INTERACTIONS BETWEEN A LEAF-GALLING WASP AND ITS INVASIVE HAWKWEED HOSTS

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I would like to dedicate this thesis to my wonderful wife, Jessica, and to my parents. Their unfailing support has allowed me to pursue my dreams in all of life's endeavors.

Abstract

This thesis aims to explore the interactions between a potential biocontrol agent, the gall-wasp *Aulacidea pilosellae*, and its invasive *Pilosella* hawkweed hosts. I discovered that increased nitrogen availability improves *P. officinarum* vegetative growth, while also interacting with *A. pilosellae* to reduce vegetative growth, as the plants cannot compensate for this herbivory. I did not detect any nitrogen effects on wasp performance. I also explored how the host species utilized for galling affects wasp performance through two generations by measuring maternal effects. These only influenced offspring performance when the mother had utilized *P. caespitosa*, but not *P. glomerata*. I discovered that *P. caespitosa* is also the better offspring host, producing significantly larger galls and heavier larvae compared to *P. glomerata*. These results may help identify, 1) if underlying site fertility may be associated with increases in agent performance and/or impact on the target weed, and 2) to indicate the appropriate *Pilosella* species to target in a biocontrol programme.

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Chapter 1: Introduction

Thesis Goals

The goals of this thesis were to identify the interactions between the gall-wasp *Aulacidea pilosellae* Kieffer. (Hymenoptera; Cynipidae) and its invasive *Pilosella* Vaill. (Asteraceae) hawkweed hosts in order to identify if this wasp would be an effective agent in a biocontrol program targeting these weeds. This was accomplished by conducting two experiments. The first used nitrogen availability and wasp presence to explore the impact and interaction of nitrogen additions and wasp galling on *P. officinarum* L. performance. Identifying if the underlying site fertility may increase agent performance and/or impact on the target weed species has important implications for present and future biocontrol programmes.

The second experiment explored how the host species utilized for galling affects wasp performance through two generations. I posited that maternal effects may influence host switching of *A. pilosellae*, as this wasp has been observed attacking several hawkweed species in their native range. Identifying the baseline performance on the different host species can help identify the specific host to target in a biocontrol programme.

Nitrogen effects on biodiversity and invasive weed species

Anthropogenic changes in nitrogen addition to the environment are negatively affecting global biodiversity (Borer et al., 2014; Powell et al., 2011). Within the last several decades there has been a significant increase in anthropogenic nitrogen deposition to the biosphere as a result of fossil fuel combustion and agricultural fertilization (Vitousek et al., 1997). Porter et al. (2013) point out that anthropogenic nitrogen addition and climate change can interact to reduce biodiversity at a much greater rate than either factor alone in both terrestrial and aquatic ecosystems. Species richness generally declines as soil nutrients (especially nitrogen) increase, with uncommon and rare species usually going locally extinct as soil resources increase (Bedford et al., 1999). This disruption to biodiversity occurs primarily because improved soil nutrients promote growth and reproduction of competitive species, mainly invasive species, more strongly than native species (Porter et al., 2013). Dukes et al. (2011) found that the highly invasive weed, yellow starthistle (*Centaurea solstitialis* L; Asteraceae), significantly increased biomass in response to increased nitrate deposition; suggesting that it may increase its invasiveness as anthropogenic nitrogen deposition continues to increase. Murphy and Romanuk (2012) determined that both nutrient additions and loss of biodiversity reduced the predictability of ecosystem resilience, meaning that after such a disturbance the communities are highly variable in their ability to maintain ecosystem integrity. As a result, we must make a greater effort to understand the effects of anthropogenic nitrogen addition on invasive species in order to preserve global biodiversity (Mattingly & Reynolds, 2014; Porter et al., 2013).

Ecosystems with high resource availability (including nitrogen) and ensuing low biodiversity are especially vulnerable to disruption from invasive species (Davis & Pelsor, 2001; Powell et al., 2011). González et al. (2010) found that high nutrient availability consistently favoured non-native (potentially invasive) species over native species. Underlying site fertility is a critical factor to measure when determining an environment's susceptibility to invasive species (Coetzee et al., 2007; Mattingly & Reynolds, 2014; Vourlitis & Kroon, 2013). Coetzee et al. (2007) found that the extremely invasive water hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) would be controlled more effectively if nutrient levels were reduced, as this weed has a greater competitive ability at high water-nutrient concentrations leading to increased invasion risk in these areas. On the other hand, the invasive common ragweed (*Ambrosia artemisiifolia* L, Asteraceae) is a very poor competitor when there is high resource availability, but is superior in environments with low nutrients (Leskovšek et al., 2012). Clearly soil fertility can have a significant effect on the vulnerability of ecosystems to invasions, with either high or low nutrient sites being susceptible to invasions from a variety of non-native weeds (Alpert et al., 2000; Eskelinen & Harrison, 2014).

Invasive plants often improve their performance under enhanced nitrogen conditions relative to native plants (Funk, 2008) due to greater inherent phenotypic plasticity (Joshi et al., 2014; Sorte et al., 2013; Van Kleunen et al., 2010). They are often very flexible in responding to nutrient and moisture fluctuations, allowing them to remain competitive with native species under a variety of environmental conditions (Vourlitis & Kroon, 2013). Suding et al. (2004) found that diffuse knapweed (*Centaurea diffusa* Lam, Asteraceae) was highly competitive under both high and low nitrogen availability. Jamieson et al. (2012) observed that the invasive weed Dalmatian toadflax (*Linaria dalmatica* L, Plantaginaceae) responds strongly to nitrogen addition with increased biomass and seed production. Vourlitis and Kroon (2013) found that the invasive grass Pampasgrass (*Cortaderia selloana* (Schultes) Asch. and Graeb.; Poaceae) performs well under low nitrogen, but responds to increased nitrogen with large boosts in physiological performance and resource use. However, not all weeds respond equally to changes in soil nutrients (Blackshaw & Brandt, 2008; Blackshaw et al., 2003). For example, Persian Darnel (*Lolium persicum* Boiss & Hohen ex Boiss; Poaceae) and Russian thistle (*Echinops exaltatus* Schrad; Asteraceae) did not increase in competitive ability versus spring wheat (AC Barrie) with nitrogen uptake or total seed yield as a response to increased nitrogen availability compared to several other co-occurring weed species (Blackshaw & Brandt, 2008). Even so, invasive species regularly perform better under increased soil fertility than native species, generally with improved growth, reproduction or competitiveness (Burns, 2004; Leger et al., 2007; Leishman & Thomson, 2005), resulting in ecosystems with increased soil nitrogen being particularly susceptible to invasion.

Nitrogen effects on insect herbivore preference, performance, and impact

The preference-performance hypothesis states that due to evolutionary selection, female insects that oviposit on hosts on which their offspring fare best will come to dominate a population (Jaenike, 1978). Female preference for 'good quality' plants or plant parts is stronger in more specialized than generalist insects, likely due to greater evolved host specificity in specialist insects which allows them to exploit these 'good quality' plants to a greater extent than generalists (Gripenberg et al., 2010). The pairing of preference and performance is genetically associated in specialists insects, and is critically important for insect population dynamics, as preference for plants that result in low insect performance can reduce insect survival and population size. Additionally, preference and performance of some insects that are particularly responsive to host plant growth can also be restricted to vigourous plant parts or vigorously growing plants, which is known as the Plant Vigour Hypothesis or PVH (Price, 1991). Vigourous plant parts are defined as those parts that grow larger and more rapidly than the mean growth rate and final size of the plant module within the same plant or the plant population (Price, 1991). Santos et al. (2008) found that the gall-midge *Schizomyia macrocapillata* Maia (Diptera: Cecidomyiidae) preferred to gall longer (vigourous) shoots of *Bauhinia brevipes* Vogel (Fabaceae) up to 10 times more often than shorter shoots on the same plant. Further, the performance of the gall-midge as measured by insect survival was increased on these longer shoots (Santos et al., 2008). Price and Hunter (2005) found that the stem-galling sawfly *Euura lasiolepis* Smith (Hymenoptera:

Tenthredinidae) on arroyo willow (*Salix lasiolepis* Bentham, Salicaceae) strongly preferred and performed better on longer (more vigourous) plant shoots.

The PVH is strongly supported with endophagous insects, namely gall-formers, shoot-borers and leaf miners, as these insects have an intimate genetic relationship with their host plants and the processes of plant growth that these insects require (Harris, 1973; Harris & Shorthouse, 1996; Price, 1991). These insect feeding guilds lay their eggs within the plant modules, with the developing larvae remaining completely enclosed within the plant tissues, feeding off the nutrients generated and moved by the plant. Craig et al. (1989) found that a shoot-galling sawfly *Euura lasiolepis* greatly prefers vigourously growing shoots for oviposition locations, and vigour as measured by plant shoot length strongly corresponds to larval survival. Heard and Cox (2009) observed that a gall-inducing moth (*Gnorimoschema gallaesolidaginis* Riley, Lepidoptera: Gelechiidae) frequently preferred larger goldenrod stems (*Solidago altissimsa* L., Asteraceae) for oviposition. De Bruyn et al. (2002) found that the leaf miner *Agromyza nigripes* Meigen (Diptera: Agromyzidae) on the grass *Holcus lanatus* L. (Poaceae) preferred and performed better on plants which had greater nutritional quality and vigour. The preference for vigourously growing plant parts can be related to the resources available in these plants or plant modules, as vigourous plant parts are stronger nutrient sinks that draw resources from other plant modules (Burstein et al., 1994).

Greater soil nutrients may boost the concentration of nutrients available or growth rates of plant modules, which can influence the quality of those organs as food for phytophagous insects (Butler et al., 2012; Center et al., 2014; Minkenberg & Ottenheim, 1990). This improved nutritional quality of plants can directly influence the performance of herbivorous insects that feed on them by improving insect fecundity, longevity, or optimizing development times (Awmack & Leather, 2002; Price, 1999). Additionally, increased soil nutrients may not influence the nutritional quality of plant organs directly, but instead may increase growth rates (vigour) of certain plant parts which may benefit specific insects which feed on them. Moran and Goolsby (2014) discovered that fertilization of giant reed (*Arundo donax* L, Poaceae) increased the relative growth rate of the plants, which subsequently improved rearing capabilities of the shoot-tip galling wasp *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) by reducing the generation times of this insect. Room and Thomas (1985) found that with *Cyrtobagous* sp. (Coleoptera: Curculionidae) there is a critical leaf nitrogen content above which the insect populations can increase and below which insect populations decrease. As such, some insects can be severely influenced by nutrient availability of their plant host, with fluctuations in nutrient availability causing changes in survival, fecundity, sex ratios and individual performance (Bownes et al., 2013a).

