrimental impacts on a biocontrol agent and their combined efficacy. Experiments both in a controlled environment and outside in field cages were carried out to test the hypotheses that *A. itadori* is susceptible to herbicides and would have a higher mortality after exposure and lower oviposition preference. The hypothesis that combining cutting, herbicide application and biocontrol of *F. japonica* would yield a greater degree of control than individual methods alone was also tested.

#### 7.1.2 Multiple biocontrol agents

Many weed biocontrol programmes release multiple biocontrol agents against the target (Julien & Griffiths 1998). There has been debate whether multiple releases improve the likelihood of success compared to single agent releases (Denoth et al. 2002) and whether multiple releases follow a Lottery model (Myers 1995). The idea of a lottery is that the more biocontrol agents released, the higher the chance that one will be effective but not necessarily with the expectation that the combination of biocontrol agents will be more effective. In many cases of releasing multiple species, most of the control is attributable to a single biocontrol agent (Myers 1995). As with combining conventional and biological control, multiple species sharing the same

resource will interact with the potential for beneficial or detrimental outcomes. Since a plant can provide multiple niches and those species targeted by classical biocontrol are usually very abundant it was thought that multiple biocontrol agents used against weeds are less likely to interfere with each other, especially compared to those used against insect pests (Denoth et al. 2002). However, Denno et al. (1995) reports competitive interactions among insects in over 90% of 45 weed biocontrol studies considered. There are examples of cases where multiple releases may have had antagonistic (Story et al. 1991), independent (Stephens & Myers 2014) or synergistic (Hoffman & Moran 1998) effects. Interactions among biocontrol agents can also be indirect. Herbivore induced changes in plant quality can have effects on other herbivores (Karban & Myers 1989) and plant damage or infection by a pathogen can alter insect fitness (Kuess 2002).

*Mycosphaerella polygoni-cuspidati* Hara, a leaf-spot pathogen, has been shortlisted as a candidate biocontrol agent for release against *F. japonica* in the UK, pending completion of host range studies. In the native range, *M. polygoni-cuspidati* is known to be damaging to *F. japonica* (Kurose et al. 2009). Symptoms include dark lesions on the leaf surface and leaf necrosis and distortion can occur at high infection rates. The life cycle of *M. polygoni-cuspidati* is complex (Fig. 7.1) and it has not been possible to complete all phases under quarantine conditions (M. Seier, personal communication). Plants may be inoculated using the spermogonia, which are present in the leaf spots. Though, suitable vectors, such as insects, are required in order to cross-fertilise spermogonia, to allow the development of the ascomata, the fruiting body of the pathogen which will contain multiple ascospores. If released, there is the potential for both biocontrol agents to have an additive impact on *F. japonica* as the periods of highest damage will likely occur at different times but also there is the potential that the presence of one could inhibit the other. In this experiment, the oviposition preferences of *A. itadori* were investigated when offered both a healthy plant and one with a light infection of *M. polygoni-cuspidati*.

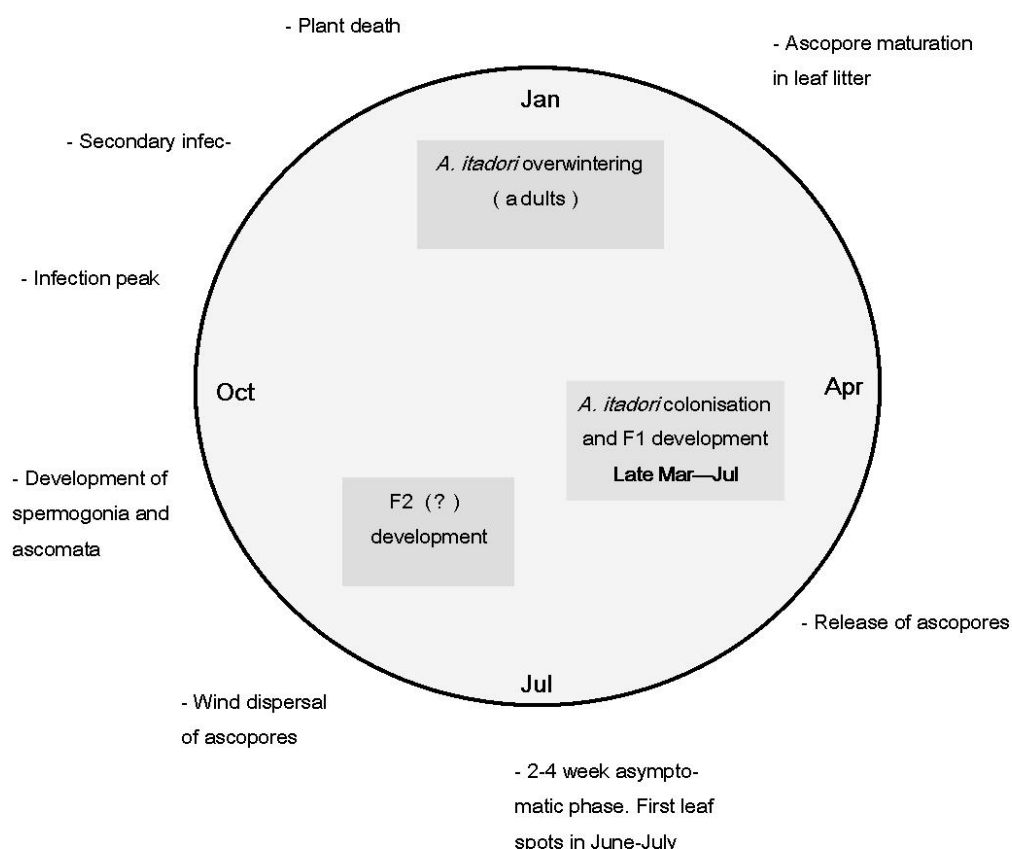


**Figure 7.1.** Overview of broad timings of potential life cycles of *A. itadori* (inside circle) and *M. polygoni-cuspidati* (outside circle) over the year. Information for *M. polygoni-cuspidati* adapted from Kurose et al. (2009).

## 7.2 Materials and methods

### 7.2.1 Conventional management

#### Toxicity trials

The survival of adults, nymphs and eggs were tested separately with two herbicides that differ in identity and concentration of active ingredient (a.i.), glyphosate (Roundup GC 120g/L a.i.; Monsanto UK, Cambridge, UK) and 2, 4-D amine (Depitox 500g/L a.i.; Nufarm, Bradford, UK). The concentrations used followed the manufacturer's advice and were 1:33.3 glyphosate: water (3.6g/L a.i.) and 1:35 2, 4-D amine: water (14.3g/L a.i.). All experiments were carried out in a controlled environment room ( $22 \pm 1^\circ\text{C}$ , 50-85% r.h. and 14:10 light:dark cycle).

For the adult survival experiment, 20 adult psyllids were placed in a tube and each randomly selected to be sprayed with three pumps from a handheld mist sprayer (4ml volume) of either tap water, glyphosate or 2, 4-D amine (n= 10 for each). Cotton wool was placed at the bottom of each tube to prevent psyllids being submerged. Each tube was then put at the base of a fresh *F. japonica* plant 15-25cm tall under individual perforated plastic cloches. Survival of psyllids was recorded for 21 days.

For the nymph survival experiment 12 first or second instar nymphs were taken from culture and placed in a 9cm Petri dish lined with filter paper and a *F. japonica* leaf. Each dish was sprayed with either tap water, glyphosate or 2, 4-D amine as above (n= 10 for each) then the nymphs were transferred to fresh *F. japonica* plants c.10cm tall under individual perforated plastic bag cloches 50cm tall. Due to space requirements, these nymphs were not followed through to adulthood but survival recorded for six days. All plants in the adult and nymph experiments were placed in plastic trays and watered as required.

Data were analysed using parametric survival models to test for differences in censored survivorship, indicating that some individuals survived beyond the end of the experiment. Models used Weibull errors which assume a non-constant hazard over time (Crawley 2007).

For the egg hatch experiment, 12 *F. japonica* plants c.20cm tall were placed under individual cloches with 20 adult psyllids taken from culture which were allowed to oviposit for seven days. Three leaves were selected from each plant and placed in 9cm Petri dishes lined with moistened filter paper. Each Petri dish was randomly assigned to be sprayed with water or either of the herbicides as above (n= 12 for each). The number of eggs on each leaf was counted using a dissecting microscope just before the treatments were applied and the subsequent numbers of first instar nymphs were recorded daily for 21 days. Nymphs were removed each day to prevent double counting.

### *Oviposition preference*

Limitations on space and materials meant that psyllid oviposition preference could only be tested for one herbicide (glyphosate) and was carried out in field cages in the CABI garden.

Two *F. japonica* plants each in ten replicates were randomly positioned at the corners of a plastic tray and covered with a gauze sleeve. One plant was a control and sprayed with water and the other was sprayed with glyphosate (at 2.4g/L a.i.) for 15s from a 1.25L Hozelock Spraymist pressure sprayer (45ml total volume applied). Plants sprayed with glyphosate were left for seven days before use and total leaf areas recorded. Thirty *A. itadori* adults were taken from culture and placed in the centre of the tray and allowed to oviposit for five days, after which all adults were removed and eggs were counted using a dissection microscope.

### *Impact trial*

A study on the combined impact of different control measures on *F. japonica* was carried out using field cages at the CABI site in Egham. Rhizome fragments containing one node, weighing between 8-18g, were planted in 3L pots at a depth of 5cm in a heated greenhouse unit for 3 weeks and watered as required. Plants were then re-potted into 6L containers and arranged outside in field cages and left for a further week. The plant height, stem diameter (1cm above soil level), leaf number and total leaf area was recorded. Each plant was randomly assigned one of three management treatments and all factorial combinations or a control (n= 10 for each). The treatments were as follows;

- *Control* – no manipulation
- *Cutting* – all aboveground material removed down to soil level at the beginning of the trial
- *Spraying* – plants sprayed with glyphosate
- *Biocontrol* – 50 *A. itadori* adults were released on the plants and left throughout.
- *Cutting + Spraying*
- *Cutting + Biocontrol* – two weeks were allowed for *F. japonica* to start to regrow before psyllids were released.
- *Spraying + Biocontrol*
- *Cutting + Spraying + Biocontrol*

In the spraying treatments, the same concentration of glyphosate was used as that used in the toxicity trials. Plants were taken to a designated area and sprayed for

15s from a 1.25L Hozelock Spraymist pressure sprayer (45ml total volume applied) and left for 30min before being returned to the field cages. The herbicide was applied 11 weeks after the start of the trial so that any differences between plants either in the presence or absence of *A. itadori* may be detected. All plants were covered individually in mesh sleeves to prevent psyllids escaping and provide a uniform environment for all treatments. All plants were harvested after 14 weeks and aboveground dried biomass and dried rhizome biomass recorded for each in addition to the plant measurements taken at the start of the experiment. Plant material was dried at 75-80°C for 72h.

### *7.2.2 Multiple biocontrol agents*

#### *Leaf spot culture*

Cultures were initiated from isolates collected from Mt. Hiko, Japan. Plugs from actively growing agar cultures were mixed with 0.2ml of sterile water and dropped into 250ml of potato dextrose broth. Flasks containing the broth were sealed with foam bungs and placed on a rotary shaker at 18°C and 170rpm for eight days. The broth was then blended for two seconds to create a more even suspension of mycelium and transferred to 30ml tubes for storage at -18°C (K. Pollard, personal communication). Frozen inoculate could be removed from storage as necessary, allowed to defrost and applied to the surface of *F. japonica* leaves using a paintbrush. Plants were then incubated in a dew chamber (Mercia Scientific, Birmingham, UK) for 48h at 22°C (four at a time and one agar plate to check viability). Inside the chamber a heat sink ensures the plants are maintained below the dew point of air, water vapour is produced from a water tray at the base. Dew then forms on the plants under high humidity and facilitates inoculation of the plants. Plants were left for 21 days before use in the experiment to allow symptoms to develop.

#### *Oviposition preference*

Oviposition choice tests were carried out in 45x45x45cm ventilated Perspex cages in a controlled environment room (24±1°C). Each replicate (n= 10) consisted of two

randomly positioned *F. japonica* plants, one control and one infected with *M. polygoni-cuspidati*. Plants were the same age and paired to be similar in size. The number of leaves and total leaf areas were recorded at the start of each trial. Thirty *A. itadori* adults were taken from culture and put in a 30ml tube. These tubes were then placed in the centre of the cage and the psyllids allowed onto the plants. After five days all adults were removed from the cage and the number of eggs on each plant counted.

## 7.3 Results

### 7.3.1 Conventional management

#### *Toxicity trials*

The application of herbicide significantly reduced the survivorship of both adults ( $\chi^2=242.21$ , d.f. = 2,598,  $P<0.001$ ) and nymphs ( $\chi^2=78.38$ , d.f. = 2,358,  $P<0.001$ ) of *A. itadori* compared with the application of tap water. Glyphosate was more toxic to the psyllids compared with 2, 4-D amine (Figs. 7.2 & 7.3). The proportion of eggs that hatched after being treated with herbicide was significantly lower than the control group ( $t = 2.23$ , d.f. = 2,33,  $P<0.05$ , GLM Quasibinomial). The application of glyphosate or 2, 4-D amine reduced hatching by 24% and 21% respectively (Fig. 7.4).

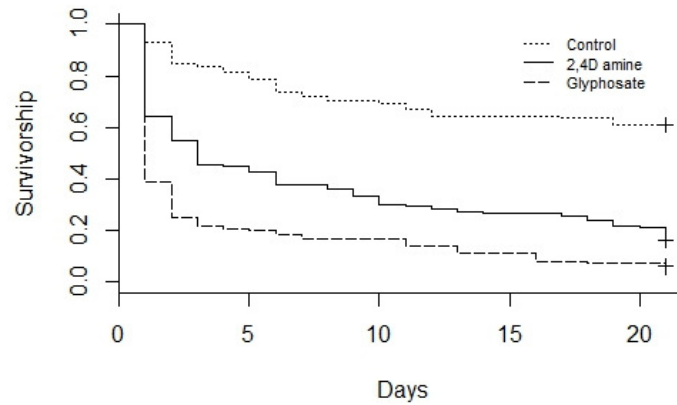


**Figure 7.2.** Kaplan-Meier survivorship curve for *A. itadori* adults sprayed with either a tap water control (dotted line), 2, 4D amine (solid line) or glyphosate (dashed line). Ten replicates of 20 psyllids were sprayed in glass tubes then released onto individual *F. japonica* plants for 21 days. + indicates that some individuals were still alive at the end of the experiment.

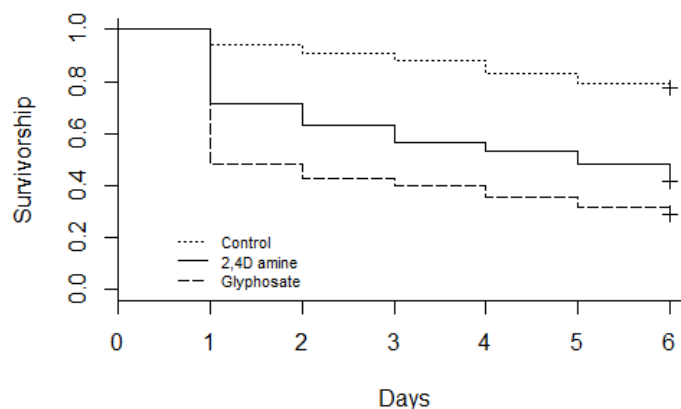


**Figure 7.3.** Kaplan-Meier survivorship curve for *A. itadori* nymphs sprayed with either a tap water control (dotted line), 2, 4D amine (solid line) or glyphosate (dashed line). Ten replicates of 12 first or second instar psyllids were sprayed in 9cm Petri dishes then transferred onto individual *F. japonica* plants for six days. + indicates that some individuals were still alive at the end of the experiment.

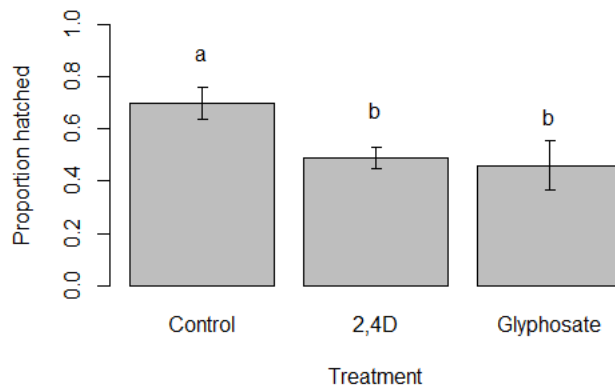


**Figure 7.4.** Mean  $\pm$  1 S.E. proportion of *A. itadori* eggs that hatched after spray application of either a tap water control, 2, 4D amine or glyphosate ( $n = 12$  each). Eggs were initially oviposited onto fresh *F. japonica* plants then individual leaves were placed in 9cm Petri dishes before being assigned to each treatment. The emergence of new first instar nymphs was recorded daily for 21days. Different letter indicate significant differences ( $P < 0.05$ ).

#### *Oviposition preference*

The proportion of eggs laid on *F. japonica* treated with glyphosate was significantly lower compared with untreated plants ( $t = 14.14$ , d.f. = 1,19,  $P < 0.001$ , GLM Quasibinomial) under choice conditions (Fig. 7.5) with a 42% reduction.

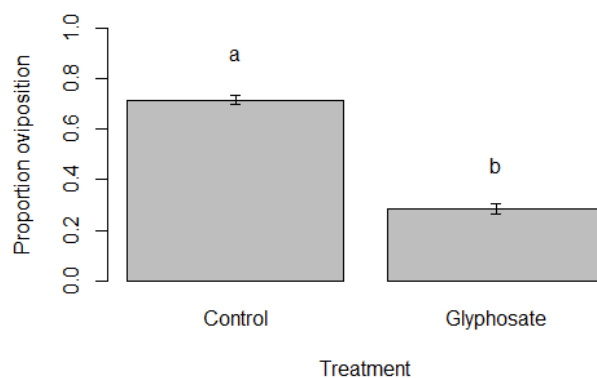


**Figure 7.5.** Mean  $\pm$  1 S.E. proportion of eggs laid on either control or herbicide treated *F. japonica* plants by thirty adult *A. itadori* psyllids allowed to oviposit for five days ( $n = 10$ ). The experiment was carried out outside in field cages. Different letters indicate significant differences ( $P < 0.05$ ).

### *Impact trial*

There were no differences between management methods for change in stem height or diameter but there were significant differences between management methods for change in leaf number, area and final above and belowground dry mass (Table 7.1). All combinations of management including cutting or spraying reduced the final leaf number and area compared to control plants and those attacked by psyllids (Fig. 7.6). The addition of psyllids alone did not significantly change the dry mass of aboveground material compared to control plants, but all other management combinations did (Fig. 7.7). Psyllids did however significantly reduce the dry mass of the rhizome compared to control plants, as did all other management combinations apart from spraying only (Fig. 7.7). There was no indication that any combination of control measures had any synergistic impacts beyond that of one of the constituent methods alone.

**Table 7.1.** Response of various plant measures of *F. japonica* to at least one method of control including cutting, spraying, biocontrol using *A. itadori* and all their combinations.

| <b>Plant measure</b>  | <b><i>F</i></b> | <b><i>d.f.</i></b> | <b><i>P</i></b>  |
|-----------------------|-----------------|--------------------|------------------|
| Change in height      | 1.62            | 7,71               | 0.15             |
| Change in diameter    | 2.13            | 7,71               | 0.052            |
| Change in leaf number | 46.46           | 7,71               | <b>&lt;0.001</b> |
| Change in leaf area   | 30.23           | 7,71               | <b>&lt;0.001</b> |
| Aboveground dry mass  | 31.66           | 7,71               | <b>&lt;0.001</b> |
| Rhizome dry mass      | 34.93           | 7,70               | <b>&lt;0.001</b> |

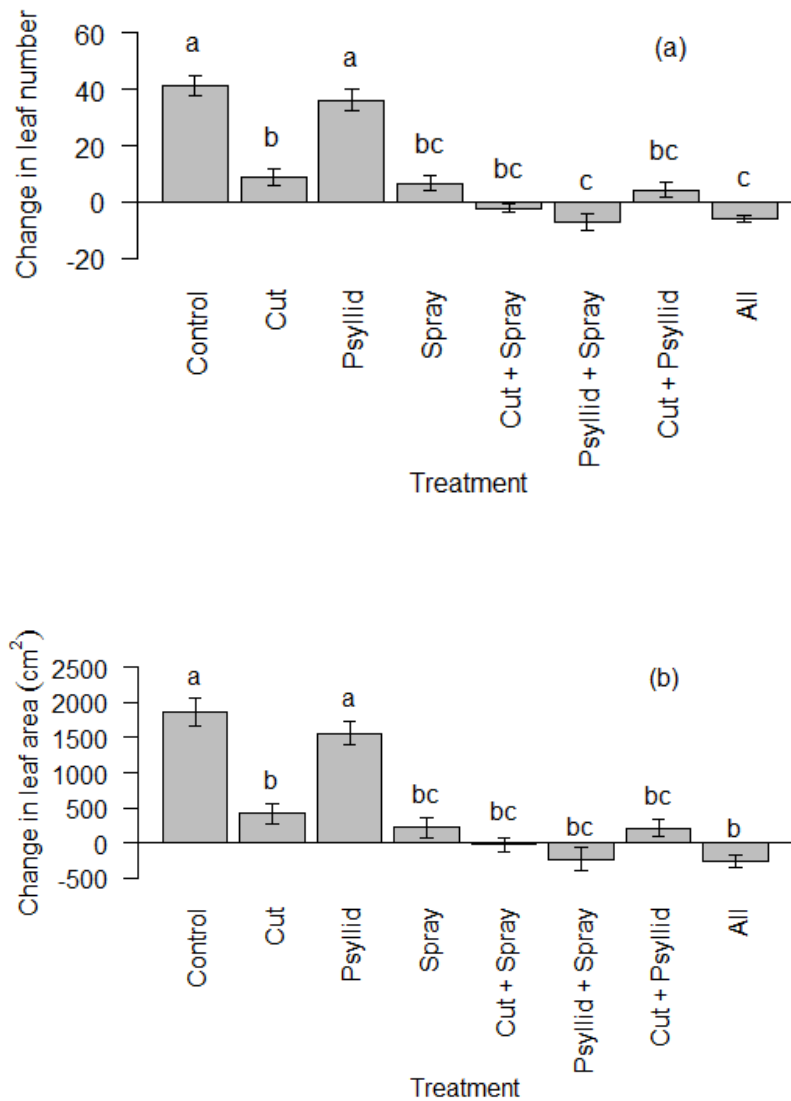


**Figure 7.6.** The change in leaf number (a) and total leaf area (b) response of *F. japonica* to different combinations of control methods (cutting at base, spraying with glyphosate or addition of 50 adult *A. itadori*). Plants were outside in field cages for 14 weeks over the summer before sampling. Boxes with different letters above are significantly different. n = 10.

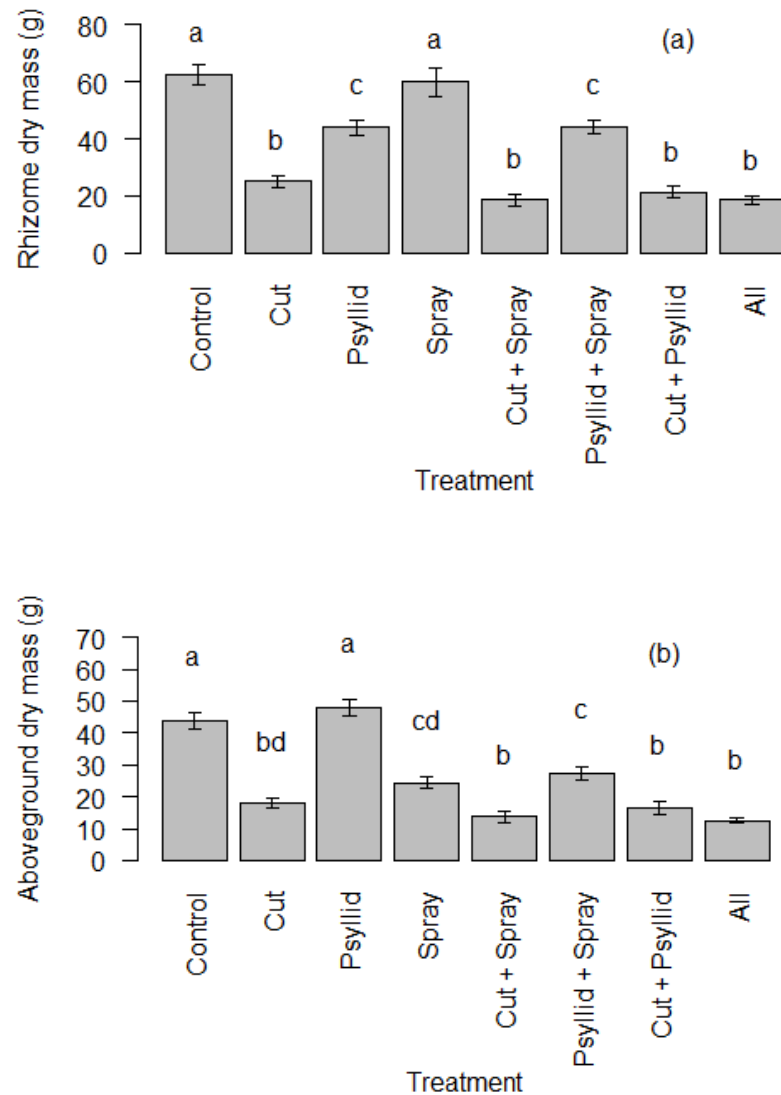


**Figure 7.7.** The rhizome dry mass (a) and aboveground material dry mass (b) of *F. japonica* under different combinations of control (cutting at base, spraying with glyphosate or addition of 50 adult *A. itadori*). Plants were outside in field cages for 14 weeks over the summer before sampling. Boxes with different letters above are significantly different.  $n = 10$ .

### 7.3.2 Multiple biocontrol agents

The proportion of eggs laid on *F. japonica* infected with *M. Polygoni-cuspidati* was significantly lower compared with uninfected plants ( $t = 2.44$ , d.f. = 1,19,  $P < 0.05$ , GLM quasibinomial) under choice conditions (Fig. 7.8).

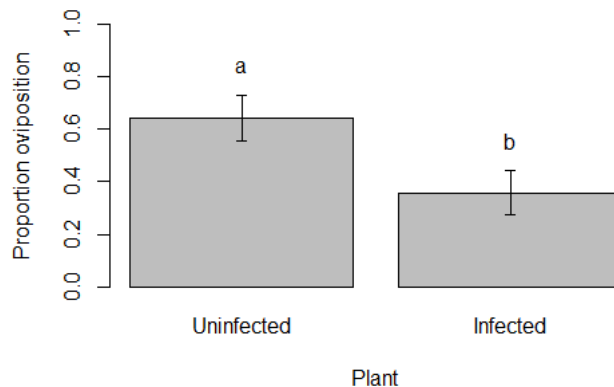


**Figure 7.8.** Mean  $\pm$  1 S.E. proportion of eggs laid on *F. japonica* plants infected or not with the leaf spot pathogen, *M. polygoni-cuspidati*. Thirty adult *A. itadori* were left to oviposit in a Perspex container with one healthy and one infected plant for five days (n = 10). Different letters indicate significant differences ( $P < 0.05$ ).

## 7.4 Discussion

### 7.4.1 Conventional management

The results of this study suggest a low compatibility between the use of *A. itadori* for biocontrol and conventional methods when directly combined, since the use of herbicides had a detrimental impact on survival at all life stages (Figs. 7.2 – 7.4). Furthermore, there appears to be no additive or synergistic impacts on *F. japonica* from combining control measures (Fig. 7.6). While at first this result appears problematic, it does not preclude the use of an integrated approach for the management of *F. japonica*. The impacts of herbicides to *A. itadori* reported here, collected under laboratory conditions, are likely to overestimate the impacts under field conditions. Additionally, integrated management can segregate the use of different control methods both spatially and across the year to minimise interference (Hatcher & Melander 2003). Timing the application of conventional methods around the life cycle of a biocontrol agent to minimise impacts can be effective. For example, Paynter (2003) demonstrates the effect of herbicide application timing on *Neurostrota gunniella* Busck (Lepidoptera: Gracillariidae), a biocontrol agent of *Mimosa pigra* L. in Australia. Three herbicides were applied at different times, post *N. gunniella* oviposition. The proportion of larvae surviving to adulthood on plants

treated nine days after oviposition was reduced from 0.5 in the control group to less than 0.1 in the herbicide treatments. However, if herbicide application was delayed until 23 days post oviposition then there were no differences between treatments. In this case, a small change in management practise avoided a considerable impact on the biocontrol agent. In the case of *F. japonica*, although some spraying regimes apply multiple herbicides throughout the growing season, autumn applications are most common. This should be emphasised where the psyllids are present as a late season use of herbicides would likely have minimal impact on immature stages of *A. itadori* compared with a spring application as they are less likely to be present. Additionally, spring applications of herbicides to *F. japonica* should be discouraged where *A. itadori* is likely to be present as it would reduce the number of suitable plants for nymph development. The two herbicides differed in their toxicity to adults and nymphs of *A. itadori* with glyphosate being more toxic. This should be considered when selecting herbicides along with other factors such as susceptibility of nearby vegetation and the persistency of the herbicide in the environment.

Timing physical control, such as cutting, to minimise the effects on the biocontrol agents, or potentially to increase their impact if for example regrowth plant material is preferred is also important. Tipping (1991) considered the combination of cutting with a seed-feeding biocontrol agent, *R. conicus* to control *Carduus thoermeri* Weinm. (syn. *Carduus nutans* L.) in the USA . Cutting at the early stages of the plant development resulted in high mortality of the biocontrol agent, whereas there was no difference in number of insects present between control and treatment groups when cutting was delayed until senescence. Furthermore, this delay facilitated *R. conicus* to significantly reduce the number of seeds produced earlier in the season. At *F. japonica* sites where an integrated approach can be used, the sequential use of different methods may well be the most effective. A spring cut of aboveground material will limit the development of the rhizome (Fig. 7.7), but leaving a small area uncut will provide a refugia for *A. itadori* until the regrowth is suitable for oviposition. A population of psyllids throughout the growing season, if undisturbed, should continue to restrict the rhizome growth (Fig. 7.7) which could be followed by an autumn application of herbicides, which at this time should have the smallest effect on *A. itadori*. Although in this study, cutting had the largest effect on *F. japonica*, it is the most labour intensive method discussed here and also the most likely to

unintentionally spread *F. japonica* through disposal of the waste material. Therefore, cutting may not be recommended for many sites and is not feasible as a control method over large areas.

The aims of land managers with a *F. japonica* invasion in an environmentally sensitive area, such as a riparian habitat in a protected area, compared with an urban development site will differ. The former will likely be ultimately looking to restore a desirable vegetation assemblage, similar to that in non-invaded areas, whereas eradication of *F. japonica* from a site would be sufficient for a developer. It depends on where the *F. japonica* invasion occurs, and the management aims, that the potential benefits of classical biocontrol differ. It is in the environmental areas, where fewer resources are available for *F. japonica* removal that biocontrol has the largest potential benefit in reducing *F. japonica* impacts on native communities and it would be more important to minimise the disruption of biocontrol from conventional methods. Furthermore, a common aim of biocontrol programmes is that the long term impacts of the biocontrol agent will reduce the costs from other management methods. This would benefit landowners regardless of which habitat has been invaded.

Integrated management can also utilise plant competition to enhance control (Manrique et al. 2009). This can be especially useful when the goal of invasive species management is the recovery of a more desired community as the removal of an invasive species alone may be insufficient (Reid et al. 2009) and active restoration efforts may be needed. Cutting & Hough-Goldstein (2013) demonstrated that the combined use of the herbivore, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), and sowing a native seed mixture had an additive impact on the performance of *Persicaria perfoliata* (L.) H. Gross, an invasive weed in the USA. The seeded treatments also had the widest diversity of establishing native plants, including species not planted. This combination of control and restoration can help to prevent secondary invasions from other non-native species, which can be a problem once free space is created following the removal of an invasive weed (Symstad 2004; Butler & Wacker 2010).

This study suffers from the same limitations as many investigating the effectiveness of invasive plant control, small scale and a relatively short time period (Kettenring &

Adams 2011). It would be useful to extend the herbicide toxicity trials to investigate susceptibility of nymphs at different instars and follow their development through to adulthood. Particularly as insect population development is likely to be restricted if exposed to herbicides. Further work is also needed to quantify the impacts of other control methods on *A. itadori* performance, such as explicitly testing the nutritional quality of *F. japonica* regrowth for *A. itadori*. Also, herbicides are not exclusively applied as a spray, stem injection treatment is also commonly used (Child & Wade 2000). If used when *A. itadori* is present then some of the direct impacts could be avoided but there may be an increase in indirect impacts, such as oviposition avoidance as seen as a response to foliar spraying in this study (Fig. 7.5), or reduced longevity.

#### 7.4.2 Multiple biocontrol agents

In this study, infection by *M. polygoni-cuspidati* had a negative impact on *A. itadori* through reduced oviposition (Fig. 7.8). If both agents were to co-occur under field conditions, the extent that *A. itadori* would be effected is unknown but should be considered. Since the symptoms of *M. polygoni-cuspidati* appear late in the season (Kurose et al. 2009), it is possible that psyllid oviposition preference in spring would not be an important factor in determining how well these two biocontrol agents could be integrated, but rather how well *A. itadori* nymphs perform on infected plants. For example, combined use of the leaf beetle *Cassida rubiginosa* Muller and pathogen *Phoma destructive* (Plowr.), two natural enemies of *C. arvensis*, would likely have antagonistic effect as insects reared on infected plants had longer development times, lower larval weight and higher pupal mortality compared to insects reared on uninfected plants (Kruess 2002).

There is always a risk of detrimental non-target effects, however small, from releasing a biocontrol agent. As such, unnecessary releases should not be made (Denoth et al. 2002), especially into systems where adequate control is already likely to be achieved or where the new biocontrol agent is likely to have a considerable impact on an existing one. However, the release of a biocontrol agent shouldn't preclude releases of additional species if there is reasonable support that they can be effectively integrated. For example, it may be possible for small but persistent

amounts of damage to an invasive weed caused by one biocontrol agent to trigger induced resistance and reduce the effectiveness of other biocontrol agents (Zidack 1999). As with any release, the damage potential of the leaf-spot, both alone and in combination with the psyllid should also be considered as there may be potential for an additive effect (Buccellato et al. 2012). It is unlikely that *M. polygoni-cuspidati* would drastically inhibit *A. itadori* populations even where they co-occur but this study has highlighted the potential for a negative interaction. Further investigation will be needed to determine how soon after *F. japonica* is infected before psyllids may be affected and also the infection rates on plants where *A. itadori* is already present. Caesar (2003) advocates the inclusion of studies on insect – pathogen interactions, especially the likelihood of synergisms, into the selection criteria of biocontrol agents.

## Chapter 8 – General discussion

### 8.1 Key findings

#### Chapter 3

- Increasing amounts of quantitative data were available from post-release monitoring programmes in the general literature. Meta-analyses indicate that recently published classical biocontrol programmes caused significant declines in target weed performance.
- Sufficient data were also available to test for the recovery of non-target plant species at release sites. After biocontrol, plant species diversity increased significantly compared with control sites. There is still not enough data available to assess non-plant taxa.