Some insects have increased access to leaf nitrogen when plants are stressed, which is known as the “Plant Stress Hypothesis” (White, 1984). Generally, those insects that feed on mature tissues (senescence feeders) will benefit from plant stress, while those that feed on young tissues (flush feeders) will suffer, as stressing plants can hasten their senescence, resulting in greater amounts of nutrients being released into the phloem tissue (i.e. easier to access for some insects) (White, 2009). During senescence, protein synthesis stops, causing a build up of easily digestible amino acids (White, 1969, 1974; White, 1984). For example, Larsson and Tenow (1984) observed that an outbreak of European pine sawfly (*Neodiprion sertifer* Geoffroy, Hymenoptera: Diprionidae) on Scots pine was precipitated by plant stress: namely drought, infertile soil, aged stands and high inter-tree competition. Waring and Pitman (1985) found that by reducing stressors of lodgepole pine (*Pinus contorta* Douglas., Pinaceae), such as improving nutrient, light, and moisture availability, plant susceptibility to mountain pine beetles (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae) was decreased. This beetle feeds on the inner bark and phloem of lodgepole pine, and thus has increased nutritional quality when plants are stressed (Waring & Pitman, 1985; White, 2009). However, flush feeding insects are negatively influenced by stress. For example, Huberty and Denno (2004) found that those insects that feed on plant sap are negatively affected by water stress, as the nutrient-rich sap that travels through the phloem of a plant is strongly affected by water stress with regard to turgor pressure, and thus has reduced movement under these conditions. This turgor pressure is required to keep plant parts rigid, and allow the free-flowing movement of plant resources throughout the plant, such as nitrogen from the roots and photoassimilates from leaves and storage organs. Thus, phloem-feeding insects are unable to access stress-induced increases in plant nitrogen due to greater tissue senescence when turgor is below a certain threshold, even though nitrogen concentrations may be elevated (Huberty & Denno, 2004). McMillin and Wagner (1995) found that the pine sawfly *Neodiprion gillettei* Rohwer (Hymenoptera: Tenthredinoidae) was unable to benefit from water stressed ponderosa pine (*Pinus ponderosa* Douglas ex Law., Pinaceae), likely due to the increased toughness of the needles when turgor pressure is low. Preszler and Price (1988) found that the shoot-galling sawfly *Euura lasiolepis* had one eighth the density, and five times the mortality in water stressed habitats, which occurred as a result of refusal to release an egg (due to poor host quality) during gall initiation or even to initiate a gall if an egg is released. Stressed plants may be more susceptible to certain feeding guilds which can exploit the increased nitrogen concentrations; however, flush feeding insects can be negatively influenced by plant stress, especially when those insects require vigourous, expanding plant tissue in order to oviposit or feed.

Improved nutritional quality of plant modules as a result of heightened soil fertility can allow some herbivorous insects to exert greater top down control (control by a biotic force in the upper trophic level on the next trophic level down) on their food sources as they can respond with increased performance and population sizes (Center & Dray, 2010; Center et al., 2014; Guenther et al., 2011; Room & Thomas, 1985). Soil nutrients have a positive effect on the abundance of several insect herbivore guilds, including gall-formers (Cornelissen & Stiling, 2006). There is evidence that top-down control of plant species can be increased when nutrients are abundant, either by increasing the palatability of plant tissues or by increasing the dominance of palatable species (Bakker & Nolet, 2014). This is especially important when managing invasive weeds, as the underlying site fertility can affect the success of phytophagous insects that are used in biocontrol (Hovick & Carson, 2015). Nutrient enrichment may also improve establishment of biocontrol agents, as insects may respond to the nutrient quality of their hosts with increased performance (Heard & Winterton, 2000; Van Hezewijk et al., 2008). Different biocontrol agents may be effective under different nutrient availabilities, with impact varying as a result of host plant nutritional quality and herbivore preference for target plant species (Center et al., 2014).

Biocontrol programmes can be negatively influenced by increased plant access to nutrients, as some invasive plants can compensate for damage caused by insect herbivores, meaning they can replace the seed/fruit or plant parts lost to herbivory through responsive growth, as predicted by the "compensatory continuum hypothesis" (Maschinski & Whitham, 1989). This hypothesis describes the range of growth responses and associated performance of plants fed upon by herbivores; at one end of the continuum the plant undercompensating (detrimental to plant, loss of growth and reproductive potential) and at the other end, the plant overcompensating via stimulated vegetative or reproductive growth (Maschinski & Whitham, 1989). Maschinski and Whitham (1989) predicted that overcompensation to herbivory, if it occurs, is most likely when nutrients are high, competition is low, and herbivory is early in the growing season. Bownes et al. (2013b) found that biocontrol efficacy of *Cornops aquaticum* Bruner. (Orthoptera: Acrididae) to control invasive water hyacinth in South Africa would improve if nutrient pollution was limited, due to water hyacinth's ability to compensate (i.e. replace lost plant parts) for damage by this herbivore and maintain biomass and vegetative reproduction rates when nutrients were abundant. A similar trend was observed with the gall-wasp *Antistrophus silphii* Gil. (Hymenoptera: Cynipidae) on rosinweed (*Silphium integrifolium* Michx, Asteraceae), with plant tolerance to galling being positively correlated to resource availability such that with added water and nutrients, biomass and reproductive output was maintained in the presence of galls (Fay et al., 1996). Sun et al. (2010) found that both invasive *Alternanthera philoxeroides* Griseb. (Amaranthaceae) and the native *Alternanthera sessilis* L. were more tolerant to herbivore damage when soil nutrient availability was high. However, not all plants can compensate for insect feeding damage when nutrients are readily available. Meyer and Root (1993) showed that feeding by a spittlebug (*Philaenus spumarius* L, Hemiptera: Aphrophoridae), beetle (*Trirhabda* sp., Coleoptera: Chrysomelidae), and aphid (*Uroleucon caligatum* Richards, Hemiptera: Aphididae) all reduced goldenrod (*Solidago altissimsa*) performance when under high soil nitrogen, but were much less effective when soil nitrogen was low. This generally occurs because the increased nutritional quality of plants improves insect performance, and thus their population levels (Center & Dray, 2010; Cornelissen & Stiling, 2006), allowing them to induce greater top-down control on their food source (Van Hezewijk et al., 2008). Considering that some invasive plant species can easily compensate for herbivory when excess nutrients are available, low-nutrient sites should be targeted for release of agents for these species (Coetzee et al., 2007). With regards to biocontrol, programmes can be improved if pre-release impact testing is undertaken with variable soil nutrient availabilities (Burns et al., 2013).

Environmental heterogeneity and maternal effects

Herbivorous insects of natural systems must navigate heterogeneous environments in order to maintain their survival and reproductive success (Cornelissen et al., 2008; Gripenberg et al., 2007; Schultz, 1983; Tews et al., 2004; Whitham, 1981). There is heterogeneity between plants within the same patch, as there are differences in plant susceptibility to insect attack between mature and young plants (Kearsley & Whitham, 1989). There can even be heterogeneity present among host plant parts within plants, as there can be differences between vigour and secondary chemical defences in young and mature plant tissues that influences herbivore preference-performance for these tissues (Cooke et al., 1984; Cornelissen et al., 2008; Höglund, 2014; Kearsley & Whitham, 1998; Weis, 2014). Morrison and Quiring (2009) discovered that gall-inducing midges (*Harmandia tremulae* Winnertz, Diptera: Cecidomyiidae) within the crown of trembling aspen (*Populus tremuloides* Michx, Salicaceae) specifically targeted young, more vigourous leaves instead of mature leaves.

Maternal effects are defined as a causal influence of maternal environment or phenotype on the performance of offspring (Marshall & Uller, 2007; Räsänen & Kruuk, 2007; Rossiter, 1996; Wolf & Wade, 2009). Maternal effects have the potential to give insect offspring a short-term boost or reduction to fitness when they are exposed to a variable environment. These effects are often expressed mainly as phenotypic plasticity (adaptive or otherwise) in the offspring (Räsänen & Kruuk, 2007). Factors that influence maternal effects include quality and quantity of resources, threats from predators, changes in temperature and climate, and the probability of mating; all of which can induce maternal effects that can contribute to overall fitness in insect offspring (Mousseau & Fox, 1998).

Different feeding guilds of herbivorous insects interact in different ways within the same environment, especially when contrasting generalists and specialists. Generalist insects have the ability to consume a variety of plant species among different plant families or within a plant family, and thus often have to deal with numerous predators, parasitoids, or defensive plant chemicals (Ali & Agrawal, 2012). On the other hand, highly specialized insects are usually constrained to feed on one or very few closely related plant species (genus level), and as such are affected by a limited range of predators, parasitoids, and plant defensive chemicals (Ali & Agrawal, 2012; Price, 1991). Cornell and Hawkins (1995) found that endophytic insects (mostly specialists) suffer from predator- or parasitoid-induced mortality much less than exophytic insects (mostly generalists). As a result, there are different selective pressures that can induce maternal effects depending on whether the insect species is exophytic or endophytic. For example, Fox et al. (1997) found that maternal host species choice in a generalist herbivore, the mother seed beetle *Stator limbatus* Horn (Coleoptera: Bruchidae), can strongly affect egg size. They determined that mother seed beetles lay much larger eggs on one host than another, which is adaptive as fitness consequences of egg size vary on each host species. Rossiter (1991) found that increased leaf defensive chemical density in the maternal diet negatively affected the pupal weights and dispersal tendency of the offspring of the polyphagous gypsy moth (*Lymantria dispar* L, Lepidoptera: Erebidae). Maternal host plant selection of specific individual plants significantly influences offspring performance in the cynipid gall-wasp *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae), with certain host plant individuals (genotypes) strongly improving offspring performance compared to neighbouring plants (Egan & Ott, 2007). However, relatively little research has been conducted on maternal effects of endophytic insects, gall-formers in particular, as they are highly specialized to one or few host species (Harris, 1973; Harris & Shorthouse, 1996).

Maternal effects may improve or diminish offspring performance when they are introduced to new environmental factors, such as diet, predator presence, or mate availability. For example, Newcombe et al. (2015) found that specialist milkweed bugs *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae) increased their egg size, while the larvae had a faster development time when they consumed a different diet than their mother. Miller (2008) found that maternal choice of host species for egg-laying by the heliconia bug *Leptoscelis tricolor* Westwood (Hemiptera: Coreidae) affects daughter performance via mating probability and fecundity, which matched seasonal changes in plant resources of each host plant species. The way that environments can influence insect offspring performance vary; from improved defense from predators (Coslovsky & Richner, 2011) to increased susceptibility to pathogens or parasites (Ben-Ami et al., 2010; Stjernman & Little, 2011). Riddick and Wu (2012) found that the food source utilized by *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) influenced egg size, which was positively correlated to hatching success. Maternal effects are increasingly being recognized as an important influence on natural selection, as they can translate maternal experience into offspring performance (Mousseau & Fox, 1998). Thus, maternal effects are an important variable to examine when attempting to determine potential insect performance when introduced to a new environment.

Identifying the environmental conditions that are most conducive to high insect performance is critical with regard to biocontrol programmes (Price, 1999). Biocontrol agents must exert such an impact on the target invasive species that there is an overall population decrease (Clewley et al., 2012; Cullen, 1992; Harris, 1973), while minimizing detrimental effects to non-target species (de Clercq et al., 2011; Pemberton, 2000; Thomas & Willis, 1998). Furthermore, another difficult step in a biocontrol programme is establishing the agent in the field (Grevstad, 1999). Maternal effects can be used to improve agent performance when rearing and subsequently releasing a biocontrol agent in the field, in order to maximise insect performance..

Gall-forming insects

One of the most commonly used guilds of insects for classical biocontrol are the gall-formers, due to their high host specificity and potential efficacy (Harris, 1973; Harris & Shorthouse, 1996). Gall-forming insects form atypical growths (galls) of existing plant tissues that nourish and protect the insect progeny as they develop (Lalonde & Shorthouse, 1985; Stone & Schönrogge, 2003). These specialist insects have the ability to manipulate host resources to their advantage (Harris & Shorthouse, 1996; Hartley & Lawton, 1992; Rehill & Schultz, 2001), as galls are physiological sinks of plant nutrients (Castro et al., 2012; Huang et al., 2014; Larson & Whitham, 1991). Gall-formers typically choose young meristematic tissues for oviposition, as these are more easily reorganized and redirected in the formation of a gall (Lalonde & Shorthouse, 1985; Shorthouse et al., 2005; West & Shorthouse, 1989). Gall-formers are highly specific to their hosts, both through preference for their host and by the ability to physiologically form galls and successfully complete their life cycle (Ghana et al., 2015). The result of gall production is that plant performance can be reduced by diverting resources from other plant parts to gall tissues, or by reducing sexual or vegetative reproductive success by terminating normal growth in the affected tissues via gall formation (Klöppel et al., 2003; Miao et al., 2011). This drain of resources from the plant via galling can reduce plant performance to the extent that the target weed populations can be successfully controlled (Harris & Shorthouse, 1996; Miao et al., 2011). Examples include the successful control of the invasive shrub *Acacia longifolia* Willd. and golden wattle *Acacia pycnantha* Benth. (Fabaceae) in South Africa by pteromalid gall wasps (Hymenoptera: Pteromalidae) (Dennill, 1990; Hoffmann et al., 2002) and control of the invasive weed *Parthenium hysterophorus* L. (Asteraceae) in Australia by a stem-galling moth (*Epiblema strenuana* Walker, Lepidoptera: Tortricidae) (Dhileepan, 2001).

Most research conducted with gall-formers and soil nitrogen simultaneously has focused on insect performance due to nitrogen addition, rather than the combined effects of galling insect and nitrogen on plant performance (Gange & Nice, 1997; Malinga et al., 2014; Moran & Goolsby, 2014). For example, Moran and Goolsby (2014) noted that fertilized giant reed (*Arundo donax*) attacked by the shoot-tip galling wasp *Tetramesa romana* produced insects that had much shorter generation times, which increased population growth rates of this insect. Similarly, Hinz and Müller-Schärer (2000) found that the potential fecundity of gall midges *Rhopalomyia* n. sp. (Diptera: Cecidomyiidae) was boosted when they were exposed to fertilized scentless chamomile (*Tripleurospermum perforatum* L, Asteraceae). Additionally, Malinga et al. (2014) determined that fertilization of *Neoboutonia macrocalyx* Pax (Euphobiaceae) host plants improved the abundance of cecidomyiid gall formers. However, some experiments have focused on the interaction between soil nitrogen and insect galling, and their impact on plant performance. For example, Klöppel et al. (2003) found that *Aulacidea subterminalis* Niblett. (Hymenoptera: Cynipidae) did not interact significantly with increased soil nitrogen availability to affect *Pilosella officinarum* (Asteraceae), though wasp galling did reduce stolon lengths under all treatments. Fay et al. (1996) found that rosinweed (*Sylphium integrifolium*) was able to tolerate galling by the apical meristem galler *Antistrophus silphii* with little loss of biomass or reproductive output when soil nutrients were supplemented. In general, gall-formers respond positively to fertilization of their host plants, often because the application of fertilizer improves the vigour of their hosts, and gall-formers are very sensitive to host plant vigour (Craig et al., 1989; Price, 1991).

A main source of environmental heterogeneity to which gall-formers are exposed stems from the timing of oviposition site selection; i.e. usually for young, vigourous plant parts (Craig et al., 1989; Faria & Fernandes, 2001; Price, 1991) that remain photoassimilate sinks for only a short time (Höglund, 2014). In some cases, gall-formers can even specialize on specific host genotypes within the same host species due to genotypic differences in growth rates (Craig et al., 2000; Höglund, 2014). These galls provide buffering effects for the insect, protecting them from changes in the outside environment by providing them with homogeneous access to nutrients and limiting interactions with plant defensive chemicals (Castro et al., 2012; Hartley & Lawton, 1992). The ovipositing insects must navigate an environment of variable plant parts in order to find this optimal oviposition site to maximize offspring performance; all the while being exposed to predators and variable conditions outside the safe environment that is the gall. Such an experience can cause maternal effects to influence offspring performance. For example, Weis and Price (1983) found that the gall-former, *Asteromyia carbonifera* O.S. (Diptera: Cecidomyiidae), varies its clutch size as a trade-off with offspring performance due to maternal response to predation risk; as predation risk increases, so does clutch size, resulting in smaller, less fecund offspring. Another factor within their variable environment is gall-former timing of attack on their specific host species. They must coordinate with their host's phenology in order to maximize fitness by choosing tissues that have enough vigour to produce the greatest larval survival (Craig et al., 1989).

Biological control of Pilosella *hawkweed species*

Pilosella (Asteraceae) hawkweeds originate from Central Europe and are invasive in many parts of the world, especially in North America and New Zealand (Makepeace, 1985a; Wilson, 2006; Wilson et al., 1997). Most species readily reproduce vegetatively from spreading stolons and rhizomes (Day & Buckley, 2011; Wilson et al., 1997), and can also produce up to 40 000 wind-dispersed seeds/m^2 hawkweed cover per year (Burton & Dellow, 2005). Additionally, most *Pilosella* hawkweeds can tolerate heat, frost and snow (Barker et al., 2006). These hawkweeds are difficult to identify in the field, which is exacerbated by the fact that they are also prone to hybridization, which influences the ecological niches in which each species occurs (Moffat et al., 2015). This may make it more difficult to control them in invaded territories as differences in genotypes may be sufficient to affect specific biocontrol agent effectiveness (Cronin & Abrahamson, 1999; Manrique et al., 2008). These different species of hawkweeds have several morphological traits that can be used for identification, including leaf and stem pubescence, as well as number of flowers per stem (Moffat et al., 2015). They have been identified as a group of species that may increase their invasiveness under climate change, resulting in extensive range shifts (Beaumont et al., 2009a). Most invasive hawkweed species can reproduce vegetatively with stolons (Wilson, 2006; Wilson et al., 1997), which are horizontal above-ground stems, which can take root and sprout daughter rosettes from their nodes. This vegetative reproduction is crucial to the maintenance of older patches (Thomas & Dale, 1975; Winkler & Stöcklin, 2002). The production of stolons and daughter rosettes via vegetative reproduction prevents other plants from germinating and encroaching into hawkweed patches. This leads to a disruption of native biodiversity that creates large, thick patches of hawkweeds that can persist for over 30 years with densities upwards of 3500 plants per m^2 (Thomas & Dale, 1975). Additionally, some species use allelopathy in the form of acidic halos around patches to inhibit or prevent other species from encroaching on patches (Dawes & Maravolo, 1973; Makepeace et al., 1985). As result of their invasiveness and impact on native biodiversity, some of these invasive hawkweed species are targets for biocontrol (Moffat et al., 2015).

One of the *Pilosella* species that has been studied extensively with respect to invasiveness, biology, and potential for control, is *P. officinarum* (mouse-ear hawkweed). This species performs well in low soil moisture and fertility (Bishop & Davy, 1994; Rose et al., 1998). Additionally, this species is especially invasive in degraded ecosystems (Syrett et al., 2012), where it can respond rapidly to pulses of nutrients, such as those caused by nearby fertilizer use (Fan & Harris, 1996). This is possibly due to the fact that *P. officinarum* undergoes facultative associations with mycorrhizal fungi, which improves its ability to obtain phosphorus and other nutrients in nutrient poor sandy soils (Höpfner et al., 2015). Further, *P. officinarum* aggressively competes with native plants to form monocultures, and hinders their growth via allelopathy (Barker et al., 2006). They can force these neighbouring plants to use nitrates and organic nitrogen instead of ammonium (Díaz-Barradas et al., 2015). This species readily takes advantage of bare soil for germination, such as in recently disturbed areas (Cipriotti et al., 2010). It can be highly competitive for phosphorus (P), which may increase its invasiveness in low P soils (Davis, 1997). There is also some research that suggests that *P. officinarum* may be effectively controlled with fertilizer treatments (Davis, 1997; Syrett et al., 2004), as many native plant species benefit more strongly from fertilization than *P. officinarum* (Davy & Bishop, 1984). This method of control is particularly effective when competition from native plant species is intense and when grazing is low or restricted, as grazing negatively affects native plant species while *P. officinarum* remains relatively ungrazed (Fan & Harris, 1996; Scott et al., 1990; Syrett et al., 2004; Winkler & Stöcklin, 2002).

A biocontrol programme for the mitigation of *Pilosella* hawkweeds in North America was begun in 2000 (Moffat, 2012). As there are several native hawkweeds (*Hieracium*) in North America, biocontrol agents for *Pilosella* hawkweeds must be sufficiently host-specific to prevent off-target effects from damaging native species. The stolon-galling wasp, *Aulacidea subterminalis*, is quite host specific, attacking only four related *Pilosella* hawkweed species: *P. officinarum, P. flagellaris* Willd*, P. aurantiaca* L. and *P. floribunda* Wimmer & Grab, and attacking no *Hieracium* species (Grosskopf et al., 2008). This wasp species has been released in North America on *P. aurantiaca* in the USA and *P. flagellaris* in Canada, but has only established on the latter species (Winston et al., 2014). It also has been released in New Zealand on *P. officinarum*, and was investigated by Klöppel et al. (2003) who found it to suppress stolon growth of its host when plants were under nutrient and water stress. In their experiment, fertilization improved stolon length and increased the number of lateral stolons produced, while wasp galling increased the production of lateral stolons while reducing length of main stolons. This results because these gall wasps terminate stolon growth by galling the apical meristematic tissue of these plant organs. However, Syrett et al. (2012) point out that although *A. subterminalis* has established at some target sites in New Zealand, this species is unlikely to be effective at reducing *P. officinarum* ground cover unless soil fertility is increased. As of 2014, over $2.9 million dollars has been spent on controlling *P. officinarum* in New Zealand alone, with a total of five agents released (Paynter et al., 2015).

The leaf-galling wasp *Aulacidea pilosellae*, is currently being investigated as a potential new biocontrol agent of select invasive *Pilosella* species (Moffat et al., 2013). This wasp species is composed of several biotypes (Moffat, 2012), which appear to have different host ranges and biology's, which make them useful potential agents for control of several specific *Pilosella* hawkweed species (Cordat et al., 2012). This species host range includes: *P. offincarum*, *P. cymosa* L., *P. echioides* Lumnitzer., *P. flagellaris*, *P. floribunda, P. piloselloides* Vill., *P. caespitosa* Dumort. and *P. glomerata* Froel. (Moffat et al., 2013). Both *P. officinarum* and *P. caespitosa* have invaded wide areas within North America, ranging from B.C. to Newfoundland in Canada and Washington to Georgia and north in the U.S.A. (Wilson et al., 2006). But *P. glomerata* is a relatively recent invader, only present in B.C. in Canada, and Washington and Idaho in the U.S.A. (Wilson et al., 2006) These species occur in mixed patches or as monocultures in their invaded North

American range (Wilson, 2006; Wilson et al., 2006). *Aulacidea pilosellae* appears to exhibit host density-dependent attack in its native European range, with galls often found on the most abundant hawkweed host in mixed-species patches (Moffat et al., 2013). This suggests a host-switching mechanism is present, though the relative wasp performance on the multiple hawkweed hosts is unknown.