#### Chapter 4

- *Aphalara itadori* were reared in cages outside and developed fully over a period of 9-14 weeks. A mean  $\pm$  SE of  $503 \pm 12$  dd above a base of  $10^{\circ}\text{C}$  was required for development. It is likely that there could be two generations in South East England.
- Fecundity of females maintained outside was lower than that under laboratory conditions. A mean  $\pm$  SE of  $156.2 \pm 22.2$  eggs per female was recorded.
- Adult *A. itadori* were able to overwinter on senesced potted *F. japonica* as well as cut trunk sections of *Q. robur* and *P. sylvestris* under no choice conditions.

#### Chapter 5

- Periods of rainfall may reduce the abundance of *A. itadori* as significantly more eggs were displaced from leaves exposed to simulated rainfall compared with control plants.
- *Aphalara itadori* showed significant oviposition preferences for plants under different watering regimes. Plants at the extremes were preferred with more eggs laid on either drought stressed or saturated plants compared with those with an intermediate soil moisture content.

- Excluding the majority of generalist predators increased the number of *A. itadori* nymphs surviving on *F. japonica* plants in a garden environment.

## Chapter 6

- *Aphalara itadori* has the potential to cause significant declines in *F. japonica* size and growth within one season. Plants with psyllids present were significantly shorter, had a smaller leaf area and had a lower rhizome dry weight compared with plants without herbivory.
- *Fallopia japonica* significantly reduced the performance of several native plant species when grown together. Allelopathic interactions were not detected as the mechanism of this negative impact.
- The performance of native species grown with *F. japonica* was not significantly different with or without the presence of *A. itadori*, which did significantly reduce the performance of *F. japonica*.

## Chapter 7

- Herbicide applications significantly reduced the survivorship of *A. itadori* at all life stages. Glyphosate was more toxic to psyllids than 2, 4-D amine.
- *Aphalara itadori* showed a higher oviposition preference under choice conditions for control plants compared with those that had been treated with glyphosate. The psyllids also preferred not to oviposit on plants which were infected with another biocontrol candidate, the leaf spot pathogen *M. Polygoni-cuspidati*.
- Combinations of conventional and biological management of *F. japonica* were tested. Individually, cutting, herbicide application or psyllid herbivory all reduced some measures of plant performance. There was no evidence for any synergistic or additive impacts in the short term by combining more than one treatment methods. Only psyllid herbivory and cutting reduced the weight of the rhizome.

## 8.2 Discussion

Invasive species remain one of the most considerable environmental and economic threats many countries face (Vitousek et al 1997; Pimentel et al. 2001). In the UK, *F. japonica* is among the most damaging invaders (Williams et al. 2010). The decision to release *A. itadori* represents a significant step in the management of invasive plants in the EU (Sheppard et al. 2006). The findings in this thesis expand on the work carried out prior to the release of *A. itadori* regarding efficacy of the biocontrol agent and factors which may affect it. Many of the experiments in this thesis used a mesocosm of large potted plants in a garden or field cage environment. This approach does have the benefit of being relatively cheap and straightforward to carry out and allows for data to be collected in a short space of time. Experiments at this middle scale provide a good interim between laboratory studies carried out pre-release and having to wait potentially many years before sufficient data can be collected from field release sites.

However, the extent to which various outcomes of weed biocontrol projects in the field can be predicted, based on data collected under controlled conditions is extremely important. As discussed throughout this thesis, it has been host range testing predominantly considered pre-release, part of the screening for safe agents. It is unsurprising then that it is for host specificity that there appears to be the greatest degree of success, highlighted by Fowler et al (2000). In recent decades, however, there has been an increase in the number of questions being asked using data collected under controlled conditions and how these relate to the outcomes of weed biocontrol efforts in the field. Broadly, these include predicting suitable climatic envelopes, estimating the likely damage caused to the target species and impacts on the agent from external pressures such as predation or weather.

The successful prediction of post-release efficacy using small scale controlled trials has been quoted as the “holy grail” of weed biocontrol (McFadyen 1998). This is due to the fact that there are many examples where agents have become established and abundant but failed to have any effective control of the target species (Julien & Griffiths 1998). Subsequently, it was advocated that efficacy testing becomes a much more integral part of the agent selection process (Raghu et al 2006). This is in part where this thesis filled a gap in the *Fallopia japonica* control programme with the

impacts results in Chapter 6. McClay & Balciunas (2005) suggest one reason why practitioners may be reluctant to carry out impact assessments is the concern of false negatives, that an agent will be rejected but may have still been effective if released. This is possible given some of the limitations of evaluating impact under artificial conditions. For example, Hough-Goldstein et al. (2008) demonstrated that *R. latipes*, a curculionid biocontrol agent of *P. perfoliata*, had only relatively minor impacts on seed production over one growing season in field cage trials when used alone, but subsequent trials combining herbivory with plant competition resulted in up to 75% target mortality. It is conceivable that in this case, *R. latipes* may have been rejected if considered in isolation only. Undoubtedly, experiments carried out under controlled conditions fail to capture the complexity of natural systems and there may well be both false negatives and false positives in agent selection. Nonetheless, I would suggest that some level of efficacy screening be included in all weed biocontrol programmes as a minimum to provide some baseline data in which field performance could be compared. Additionally, the use of data collected under controlled conditions can help calibrate expectations of performance, be it impact to the host or survival etc. by comparing changes in response between different increments each more resembling natural conditions (e.g. Manrique et al. 2011). Since the goal of a biocontrol programme will include the control of the target species at the field scale, a biocontrol agent that is effective only in an artificial environment would not be deemed successful, though, field cage studies can be very useful for predicting expected outcomes and informing best practise. It is also worth remembering that even relatively minor impacts on *F. japonica* cause by *A. itadori* in the field could be considered successful if considered under a cost benefit framework as the disruption caused by *F. japonica* is considerable.

*Aphalara itadori*, as a biocontrol agent of *F. japonica*, has thus far failed to establish a persistent population or cause noticeable damage to its host in the field (R. Shaw, personal communication). This is despite the successful reproduction and impacts on *F. japonica* demonstrated in this thesis, that suggest that *A. itadori* has the potential to establish in the south of England and be a successful biocontrol agent. Competition, predation and plant quality were all shown to reduce the performance of *A. itadori* but it is unclear whether it is any of these factors, individually or in combination, alongside abiotic conditions that explain the failure thus far for *A. itadori*

to establish in the field. These are important considerations as the outcome of the classical biocontrol efforts of *F. japonica* may have some considerable consequences for weed biocontrol in Europe more generally. As discussed in this thesis the regulation and release of *A. itadori* in the UK represents a step-change in European invasive weed management as it widens the available tools which can be used, in line with many other countries outside Europe. It is perhaps both surprising and unsurprising that Europe seems so far behind the rest of the world as a recipient of weed biocontrol, on the one hand, there has been a long history of releasing biocontrol agents against arthropod pests in European countries (Greathead & Greathead 1992). This widespread use of biocontrol for arthropod pests is in contrast to the lack of clear legislation and general public unease regarding releases against plant targets. Perhaps the risks for arthropod control have been perceived to be lower given its prevalence in protected cropping or horticultural settings, nonetheless there appears to have been a political and public blind-spot to plant pathogens and herbivores as beneficial species. On the other side, unlike some of the other countries which had led the way for weed biocontrol, Europe is an intensively managed, fragmented area with a dense population. This may have limited the scope for relatively few invasive weeds from occupying vast areas of land and having a very visible impact on the landscape, coupled with the strong horticultural heritage in many European countries and the UK in particular, it is perhaps unsurprising there has been some degree of stagnation to manage invasive plants at a landscape scale. Nevertheless, the detrimental impacts of invasive weeds have been more widely recognised in recent decades and irrespective of the previous impetus for weed biocontrol, there will most likely be a need to integrate every available method, including classical biocontrol, for effective weed management in the future.

By setting a precedent, the *F. japonica* biocontrol programme should be able to open the way for similar biocontrol efforts against other candidate weeds in Europe, which are not few or trivial (Sheppard et al. 2006). The release of *A. itadori* does demonstrate that it is possible to carry out classical weed biocontrol under the current legislative framework in the UK, but also serves to highlight that while possible, the regulatory process is far from straight forward. Shaw et al. (2011) outlined some of the lessons to be learnt from the *F. japonica* programme for a pre-

release phase, such as more tailored legislation. Streamlining the regulation, perhaps by following the model of countries such as New Zealand, is certainly one way to help facilitate the uptake and will be especially important if the use of plant pathogens as biocontrol agents is to move forward. However, it will also be important to set in place the necessary support for complete biocontrol programmes, beyond just the identification and release of agents. This has been a common theme throughout this thesis that delivering and understanding effecting weed control relies of investment during the post-release phase. It has previously been suggested it is up to biocontrol practitioners to communicate the importance of post-release work (Morin et al. 2009) and this is still largely true. Not only must the science behind a control programme be sound, but considerable effort must be placed into how the work is communicated and implemented as rarely are the challenges purely ecological.

Several years after the initial release, it is still very unclear whether *A. itadori* will prove to be a persistent and effect biocontrol agent. While there have been no observed non-target effects in the field, which should serve to give confidence in the host-range testing to those unfamiliar with biocontrol, there has also been no demonstrable impacts in the field to *F. japonica*. It is now well established that ineffective biocontrol agents should not be released wherever possible (McClay & Balciunas 2005) and though this thesis does serve to establish that *A. itadori* can fundamentally be an effective agent, ultimately there is still the missing information collected at the open field scale. Given the results of this thesis, it is unlikely that *A. itadori* would establish large self-sustaining natural populations if continued to be released in the existing way. Ideally, a *more, bigger, better* approach to release would be possible by releasing an adequate number of individuals (which is likely true for the current programme) but over more sites and providing some initial protection against natural enemies. We do know that *A. itadori* can reproduce in the UK, restrict the growth of *F. japonica*, overwinter successfully but until a more thorough release effort can be permitted and supported I would not suggest ruling out *A. itadori* as an effective biocontrol agent. There are still some fundamental aspects of the biology of *A. itadori* which are still unknown and would warrant further investigation, especially if self-sustaining field populations are identified. In particular, the dispersal capability of the psyllid could have a considerable effect on its

effectiveness as a control agent and how quickly new patches of *F. japonica* could be colonised and whether movement may be in the region of a few kilometres a year, similar to other psyllid agents (Center et al. 2006) or otherwise.

Psyllids are palatable to a wide range of predators (Hodek & Honek 2009) so it is likely that the top-down predation pressure could be severely inhibiting establishment, particularly when psyllid populations are only present at low densities. Field studies manipulating predator presence or density, similar to that detailed in Chapter 6 but at a larger scale, would quickly reveal whether generalist predation is a barrier to establishment. Additionally, a treatment that initially contains psyllids on a patch before allowing dispersal and predators to immigrate would assess the effectiveness of a soft release for *A. itadori* should be considered as part of the strategy for future releases. Further investigation into how *A. itadori* interacts with predators in its released range would be very interesting, not only in terms of the efficacy of control but also ecologically. *Fallopia japonica* possesses extrafloral nectaries, structures which serve to attract natural enemies when the plant is suffering from a high herbivore load, which has been demonstrated in the native range (Kawano et al. 1999). It is unclear how these interactions may be structured in the UK if *A. itadori* becomes established, but the potential is there for extrafloral nectaries to become attractants to natural enemies. In particular, as a common ant species, *L. niger*, was frequently seen investigating new patches of knotweed (*personal observation*) and may interact with *A. itadori* which would be of interest more generally to understand any potential food web effects.

Despite the benefits of carrying out small-scale experiments immediately post-release, such as those included in this thesis, there are limitations. Particularly with *F. japonica*, plants grown for field cage studies can suffer from pot effects if used for more than one season. This makes it difficult to attribute changes in plant performance to an experimental treatment and therefore, impacts from psyllid herbivory were not determined for cumulative years. Consequently, there is little substitute for well designed, manipulative field studies. Invasion biology generally has suffered from a lack of manipulative studies (Kareiva 1996) so it is difficult to make predictions on how well different species will perform. The same is also true of classical biocontrol releases, which are essentially intentional and, initially managed, biological invasions.

How various factors affect the survival and performance of *A. itadori* are presented here, yet each importance of each of these factors can be modified by complex interactions under natural conditions and are an important consideration. For example, Jamieson et al. (2012) recently carried out an experimental study alongside impact monitoring using the *Linaria dalmatica* (L.) P. Mill. (Plantaginaceae) biocontrol system. Levels of nitrogen availability and interspecific plant competition were manipulated to determine their effects on herbivore damage by *Mecinus janthinus* Germar (Coleoptera: Curculionidae). It was determined that *M. janthinus* has the potential to cause significant damage to its host if present in sufficient density, however, *L. dalmatica* showed a marked positive response to the addition of nitrogen and top-down control of this species may well be reduced in high nutrient areas.

Understanding the outcome of potential interactions between factors such as predation, plant quality, competition and so on, may have implications for how biocontrol is implemented and also perceived. Data collected under controlled or partially controlled conditions (field cage), as it was for the majority of work in this thesis, undoubtedly does have a role to play in identifying which factors are most likely to be important or some fundamental aspect of the study system and can be gathered relatively quickly and cheaply. However, the artificial nature of these types of study means there will always be more uncertainty about interactions compared with factorial field experiments. Ideally, releases would be made as part of a large scale manipulative field experiment, incorporating additional factors of likely importance and in the case of *A. itadori* the role of natural enemies and alternative control methods have been identified. It may be the case that owing to how different factors interact at different scales that effective control in one place may not predict effective control elsewhere, even for the same biocontrol agents and target combinations. This may lead to context dependent control, for example Shea et al. (2005) suggested that population level variation in plant life history traits may explain the mixed control success of Nodding thistle, but other cases where different predator assemblages, nutrient levels, management practises etc. and their combinations could equally determine the outcome of biocontrol.

Looking forward from this thesis. For the biocontrol programme of *F. japonica* in the UK, it seems prudent to persist with attempts to establish *A. itadori* at least until

every effort to aid establishment has been taken considering the problem of few release sites and top-down natural enemy pressures. Perhaps if there is still no establishment after these additional efforts, only then would diverting resources into an alternative agent, *M. polygoni-cuspidati*, or even other targets. The results from this thesis have served to inform several important aspects of the control and release strategy but also highlighted the limitations of solely relying on data collected under control conditions and not paired with factorial experiments under natural conditions, without which it is extremely difficult to understand the system with confidence. The lack of evident control in the first few years of the *F. japonica* control programme may have been a setback to propelling the use of weed biocontrol in Europe but regardless, this project has been extremely successful in increasing public and political awareness of the costs of invasive species and how they can be better controlled. It is hoped that weed biocontrol in general, will continue on an upward trajectory in Europe following on from the release of *A. itadori* and take note of the lessons to be learnt regarding the improvement of regulation and scale of research to assess and improve effectiveness. There is an opportunity in Europe not only to catch up with the rest of the world but move ahead. For this to happen there needs to be continued (and expanded) collaboration between the relatively few deliverers of classical weed biocontrol in Europe and academic institutions and additionally, utilisation of the expertise of North American and Australasian organisations whom have centres in Europe as part of their 'native' range work.

Integrating biocontrol systems and experimental ecology is certainly not a new idea and has been advocated by Crawley (1989) and again more recently by Fowler et al. (2012) but is still underutilised. The potential for collaborations are not restricted to applied biologists either. There has been a vast amount of data collected for exploratory surveys and host range testing about the host specificity of insect faunas associated with particular plants. This bank of information regarding the numbers of specific species recorded for different hosts, as well as the response of monophagous species once released into a new environment could be more widely utilised by evolutionary ecologists interested in feeding specialisation. This would increase the value of data collected routinely as part of a biocontrol programme (Schaffner 2001).

As discussed above, the work carried out in this thesis was conducted as part of a collaborative effort to the benefit of all parties involved, including academic partners, biocontrol practitioners, the funding bodies of the *F. japonica* biocontrol programme in the UK as well as other interested stakeholders. Evidence supporting the use of classical biocontrol of weeds, as an effective management method, was presented in Chapter 3. Also, by expanding on the work carried out prior to the release of *A. itadori*, this thesis contributes to the applied field of weed biocontrol and is useful for informing the biocontrol programme's release strategy. In particular, the fundamental development and impact data support that *A. itadori* could be an effective biocontrol agent of *F. japonica* in the UK and changes to the release strategy are advocated, such as the use of predator exclusion measures, which may increase the likelihood of establishment success. In addition, this work provides the basis for future research, relevant to both applied and pure ecology.

## References

- Adachi, N., Terashima, I., & Takahashi, M. (1996).** Central die-back of monoclonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. *Annals of Botany*, 77, 477-486.
- Aguilera, A.G., Alpert, P., Dukes, J.S., & Harrington, R. (2010).** Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. *Biological Invasions*, 12, 1243– 1252.
- Ainsworth, N. (1999).** Potential for integrated control of horehound *Marrubium vulgare* L. by the plume moth *Wheeleria spilodactylus* (Curtis) and 2, 4-D. *Biocontrol Science and Technology*, 9, 355-364.
- Ainsworth, N. (2003).** Integration of herbicides with arthropod biocontrol agents for weed control. *Biocontrol Science and Technology*, 13, 547-570.
- Aldridge, E. (2012).** Effects of water-stress and rainfall on the potential establishment and impact of a classical biological control agent of Japanese knotweed. Unpublished MSc thesis, Imperial College London.
- Ashton, F.L. (1936).** Selenium as a catalyst in the Kjeldahl method as applied to soil and grass analysis. *Journal of Agricultural Science*, 26, 239-248.
- Awmack, C.S. & Leather, S.R. (2002).** Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, 47, 817-844.
- Baba, K. & Miyatake, Y. (1982).** Survey of adult psyllids in winter in Niigata prefecture. *Transactions of the Entomological Society of Japan*, 54, 55–62.
- Bailey, J.P. & Conolly, A.P. (2000).** Prize-winners to pariahs - A history of Japanese Knotweed s.l. (Polygonaceae) in the British Isles. *Watsonia*, 23, 93-110.