Experimental objectives

The goal of this Thesis was to determine if *A. pilosellae* will be an effective agent for invasive hawkweeds. In the first experiment we explored how *A. pilosellae* galling affects *P. officinarum* performance under several nitrogen treatments to determine what soil fertility would improve the probability of successful control of this invasive weed species. With several *A. pilosellae* biotypes that attack multiple *Pilosella* species (Moffat, 2012; Moffat et al., 2013), we hope that these results can improve the success of future biocontrol programmes targeting *Pilosella* spp. We used the *A. pilosellae – P. officinarum* system for this experiment as much previous research has been conducted with this plant species with regards to biocontrol and nutrient regimes in New Zealand (Klöppel et al., 2003; Steer & Norton, 2013; Syrett et al., 2012). However, there is limited research on the interaction between nitrogen and gall-former impact on plant performance. We tested four predictions: 1) Increased nitrogen availability will improve *P. officinarum* performance, 2) *Aulacidea pilosellae* (i.e., gall formation) will reduce *P. officinarum* performance, 3) There will be an interaction between nitrogen availability and *A. pilosellae* presence on the performance of *P. officinarum*, with the effect of nitrogen counteracting the damage caused by the wasp under high nitrogen availability, and 4) enhanced nitrogen will increase *A. pilosellae* performance via plant mediated interactions. There have been few studies that combine the effects of nitrogen and gall-formers simultaneously on their host plant (Hinz & Müller-Schärer, 2000; Malinga et al., 2014). Generally when these factors are studied in conjunction, it is to measure the plant-mediated effects of nitrogen on the performance of the insect agent (Hinz & Müller-Schärer, 2000). This makes sense because in biocontrol, agent survival, performance and establishment are of utmost importance (Grevstad, 1999; Shea & Possingham, 2000). However, it is crucial to understand how different biotic and abiotic factors interact with biological control efforts, as they can either act in a complementary fashion (i.e., additively or synergistically) to increase impact on the target plant, or they can act antagonistically, potentially reducing overall impact on the target plant (Jamieson et al., 2012).

In a second experiment, we aimed to identify *A. pilosellae*'s performance on two common European field hosts, meadow and yellowdevil hawkweeds (*P. caespitosa* and *P. glomerata*, respectively), which are currently present in North America (Wilson, 2006) and targeted for biocontrol, especially in British Columbia where they are invasive. As this insect exhibits host density-dependent attack in the native range (Moffat et al., 2013), the baseline performance on the different hosts should be identified before a biological control programme is initiated. We predicted that: 1) maternal effects through maternal host species will improve offspring performance when exposed to the same host as the mother; and 2) offspring host effects will be stronger than maternal effects in determining wasp performance, due to evolved host specialization. It would be particularly useful if one agent can be utilized to control multiple target species, especially with regards to the many related *Pilosella* hawkweed species that are all considered invasive, with many being potential candidates for a biocontrol programme.

Chapter 2: Interactive effects of a leaf-galling wasp and nitrogen on an invasive hawkweed species

Introduction

In addition to increasing ecosystem susceptibility to invasive species (Davis & Pelsor, 2001), increased soil nitrogen tends to decrease species richness (Bedford et al., 1999; Powell et al., 2011). Within the last several decades there has been a significant increase in anthropogenic nitrogen deposition to the biosphere as a result of fossil fuel combustion and agricultural fertilization (Vitousek et al., 1997). Furthermore, ecosystems with high resource availability and low biodiversity are especially vulnerable to disruption from invasive species (Davis & Pelsor, 2001; Powell et al., 2011). Invasive plants often improve their performance under enhanced nitrogen conditions relative to native plants (Funk, 2008) due to greater inherent phenotypic plasticity, which allows these plants to respond to a variety of different environments (Joshi et al., 2014; Sorte et al., 2013; Van Kleunen et al., 2010). As such, underlying site fertility may be a critical factor to measure when determining an environment's susceptibility to invasive species (Coetzee et al., 2007; Mattingly & Reynolds, 2014; Vourlitis & Kroon, 2013).

Changes in soil nutrients may boost the concentration of nutrients available in plant organs, which can influence the quality of those organs as food for phytophagous insects (Butler et al., 2012; Center et al., 2014; Minkenberg & Ottenheim, 1990). Some insects can be positively influenced by the availability of nutrients to their plant host, with variation in soil nutrients influencing insect survival, fecundity, and sex ratios via plant mediated effects (Bownes et al., 2013a). Moran and Goolsby (2014) noted that fertilized giant reed (*Arundo donax*) attacked by the shoot-tip galling wasp *Tetramesa romana* produced insects that had much shorter generation times, which increased population growth rates of this insect. However, some insects have increased access to plant nitrogen when plants are stressed, as senescing plant parts tend to increase in nutritional quality as a response to nutrient stress (Waring & Cobb, 1992; White, 1984). The insects that benefit from feeding on stressed plants are also commonly known as "senescence-feeders", as they feed preferentially on mature plant tissues (White, 2009). Stressed plants have increased levels of accessible nitrogen in the form of amino acids due to reduced protein synthesis (White, 1969, 1974; White, 1984). However, "flush-feeders" or those that feed on young tissue, tend to benefit from healthy plants rather than stressed plants (White, 2009)~~.~~

To the detriment of some weed biocontrol programmes, invasive plants may compensate for damage caused by insect herbivores when they have increased access to nutrients, as predicted by the "compensatory continuum hypothesis" (Maschinski & Whitham, 1989). This hypothesis describes how insect herbivory influences the performance of the plant eaten; increased vegetative or reproductive growth compared to ungrazed plants (overcompensation), through equal or replacement growth (compensation) to restricted vegetative or reproductive capacity (undercompensation)

(Maschinski & Whitham, 1989). Indeed, Maschinski and Whitham (1989) go on to predict that overcompensation to herbivory is most likely when nutrient availabilities are high, competition is low, and herbivory is early in the growing season. Accordingly, Bownes et al. (2013b) found that biocontrol efficacy of *Cornops aquaticum* in controlling invasive water hyacinth (*Eichhornia crassipes*) in South Africa would improve if nutrient pollution was limited. They state this is due to water hyacinth's ability to compensate for damage by this herbivore to maintain biomass and vegetative reproduction rates when nutrients are abundant. A similar trend was observed with the gall-wasp *Antistrophus silphii* on rosinweed (*Silphium integrifolium*), with plant tolerance to galling being highly positively dependent on resource availability (Fay et al., 1996). However, not all plants are able to compensate for insect damage via herbivory when nutrients are readily available. Meyer and Root (1993) showed that feeding by a spittlebug (*Philaenus spumarius*), beetle (*Trirhabda* sp), and aphid (*Uroleucon caligatum*) all reduced goldenrod (*Solidago altissimsa*) performance when under high soil nitrogen, but were much less effective when soil nitrogen was low. Thus, insect preference and performance should be evaluated under various soil fertilities, as plant-mediated effects may reduce the effectiveness of biocontrol programmes in controlling their target plant (Hovick & Carson, 2015).

Identifying the environmental conditions that are most conducive to high insect performance is critical with regard to biocontrol programmes (Price, 1999). To be effective, biocontrol agents must induce a sufficient impact on the target invasive species such that their populations decrease (Clewley et al., 2012; Cullen, 1992; Harris, 1973). It is also difficult in a biocontrol programme to establish the agent in the field (Grevstad, 1999), as its performance in a new environment is difficult to predict (McFadyen, 1998). In fact, agent establishment may take several years (Hoffmann, 1995), with control of the target plant occurring up to 10 years later (McFadyen, 1998). Biocontrol success can be improved if agents are released in areas favourable to insect performance, as establishment success can be enhanced and insect performance can be maximized, increasing the probability of control of the target plant. Considering that some invasive plant species can compensate for herbivory when excess nutrients are available, low nutrient sites should be targeted for release of agents for these species (Coetzee et al., 2007). Underlying site fertility can have strong implications for biocontrol programmes as they relate to insect performance and subsequent impact on the target invasive plant (Bakker & Nolet, 2014; Hovick & Carson, 2015).

One of the most commonly deployed guilds of insects for classical biocontrol are the gall-formers, due to their high host specificity and potential efficacy (Dennill & Gordon, 1990; Fay et al., 1996; Harris, 1973; Harris & Shorthouse, 1996). Gall-forming insects form atypical growths (galls) of plant tissue, inside which the insect is nourished and protected throughout development (Lalonde & Shorthouse, 1985; Stone & Schönrogge, 2003). These specialist insects have the ability to manipulate host resources to their advantage (Harris & Shorthouse, 1996; Hartley & Lawton, 1992; Rehill & Schultz, 2001), as their galls are physiological sinks of plant nutrients that are used to form the gall as well as feed the contained insect (Castro et al., 2012; Huang et al., 2014; Larson & Whitham, 1991). This drain of resources can reduce plant performance to the extent that the target weed populations can be successfully controlled (Harris & Shorthouse, 1996; Miao et al., 2011). Examples include the successful control of the invasive shrub *Acacia longifolia* and golden wattle *Acacia pycnantha* in South Africa by pteromalid gall wasps (Dennill, 1990; Hoffmann et al., 2002) and control of the invasive weed *Parthenium hysterophorus* in Australia by a stem-galling moth (*Epiblema strenuana*) (Dhileepan, 2001). However, there has been little research conducted on the interaction between soil nutrients, gall-formers, and their hosts.

A leaf-galling wasp, *Aulacidea pilosellae*, is being investigated as a potential new biocontrol agent of invasive *Pilosella* hawkweed species (Moffat et al., 2013). This wasp has several biotypes which are specific to a number of invasive hawkweed species (Moffat, 2012), including mouse-ear (*P. officinarum*), meadow (*P. caespitosa*) and yellowdevil hawkweed (*P. glomerata*). These hawkweeds are endemic to Central Europe and are invasive in many parts of the world, especially in North America and New Zealand (Makepeace, 1985a; Wilson, 2006; Wilson et al., 1997). Most hawkweed species perform well in low soil moisture and fertility, often with preferences for degraded ecosystems, such as recently disturbed areas or permanent pastures (Syrett et al., 2012; Wilson, 2006). *Pilosella officinarum*, in particular, performs well in these low-nutrient areas (Bishop & Davy, 1994; Rose et al., 1998), where it can respond rapidly to pulses of nutrients, such as those caused by nearby fertilizer use on crop- and rangelands (Fan &

Harris, 1996). However, *P. officinarum* does not perform well when under intense competition from native plants or when grazing is low or restricted, as grazing generally reduces competitive neighbouring plant species biomass more than *P. officinarum* (Scott et al., 1990). *Aulacidea pilosellae* is a potential candidate agent for control of several *Pilosella* hawkweeds, identified because of its ability to gall several species of hawkweeds in their native range.

It is crucial to understand how different biotic and abiotic factors interact in biological control, as they can either act in a complementary fashion (e.g., additively or synergistically) to increase impact on the target plant, or they can act antagonistically, potentially reducing overall impact on the target (Jamieson et al., 2012). We used the *A. pilosellae* – *P. officinarum* system for this experiment as much previous research has been conducted with *P. officinarum* with regards to biocontrol and nutrient regimes in New Zealand (Klöppel et al., 2003; Steer & Norton, 2013; Syrett et al., 2012). With several *A. pilosellae* biotypes that attack multiple *Pilosella* species (Moffat, 2012; Moffat et al., 2013), we also hope that our study will help improve the success of future biocontrol programmes targeting various *Pilosella* spp. The objective of this greenhouse experiment was to identify the joint effects of nitrogen availability and of *A. pilosellae* galling on the performance of invasive *P. officinarum*. We tested four predictions: 1) Increased nitrogen availability will improve *P. officinarum* performance, 2) *Aulacidea pilosellae* (i.e., gall formation) will reduce *P. officinarum* performance, 3) There will be an interaction between nitrogen availability and *A. pilosellae* presence on the performance of *P. officinarum*, with the effect of nitrogen counteracting the damage caused by the wasp under high nitrogen availability, and 4) enhanced nitrogen will increase *A. pilosellae* performance via plant-mediated interactions.

Materials and Methods

Plants

The plant species used for this experiment was the perennial herbaceous weed *P. officinarum* (mouse-ear hawkweed). This species reproduces using seeds, and also vegetatively with stolons that can take root and grow daughter rosettes at nodes, with lateral stolons branching off the main stolons that emerge from the primary rosette. Plants were grown from seed collected in 2004 in Thurston Co., WA, and stored at 5°C. Seeds were germinated on water-saturated vermiculite in sealed glass Petri dishes, then transferred to root trainers containing heat-sterilized (48 hrs at 72°C) 1:1:1 Cornell mix:sand:loam (Boodley & Sheldrake, 1977). Plants were allowed to grow in a greenhouse for four weeks, at day/night temperatures of 22/18°C and 16 h/day natural light supplemented with high-pressure sodium bulbs. Root-trainer plants were watered when soil was dry by soaking for several hours before draining. The plants were transplanted into 15 cm diameter pots containing heat-sterilized (48 hrs at 72°C) 1:1 sand:loam. Soil sterilization was used to prevent microbial, insect and fungal contamination, (e.g. mycorrhizal fungi). Watering was automated using drip irrigation at 60, 80, 100 or 120 mL per day, depending on the size of the plants. The plants were allowed to grow under the same greenhouse conditions for a further 1-2 weeks prior to experimental use.

Insects

Aulacidea pilosellae wasps create unilocular galls (i.e., one larva per gall) on the midrib of hawkweed leaves, generally towards the leaf base, or on stolons. Galls are 2-4 mm in length and ellipsoid, with the body length of emerging adults about 1-2 mm. The wasps of this study were multivoltine and parthenogenetic, with proovigenic females (eggs fully developed at emergence). They develop with three larval instars and an adult lifespan of 7-10 days, with 1-2 generations per year in the field, though up to four per year in the lab. The *A. pilosellae* wasps used for the experiment were imported from the Centre for Agriculture and Biosciences International (CABI) in Switzerland in 2012 as final instar larvae within galls on their host, *P. officinarum*. They were reared for three generations in 2013 within containment at the Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, Alberta. Larvae were overwintered (in galls) at 5°C for five months, and moved to 10/8°C (16/8 hr day/night) for one week, then to 22/18°C (16/8 hr day/night) in a mesh-covered container for emergence. To maintain humidity at 100%, the container was placed under a 50x24x16 cm plastic cover containing potted *P. officinarum*. Each container was checked daily for wasp emergence. Wasps that emerged were placed on 0.25 mm Dacron mesh-covered plants immediately (approximate volume of covered plant cage: 1200 cm^3, cone-shaped) and reared for one generation after overwintering, before experiment setup. Plants were covered for two weeks after wasp introduction.

Experimental design

To determine the effects that nitrogen and *A. pilosellae* galling had on *P. officinarum* performance, three nitrogen treatments were crossed with two wasp treatments (present or absent), with five spatiotemporal blocks consisting of 24 plants each; four replicates of each of the six treatment combinations. Plant positions within blocks were randomized using R (2015) with blocks set up on five consecutive days in accordance with wasp emergence. *Aulacidea pilosellae* were checked daily for emergence, and released on plants as available. Five female wasps were introduced to each wasp treatment plant over two days per block (six days total), with wasps selected after two days of initial emergence. Nitrogen was added in the form of ammonium nitrate (NH_4NO_3) in one of three amounts (none, low, high): 0, 75 and 150 mg/kg of soil per pot. The nitrogen densities used were determined to be practical levels present within natural environments where hawkweeds can occur (Blackshaw & Brandt, 2008; Blackshaw et al., 2003; Purton et al., 2015), with our low nitrogen treatment (75 mg NH_4NO_3/kg soil) being comparable to a fertilizer level used in a similar experiment (Klöppel et al., 2003). The weight of the soil was calculated dry (three days at 30°C with 0% humidity), by averaging the weight of 20 soil-filled pots. Ammonium nitrate was dissolved in the appropriate amount of water for the plants each day in lieu of the automated delivered water, and applied by pouring evenly on the top of the soil.

Nitrogen was applied at zero, three, and six weeks after initial wasp introduction. The first application was immediately prior to the introduction of the first wasps to the plant (zero weeks).

Non-destructive plant performance measurements

The number of leaves and main stolons were counted at the initial introduction of wasps to plants. Two weeks later, when cages were removed, a main stolon was randomly selected and total main stolon length and number of lateral stolons was recorded, with a twist tie loosely wrapped around the stolon for repeated measures four, six, and seven weeks after final wasp introduction. Plants were harvested at seven weeks. The number of main and lateral stolons, flower stems, and senesced leaves per plant were measured at this time.

Destructive plant performance measurements

Seven weeks after final wasp introduction, the plant shoots were harvested by severing the plant at the soil level. All main stolons and rhizomes were counted, and each stolon and rhizome had the following measured: length, number of lateral stolons, gall position (leaf, lateral or main stolon), and number of galls, as well as whether the stolon was sexually reproductive (flowering) or vegetative. For each plant, the number of flowering stems also was recorded. Plants with galls were placed into paper towel-lined 1 L plastic containers and set in a rearing room at 22/18°C (day/night) under artificial lights (16/8 hr day/night) for emergence after destructive plant measurements were recorded. After wasp emergence and gall content removal, all plants were dried on the greenhouse bench in paper bags before being sterilized at 72°C for 48 hrs and dried for a further seven days at 0% humidity and 30°C in a drying oven before being weighed.

Wasp performance measurements

Plants were checked daily for wasp emergence, at which point a consistent humidity (~100%) was maintained within containers. The number of emerged wasps was recorded (emergence success), and the wasps not used for rearing were stored in 95% ethanol. After emergence had ceased for 2-3 days, the remaining galls of each plant were dissected and the contents recorded and removed as either immature larva (first-second instar), mature third instar larva, pupa, adult (living or dead), or empty/dead of unknown stage. Development success was measured as the sum of third instar larvae, pupae and adults (emerged or dead)/total galls. Insects were removed at this time such that they were not included in the plant dry weights. Wasps stored in 95% ethanol had abdomens dissected for mature egg counts. Ten wasps from each nitrogen treatment from each block were randomly selected and dissected under a dissecting microscope (n = 150).

Nitrogen analysis

In each block-by-treatment combination, additional plants were predesignated for harvest throughout the experiment to have their leaves removed and dried (as above) for determination of percent nitrogen content. Leaves were harvested at zero, four, and seven weeks after wasp introduction, with four plants per treatment. Galled and non-galled leaves were separated for wasp treatment plants, and all ungalled leaves were pooled for measurements. All dried leaf tissue was finely ground before Kjeldahl-Nitrogen analysis (performed by D. Messer, Swift Current Agriculture and Agri-food Canada) (Sáez-Plaza et al., 2013). The galled leaves were dissected and the insects removed before drying so that insects were not included in the nitrogen analysis.

Statistical analysis

R (2015) was used for all statistical tests. Two pots were removed from analysis as they contained two *P. officinarum* plants instead of one. Analysis of Variance (ANOVA) was used on continuous variables, generalized linear models with Poisson distribution (GLM-P) were used on count variables (Nelder & Wedderburn, 1972), and negative binomial generalized linear models (GLM-NB) were used on over-dispersed count data (Zuur et al., 2009). Repeated-measures ANOVA was carried out on biweekly measurements of random stolon lengths, total number of lateral stolons, and total number of senesced leaves. Block was used as a factor in all tests, along with the main and interaction effects of nitrogen treatment crossed with wasp treatment.

Results

Dry weights and leaf senescence

Increased nitrogen availability had a significant positive effect on above-ground plant dry weight, while insect galling had a significant negative effect on above-ground plant dry weight (Figure 2.1, Table 2.2). We detected a significant interaction between nitrogen treatment and wasp galling on above-ground dry weight with a greater negative impact due to galling when plants were exposed to higher amounts of nitrogen (Figure 2.1, Table 2.2).

Total leaf senescence was unaffected by nitrogen treatments, but was significantly increased as a result of wasp galling (Table 2.2, 2.3). There was also a significant interaction between week and wasp, with more leaves senescing between weeks two and four when wasps were present (Table 2.3).

Vegetative and reproductive growth

Increased nitrogen availability had a significant positive effect, whereas wasp presence had a significant negative effect on the mean total number of lateral stolons between treatments (Figure 2.2, Table 2.2) at week 7 and throughout the experiment (Table 2.3). In addition, there was an interactive effect between nitrogen and wasp galling, with wasps only reducing mean number of lateral stolons when nitrogen treatments were high or low compared to controls (Figure 2.2, Table 2.2). Under no nitrogen, wasp presence did not affect the number of lateral stolons produced (paired t-test, $p = 0.78577$) (Figure 2.2, Table 2.2). There was an interaction between wasps, nitrogen and week (Table 2.3), with galling delaying the production of lateral stolons under high and low nitrogen treatments (Figure 2.2). Lateral stolon growth was initiated between weeks 2 and 4 when wasps were absent and between weeks 4 and 6 when wasps were present (Figure 2.2).

Most main stolons were initiated by experiment setup at week 0 (451 main stolons at week 0 out of a total of 685 at week 7 from a total of 118 plants); however, the longest main stolon at week 0 in blocks 1, 2, 3, 4, and 5 were less than 30, 50, 60, 80 and 70 mm, respectively. All plants at week 0 had been in pots for 6-11 days (and in root trainers for 4 weeks) prior to experiment setup. Galling had a strong negative effect on randomly selected main stolon length (Table 2.3), with shorter main stolons observed at all nitrogen treatments at two weeks after initial wasp introduction and continuing thereafter (Figure 2.3). There was also an effect due to nitrogen (Table 2.3) with high nitrogen treatment plants having shorter stolons than low or no nitrogen treatment plants, and main stolon growth slowing drastically after four weeks (Figure 2.3). There were no significant effects detected for number of main stolons or reproductive stems at week 7 as a response to nitrogen, wasps, or interactive effects between any factors (Table 2.2).