**Bailey, J. P., Bímová, K., & Mandák, B. (2007).** The potential role of polyploidy and hybridisation in the further evolution of the highly invasive *Fallopia* taxa in Europe. *Ecological research*, 22, 920-928.

**Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003).** Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, 301, 1377–1380.

**Balciunas, J.K. (2000).** A proposed Code of Best Practices for classical biological control of weeds. In *Proceedings of the X International Symposium on Biological Control of Weeds* (ed N.R. Spencer), 4-14 July 1999, pp. 435–436. United States Department of Agriculture, Agricultural Research Services, Sidney, MT and Montana State University, Bozeman, MT, USA.

**Bale, J. (2002).** Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 357, 849–62.

**Bale, J. (2011).** Harmonization of regulations for invertebrate biocontrol agents in Europe: progress, problems and solutions. *Journal of Applied Entomology*, 135, 503-513.

**Barton, J., Fowler, S., Gianotti, A., Winks, C., Debeurs, M., Arnold, G. & Forrester, G. (2007).** Successful biological control of mist flower (*Ageratina riparia*) in New Zealand: Agent establishment, impact and benefits to the native flora. *Biological Control*, 40, 370-385.

**Bashtanova, U.B., Beckett, K.P. & Flowers, T.J. (2009).** Review: Physiological Approaches to the Improvement of Chemical Control of Japanese Knotweed (*Fallopia japonica*). *Weed Science*, 57, 584–592.

**Beck, S.D. (1983).** Insect thermoperiodism. *Annual review of entomology*, 28, 91-108.

**Beerling, D.J., Bailey, J.P. & Conolly, A.P. (1994).** *Fallopia japonica* (Houtt.) Ronse Decraene. *Journal of Ecology*, 82, 959-979.

**Bímová, K., Mandák, B., & Kašparová, I. (2004).** How does Reynoutria invasion fit the various theories of invisibility? *Journal of Vegetation Science*, 15, 495-504.

**Birkin, L. (2008).** Thermal tolerances of the psyllid *Aphalara itadori* and its establishment potential as a biological control agent for Japanese knotweed (*Fallopia japonica*) in the UK. Unpublished MSc thesis, Imperial College London, UK.

**Blossey, B. & Notzold, R. (1995).** Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. *Journal of Ecology*, 83, 887-889.

**Blossey, B. & Skinner, L. (2000).** Design and Importance of Post-Release Monitoring. In *Proceedings of the X International Symposium on Biological Control of Weeds* (ed N.R. Spencer), 4-14 July 1999, pp. 693-706. United States Department of Agriculture, Agricultural Research Services, Sidney, MT and Montana State University, Bozeman, MT, USA.

**Blumenthal, D. (2005).** Interrelated causes of plant invasion. *Science*, 310, 243-244.

**Briese, D. T. (2005).** Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. *Biological Control*, 35, 208-214.

**Broom, J.W. (2009).** Secondary Competition and Oviposition Studies of the Psyllid *Aphalara itadori*, a Novel Biological Control Agent. Unpublished MSc Thesis, Imperial College London, UK.

**Buccellato, L. (2012).** An insect-plant pathogen interaction between two biocontrol agents, the stem-gall fly and leaf-spot pathogen released against crofton weed, *Ageratina adenophora*. Unpublished Doctoral dissertation, University of the Witwatersrand, South Africa.

**Burckhardt, D. & Lauterer, P. (1997).** Systematics and biology of the *Aphalara exilis* (Weber and Mohr) species assemblage (Hemiptera: Psyllidae). *Entomological Scandinavica*, 28, 271–305.

**Butler, J.L. & Wacker, S.D. (2010).** Lack of native vegetation recovery following biological control of leafy spurge. *Rangeland Ecology and Management*, 63, 553–563.

**Byrne, M. J., Coetzee, J., McConnachie, A. J., Parasram, W., & Hill, M. P. (2004).** Predicting climate compatibility of biological control agents in their region of introduction. In *Proceedings of the XI International Symposium on Biological Control of Weeds* (eds Cullen, J.M., Briese, D.T. Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K.), 27 April – 2 May 2003, pp. 28-35. CSIRO Entomology, Canberra, Australia.

**CABI (2007).** Natural control of Japanese knotweed. CABI Europe, Ascot, UK

**Caesar, A.J. (2003).** Synergistic interaction of soilborne plant pathogens and root-attacking insects in classical biological control of an exotic rangeland weed. *Biological Control*, 28, 144-153.

**Callaway, R.M. & Aschehoug, E.T. (2000).** Invasive Plants Versus Their New and Old Neighbours: A Mechanism for Exotic Invasion. *Science*, 290, 521-523.

**Callaway, R.M. & Maron, J.L. (2006).** What have exotic plant invasions taught us over the past 20 years? *Trends in Ecology & Evolution*, 21, 369-74.

**Callaway, R.M. & Ridenour, W.M. (2004).** Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, 2, 436–443.

**Cameron, P.J., Hill, R.L., Bain, J. and Thomas, W.P. (1993).** Analysis of importations for biological control of insect pests and weeds in New Zealand. *Biocontrol Science and Technology*, 3, 387–404.

**Carson, W.P., Hovick, S.M., Baumert, A.J., Bunker, D.E. & Pendergast, T.H. (2008).** Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. *Arthropod-Plant Interactions*, 2, 77-86.

**Carvalho, L.G., Buckley, Y.M., Ventim, R., Fowler, S.V. & Memmott, J. (2008).** Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters*, 11, 690-700.

**Carvalho, L.G., Seymour, C.L., Veldtman, R., & Nicolson, S.W. (2010).** Pollination services decline with distance from natural habitat even in biodiversity-rich areas. *Journal of Applied Ecology*, 47, 810-820.

**Catford, J.A., Jansson, R. & Nilsson, C. (2009).** Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions*, 15, 22–40.

**Center, T.D., Dray, F.A., Jubinsky, G., & Grodowitz, M.J. (1999).** Biological control of waterhyacinth under conditions of maintenance management: Can herbicides and insects be integrated? *Environmental Management*, 23, 241-256.

**Center, T.D., Pratt, P.D., Tipping, P.W., Rayamajhi, M.B., Van, T.K., Wineriter, S.A., Dray Jr, F.A. & Purcell, M. (2006).** Field colonization, population growth, and dispersal of *Boreioglycaspis melaleucae* Moore, a biological control agent of the invasive tree, *Melaleuca quinquenervia* (Cav.) Blake. *Biological Control*, 39, 363-374.

**Center, T.D., Purcell, M.F., Pratt, P.D., Rayamajhi, M.B., Tipping, P.W., Wright, S.A. & Dray, F.A. (2012).** Biological control of *Melaleuca quinquenervia*: an Everglades invader. *BioControl*, 57, 151-165.

**Chapman, E.G., Romero, S.A. & Harwood, J.D. (2010).** Maximizing collection and minimizing risk: does vacuum suction sampling increase the likelihood for misinterpretation of food web connections? *Molecular ecology resources*, 10, 1023-1033.

**Chen, S., Xiao, S. & Callaway, R.M. (2012).** Light intensity alters the allelopathic effects of an exotic invader. *Plant Ecology & Diversity*, 5, 521–526.

**Child, L. & Wade, M. (2000).** *The Japanese Knotweed Manual*. Packard Publishing Limited. Chichester, UK.

**Colautti, R.I., Ricciardi, A., Grigorovich, I.A., & MacIsaac, H.J. (2004).** Is invasion success explained by the enemy release hypothesis? *Ecology letters*, 7, 721-733.

**Coley, P.D., Bateman, M.L. & Kursar, T.A. (2006).** The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos*, 115, 219-228.

**Crawley, M. J. (1989).** The successes and failures of weed biocontrol using insects. *Biocontrol News and Information*, 10, 213-223.

**Crawley, M. J. (1990).** Plant life-history and the success of weed biological control projects. In *Proceedings of the VII International Symposium on Biological Control of Weeds* (ed Delfosse, E.), 6-11 March 1988, pp. 17-26. Ministry of Agriculture, Forestry, Rome, Italy & CSIRO, Melbourne, Australia.

**Crawley, M. J. (2007).** The R book Chichester. UK: Wiley.

**Crider, K. (2011).** Predator interference with the cinnabar moth (*Tyria jacobaeae*) for the biological control of tansy ragwort (*Senecio jacobaea*). *Invasive Plant Science and Management*, 4, 332-340.

**Crooks, J. A. (2005).** Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Ecoscience*, 12, 316-329.

**Culliney, T.W. (2005).** Benefits of classical biological control for managing invasive plants. *Critical Reviews in Plant Sciences*, 24, 131-150.

**Cutting, K.J. & Hough-Goldstein, J. (2013).** Integration of biological control and native seeding to restore invaded plant communities. *Restoration Ecology*, 21, 648-655.

**DAISIE (2013).** Delivering alien species inventories for Europe. Accessed from <http://www.europe-aliens.org/> March 2013.

**Dassonville, N., Vanderhoeven, S., Gruber, W. & Meerts, P. (2007).** Invasion by *Fallopia japonica* increases topsoil mineral nutrient concentrations. *Ecoscience*, 14, 230–240.

**De Bach, P. (1964).** Biological control of insects, pests and weeds. Chapman & Hall, London, UK.

**De Clerck-Floate, R.A., Mason, P.G., Parker, D.J., Gillespie, D.R., Broadbent, A.B. & Boivin, G. (2006).** Guide for the importation and release of arthropod biological control agents in Canada. *Agriculture and Agri-Food Canada*, Ottawa, Canada.

**Defra (2003).** Review of non-native species policy-report of the working group. PB8072

**Dennis, B. (1989).** Allee effects: population growth, critical density, and the chance of extinction. *Natural Resource Modelling*, 3, 481–538.

**Denno, R.F., McClure, M.S. & Ott, J.R. (1995).** Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual review of entomology*, 40, 297-331.

**Denno, R.F., Peterson, M.A., Gratton, C., Cheng, J.A., Langellotto, G.A., Huberty, A.F. & Finke, D.L. (2000).** Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology*, 81, 1814-1827.

**Denoth, M., Frid, L. & Myers, J.H. (2002).** Multiple agents in biological control: improving the odds? *Biological control*, 24, 20-30.

**Denslow, J.S. & D'Antonio, C.M. (2005).** After biocontrol: assessing indirect effects of insect releases. *Biological Control*, 35, 307–318.

**Derraik, J.G. (2007).** *Heracleum mantegazzianum* and *Toxicodendron succedaneum*: plants of human health significance in New Zealand and the National Pest Plant Accord. *Journal of the New Zealand Medical Association*, 120.

**Diaz, R., Overholt, W.A., Hahn, D. & Samayoa, A.C. (2011).** Diapause Induction in *Gratiana boliviana* (Coleoptera: Chrysomelidae), a Biological Control Agent of Tropical Soda Apple in Florida. *Annals of the Entomological Society of America*, 104, 1319–1326.

**Dodd, A.P. (1959).** The biological control of prickly pear in Australia. In *Biogeography and ecology in Australia* (pp. 565-577). Springer Netherlands.

**Dray, M.W. (2010).** Fighting the alien invasion: An investigation into the biological control of Japanese knotweed by *Aphalara itadori*. Unpublished MSc Thesis, Imperial College London, UK.

**Dudley, T.L., & Bean, D.W. (2012).** Tamarisk biocontrol, endangered species risk and resolution of conflict through riparian restoration. *BioControl*, 57, 331-347.

**Duncan, R.P., & Williams, P.A. (2002).** Ecology: Darwin's naturalization hypothesis challenged. *Nature*, 417, 608-609.

**Ehrenfeld, J.G. (2003).** Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, 6, 503-523.

**Environment Agency (2007).** Managing Japanese knotweed on development sites. [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/296930/LIT\\_2695\\_df1209.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/296930/LIT_2695_df1209.pdf). Accessed March 2014.

**EPPO (2010).** PM 6/2 (2): Import and release of non-indigenous biological control agents. *EPPO Bulletin*, 40: 335–344.

**Esler, K.J., Van Wilgen, B.W., Roller, K S., Wood, A.R. & Van Der Merwe, J.H. (2010).** A landscape-scale assessment of the long-term integrated control of an invasive shrub in South Africa. *Biological invasions*, 12, 211-218.

**Evans, H.C. (2008).** The endophyte-enemy release hypothesis: implications for classical biological control and plant invasions. In *Proceedings of the XII International Symposium on Biological Control of Weeds* (eds Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L. & Rector, B.G.), 23-27 April 2007, pp 20-25. CAB International, Wallingford, UK.

**Ewel, J.J. & Putz, F.E. (2004).** A place for alien species in ecosystem restoration. *Frontiers in Ecology and the Environment*, 2, 354-360.

**Fan, P., Hostettmann, K. & Lou, H. (2010).** Allelochemicals of the invasive neophyte *Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae). *Chemoecology*, 20, 223–227.

**Fauvergue, X., Vercken, E., Malausa, T. & Hufbauer, R.A. (2012).** The biology of small, introduced populations, with special reference to biological control. *Evolutionary applications*, 5, 424-443.

**Ferran, A. & Dixon, A. F. G. (1993).** Foraging behaviour of ladybird larvae (Coleoptera: Coccinellidae). *European Journal of Entomology*, 90, 383-402.

**Fiedler, A.K., Landis, D.A. & Arduser, M. (2011).** Rapid shift in pollinator communities following invasive species removal. *Restoration Ecology*, 20, 593–602.

**Florens, F.B.V., Mauremootoo, J.R., Fowler, S.V., Winder, L. & Baider, C. (2010).** Recovery of indigenous butterfly community following control of invasive alien plants in a tropical island's wet forests. *Biodiversity and Conservation*, 19, 3835–3848.

**Fowler, S.V., Syrett, P. & Hill, R.L. (2000).** Success and safety in the biological control of environmental weeds in New Zealand. *Austral Ecology*, 25, 553-562.

**Fowler, S.V., Paynter, Q., Hayes, L., Dodd, S., & Groenteman, R. (2010).** Biocontrol of weeds in New Zealand: an overview of nearly 85 years. In *Seventeenth Australasian Weeds Conference* (ed Zydenbos, S.M.), 26-30 September 2010, pp. 26-30. New Zealand Plant Protection Society, Christchurch, New Zealand.

**Fowler, S.V., Paynter, Q., Dodd, S., & Groenteman, R. (2012).** How can ecologists help practitioners minimize non-target effects in weed biocontrol? *Journal of Applied Ecology*, 49, 307-310.

**Freckleton, R.P. (2000).** Biological control as a learning process. *Trends in Ecology and Evolution*, 15, 263-264.

**García-Ruiz, E., Marco, V., & Pérez-Moreno, I. (2011).** Effects of variable and constant temperatures on the embryonic development and survival of a new grape pest, *Xylotrechus arvicola* (Coleoptera: Cerambycidae). *Environmental Entomology*, 40, 939-947.

**Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R. & Schaffner, U (2008).** Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biological Conservation*, 141, 646-654.

**Glinwood, R., Ninkovic, V. & Pettersson, J. (2011).** Chemical interaction between undamaged plants - Effects on herbivores and natural enemies. *Phytochemistry*, 72, 1683–1689.

**Greathead, D.J. & Greathead, A.H. (1992).** Biological control agents of insect pests by insect parasitoids and predators: the BIOCAT database. *Biocontrol News and Information*, 13, 61-68.

**Green S & Bailey KL (2000).** Effects of leaf maturity, infection site, and application rate of *Alternaria cirsinoxia* conidia on infection of Canada thistle (*Cirsium arvense*). *Biological Control*, 19, 167–174.

**Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E. & Hawthorne, D.J. (2007).** Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research*, 97, 201-209.

**Greenstone, M.H., Szendrei, Z., Payton, M.E., Rowley, D.L., Coudron, T.C. & Weber, D.C. (2010).** Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. *Entomologia Experimentalis et Applicata*, 136, 97-107.

**Greenstone, M.H., Weber, D.C., Coudron, T.C. & Payton, M.E. (2011).** Unnecessary roughness? Testing the hypothesis that predators destined for molecular gut-content analysis must be hand-collected to avoid cross-contamination. *Molecular Ecology resources*, 11, 286-293.

**Greenstone, M.H., Weber, D.C., Coudron, T.A., Payton, M.E., & Hu, J.S. (2012).**

Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. *Molecular ecology resources*, 12, 464-469.

**Grevstad, F.S., (1999).** Factors influencing the chance of population establishment:

Implications for release strategies in biocontrol. *Ecological Applications*, 9, 1439-1447.

**Grevstad, F.S., Coombs, E.M. & McEvoy, P.B. (2011).** Revisiting release strategies

in biological control of weeds: are we using enough releases? In *Proceedings of the XIII International Symposium on the Biological Control of Weeds* (eds Wu, Y., Johnson, T., Sing, S., Raghu, S., Wheeler, G., Pratt, P., Warner, K., Center, T., Goolsby, J. & Reardon, R.) 11-16 September 2011, pp. 368-376. US Forest Service, Waikoloa, USA.

**Grevstad, F., Shaw, R., Bouchier, R., Sanguankee, P., Cortat, G. & Reardon, R.C.**

**(2013).** Efficacy and host specificity compared between two populations of the psyllid *Aphalara itadori*, candidates for biological control of invasive knotweeds in North America. *Biological Control*, 65, 53–62.

**Grimsby, J.L., Tsirelson, D., Gammon, M.A. & Kesseli, R. (2007).** Genetic diversity

and clonal vs. sexual reproduction in *Fallopia* spp.(Polygonaceae). *American Journal of Botany*, 94, 957-964.

**Groenteman, R., Fowler, S.V., & Sullivan, J.J. (2011).** St. John's wort beetles would

not have been introduced to New Zealand now: a retrospective host range test of New Zealand's most successful weed biocontrol agents. *Biological Control*, 57, 50-58.