Leaf nitrogen content

Increased nitrogen availability had a significant positive effect on the leaf nitrogen content per plant (Figure 2.4, Table 2.2). Wasp presence had differing effects on leaf nitrogen content in galled and ungalled leaves within the same plants. At seven weeks, ungalled leaves had more nitrogen than control leaves (wasp absent) when nitrogen was high, but not under no or low nitrogen treatments (Figure 2.4). At the same time, galled leaves under all nitrogen treatments had significantly less nitrogen than ungalled leaves and control leaves (Figure 2.4).

Aulacidea pilosellae *performance*

There were no significant effects detected on wasp performance due to nitrogen treatments as measured by gall number, emergence success, development success, or number of eggs per adult (Tables 2.1, 2.4). Wasps began developing into pupae four days before plants were harvested at week 7, and emergence started immediately after harvest. Adult emergence lasted for 16 days, from 51-67 days after wasp introduction, with peak emergence between 52-60 days. Among all treatments, there was an average of 71.8 ± 4.3 wasps (mean ± SE) emerged per plant from an average of 155.1 ± 6.6 galls per plant. Most galls (51.9 ± 11.7%) contained final instar (L3) larvae after adult emergence, while the adults did not have significantly different numbers of eggs as a result of nitrogen treatment (Table 2.4).

Table 2. 1. Summary table of response variables associated with *A. Pilosellae* performance on *P. officinarum* per plant. Means ± standard error (n=) are shown. None of the values are significantly different between nitrogen treatments (at P < 0.05).

Response variable	None	Low	High
Total Number of Galls	157.9±10.85(20)	153.0±10.62(20)	152.8±13.68(19)
Emergence Success (wasps emerged)	70.0±7.19(20)	71.9±7.15(20)	72.8±8.68(19)
Development Success to 3rd Instar (3rd instar larvae) (%)*	82.4±6.62(20)	76.3±5.13(20)	74.8±5.75(19)
Number of Eggs per female	76.8±1.53(50)	75.8±1.64(50)	75.8±1.21(50)

*after emergence of adults

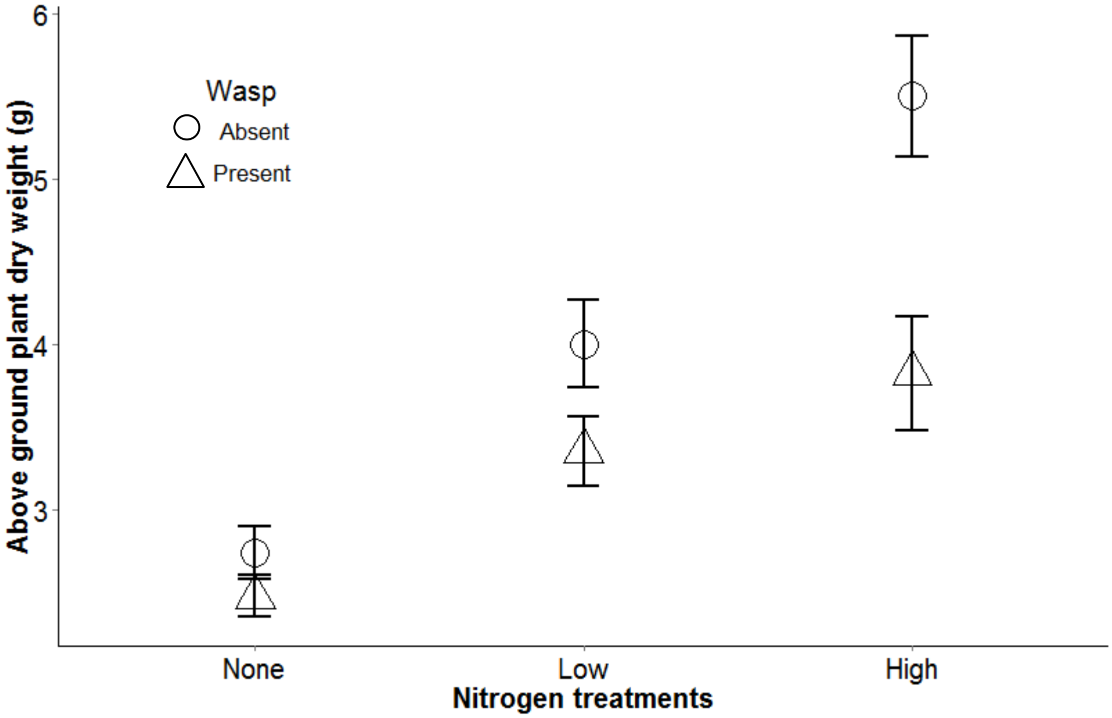

Figure 2.1. Response at week 7 of mean above-ground *P. officinarum* dry weight to nitrogen treatments of no, low, or high nitrogen (0, 75, 150 mg/kg soil NH_4NO_3) and *A. pilosellae* wasp absence or presence. Means are shown ± standard error, n = 118.

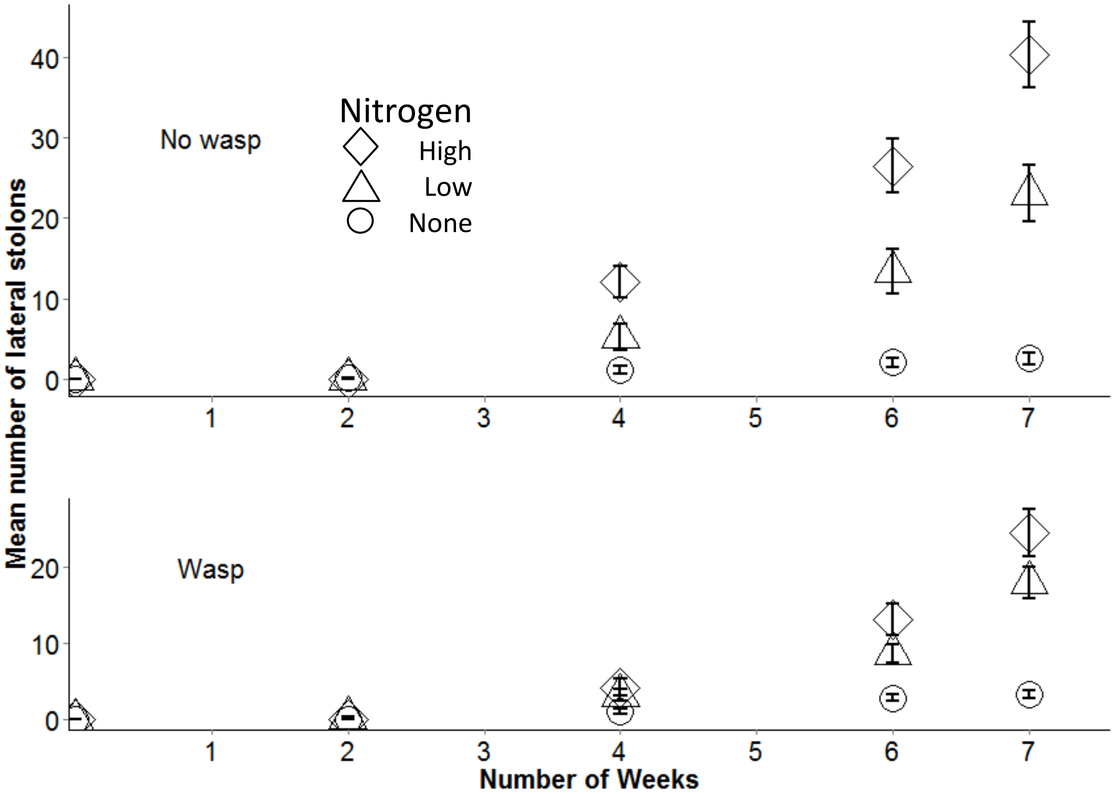

Figure 2.2. Mean number of lateral stolons produced per *P. officinarum* plant at weeks 0, 2, 4, 6, and 7 as a response to nitrogen treatments of no, low, or high nitrogen (0, 75, 150 mg/kg soil NH_4NO_3) and *A. pilosellae* wasp absence or presence. Means are shown ± standard error, n = 590.

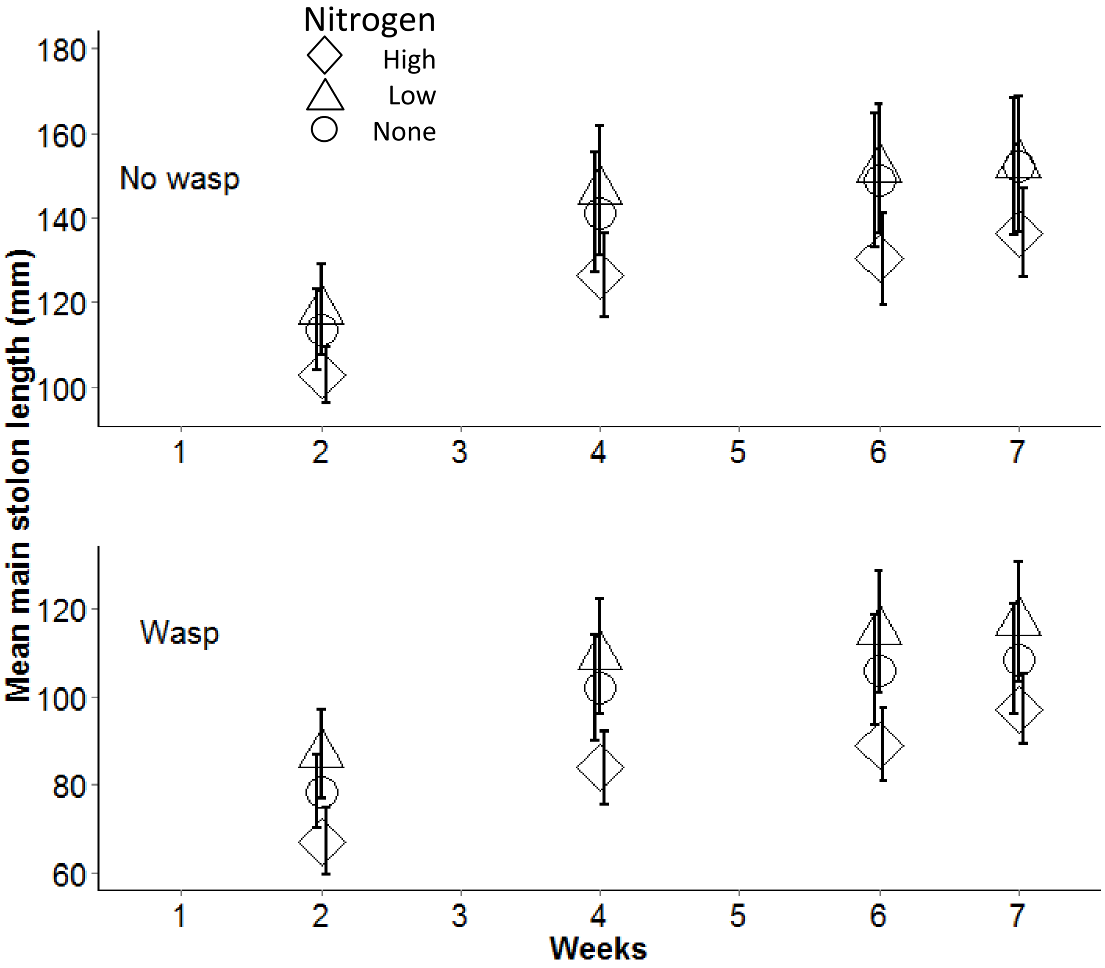

Figure 2.3. Mean length of randomly selected main stolons produced per *P. officinarum* plant at weeks 2, 4, 6, and 7 as a response to nitrogen treatments of no, low, or high nitrogen (0, 75, 150 mg/kg soil NH_4NO_3) and *A. pilosellae* wasp absence or presence. Means are shown ± standard error, n = 472.

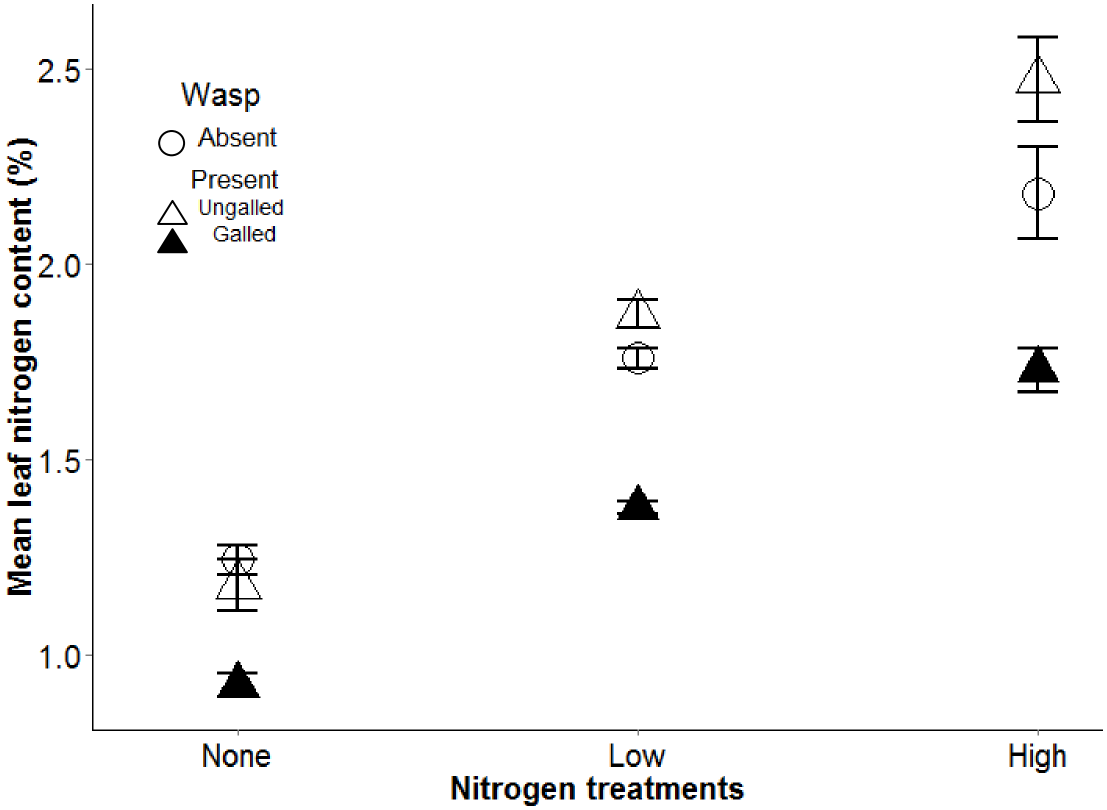

Figure 2.4. Response of mean percent nitrogen content in leaves at week 7 of *P. officinarum* to nitrogen treatments of no, low, or high nitrogen (0, 75, 150 mg/kg soil NH_4NO_3) and *A. pilosellae* wasp absence or presence, with galled leaves and ungalled leaves from within the same wasp treatment plants. Means are shown ± standard error, n = 36.

Table 2. 2. Statistical test summary tables for *P. officinarum* plant responses to nitrogen (N) and *A. pilosellae* (W) treatments at week 7 post final wasp introduction. Reference column refers to supplemental statistical test tables. Significance denoted as: *p < 0.05, **p < 0.001, ***p < 0.0001.

Response variable	Test	Block	N	W	N*W	Reference
Number of Main Stolons	GLM-P	ns	ns	ns	ns	Table S2.1
Number of Lateral Stolons	GLM-NB	***	***	*	*	Table S2.2
Randomly Selected Main Stolon Length	ANOVA	ns	ns	***	ns	Table S2.3
Mean Length of All Main Stolons	ANOVA	ns	ns	***	ns	Table S2.4
Number of Flowering Stems	GLM	ns	ns	ns	ns	Table S2.5
Above Ground Dry Plant Weight	ANOVA	*	***	***	*	Table S2.6
Total Number of Senesced Leaves	GLM-NB	ns	ns	*	ns	Table S2.7
Leaf Nitrogen Content	ANOVA	***	***	**	ns	Table S2.8

Table 2.3. Statistical test summary table for repeated-measures ANOVA conducted on main stolon length, total number of lateral stolons, and total number of senesced leaves. Error of block within each week was used. Significance denoted as: *p < 0.05, **p < 0.001, ***p < 0.0001. Supplemental statistical tables for mean stolon length, number of lateral stolons and number of senesced leaves are tables: S2.14, S2.15 and S2.16, respectively.

Source	P-value					
Error of Block	Wasp	Nitrogen				
Main stolon length	ns	ns				
Number of lateral stolons	ns	ns				
Number of senesced leaves	ns	ns				
Error of Block within Week	Week	Week*Wasp	Week*Nitrogen			
Main stolon length	*	ns	ns			
Number of lateral stolons	*	ns	ns			
Number of senesced leaves	*	ns	ns			
Within Factors	Wasp	Nitrogen	Week*Wasp	Week*Nitrogen	Wasp*Nitrogen	Week*Wasp*Nitrogen
Main stolon length	***	*	ns	ns	ns	ns
Number of lateral stolons	***	***	***	***	***	***
Number of senesced leaves	*	ns	*	ns	ns	ns

Table 2.4. Statistical test summary tables for *A. pilosellae* performance responses to nitrogen treatments. Reference column refers to supplemental statistical test tables. Significance denoted as: *<0.05, **<0.001, ***<0.0001.

Response variable	Test	Block	N	Reference
Total Number of Galls	GLM-NB	ns	ns	Table S2.9
Emergence Dates[1]	Repeated Measures ANOVA	ns	ns	Table S2.10
Emergence Success	GLM-NB	ns	ns	Table S2.11
Development Success	GLM-NB	*	ns	Table S2.12
Number of Eggs	GLM-NB	*	ns	Table S2.13

[1] There was an effect of date, with peak emergence of all nitrogen treatments between 56-58 days.

Discussion

As we predicted, increased nitrogen availability for *P. officinarum* resulted in improved plant performance with an increase in number of lateral stolons and above-ground plant dry weight (Figures 2.1, 2.2). *Pilosella officinarum* has been previously found to respond positively to nitrogen addition (Fan & Harris, 1996; Makepeace, 1985b). Similar to our results, Klöppel et al. (2003) observed increased mean main stolon length and number of lateral stolons per plant of *P. officinarum* when nutrient availability was comparable to our low nitrogen treatment, in comparison to a nutrient stressed treatment (our no nitrogen treatment). Indeed, main stolons were significantly longer in low nitrogen treatment plants compared to no nitrogen, while high nitrogen treatment plants were significantly shorter (Figure 2.3). Excess nitrogen may not be beneficial for *P. officinarum* with regards to stolon length, even though it increases lateral stolon production and above ground biomass.

The increased number of lateral stolons, and subsequent increased dry weight associated with improved soil nitrogen, indicates that *P. officinarum* may benefit from increased competitiveness, as their stolons and resulting daughter rosettes prevent neighbouring plants from encroaching into hawkweed patches (Makepeace, 1985a; Winkler & Stöcklin, 2002). Addition of nitrogen has been used to reduce *P. officinarum* populations in the presence of taller, more vigourous and potentially more competitive species like grasses (Bishop & Davy, 1994), as they tend to respond more strongly to nutrient additions than *P. officinarum* (Davis, 1997), while also being effective competitors for light (Fan & Harris, 1996). This method of control is especially effective when grazing by mammals is reduced or removed, as *P. officinarum* is often too close to the ground to be negatively affected by these grazers, while native species are competitively inhibited by such activity (Davy & Bishop, 1984; Scott et al., 1990). But, in the absence of tall neighbouring plants or when grazing is high, *P. officinarum* may be a superior competitor compared to native plants.

We accounted for mycorrhizal associations with *P. officinarum* by removing them via soil sterilization, as these associations have previously been observed in the field, and may help this weed species exploit soil nutrient additions (Höpfner et al., 2015). These mycorrhizal associations allow nutrients to be rapidly absorbed, especially when soil nutrients are limiting (Höpfner et al., 2015; Johnson et al., 2010; Smith & Gianinazzi-Pearson, 1988), which can influence the magnitude of plant performance affected by various nutrient regimes and how insects interact with their host plants. For example, Kempel et al. (2010) found that mycorrhizal associations can positively influence plant resources dedicated to resistance to herbivory, which may restrict the amount of damage incurred by herbivores. On the other hand, Koricheva et al. (2009) noted that although mycorrhizal relationships can influence herbivore performance, the magnitude and direction depends on a number of disparate factors, including insect feeding mode, diet breadth, and the identity of the fungi. Since we removed mycorrhizal associations from our experiment, we can state that the effects detected due to nitrogen availability and *A. pilosellae* presence are due to the factors alone, and not interactions with mycorrhizal fungi.