**Gurevitch, J. & Hedges, L.V. (1993).** Meta-analysis: combining the results of

independent experiments. In *Design and Analysis of Ecological Experiments* (eds Scheiner, S.M. & Gurevitch, J). pp. 378-398. Chapman and Hall, New York, USA.

**Gurevitch, J., Morrison, J.A. & Hedges, L.V. (2000).** The interaction between competition and predation: a meta-analysis of field experiments. *The American Naturalist*, 155, 435-453.

**Gurevitch, J., Fox, G.A., Wardle, G.M. & Taub, D. (2011).** Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters*, 14, 407-418.

**Hall, D.G., Richardson, M.L., Ammar, E.D. & Halbert, S.E. (2013).** Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. *Entomologia Experimentalis et Applicata*, 146, 207-223.

**Harris, P. (1991).** Classical biocontrol of weeds: Its definition, selection of effective agents and administrative-political problems. *The Canadian Entomologist*, 123, 827-849.

**Harrison, F. (2011).** Getting started with meta-analysis. *Methods in Ecology and Evolution*, 2, 1-10.

**Hatcher, P.E. & Melander, B. (2003).** Combining physical, cultural and biological methods: prospects for integrated non-chemical weed management strategies. *Weed Research*, 43, 303-322.

**Hatherly, I.S., Hart, A.J., Tullett, A.G. & Bale, J.S. (2005).** Use of thermal data as a screen for the establishment potential of non-native biological control agents in the UK. *BioControl*, 50, 687-698.

**Hayes, L.M. (2000).** Technology transfer programmes for biological control of weeds – the New Zealand experience. In *Proceedings of the X International Symposium on Biological Control of Weeds* (ed Spencer, N.R.). 4-14 July 1999, pp. 719–727. Montana State University, Bozeman, USA.

**Hedges, L.V. & Vevea, J.L. (1998).** Fixed- and Random-Effects Models in Meta-Analysis. *Psychological Methods*, 3, 486-504.

**Herrera-Reddy, A.M., Carruthers, R.I. & Mills, N.J. (2012).** Integrated management of Scotch Broom (*Cytisus scoparius*) using biological control. *Invasive Plant Science and Management*, 5, 69-82.

**Hinz, H.L. & Schwarzaender, M. (2004).** Comparing Invasive Plants from Their Native and Exotic Range: What Can We Learn for Biological Control? Symposium Comparing Invasive Plants from Their Native and Exotic Range: What Can We Learn for Biological Control? *Weed Technology*, 18, 1533–1541.

**Hodek, I. & Honěk, A. (2009).** Scale insects, mealybugs, whiteflies and psyllids (Hemiptera, Sternorrhyncha) as prey of ladybirds. *Biological Control*, 51, 232-243.

**Hodkinson, I.D. & White, I.M. (1979).** Homoptera Psylloidea. *Handbooks for the identification of British insects*, 2(5 (a)). Royal Entomological Society.

**Hodkinson, I.D. (1974).** The biology of the Psylloidea (Homoptera): a review. *Bulletin of Entomological Research*, 64, 325-338.

**Hodkinson, I.D. (2009).** Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. *Journal of Natural History*, 43, 65-179.

**Hoffmann, J.H. & Moran, V.C. (1998).** The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia*, 114, 343-348.

**Hoffmann, J.H. (1995).** Biological control of weeds: the way forward, a South African perspective. In *Weeds in a Changing World* (eds J. K. Waage). British Crop Protection Council Symposium Proceedings no. 64. pp. 77-98. The British Crop Protection Council, Farnham, UK.

**Hollingsworth, M.L. & Bailey, J.P. (2000).** Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese Knotweed). *Botanical Journal of the Linnean Society*, 133, 463–472

**Holt R.D. & Lawton, J.H. (1994).** The ecological consequences of shared natural enemies. *Annual Review of Ecology, Evolution and Systematics*, 25, 495-520.

**Hoogendoorn, M. & Heimpel, G.E. (2001).** PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular Ecology*, 10, 2059-2067.

**Hopper, K.R. & Roush, R.T. (1993).** Mate finding, dispersal, number released, and the success of biological control introductions. *Ecological Entomology*, 18, 321–330.

**Hough-Goldstein, J., Schiff, M., Lake, E. & Butterworth, B. (2008).** Impact of the biological control agent *Rhinoconcomimus latipes* Coleoptera: Curculionidae) on mile-a-minute weed, *Persicaria perfoliata*, in field cages. *Biological Control*, 46, 417-423.

**Huberty, A.F. & Denno, R.F. (2004).** Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, 85, 1383-1398.

**Hulme, P.E. & Bremner, E.T. (2006).** Assessing the impact of *Impatiens glandulifera* on riparian habitats: partitioning diversity components following species removal. *Journal of Applied Ecology*, 43, 43-50.

**Impson, F.A., Kleinjan, C.A., Hoffmann, J.H., Post, J.A. & Wood, A.R. (2011).** Biological control of Australian Acacia species and *Paraserianthes lophantha* (Willd.) Nielsen (Mimosaceae) in South Africa. *African Entomology: Biological control of invasive alien plants in South Africa (1999-2010): Special Issue 2*, 19, 186-207.

**Inderjit & Callaway, R.M. (2003).** Experimental designs for the study of allelopathy. *Plant and Soil*, 256, 1–11.

**Inderjit, Seastedt, T.R., Callaway, R.M., Pollock, J.L. & Kaur, J. (2008).** Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. *Biological Invasions*, 10, 875–890.

**Inoue, M., Nishimura, H., Li, H. & Mizutani, J. (1992).** Allelochemicals from *Polygonum sachalinense* FR. SCHM. (Polygonaceae). *Journal of Chemical Ecology*, 18, 1833–1840.

**Jamieson, M.A., Knochel, D., Manrique, A. & Seastedt, T.R. (2012).** Top-down and bottom-up controls on Dalmatian toadflax (*Linaria dalmatica*) performance along the Colorado Front Range, USA. *Plant Ecology*, 213, 185-195.

**Jarić, I. & Cvijanović, G. (2012).** The Tens Rule in invasion biology: measure of a true impact or our lack of knowledge and understanding? *Environmental management*, 50, 979-981.

**Jeschke, J.M. & Strayer, D.L. (2005).** Invasion success of vertebrates in Europe and North America. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 7198-7202.

**Joshi, J. & Vrieling, K. (2005).** The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters*, 8, 704-714.

**Julien, M.H. & Griffiths, M.W. (1998).** *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds* 4<sup>th</sup> ed. CABI, Wallingford, UK.

**Julien, M.H., Kerr, J.D. & Chan, R.R. (1984).** Biological-control of weeds: an evaluation. *Protection Ecology*, 7, 3-25.

**Kabat, T.J., Stewart, G.B. & Pullin, A.S. (2006).** Are Japanese Knotweed (*Fallopia japonica*) Control and Eradication Interventions Effective? Systematic Review No. 21. *Centre for Evidence-based Conservation*. Birmingham, UK.

**Kajita, Y. & Evans, E.E. (2010).** Relationships of Body Size, Fecundity, and Invasion Success Among Predatory Lady Beetles (Coleoptera: Coccinellidae) Inhabiting Alfalfa Field. *Annals of the Entomological Society of America*, 103, 750-756.

**Kappes, H., Lay, R. & Topp, W. (2007).** Changes in different trophic levels of litter-dwelling macrofauna associated with giant knotweed invasion. *Ecosystems*, 10, 734-744.

**Karban, R. & Myers, J.H. (1989).** Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, 20, 331-348.

**Kareiva (1996).** Developing a predictive ecology for non-indigenous species and ecological invasions. *Ecology*, 77, 1651-1652.

**Katembo, N., Hill, M.P. & Byrne, M.J. (2013).** Impacts of a sub-lethal dose of glyphosate on water hyacinth nutrients and its indirect effects on *Neochetina* weevils. *Biocontrol Science and Technology*, 23, 1412-1426.

**Kawano, S., Azuma, H., Ito, M. & Suzuki, K. (1999).** Extrafloral nectaries and chemical signals of *Fallopia japonica* and *Fallopia sachalinensis* (Polygonaceae), and their roles as defense systems against insect herbivory. *Plant Species Biology*, 14, 167-178.

**Keane, R.M. & Crawley, M.J. (2002).** Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164-170.

**Kettenring, K.M. & Adams, C.R. (2011).** Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. *Journal of Applied Ecology*, 48, 970-979.

**King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C. (2008).** Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, 17, 947-963.

**Kjeldahl, J. (1883).** A new method for the determination of nitrogen in organic matter. *Zeitschreft fur Analytische Chemie*, 22, 366.

**Klein, H. (2011).** A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. *African Entomology*, 19, 515–549.

**Kong, C., Hu, F. & Xu, X. (2002).** Allelopathic potential and chemical constituents of volatiles from *Ageratum conyzoides* under stress. *Journal of Chemical Ecology*, 28, 1173–1182.

**Koricheva, J., Larsson, S. & Haukioja, E. (1998).** Insect performance on experimentally stressed woody plants: A meta-analysis. *Annual Review of Entomology*, 43, 195-216.

**Krebs, C., Mahy, G., Matthies, D., Schaffner, U., Tiébré, M.S. & Bizoux, J.P. (2010).** Taxa distribution and RAPD markers indicate different origin and regional differentiation of hybrids in the invasive *Fallopia* complex in central-western Europe. *Plant Biology*, 12, 215-223.

**Kristoffersen, L. & Anderbrant, O. (2006).** Carrot psyllid (*Trioza apicalis*) winter habitats – insights in shelter plant preference and migratory capacity. *Journal of Applied Entomology*, 131, 174-178.

**Kruess, A. (2002).** Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*. *Oecologia*, 130, 563-569

**Kurose, D., Furuya, N., Tsuchiya, K., Evans, H.C., Djeddour, D.H. & Cannon, P.F. (2009).** Systematics of *Mycosphaerella* species associated with the invasive weed *Fallopia japonica*, including the potential biological control agent *M. polygoni-cuspidati*. *Mycoscience*, 50, 179-189.

**Larson, D.L., Grace, J.B., Rabie, P.A. & Andersen, P. (2007).** Short-term disruption of a leafy spurge (*Euphorbia esula*) biocontrol program following herbicide application. *Biological Control*, 40, 1-8.

**Lau, J.A., Puliafico, K.P., Kopshever, J.A., Steltzer, H., Jarvis, E.P., Schwarzländer, M., Strauss, S.Y. & Hufbauer, R.A. (2008).** Inference of allelopathy is complicated by effects of activated carbon on plant growth. *The New Phytologist*, 178, 412–23.

**Lei, Y., Xiao, F. & Feng, Y. (2010).** Impacts of alien plant invasions on biodiversity and evolutionary responses of native species. *Biodiversity Science*, 18, 622-630.

**Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004).** A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, 7, 975-989.

**Lindgren, C.J., Gabor, T.S. & Murkin, H.R. (1999).** Compatibility of glyphosate with *Galerucella californiensis*; a biological control agent for purple loosestrife (*Lythrum salicaria*). *Journal of Aquatic Plant Management*, 37, 44-48.

**Lockwood, J.L., Cassey, P. & Blackburn, T. (2005).** The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, 20, 223-228.

**Louda, S.M., Arnett, A.E., Rand, T.A., & Russell, F.L. (2003).** Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. *Conservation Biology*, 17, 73-82

**Lowe S., Browne M., Boudjelas S., De Poorter M. (2000).** *100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database.*

Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. First published as special lift-out in *Aliens* 12, December 2000. Updated and reprinted version: November 2004.

**Lucas, E. (2005).** Intraguild predation among aphidophagous predators. *European Journal of Entomology*, 102, 351-364.

**Lucas, E., Labrie, G., Vincent, C. & Kovach, J. (2007).** The multicolored Asian ladybeetle, *Harmonia axyridis*— beneficial or nuisance organism? In *Biological control: a global perspective* (eds Vincent, C., Goettel, M., Lazarovitz, G.). CABI, Wallingford, UK.

**Lym, R.G. (2005).** Integration of biological control agents with other weed management technologies: Successes from the leafy spurge (*Euphorbia esula*) IPM program. *Biological Control*, 35, 366-375.

**MacDougall, A.S. & Turkington, R. (2005).** Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, 86, 42-55.

**Maerz, J.C., Blossey, B. & Nuzzo, V. (2005).** Green frogs show reduced foraging success in habitats invaded by Japanese knotweed. *Biodiversity and Conservation*, 14, 2901–2912.

**Mangan, R. & Baars, J. R. (2013).** Use of life table statistics and degree day values to predict the colonisation success of *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a leaf mining fly of *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae), in Ireland and the rest of Europe. *Biological Control*, 64, 143-151.

**Manrique, V., Cuda, J.P., Overholt, W.A. & Ewe, S.M.L. (2009).** Synergistic effect of insect herbivory and plant parasitism on the performance of the invasive tree *Schinus terebinthifolius*. *Entomologia Experimentalis et Applicata*, 132, 118-125.

**Manrique, V., Diaz, R., Cuda, J.P., & Overholt, W.A. (2011).** Suitability of a new plant invader as a target for biological control in Florida. *Invasive Plant Science and Management*, 4, 1-10.

**Maruta, E. (1976).** Seedling establishment of *Polygonum cuspidatum* on Mt. Fuji. *Japanese Journal of Ecology*, 26, 101-105.

**Masters, G.J., Brown, V.K., Clarke, I.P., Whittaker, J.B. & Hollier, J.A. (1998).** Direct and indirect effects of climate change on insect herbivores: Auchenorrhyncha (Homoptera). *Ecological Entomology*, 23, 45-52.

**McClay, A.S. (1996).** Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents. In *Proceedings of the IX International Symposium on Biological Control of Weeds* (eds Moran, V.C. & Hoffman, J.H.), 19-26 January 1996, pp. 377–383. University of Cape Town, Stellenbosch, South Africa.

**McClay, A. S. & Balciunas, J. K. (2005).** The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. *Biological control*, 35, 197-207.

**McEvoy, P.B. & Coombs, E.M. (1999).** Biological Control of Plant Invaders: Regional Patterns, Field Experiments, and Structured Population Models. *Ecological Applications*, 9, 387-401.

**McEvoy, P.B. & Coombs, E.M. (2001).** Why things bite back: unintended consequences of biological control. In *Nontarget Effects of Biological Control* (eds Follett, P.A. & Duan, J.J), pp. 167–194. Kluwer Academic Publishers, Boston, USA.

**McFadyen, R.E. (1998).** Biological control of weeds. *Annual Review of Entomology*, 43, 369-93.

**McFadyen, R. (2000).** Successes in biological control of weeds. In *Proceedings of the X International Symposium on Biological Control of Weeds* (ed N.R. Spencer), 4-14 July 1999, pp. 3-14. United States Department of Agriculture, Agricultural Research Services, Sidney, MT and Montana State University, Bozeman, MT, USA.

**Memmott, J., Fowler, S.V. & Hill, R.L. (1998).** The effect of release size on the probability of establishment of biological control agents: gorse thrips (*Sericothrips staphylinus*) released against gorse (*Ulex europeus*) in New Zealand. *Biocontrol Science and Technology*, 8, 103-115.

**Memmott, J., Craze, P.G., Harman, H.M., Syrett, P. & Fowler, S.V. (2005).** The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, 74, 50-62.

**Messersmith, C.G. & Adkins, S.W. (1995).** Integrating weed-feeding insects and herbicides for weed control. *Weed Technology*, 9, 199-208.

**Miyatake, Y. (1973).** Psyllids and their life. *Nature Study*, 19, 5–11.

**Miyatake, Y. (2001).** A new species of the genus *Aphalara* from the cool temperate zone of Honshu and Shikoku, Japan (Homoptera: Psylloidea: Aphalaridae). *Special Publication of the Japan Coleopterological Society*, 123–127.

**Moran, V.C. & Hoffman, J.H. (1987).** The effects of simulated and natural rainfall on cochineal insects (Homoptera: Dactylopiidae): colony distribution and survival on cactus cladodes. *Ecological Entomology*, 12, 61-68.

**Moran, V.C. & Hoffmann, J.H. (2012).** Conservation of the fynbos biome in the Cape Floral Region: The role of biological control in the management of invasive alien trees. *BioControl*, 57, 139-149.

**Moravcová, L., Pyšek, P., Jarošík, V. & Zákrevský, P. (2011).** Potential phytotoxic and shading effects of invasive *Fallopia* (Polygonaceae) taxa on the germination of native dominant species. *NeoBiota*, 9, 31.

**Morin, L., Reid, A.M., Sims-Chilton, N.M., Buckley, Y.M., Dhileepan, K., Hastwell, G.T., Nordblom, T.L. & Raghu, S. (2009).** Review of approaches to evaluate the effectiveness of weed biological control agents. *Biological Control*, 51, 1-15.

**Müller-Schärer, H., Lommen, S.T.E., Rossinelli, M., Bonini, M., Boriani, M., Bosio, G. & Schaffner, U. (2014).** *Ophraella communa*, the ragweed leaf beetle, has successfully landed in Europe: fortunate coincidence or threat? *Weed Research*, 54, 109-119.

**Murrell, C., Gerber, E., Krebs, C., Parepa, M., Schaffner, U. & Bossdorf, O. (2011).** Invasive knotweed affects native plants through allelopathy. *American Journal of Botany*, 98, 38-43.

**Musolin, D.L. & Saulich, A.H. (1999).** Diversity of seasonal adaptations in terrestrial true bugs (Heteroptera) from the temperate zone. *Entomological Science*, 2, 623-639.

**Myers, J.H. (1985).** How many insect species are necessary for successful biocontrol of weeds? In *Proceedings of the VI International Symposium on the Biological Control of Weeds* (ed Delfosse, E.S), pp. 77-82. Agriculture Canada Ottawa, Canada.

**Myint, Y.Y., Nakahira, K., Takagi, M., Furuya, N. & Shaw, R.H. (2012).** Using life-history parameters and a degree-day model to predict climate suitability in England for the Japanese knotweed psyllid *Aphalara itadori* Shinji (Hemiptera: Psyllidae). *Biological Control*, 63, 129–134.

**Norambuena, H. & Piper, G.L. (2000).** Impact of *Apion ulicis* Forster on *Ulex europaeus* L. Seed Dispersal. *Biological Control*, 17, 267-271.

**Norris, R.J., Memmott, J. & Lovell, D.J. (2002).** The effect of rainfall on the survivorship and establishment of a biocontrol agent. *Journal of Applied Ecology*, 39, 226-234.

**Norton, A.P., Blair, A.C., Hardin, J.G., Nissen, S.J. & Brunk, G.R. (2008).** Herbivory and novel weapons: no evidence for enhanced competitive ability or allelopathy induction of *Centaurea diffusa* by biological control. *Biological Invasions*, 10, 79–88.

**Page A.R. & Lacey K.L. (2006).** Economic impact assessment of Australian weed biological control. Technical series report prepared by the AEC group for the CRC for Australian Weed Management, Waite Campus, University of Adelaide

**Parepa, M., Schaffner, U. & Bossdorf, O. (2012).** Sources and modes of action of invasive knotweed allelopathy: the effects of leaf litter and trained soil on the germination and growth of native plants. *NeoBiota*, 13, 13–30.

**Paterson, I.D., Coetzee, J.A., Hill, M.P. & Downie, D.D. (2011).** A pre-release assessment of the relationship between the invasive alien plant, *Pereskia aculeata* Miller (Cactaceae), and native plant biodiversity in South Africa. *Biological Control*, 57, 59-65.

**Paynter, Q. (2003).** Integrated weed management: effect of herbicide choice and timing of application on the survival of a biological control agent of the tropical wetland weed, *Mimosa pigra*. *Biological Control*, 26, 162–167

**Paynter, Q., Fowler, S.V., Gourlay, A.H., Haines, M.L., Harman, H.M., Hona, S.R., Peterson, P.G., Smith, L.A., Wilson-Davey, J.R.A., Winks, C.J. & Withers, T. M. (2004).** Safety in New Zealand weed biocontrol: a nationwide survey for impacts on nontarget plants. *New Zealand Plant Protection*, 57, 102-107.

**Paynter, Q., Fowler, S.V., Gourlay, A.H., Groenteman, R., Peterson, P.G., Smith, L. & Winks, C.J. (2010).** Predicting parasitoid accumulation on biological control agents of weeds. *Journal of Applied Ecology*, 47, 575-582.

**Paynter, Q., Overton, J.M., Hill, R.L., Bellgard, S.E. & Dawson, M.I. (2012).** Plant traits predict the success of weed biocontrol. *Journal of Applied Ecology*, 49, 1140-1148.

**Pearson, D.E. & Callaway, R.M. (2003).** Indirect effects of host-specific biological control agents. *Trends in Ecology & Evolution*, 18, 456-461.

**Pearson, D.E. & Callaway, R.M. (2008).** Weed-biocontrol insects reduce native-plant recruitment through second-order apparent competition. *Ecological Applications*, 18, 1489-1500.

**Pilkington, L.J. & Hoddle, M.S. (2006).** Use of life table statistics and degree-day values to predict the invasion success of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitoid of *Homalodisca coagulate* (Hemiptera: Cicadellidae), in California. *Biological Control*, 37, 276-283.

**Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. & Tsomondo, T. (2001).** Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84, 1-20.

**Pimentel, D., Zuniga, R. & Morrison, D. (2005).** Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273 - 288.

**Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. (2012).** Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, 21, 1931-1950.

**Price, P.W. (1991).** The plant vigour hypothesis and herbivore attack. *Oikos*, 62, 244-251.

**Qin, B., Lau, J.A., Kopshever, J., Callaway, R.M., McGray, H., Perry, L.G., Weir, T.L., Paschke, M.W., Hierro, J.L., Yoder, J., Vivanco, J.M. & Strauss, S. (2007).** No evidence for root-mediated allelopathy in *Centaurea solstitialis*, a species in a commonly allelopathic genus. *Biological Invasions*, 9, 897–907.

**R Development Core Team (2013).** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

**Raghu, S. & Walton, C. (2007).** Understanding the ghost of Cactoblastis past: historical clarifications on a poster child of classical biological control. *BioScience*, 57, 699-705.

**Raghu, S., Wilson, J. R. & Dhileepan, K. (2006).** Refining the process of agent selection through understanding plant demography and plant response to herbivory. *Australian Journal of Entomology*, 45, 308-316.

**Reid, A.M., Morin, L., Downey, P.O., French, K. & Virtue, J.G. (2009).** Does invasive plant management aid the restoration of natural ecosystems? *Biological Conservation*, 142, 2342–2349.

**Reilly, T., Spafford Jacob, H. & Batchelor, K. (2004).** The effectiveness of ant baiting to reduce predation of *Crioceris* sp. (Coleoptera: Chrysomelidae), a biological control agent for bridal creeper. In *Proceedings of the 14<sup>th</sup> Australian Weeds Conference* (eds Sindel, B.M. & Johnson, S.B.), 6-9 September 2004, pp. 391-394. Weed Society of New South Wales, Australia.

**Ricciardi, A. (2007).** Are modern biological invasions an unprecedented form of global change? *Conservation Biology*, 21, 329-336.

**Richardson, D. M. & Pyšek, P. (2006).** Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, 30, 409-431.

**Rosenberg, M.S. (2005).** The File-Drawer Problem Revisited: a General Weighted Method for Calculating Fail-Safe Numbers in Meta-Analysis. *Evolution*, 59, 464-468.

**Rosenthal, R. (1979).** The “file drawer problem” and tolerance for null results. *Psychological Bulletin*, 86, 638-641.

**Rouifed, S., Byczek, C., Laffray, D. & Piola, F. (2012).** Invasive Knotweeds are Highly Tolerant to Salt Stress. *Environmental management*, 50, 1027-1034.

**Sagar, D. & Balikai, R.A. (2013).** Psyllid pests of horticultural and forage crops: taxonomy, biology and their management. *Journal of Experimental Zoology, India*, 16, 1-18.

**Sasaki, K. (1954).** A list of the known species and their host plants of the Psyllidae of Japan (Homoptera). *Scientific Reports of the Matsuyama Agricultural College*, 14, 29-39.

**Schaffner, U. (2001).** Host Range Testing of Insects for Biological Weed Control: How Can It Be Better Interpreted? Data on the host range of biocontrol candidates are particularly relevant in assessing potential detrimental effects to nontarget organisms. *BioScience*, 51, 951-959.

**Seabloom, E.W., Harpole, W.S., Reichman, O.J. & Tilman, D. (2003).** Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 13384–13389.

**Seawright, E.K. (2009).** Select economic implications for the biological control of *Arundo donax* along the Rio Grande. Unpublished MSc thesis, Texas A&M University, USA.

**Seymour, C.L. & Veldtman, R. (2010).** Ecological role of control agent, and not just host-specificity, determine risks of biological control. *Austral ecology*, 35, 704-711.

**Shaw, R.H. (2007).** Classical Biological Control of Weeds in Europe: Principles and Practice. Unpublished PhD thesis, University of London, UK.

**Shaw, R.H., Bryner, S. & Tanner, R. (2009).** The life history and host range of the Japanese knotweed psyllid, *Aphalara itadori* Shinji: Potentially the first classical biological weed control agent for the European Union. *Biological Control*, 49, 105-113.

**Shaw, R.H., Tanner, R., Djeddour, D. & Cortat, G. (2011).** Classical biological control of *Fallopia japonica* in the United Kingdom - lessons for Europe. *Weed Research*, 51, 552-558.

**Shea, K. & Possingham, H.P. (2000).** Optimal release strategies for biological control agents: an application of stochastic dynamic programming to population management. *Journal of Applied Ecology*, 37, 77-86.

**Shea, K., Kelly, D., Sheppard, A. W. & Woodburn, T. L. (2005).** Context-dependent biological control of an invasive thistle. *Ecology*, 86, 3174-3181.

**Shea, K., Jongejans, E., Skarpaas, O., Kelly, D. & Sheppard, A.W. (2010).** Optimal management strategies to control local population growth or population spread may not be the same. *Ecological Applications*, 20, 1148–1161.

**Sheppard, S.K. & Harwood, J.D. (2005).** Advances in molecular ecology: tracking trophic links through predator–prey food-webs. *Functional Ecology*, 19, 751-762.

**Sheppard, A.W., Shaw, R.H. & Sforza, R. (2006).** Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Research*, 46, 1–25.

**Shine, R. (2010).** The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology*, 85, 253-291.

**Shure, D.J., Mooreside, P.D. & Ogle, S.M. (1998).** Rainfall effects on plant-herbivore processes in an upland oak forest. *Ecology*, 79, 604-617.

**Siemens, T.J. & Blossey, B. (2007).** An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian knotweed (*Fallopia× bohemica*, Polygonaceae). *American Journal of Botany*, 94, 776-783.

**Sigsgaard, L. (2010).** Habitat and prey preferences of the two predatory bugs *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera). *Biological Control*, 53, 46-54.

**Simberloff, D. & Stiling, P. (1996).** How risky is biological control? *Ecology*, 77, 1965-1974.

**Simberloff, D. (1989).** Which insect introductions succeed and which fail? Pages 61–75 In *Biological invasions: a global perspective* (eds Drake, J.A., Mooney, H.A., di Castri, F., Groves, R.H., Kruger, F.J., Rejmanek, M. & Williamson, M.), pp. 61-75. John Wiley and Sons, Chichester, UK.

**Simelane, D.O. & Phenyne, M.S. (2005).** Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *Biocontrol Science and Technology*, 15, 163–163.

**Sint, D., Raso, L., Kaufmann, R. & Traugott, M. (2011).** Optimizing methods for PCR-based analysis of predation. *Molecular Ecology Resources*, 11, 795-801.

**Stephens, A.E. & Myers, J.H. (2014).** Testing biological control agent compatibility: *Cyphocleonus achates* and *Larinus minutus* on diffuse knapweed. *Biological Control*, 70, 48-53.

**Stiling, P. & Cornelissen, T. (2005).** What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, 34, 236-246.

**Story, J.M., Boggs, K.W., Good, W.R., Harris, P. & Nowierski, R.M. (1991).** *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae), A moth introduced against spotted knapweed: Its feeding strategy and impact on two introduced *Urophora* spp. (Diptera: Tephritidae). *The Canadian Entomologist*, 123, 1001-1007.

**Sutherland, S. (2004).** What makes a weed a weed: life history traits of native and exotic plants in the USA. *Oecologia*, 141, 24–39.

**Symondson W.O.C. (2002).** Molecular identification of prey in predator diets. *Molecular Ecology*, 11, 627–641.

**Symstad, A.J. (2004).** Secondary Invasion Following the Reduction of *Coronilla varia* (Crownvetch) in Sand Prairie. *The American Midland Naturalist*, 152, 183-189.

**Syrett, P., Briese, D.T. & Hoffmann, J.H. (2000).** Success in biological control of terrestrial weeds by arthropods. In *Biological Control: Measures of Success* (eds Gurr, G. and Wratten, S.), pp. 189-230. Kluwer, Dordrecht, The Netherlands.

**Tanaka, H. (1966).** The insect visitors of *Polygonum cuspidatum* Sieb. et Zucc. *Collecting and Breeding*, 28, 141-143.

**Tanner, R. A. & Gange, A.C. (2013).** The impact of two non-native plant species on native flora performance: potential implications for habitat restoration. *Plant Ecology*, 214, 423–432.

**Thelen, G.C., Vivanco, J.M., Newingham, B., Good, W., Bais, H.P., Landres, P., Caesar, A. & Callaway, R.M. (2005).** Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecology Letters*, 8, 209–217.

**Thomas, M. & Reid, A. (2007).** Are exotic natural enemies an effective way of control invasive plants? *Trends in Ecology and Evolution*, 22, 447-453.

**Tipping, P. W. (1991).** Effects of mowing or spraying *Carduus thoermeri* on *Rhinocyllus conicus*. *Weed Technology*, 5, 628-631.

**Tipping, P.W., Center, T.D. & Pratt, P.D. (2011).** Biological Control, Prey Subsidies and Food Webs: One Plant, Two Insects and Two Outcomes. In *Proceedings of the XIII International Symposium on the Biological Control of Weeds* (eds Wu, Y., Johnson, T., Sing, S., Raghu, S., Wheeler, G., Pratt, P., Warner, K., Center, T., Goolsby, J. & Reardon, R). 11-16 September 2011, pp. 302. US Forest Service, Waikoloa, USA.

**Tipping, P.W., Martin, M.R., Pratt, P.D., Rayamajhi, M.B., Center, T.D. (2013).** An abundant biological control agent does not provide a significant predator subsidy. *Biological Control*, 67, 212-219.

**United Nations (1992).** Convention on Biological Diversity.  
<http://www.cbd.int/convention/text/>. Accessed Jan 12<sup>th</sup> 2012.

**Urgenson, L., Reichard, S. & Halpern, C. (2012).** Multiple competitive mechanisms underlie the effects of a strong invader on early- to late-seral tree seedlings. *Journal of Ecology*, 100, 1204–1215.

**van Kleunen, M., Weber, E., & Fischer, M. (2010).** A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, 13, 235-245.

**van Klinken, R.D. & Raghu, S. (2006).** A scientific approach to agent selection. *Australian Journal of Entomology*, 45, 253-25.

**van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T. & Loomans, A.J.M. (2006).** Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology*, 51, 609-634.

**Viechtbauer, W. (2010).** Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*, 36, 1-48.

**Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y. & Pyšek, P. (2011).** Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, 14, 702-708.

**Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997).** Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 21, 1-16.

**Vrchotová, N. & Šerá, B. (2008).** Allelopathic properties of knotweed rhizome extracts. *Plant, Soil and Environment*, 54, 301–303.

**Vrchotová, N., Sera, B. & Triska, J. (2007).** The Stilbene and Catechin Content of the Spring Sprouts of *Reynoutria* Species. *Acta Chromatographica*, 19, 21–28.

**Walling, L.L. (2000).** The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, 19, 195-216.

**Wapshere, A.J. (1974).** Strategy for evaluating safety of organisms for biological weed control. *Annals of Applied Biology*, 77, 201-211.

**Wheeler, G. S. (2001).** Host Plant Quality Factors That Influence the Growth and Development of *Oxyops vitiosa*, a Biological Control Agent of *Melaleuca quinquenervia*. *Biological Control*, 22, 256-264.

- White, T.C.R. (1969).** An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology*, 50, 905-909.
- White, S.D. (2007).** The efficacy of *Aphalara itadori* as a biological control agent of Japanese knotweed (*Fallopia japonica*). Unpublished MSc Thesis, Imperial College London.
- Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R. S., Varia, S., Lamontagne-Godwin, J., Thomas, S. E. & Murphy, S. T. (2010).** *The Economic Cost of Invasive Non-Native Species on Great Britain*. CABI. Wallingford, UK.
- Williamson, M. (1996).** Biological Invasions. *Chapman & Hall, London*.
- Willis, A.J. & Memmott, J. (2005).** The potential for indirect effects between a weed, one of its biocontrol agents and native herbivores: A food web approach. *Biological Control*, 35, 299-306.
- Wood, A.R. & Morris, M.J. (2007).** Impact of the gall-forming rust fungus *Uromycladium tepperianum* on the invasive tree *Acacia saligna* in South Africa: 15 years of monitoring. *Biological Control*, 41, 68-77.
- Wurst, S., Vender, V. & Rillig, M.C. (2010).** Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology*, 211, 19–26.
- Zhou, Z. S., Guo, J. Y., Michaud, J. P., Li, M., & Wan, F.H. (2010).** Variation in cold hardiness among geographic populations of the ragweed beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), a biological control agent of *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), in China. *Biological Invasions*, 13, 659–667
- Zhou, Z.S., Guo, J.Y., Zheng, X.W., Luo, M., Chen, H.S. & Wan, F.H. (2011).** Reevaluation of biosecurity of *Ophraella communa* against sunflower (*Helianthus annuus*). *Biocontrol Science and Technology*, 21, 1147-1160.

**Zidack, N.K. (1999).** Implications of induced resistance to pathogens and herbivores for biological weed control. In *Induced Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds Agrawal, A., Tuzun, S. & Bent, B.), pp. 371–378. APS Press, St Paul, USA.

**Zvereva, E.L., Lanta, V. & Kozlov, M.V. (2010).** Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia*, 163, 949-960.

## Appendices

Appendix 1. Summary of the studies included in the meta-analyses (Chapter 3), including the characteristics used to explain heterogeneity in effect sizes. 'Multiple' in the agent guild column refers to either the presence of multiple control agents (where relative impact was not assessed separately) or when a single agent has different attack strategies during different life stages. Target growth forms follow those defined by the United States Department of Agriculture with the addition of 'Aquatic' as a classification. Global regions are based on a continental scale but only Australia and New Zealand are represented under 'Oceania'. For studies included in the non-target recovery analysis, whether the non-target species were native (N) or invasive (I) is detailed in the response variable column. If blank then this information was not available.

| Author                                               | Target                          | Target growth form | Agent                                                                                                               | Agent guild           | Invasive region | Native Region | Duration (years) | Study conditions | Response variable                                            |
|------------------------------------------------------|---------------------------------|--------------------|---------------------------------------------------------------------------------------------------------------------|-----------------------|-----------------|---------------|------------------|------------------|--------------------------------------------------------------|
| <b>Biocontrol agent presence/absence comparisons</b> |                                 |                    |                                                                                                                     |                       |                 |               |                  |                  |                                                              |
| Abu-dieyeh and Watson (2007)                         | <i>Taraxacum officinale</i>     | Forb, perennial    | <i>Sclerotinia minor</i> (Helotiales: Sclerotiniaceae)                                                              | Pathogen              | N. America      | Europe        | 1                | Field            | Plants/m <sup>2</sup> , % Cover                              |
| Ajuonu et al. (2009)                                 | <i>Eichhornia crassipes</i>     | Aquatic, perennial | <i>Eccritotarsus catarinensis</i> (Hemiptera: Miridae)<br><i>Neochetina eichhorniae</i> (Coleoptera: Curculionidae) | Sap feeder, Folivore  | Africa          | S. America    | 1                | Lab              | Leaf length, Total biomass                                   |
| Barton (2004)                                        | <i>Ageratina riparia</i>        | Forb, perennial    | <i>Entyloma ageratinae</i> (Entylomatales: Entylomataceae),<br><i>Procecidochares alani</i> (Diptera: Tephritid)    | Pathogen, Gall former | Oceania         | S. America    | 1                | Field            | Species no. (N)                                              |
| Barton et al. (2007)                                 | <i>Ageratina riparia</i>        | Forb, perennial    | <i>Entyloma ageratinae</i> (Entylomatales: Entylomataceae)<br><i>Procecidochares alani</i> (Diptera: Tephritid)     | Pathogen, Gall former | Oceania         | C. America    | 6                | Field            | Species richness (N)                                         |
| Bownes et al. (2010)                                 | <i>Eichhornia crassipes</i>     | Aquatic, perennial | <i>Cornops aquaticum</i> (Orthoptera: Acrididae)                                                                    | Folivore              | Africa          | S. America    | 1                | Lab              | Total biomass                                                |
| Boydston and Williams (2004)                         | <i>Convolvulus arvensis</i>     | Vine, perennial    | <i>Aceria malherbae</i> (Acari: Eriophyidae)                                                                        | Gall former           | N. America      | Europe        | 1                | Lab              | Plant height, Shoot mass                                     |
| Butler and Wacker (2010)                             | <i>Euphorbia esula</i>          | Forb, perennial    | <i>Aphthona spp</i> (Coleoptera: Chrysomelidae)                                                                     | Folivore              | N. America      | Europe        | 6                | Field            | % Cover (N,I)                                                |
| Coetzee et al. (2007)                                | <i>Eichhornia crassipes</i>     | Aquatic, perennial | <i>Eccritotarsus catarinensis</i> (Hemiptera: Miridae)                                                              | Sap feeder            | Africa          | S. America    | 1                | Field            | Petiole length, Total biomass, Seedling no., Flower no.      |
| Collier et al. (2008)                                | <i>Cirsium arvense</i>          | Forb, perennial    | <i>Ceutorhynchus litura</i> (Coleoptera: Curculionidae)                                                             | Miner/borer           | N. America      | Europe        | 1                | Lab              | Total biomass                                                |
| Corn et al. (2006)                                   | <i>C. maculosa*</i>             | Forb, perennial    | <i>Cyphocleonus achates</i> (Coleoptera: Curculionidae)                                                             | Root feeder           | N. America      | Europe        | 3                | Field            | Stem length, Total biomass, Seed head no.                    |
| Davies et al. (2005)                                 | <i>Ulex europaeus</i>           | Shrub, perennial   | <i>Sericothrips staphylinus</i> (Thysanoptera: Thripidae)                                                           | Folivore              | Oceania         | Europe        | 1                | Lab              | Total biomass                                                |
| De Clerck-Floate and Wikeem (2009)                   | <i>Cynoglossum officinale</i>   | Forb, annual       | <i>Mogulones cruciger</i> (Coleoptera: Curculionidae)                                                               | Root feeder           | N. America      | Europe        | 2                | Field            | Plants/1.25m <sup>2</sup>                                    |
| Dhileepan (2001)                                     | <i>Parthenium hysterophorus</i> | Forb, annual       | Multiple                                                                                                            | Multiple              | Oceania         | S. America    | 3                | Field            | Plant height, Seed no., Flower no., Plants/m <sup>2</sup>    |
| Dhileepan (2003)                                     | <i>Parthenium hysterophorus</i> | Forb, annual       | <i>Zygogramma bicolorata</i> (Coleoptera: Chrysomelidae)<br><i>Epiblema</i>                                         | Folivore, Gall former | Oceania         | S. America    | 4                | Field            | Plant height, Total biomass, Seed no., Plants/m <sup>2</sup> |

|                               |                                 |                    |                                                                                                                                 |                       |            |            |    |       |                                                          |
|-------------------------------|---------------------------------|--------------------|---------------------------------------------------------------------------------------------------------------------------------|-----------------------|------------|------------|----|-------|----------------------------------------------------------|
| Dhileepan et al. (2000)       | <i>Parthenium hysterophorus</i> | Forb, annual       | <i>strenuana</i> (Lepidoptera: Tortricidae)<br><i>Zygogramma bicolorata</i> (Coleoptera: Chrysomelidae)                         | Folivore              | Oceania    | S. America | 1  | Cage  | Height, Total biomass, Seed bank, Flower no.             |
| Doyle et al. (2002)           | <i>Hydrilla verticillata</i>    | Aquatic, perennial | <i>Hydrellia pakistanae</i> (Diptera: Ephydriidae)                                                                              | Miner/borer           | N. America | Asia       | 1  | Lab   | Tuber mass, Flower no.                                   |
| Doyle et al. (2007)           | <i>Hydrilla verticillata</i>    | Aquatic, perennial | <i>Hydrellia pakistanae</i> (Diptera: Ephydriidae)                                                                              | Miner/borer           | N. America | Asia       | 2  | Lab   | Total biomass                                            |
| Franks et al. (2006)          | <i>Melaleuca quinquenervia</i>  | Tree, perennial    | <i>Boreioglycaspis melaleucae</i> (Hemiptera: Psyllidae)                                                                        | Sap feeder            | N. America | Oceania    | 1  | Cage  | Plant height, Total biomass                              |
| Garren and Strauss (2009)     | <i>Centaurea solstitialis</i>   | Forb, perennial    | <i>Eustenopus villosus</i> (Coleoptera: Curculionidae)<br><i>Chaetorellia succinea</i> (Diptera: Tephritidae)                   | Seed feeder           | N. America | Europe     | 2  | Field | Seed no., Plants/m <sup>2</sup>                          |
| Gopalakrishnan et al. (2011)  | <i>Eichhornia crassipes</i>     | Aquatic, perennial | <i>Neochetina spp</i> (Coleoptera: Curculionidae)                                                                               | Folivore              | Asia       | S. America | 2  | Cage  | Leaf no., Total biomass, Flower no., Plant no.           |
| Grevstad (2006)               | <i>Lythrum salicaria</i>        | Forb, perennial    | <i>Galerucella pusilla</i> , <i>G. californiensis</i> (Coleoptera: Chrysomelidae)                                               | Folivore              | N. America | Europe     | 11 | Field | Stem height, Stand area                                  |
| Grevstad et al. (2003)        | <i>Spartina alterniflora</i>    | Grass, perennial   | <i>Prokelisia marginata</i> (Hemiptera: Delphacidae)                                                                            | Sap feeder            | N. America | S. America | 3  | Cage  | Plant height, Total biomass                              |
| Guo et al. (2010)             | <i>Persicaria perfoliata</i>    | Vine, annual       | <i>Rhinoncomimus latipes</i> (Coleoptera: Curculionidae)                                                                        | Miner/borer           | N. America | Asia       | 1  | Cage  | Plant height, Total biomass, Seed no., Inflorescence no. |
| Heard and Winterton (2000)    | <i>Eichhornia crassipes</i>     | Aquatic, perennial | <i>Neochetina bruchi</i> , <i>N. eichhorni</i> (Coleoptera: Curculionidae)                                                      | Folivore              | Africa     | S. America | 1  | Lab   | Petiole length, Total biomass                            |
| Hoffman et al. (2002)         | <i>Acacia pycnantha</i>         | Tree, perennial    | <i>Trichilogaster sp.</i> (Hymenoptera: Pteromalidae)                                                                           | Gall former           | Africa     | Oceania    | 2  | Field | Seedpod no.                                              |
| Hough-Goldstein et al. (2008) | <i>Persicaria perfoliata</i>    | Vine, annual       | <i>Rhinoncomimus latipes</i> (Coleoptera: Curculionidae)                                                                        | Miner/borer           | N. America | Asia       | 1  | Cage  | Stem circumference, Total biomass, Achene size           |
| Hough-Goldstein et al. (2009) | <i>Persicaria perfoliata</i>    | Vine, annual       | <i>Rhinoncomimus latipes</i> (Coleoptera: Curculionidae)                                                                        | Miner/borer           | N. America | Asia       | 4  | Field | % Cover                                                  |
| Hudgeons et al. (2008)        | <i>Tamarix sp.</i>              | Tree, perennial    | <i>Diorhabda elongata</i> (Coleoptera: Chrysomelidae)                                                                           | Folivore              | N. America | Europe     | 2  | Cage  | Regrowth (mass)                                          |
| Hunt-Joshi et al. (2004)      | <i>Lythrum salicaria</i>        | Forb, perennial    | <i>Galerucella californiensis</i> (Coleoptera: Chrysomelidae)<br><i>Hylobius transversovittatus</i> (Coleoptera: Curculionidae) | Folivore, Root feeder | N. America | Europe     | 4  | Cage  | Stem height, Flower no., Species richness                |

|                             |                                 |                  |                                                                                                               |                               |            |            |   |       |                                                                     |
|-----------------------------|---------------------------------|------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------|------------|------------|---|-------|---------------------------------------------------------------------|
| Jacobs et al. (2006)        | <i>Centaurea stoebe</i>         | Forb, biennial   | <i>Cyphocleonus achates</i> (Coleoptera: Curculionidae)                                                       | Folivore and root feeder      | N. America | Europe     | 8 | Field | Stem length, Total biomass, Flower no.                              |
| Knochel et al. (2010)       | <i>Centaurea stoebe</i>         | Forb, biennial   | <i>Cyphocleonus achates</i> , <i>Larinus minutus</i> (Coleoptera: Curculionidae)                              | Flower, root and seed feeders | N. America | Europe     | 2 | Field | Total biomass, Flower no.                                           |
| Landis et al. (2003)        | <i>Lythrum salicaria</i>        | Forb, perennial  | <i>Galerucella pusilla</i> , <i>G. californiensis</i> (Coleoptera: Chrysomelidae)                             | Folivore                      | N. America | Europe     | 7 | Field | Stem height, Species richness                                       |
| Lesica and Hanna (2004)     | <i>Euphorbia esula</i>          | Forb, perennial  | <i>Aphthona nigricutis</i> (Coleoptera: Chrysomelidae)                                                        | Folivore                      | N. America | Europe     | 6 | Field | Total biomass, Flower no., Plant/m <sup>2</sup> , Species no. (N,I) |
| Martin et al. (2010)        | <i>Melaleuca quinquener via</i> | Tree, perennial  | <i>Oxyops vitiosa</i> (Col.: Curculionidae)<br><br><i>Boreioglycaspis melaleucae</i> (Hemiptera: Psyllidae)   | Folivore, Sap feeder          | N. America | Oceania    | 3 | Field | Total biomass                                                       |
| McAvoy et al. (2002)        | <i>Lythrum salicaria</i>        | Forb, perennial  | <i>Hylobius transversovittatus</i> (Coleoptera: Curculionidae)                                                | Root feeder                   | N. America | Europe     | 5 | Field | Root biomass                                                        |
| Medal and Cuda (2010)       | <i>Solanum viarum</i>           | Shrub, perennial | <i>Gratiana boliviana</i> (Coleoptera: Chrysomelidae)                                                         | Folivore                      | N. America | S. America | 3 | Field | Plant height, % Cover (N,I)                                         |
| Mejri et al. (2010)         | <i>Bromus diandrus</i>          | Grass, annual    | <i>Pseudomons trivialis</i> (Pseudomonadaceae)                                                                | Pathogen                      | Africa     | Europe     | 1 | Lab   | Total biomass                                                       |
| Milbrath and Nechols (2004) | <i>Carduus nutans</i>           | Forb, biennial   | <i>Rhinocyllus conicus</i> , <i>Trichosirocalus horridus</i> (Coleoptera: Curculionidae)                      | Seed feeder, Folivore         | N. America | Europe     | 1 | Cage  | Flower no.                                                          |
| Myers et al. (2009)         | <i>Centaurea diffusa</i>        | Forb, perennial  | <i>Larinus minutus</i> (Coleoptera: Curculionidae)                                                            | Flower and seed feeders       | N. America | Europe     | 1 | Field | Seed head no., Plants/0.25m <sup>2</sup>                            |
| Norambuena and Piper (2000) | <i>Ulex europaeus</i>           | Shrub, perennial | <i>Apion ulicis</i> (Coleoptera: Apionidae)                                                                   | Seed feeder                   | S. America | Europe     | 2 | Field | Seed no.                                                            |
| O'Brien et al. (2010)       | <i>Centaurea solstitialis</i>   | Forb, annual     | <i>Puccinia jaceae</i> var. <i>solstitialis</i> (Basidiomycota: Uredinales)                                   | Pathogen                      | N. America | Europe     | 2 | Field | Seed no.                                                            |
| Overholt et al. (2010)      | <i>Solanum viarum</i>           | Shrub, perennial | <i>Gratiana boliviana</i> (Coleoptera: Chrysomelidae)                                                         | Folivore                      | N. America | S. America | 4 | Field | Plants/4m <sup>2</sup>                                              |
| Paynter (2005)              | <i>Mimosa pigra</i>             | Shrub, perennial | <i>Carmenta mimosa</i> (Lepidoptera: Sesiiidae)                                                               | Miner/borer                   | Oceania    | S. America | 4 | Field | Seed no., Plants/m <sup>2</sup>                                     |
| Paynter et al. (2006)       | <i>Clematis vitalba</i>         | Vine, perennial  | <i>Phoma clematidina</i> (Pleosporaceae: Dothideales)<br><br><i>Phytomyza vitalbae</i> (Diptera: Agromyzidae) | Pathogen, Miner/borer         | Oceania    | Europe     | 2 | Field | % Cover                                                             |

|                                         |                                             |                            |                                                                                                                   |                         |            |            |    |       |                                        |
|-----------------------------------------|---------------------------------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------|-------------------------|------------|------------|----|-------|----------------------------------------|
| Pratt et al. (2005)                     | <i>Melaleuca quinquener via</i>             | Tree, perennial            | <i>Oxyops vitiosa</i> (Coleoptera: Curculionidae)                                                                 | Folivore                | N. America | Oceania    | 1  | Field | Inflorescence no.                      |
| Ripley et al. (2006)                    | <i>Eichhornia crassipes</i>                 | Aquatic, perennial         | <i>Ecritotarsus catarinensis</i> (Hemiptera: Miridae)                                                             | Sap feeder              | Africa     | S. America | 1  | Lab   | Total biomass                          |
| Roche et al. (2001)                     | <i>Centaurea solstitialis</i>               | Forb, annual               | <i>Eustenopus villosus</i> (Coleoptera: Curculionidae)                                                            | Seed feeder             | N. America | Europe     | 1  | Lab   | Achene no.                             |
| Seastedt et al. (2008)                  | <i>Centaurea diffusa</i> , <i>C. stoebe</i> | Forb, biennial & perennial | <i>Urophora sp.</i> (Diptera: Tephritidae)                                                                        | Seed feeder             | N. America | Europe     | 10 | Field | Seed no.                               |
| Sheppard et al. (2001)                  | <i>Echium plantagineum</i>                  | Forb, annual               | <i>Mogulones larvatus</i> (Coleoptera: Curculionidae)                                                             | Root feeder             | Oceania    | Europe     | 2  | Cage  | Total biomass, Seed no., Flower no.    |
| Simelane and Phenye (2005)              | <i>Lantana camara</i>                       | Shrub, perennial           | <i>Teleonemia scrupulosa</i> (Hemiptera: Tingidae)<br><br><i>Ophiomyia camarae</i> (Diptera: Agromyzidae)         | Sap feeder, Miner/borer | Africa     | S. America | 2  | Field | Stem height, Total biomass, Flower no. |
| Tipping et al. (2008)                   | <i>Melaleuca quinquener via</i>             | Tree, perennial            | <i>Oxyops vitiosa</i> (Coleoptera: Curculionidae)<br><br><i>Boreioglycaspis melaleucae</i> (Hemiptera: Psyllidae) | Sap feeder              | N. America | Oceania    | 3  | Field | Total biomass, Seed no.                |
| Tipping et al. (2009)                   | <i>Melaleuca quinquener via</i>             | Tree, perennial            | <i>Boreioglycaspis melaleucae</i> (Hemiptera: Psyllidae)                                                          | Sap feeder              | N. America | Oceania    | 4  | Field | % Cover (N)                            |
| Turner et al. (2010)                    | <i>Asparagus asparagoides</i>               | Vine, perennial            | <i>Puccinia myrsiphylli</i> (Basidiomycota: Uredinales)<br><br><i>Zygina sp</i> (Hemiptera: Cicadellidae)         | Pathogen, Sap feeder    | Oceania    | Africa     | 1  | Lab   | Tuber length, Total biomass            |
| Van Hezewijk et al. (2010)              | <i>Linaria dalmatica</i>                    | Forb, perennial            | <i>Mecinus janthinus</i> (Coleoptera: Curculionidae)                                                              | Miner/borer             | N. America | Europe     | 9  | Field | Stem length                            |
| Wirf (2006)                             | <i>Mimosa pigra</i>                         | Shrub, perennial           | <i>Macaria pallidata</i> (Lepidoptera: Geometridae)                                                               | Folivore                | Oceania    | C. America | 1  | Field | Plant height, Aboveground biomass      |
| <b>Pre and post-release comparisons</b> |                                             |                            |                                                                                                                   |                         |            |            |    |       |                                        |
| Albright et al. (2004)                  | <i>Lythrum salicaria</i>                    | Forb, perennial            | <i>Galerucella spp.</i> (Coleoptera: Chrysomelidae)                                                               | Folivore                | N. America | Europe     | 4  | Field | % cover                                |
| Cline et al. (2008)                     | <i>Euphorbia esula</i>                      | Forb, perennial            | <i>Aphthona spp.</i> (Coleoptera: Chrysomelidae)                                                                  | Folivore                | N. America | Europe     | 5  | Field | % emergence (N, I)                     |
| Lindgren (2003)                         | <i>Lythrum salicaria</i>                    | Forb, perennial            | <i>Galerucella californiensis</i> (Coleoptera: Chrysomelidae)                                                     | Folivore                | N. America | Europe     | 5  | Field | Plants/m <sup>2</sup>                  |
| Sing et al. (2008)                      | <i>Linaria dalmatica</i>                    | Forb, perennial            | <i>Mecinus janthinus</i> (Coleoptera: Curculionidae)                                                              | Miner/borer             | N. America | Europe     | 14 | Field | % Cover                                |

|                                                                                                            |                         |                |                                                         |                          |            |        |   |       |                                 |
|------------------------------------------------------------------------------------------------------------|-------------------------|----------------|---------------------------------------------------------|--------------------------|------------|--------|---|-------|---------------------------------|
| Stephens et al. (2009)                                                                                     | <i>Centaurea stoebe</i> | Forb, biennial | <i>Larinus minutus</i> (Coleoptera: Curculionidae)      | Flower and seed feeders  | N. America | Europe | 5 | Field | Species richness, % cover (N,I) |
| Story et al. (2006)                                                                                        | <i>Centaurea stoebe</i> | Forb, biennial | <i>Cyphocleonus achates</i> (Coleoptera: Curculionidae) | Folivore and root feeder | N. America | Europe | 3 | Field | Plants/m <sup>2</sup> (N,I)     |
| * <i>Centaurea maculosa</i> is synonymous with <i>C. stoebe</i> such they were treated as the same species |                         |                |                                                         |                          |            |        |   |       |                                 |

## References used in the meta-analysis

**Abu-Dieyeh, M.H. & Watson, A.K. (2007).** Grass overseeding and a fungus combine to control *Taraxacum officinale*. *Journal of Applied Ecology*, **44**, 115-124.

**Ajuonu, O., Byrne, M., Hill, M., Neuenschwander, P. & Korie, S. (2009).** The effect of two biological control agents, the weevil *Neochetina eichhorniae* and the mirid *Eccritotarsus catarinensis* on water hyacinth, *Eichhornia crassipes*, grown in culture with water lettuce, *Pistia stratiotes*. *BioControl*, **54**, 155-162.

**Albright, M.F., Harman, W.N., Fickbohm, S.S., Meehan, H., Groff, S. & Austin, T. (2004).** Recovery of Native Flora and Behavioral Responses by *Galerucella* spp. Following Biocontrol of Purple Loosestrife. *The American Midland Naturalist*, **152**, 248-254.

**Barton, J., Boow, J., Ragiel, K., Edenborough, K. & Whaley, K. (2004).** Evaluating the flow-on effects of the biological control agents for *Ageratina riparia* (mist flower) on plant succession. In: *Proceedings of the XI International Symposium on Biological Control of Weeds* (eds Cullen, J.M., Briese, D.T. Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K.), 27 April – 2 May 2003, pp. 487-492. CSIRO Entomology, Canberra, Australia.

**Barton, J., Fowler, S., Gianotti, A., Winks, C., Debeurs, M., Arnold, G. & Forrester, G. (2007).** Successful biological control of mist flower (*Ageratina riparia*)

in New Zealand: Agent establishment, impact and benefits to the native flora.

*Biological Control*, **40**, 370-385.

**Bownes, A., Hill, M.P. & Byrne, M.J. (2010).** Evaluating the impact of herbivory by a grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae), on the competitive performance and biomass accumulation of water hyacinth, *Eichhornia crassipes* (Pontederiaceae). *Biological Control*, **53**, 297-303.

**Boydston, R. A. & Williams, M.M. (2004).** Combined effects of *Aceria malherbae* and herbicides on field bindweed (*Convolvulus arvensis*) growth. *Weed Science*, **52**, 297-301.

**Butler, J.L. & Wacker, S.D. (2010).** Lack of Native Vegetation Recovery Following Biological Control of Leafy Spurge. *Rangeland Ecology & Management*, **63**, 553-563.

**Cline, D., Juricek, C., Lym, R.G. & Kirby, D.R. (2008).** Leafy Spurge (*Euphorbia esula*) Control with *Aphthona* spp. Affects Seedbank Composition and Native Grass Reestablishment. *Invasive Plant Science and Management*, **1**, 120-132.

**Coetzee, J. A., Byrne, M.J. & Hill, M.P. (2007).** Impact of nutrients and herbivory by *Eccritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia crassipes*. *Aquatic Botany*, **86**, 179-186.

**Collier, T.R., Enloe, S.F., Sciegienka, J.K. & Menalled, F.D. (2007).** Combined impacts of *Ceutorhynchus litura* and herbicide treatments for Canada thistle suppression. *Biological Control*, **43**, 231-236.

**Corn, J.G., Story, J.M. & White, L.J. (2006).** Impacts of the biological control agent *Cyphocleonus achates* on spotted knapweed, *Centaurea maculosa*, in experimental plots. *Biological Control*, **37**, 75-81.

**Davies, J.T., Ireson, J.E. & Allen, G.R. (2005).** The impact of gorse thrips, ryegrass competition, and simulated grazing on gorse seedling performance in a controlled environment. *Biological Control*, **32**, 280-286.

**De Clerck-Floate, R. & Wikeem, B. (2009).** Influence of release size on establishment and impact of a root weevil for the biocontrol of houndstongue (*Cynoglossum officinale*). *Biocontrol Science and Technology*, **19**, 169-183.

**Dhileepan, K. (2001).** Effectiveness of introduced biocontrol insects on the weed *Parthenium hysterophorus* ( Asteraceae ) in Australia. *Bulletin of Entomological Research*, **91**, 167-176.

**Dhileepan, K. (2007).** Seasonal variation in the effectiveness of the leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) and stem-galling moth *Epiblema strenuana* (Lepidoptera: Tortricidae) as biocontrol agents on the weed *Parthenium hysterophorus* (Asteraceae). *Bulletin of Entomological Research*, **93**, 393-401.

**Dhileepan, K., Setter, S. & McFadyen, R. (2000).** Response of the Weed *Parthenium hysterophorus* (Asteraceae) to Defoliation by the Introduced Biocontrol Agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae). *Biological Control*, **19**, 9-16.

**Doyle, R.D., Grodowitz, M., Michael Smart, R. & Owens, C. (2002).** Impact of herbivory by *Hydrellia pakistanae* (Diptera: Ephydriidae) on growth and photosynthetic potential of *Hydrilla verticillata*. *Biological Control*, **24**, 221-229.

**Doyle, R., Grodowitz, M., Smart, M. & Owens, C. (2007).** Separate and interactive effects of competition and herbivory on the growth, expansion, and tuber formation of *Hydrilla verticillata*. *Biological Control*, **41**, 327-338.

**Franks, S.J., Kral, A.M. & Pratt, P.D. (2006).** Herbivory by Introduced Insects Reduces Growth and Survival of *Melaleuca quinquenervia* Seedlings. *Environmental Entomology*, **35**, 366-372.

**Garren, J.M. & Strauss, S.Y. (2009).** Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecological Applications*, **19**, 709-21.

**Gopalakrishnan, A., Rajkumar, M., Sun, J., Parida, A. & Venmathi Maran, B.A. (2011).** Integrated biological control of water hyacinths, *Eichhornia crassipes* by a novel combination of grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), and the weevil, *Neochetina* spp. *Chinese Journal of Oceanology and Limnology*, **29**, 162-166.

**Grevstad, F.S. (2006).** Ten-year impacts of the biological control agents *Galerucella pusilla* and *G. californiensis* (Coleoptera: Chrysomelidae) on purple loosestrife (*Lythrum salicaria*) in Central New York State. *Biological Control*, **39**, 1-8.

**Grevstad, F.S., Strong, D.R., Garcia-Rossi, D., Switzer, R.W. & Wecker, M.S. (2003).** Biological control of *Spartina alterniflora* in Willapa Bay, Washington using the planthopper *Prokelisia marginata*: agent specificity and early results. *Biological Control*, **27**, 32-42.

**Guo, W., Li, X., Guo, X. & Ding, J. (2010).** Effects of *Rhinoncomimus latipes* on the growth and reproduction of *Persicaria perfoliata*, an invasive plant in North America. *Biocontrol Science and Technology*, **21**, 35-45.

**Heard, T.A. & Winterton, S.L. (2011).** Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology*, **37**, 117-127.

**Hoffmann, J.H., Impson, F. A.C., Moran, V.C. & Donnelly, D. (2002).** Biological control of invasive golden wattle trees (*Acacia pycnantha*) by a gall wasp, *Trichilogaster* sp. (Hymenoptera: Pteromalidae), in South Africa. *Biological Control*, **25**, 64-73.

**Hough-Goldstein, J., Mayer, M. a., Hudson, W., Robbins, G., Morrison, P. & Reardon, R. (2009).** Monitored releases of *Rhinoncomimus latipes* (Coleoptera: Curculionidae), a biological control agent of mile-a-minute weed (*Persicaria perfoliata*), 2004–2008. *Biological Control*, **51**, 450-457.

**Hough-Goldstein, J., Schiff, M., Lake, E. & Butterworth, B. (2008).** Impact of the biological control agent *Rhinoncomimus latipes* (Coleoptera: Curculionidae) on mile-a-minute weed, *Persicaria perfoliata*, in field cages. *Biological Control*, **46**, 417-423.

**Hudgeons, J.L., Knutson, A.E., Heinz, K.M., DeLoach, C.J., Dudley, T.L., Pattison, R.R. & Kiniry, J.R. (2007).** Defoliation by introduced *Diorhabda elongata* leaf beetles (Coleoptera: Chrysomelidae) reduces carbohydrate reserves and regrowth of *Tamarix* (Tamaricaceae). *Biological Control*, **43**, 213-221.

- Hunt-Joshi, T.R., Blossey, B., Root, R.B. (2004).** Root and leaf herbivory on *Lythrum salicaria*: Implications for plant performance and communities. *Ecological Applications*, **14**, 1574-1589.
- Jacobs, J.S., Sing, S.E. & Martin, J.M. (2006).** Influence of Herbivory and Competition on Invasive Weed Fitness: Observed Effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and Grass-Seeding Treatments on Spotted Knapweed Performance Influence of Herbivory and Competition on Invasive Weed. *Environmental Entomology*, **35**, 1590-1596.
- Knoche, D.G., Monson, N.D. & Seastedt, T.R. (2010).** Additive effects of aboveground and belowground herbivores on the dominance of spotted knapweed (*Centaurea stoebe*). *Oecologia*, **164**, 701-12.
- Landis, D. a, Sebolt, D.C., Haas, M.J. & Klepinger, M. (2003).** Establishment and impact of *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) on *Lythrum salicaria* L. and associated plant communities in Michigan. *Biological Control*, **28**, 78-91.
- Lesica, P. & Hanna, D. (2004).** Indirect Effects of Biological Control on Plant Diversity Vary across Sites in Montana Grasslands. *Conservation Biology*, **18**, 444-454.
- Lindgren, C.J. (2003).** Using 1-min scans and stem height data in a post-release monitoring strategy for *Galerucella californiensis* (L.) (Coleoptera: Chrysomelidae) on purple loosestrife, *Lythrum salicaria* L. (Lythraceae), in Manitoba. *Biological Control*, **27**, 201-209.
- Martin, M.R., Tipping, P.W., Reddy, K.R., Daroub, S.H. & Roberts, K.M. (2010).** Interactions of biological and herbicidal management of *Melaleuca quinquenervia* with fire: Consequences for ecosystem services. *Biological Control*, **54**, 307-315.
- McAvoy, T.J., Kok, L.T. & Mays, W.T. (2002).** Establishment of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife, in Virginia. *Biological Control*, **24**, 245-250.

**Medal, J.C. & Cuda, J.P. (2010).** Establishment and Initial Impact of the Leaf-Beetle *Gratiana boliviana* (Chrysomelidae), First Biocontrol Agent Released Against Tropical Soda Apple in Florida. *Florida Entomologist*, **93**, 493-500.

**Mejri, D., Gamalero, E., Tombolini, R., Musso, C., Massa, N., Berta, G. & Souissi, T. (2010).** Biological control of great brome (*Bromus diandrus*) in durum wheat (*Triticum durum*): specificity, physiological traits and impact on plant growth and root architecture of the fluorescent pseudomonad strain X33d. *BioControl*, **55**, 561-572.

**Milbrath, L.R. & Nechols, J.R. (2004).** Individual and combined effects of *Trichosirocalus horridus* and *Rhinocyllus conicus* (Coleoptera: Curculionidae) on musk thistle. *Biological Control*, **30**, 418-429.

**Myers, J.H., Jackson, C., Quinn, H., White, S.R. & Cory, J.S. (2009).** Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. *Biological Control*, **50**, 66-72.

**Norambuena, H. & Piper, G.L. (2000).** Impact of *Apion ulicis* Forster on *Ulex europaeus* L. Seed Dispersal. *Biological Control*, **17**, 267-271.

**Overholt, W. A., Diaz, R., Markle, L. & Medal, J.C. (2010).** The effect of *Gratiana boliviana* (Coleoptera: Chrysomelidae) herbivory on growth and population density of tropical soda apple (*Solanum viarum*) in Florida. *Biocontrol Science and Technology*, **20**, 791-807.

**O'Brien, J.M., Kyser, G.B., Woods, D.M. & DiTomaso, J.M. (2010).** Effects of the rust *Puccinia jaceae* var. *solstitialis* on *Centaurea solstitialis* (yellow starthistle) growth and competition. *Biological Control*, **52**, 174-181.

**Paynter, Q. (2005).** Evaluating the impact of a biological control agent *Carmentis mimosa* on the woody wetland weed *Mimosa pigra* in Australia. *Journal of Applied Ecology*, **42**, 1054-1062.

**Paynter, Q., Waipara, N., Peterson, P., Hona, S., Fowler, S., Gianotti, A. & Wilkie, P. (2006).** The impact of two introduced biocontrol agents, *Phytomyza vitalbae* and *Phoma clematidina*, on *Clematis vitalba* in New Zealand. *Biological Control*, **36**, 350-357.

- Pratt, P.D., Rayamajhi, M.B., Van, T.K., Center, T.D. & Tipping, P.W. (2005).** Herbivory alters resource allocation and compensation in the invasive tree *Melaleuca quinquenervia*. *Ecological Entomology*, **30**, 316-326.
- Ripley, B.S., Muller, E., Behenna, M., Whittington-Jones, G.M. & Hill, M.P. (2006).** Biomass and photosynthetic productivity of water hyacinth (*Eichhornia crassipes*) as affected by nutrient supply and mirid (*Eccritotarus catarinensis*) biocontrol. *Biological Control*, **39**, 392-400.
- Roché, C.T., Harmon, B.L., Wilson, L.M. & McCaffrey, J.P. (2001).** *Eustenopus villosus* (Coleoptera: Curculionidae) Feeding of Herbicide-Resistant Yellow Starthistle (*Centaurea solstitialis* L.). *Biological Control*, **20**, 279-286.
- Seastedt, T.R., Knochel, D.G., Garmoe, M. & Shosky, S. A. (2007).** Interactions and effects of multiple biological control insects on diffuse and spotted knapweed in the Front Range of Colorado. *Biological Control*, **42**, 345-354.
- Sheppard, A.W., Smyth, M.J. & Swirepik, A. (2001).** The impact of a root-crown weevil and pasture competition on the winter annual *Echium plantagineum*. *Journal of Applied Ecology*, **38**, 291-300.
- Simelane, D.O. & Phenyne, M.S. (2005).** Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *Biocontrol Science and Technology*, **15**, 153-163.
- Sing, S.E., Weaver, D.K., Nowierski, R.M. & Markin, G.P. (2008).** Long-term field evaluation of *Mecinus janthinus* releases against Dalmatian toadflax in Montana (USA). In *Proceedings of the XII International Symposium on Biological Control of Weeds* (eds. Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L. and Rector, B.G.), 23-27 April 2007, pp. 620 - 624. CAB International Wallingford, UK.
- Stephens, A.E. A., Krannitz, P.G. & Myers, J.H. (2009).** Plant community changes after the reduction of an invasive rangeland weed, diffuse knapweed, *Centaurea diffusa*. *Biological Control*, **51**, 140-146.

**Story, J.M., Callan, N.W., Corn, J.G. & White, L.J. (2006).** Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). *Biological Control*, **38**, 227-232.

**Tipping, P.W., Martin, M.R., Nimmo, K.R., Pierce, R.M., Smart, M.D., White, E., Madeira, P.T. & Center, T.D. (2009).** Invasion of a West Everglades wetland by *Melaleuca quinquenervia* countered by classical biological control. *Biological Control*, **48**, 73-78.

**Tipping, P.W., Martin, M.R., Pratt, P.D., Center, T.D. & Rayamajhi, M.B. (2008).** Suppression of growth and reproduction of an exotic invasive tree by two introduced insects. *Biological Control*, **44**, 235-241.

**Turner, P.J., Morin, L., Williams, D.G. & Kriticos, D.J. (2010).** Interactions between a leafhopper and rust fungus on the invasive plant *Asparagus asparagoides* in Australia: A case of two agents being better than one for biological control. *Biological Control*, **54**, 322-330.

**Van Hezewijk, B.H., Bouchier, R.S. & De Clerck-Floate, R. A. (2010).** Regional-scale impact of the weed biocontrol agent *Mecinus janthinus* on Dalmatian toadflax (*Linaria dalmatica*). *Biological Control*, **55**, 197-202.

**Wirf, L. A. (2006).** The effect of manual defoliation and *Macaria pallidata* (Geometridae) herbivory on *Mimosa pigra*: Implications for biological control. *Biological Control*, **37**, 346-353.

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