Aulacidea pilosellae galling negatively affected plant performance resulting in reduced main stolon length, mean total lateral stolons, and above-ground dry weight through an interaction with increased nitrogen availability (Table 2.2); all of which suggest that *A. pilosellae* has potential to be a successful agent at controlling *P. officinarum* populations. Similar results were obtained by Klöppel et al. (2003) with *A. subterminalis*, a congeneric stolon-galling wasp specific to *P. officinarum*. However, in Klöppel et al. (2003), main stolons were reduced in length because the galls terminate stolon growth, whereas *A. pilosellae* does not terminate stolon nor leaf growth. The reduction in stolon length is quite important with regards to biocontrol because, as Winkler and Stöcklin (2002) showed, phenotypic plasticity of stolon length of *P. officinarum* is a pivotal factor for the maintenance of populations, as this allows them to perform well under a variety of environmental conditions. Main and lateral stolons generate daughter rosettes at nodes, which allow *P. officinarum* to maintain ground cover in the midst of interspecific competition and space limitations (Winkler & Stöcklin, 2002). Thus, the production of shorter main stolons and fewer lateral stolons as an impact of galling suggests a reduction in competitive ability of *P. officinarum*, as its ability to exclude other plants via vegetative growth and spread daughter rosettes is diminished. The impact of *A. pilosellae* galling on *P. officinarum* stolon proliferation should allow regrowth and recolonization of native plants as the hawkweed populations (via daughter rosettes) are reduced, as long as native plants are present (Davy & Bishop, 1984; Winkler & Stöcklin, 2002). *Aulacidea pilosellae* impact on stolon length was first detected at two weeks after wasp introduction (Figure 2.3), indicating that this effect was primarily due to early gall development and not larval feeding within the developed galls. Gall development is usually split into three phases: initiation, development and maturation (Lalonde & Shorthouse, 1985). In *A. pilosellae*, galls are at their full size (but not full maturity) at 2-4 weeks after oviposition , while the larvae are only at 1st and 2nd instars (pers. obs.). The impact on *P. officinarum* of *A. pilosellae* galling on main stolon length took place within the first two weeks of the experiment: oviposition, gall initiation and early gall development. There were minimal effects detected for the remainder of the experiment on stolon length as a result of galling, likely because *P. officinarum* switched from main stolon to lateral stolon growth. As such, it appears that the timing of gall induction is important for impact on the host plant. Galling during main stolon growth seems to reduce main stolon length. We predict that if plants have already switched to lateral stolon growth, galling at this time would primarily limit the production of lateral stolons, as plant resources would be redirected towards gall development.

Galling and subsequent larval feeding caused *P. officinarum* to switch from main stolon growth to lateral stolon growth later than in ungalled treatments: between 2-4 weeks in the absence of *A. pilosellae* galls, and between 4-6 weeks with galls (Figure 2.2). Leaf nitrogen content results suggest that larval presence influenced the nitrogen content of the gall tissue under all nitrogen treatments (Figure 2.4), which may have continued to drain resources from the plant, and may explain why fewer lateral stolons were produced on wasp treated plants. Larval feeding consumes plant nutrients while maintaining a relatively stable gall environment for the contained insect (Harris & Shorthouse, 1996; St John & Shorthouse, 2000). Additionally, shorter stolons as a result of galling tended to have fewer nodes for lateral stolon production, which could also lead to the production of fewer lateral stolons. Thus, both gall induction and larval feeding had a negative impact on the ability of *P. officinarum* to expand via vegetative growth, indicating that *A. pilosellae* may exert an impact beyond just the development of the gall.

In their native range, the *A. pilosellae* biotype used generally has 1-2 generations per year (Moffat et al., 2013). With regards to biocontrol, this means that the impact due to galling on *P. officinarum* can potentially be exerted multiple times each year. Galls are known to be nutrient sinks that are formed by the host plant at the cost of the plant's resources (Dsouza & Ravishankar, 2014; Huang et al., 2014). The developing larvae can also continue to redirect newly created plant resources towards the gall tissues, thus diverting these nutrients from plant growth and reproduction throughout the insects growth stages (Hartley, 1998). We detected significant reductions in plant performance as a result of *A. pilosellae* gall formation and larval feeding, indicating this may be an effective biocontrol agent for hawkweeds.

Some plants can compensate for insect damage under varying environmental conditions, such as when soil nutrients are high, which can effectively reduce the impact of the herbivores (McNaughton, 1983). Klöppel et al. (2003) observed that lateral stolon production in *P. officinarum* was stimulated by *A. subterminalis* galling. As these are stolon-galling wasps that target the apical meristem of these tissues, this caused the release of lateral bud growth. However in our experiment, *P. officinarum* plants were unable to compensate for the lost resources due to *A. pilosellae* galling through the production of lateral stolons, as the growth of these lateral stolons was inhibited by galling and delayed by larval feeding. This was still true when nitrogen availability was high, as there was an interactive effect that actually increased the impact of *A. pilosellae* on *P. officinarum* when more nitrogen was available. Meyer and Root (1993) showed that feeding by a spittlebug (*Philaenus spumarius*), beetle (*Trirhabda* sp.), and aphid (*Uroleucon caligatum*) all reduced goldenrod (*Solidago altissimsa*) performance when under high soil nitrogen, but were much less effective when soil nitrogen was low. However, other systems have shown that compensation is possible when nutrients are readily available, as with water hyacinth (*Eichhornia crassipes*)(Bownes et al., 2013b) and rosinweed (*Silphium integrifolium*) (Fay et al., 1996). Thus, improved nitrogen availability may facilitate or hinder biocontrol programmes, depending on the specific weed being targeted. Indeed, site fertility may play a critical role in managing and maintaining top-down control of invasive weeds targeted for biocontrol, either by affecting the impact on the target weed or influencing performance of the biocontrol agent (Hovick & Carson, 2015).

We did not detect any performance benefits to *A. pilosellae* as a result of *P. officinarum* nitrogen availabilities, though it is not certain whether this would be true through multiple generations. Even though wasp egg load was not significantly different as a result of the nitrogen treatments, this is not always indicative of insect fecundity (Leather, 1988). Often insect size as it contributes to adult longevity is used as a measure of overall insect fecundity (Leather, 1988). However, as it relates to gall-formers, fecundity can be dependent upon insect preference for host quality, which in turn can be influenced by soil nutrients (Minkenberg et al., 1992). As such, the different nitrogen treatments did not affect wasp performance for the first generation; though the increased leaf nitrogen content of ungalled leaves on wasp-treated plants (Figure 2.4) may improve performance in subsequent generations, as gall-formers are sensitive to the vigour of their host plants which in turn can be influenced by soil fertility.. Additionally, improved soil nitrogen can increased the number of desired plant parts, i.e. young vigourous leaves, for *A. pilosellae* to gall. This alteration in leaf nitrogen content due to galling and nitrogen availability could improve *A. pilosellae* performance through subsequent generations (Aoyama et al., 2012; Moran & Goolsby, 2014). Tipping et al. (2015) observed that unrestricted herbivory by a defoliating weevil on its host plant actually promoted growth of leaves that were more suitable to feeding by the following generation of weevils. Thus, galling and other herbivory can influence plant growth patterns and resource movement in order to promote offspring performance of the insects, either through production of new shoots or sequestration of resources to specific plant organs (Craig & Price, 1986; Paulo Inácio & Vieira, 1999). This is known as the “resource regulation hypothesis” (Craig & Price, 1986), in which certain herbivores maintain or increase the production of plant modules which favour further herbivory. In our system, increased nitrogen availability and galling resulted in nitrogen being increased in leaves that were ungalled by *A. pilosellae*, though this may be due to the pooled leaves of all ages in the ungalled samples. If nitrogen and galling did improve leaf nitrogen in ungalled leaves, it may improve the desirability of these leaves to the following generation of wasps, possibly improving their performance (Minkenberg & Ottenheim, 1990).

The interaction between nitrogen availability and *A. pilosellae* on *P. officinarum* performance can have important implications for biocontrol, as wasp galling reduces main stolon length and lateral stolon production, resulting in reduced ground cover under various soil fertilities. *Pilosella officinarum* is a superior competitor in low fertility soils (Díaz-Barradas et al., 2015), and thus can outcompete native plants in invaded ranges when nutrients are scarce (Fan & Harris, 1996; Makepeace, 1985b). Indeed, soil fertility has been shown to be highly influential on biocontrol agent success at suppressing invasive weeds (Hovick & Carson, 2015). Additionally, *P. officinarum* has been identified as a ‘sleeper weed’ (Groves, 2006) in New Zealand (Day & Buckley, 2011), which is when a weed becomes invasive after already being naturalized in an area for many years. Altered nutrient regimes and environmental degradation may cause some of these ‘sleeper weeds’ to awaken, resulting in an even greater threat to biodiversity (Groves, 2006). As such, *P. officinarum* and other *Pilosella* species may become invasive in new environments in the future. Understanding how these weeds will respond to new nutrient availabilities and natural enemies will better enable us to predict their spread and control them before they become a greater threat.

Chapter 3: Maternal and offspring host effects on the performance of a leaf-galling wasp

Introduction

Maternal effects have the potential to influence offspring fitness when the mother is exposed to a variable environment. In particular, maternal effects are defined as a causal influence of maternal environment or phenotype on the performance of offspring (Marshall & Uller, 2007; Räsänen & Kruuk, 2007; Rossiter, 1996; Wolf & Wade, 2009). They are a response to a variable and heterogeneous environment, expressed mainly as phenotypic plasticity (adaptive or otherwise) in the offspring (Plaistow & Benton, 2009; Räsänen & Kruuk, 2007). The relevant environmental heterogeneity that herbivorous insects experience can include variation in host species or part of plant fed upon, or any change in resource availability, threats from predation, temperature, moisture, or probability of mating (Mousseau & Fox, 1998; Whitham, 1981). For example, Storm and Lima (2010) found that field crickets (*Gryllus pennsylvanicus* Burmeister, Orthoptera: Gryllidae) that are exposed to their wolf spider (*Hogna helluo* Walckenaer, Araneae: Lycosidae) predator produce offspring that are more responsive to spider cues than other crickets, which significantly increases their survival in the presence of these predators. Miller (2008) found that maternal choice of host species for egg-laying of the heliconia bug *Leptoscelis tricolor* Westwood (Hemiptera: Coreidae) affected daughter performance via mating probability and fecundity.

Maternal effects may be adaptive if offspring performance is improved as a parental response to changes in the environment (Mousseau & Fox, 1998). For example, Fox et al. (1997) found that maternal host choice in a generalist herbivore, *Stator limbatus*, can affect egg size, as the mother seed beetles lay larger eggs on one host than another, which is adaptive as fitness consequences of egg size vary on each host. Newcombe et al. (2015) found that specialist milkweed bugs *Oncopeltus fasciatus* increased their egg size and larvae had a faster development time when they consumed a different diet than their mother. In fact, certain insects can experience maternal effects as a result of oviposition site preference, as their offspring may be exposed to different hosts. For the cynipid gall-wasp *Belonocnema treatae* (Hymenoptera), maternal host plant selection significantly influenced offspring performance, with specific genotypes within host species significantly improving offspring performance more than neighbouring plants (Egan & Ott, 2007).

Gall-forming insects are generally not studied with regards to maternal effects, as there is a close genetic relationship between gall-former and their host, such that they are typically restricted to one or very few related host species (genus level) (Hardy & Cook, 2010). Thus, they do not typically experience multiple hosts through subsequent generations. Gall-forming insects physiologically alter and reorganize existing plant tissues in the formation of a novel plant structure called a ‘gall’, which provides an environment optimum to insect growth and development, by providing it with nutrients and protection (Hartley & Lawton, 1992; Koyama et al., 2004). A main source of environmental heterogeneity to which gall-formers are exposed stems from the timing of oviposition site selection; they require vigourous meristematic plant tissues at the correct stage of growth in order to successfully form their galls (Shorthouse et al., 2005). They must navigate an environment of plant parts with various growth rates and nutritional qualities in order to find an optimal oviposition site to maximize offspring performance. Such a wide array of choice for an ovipositing insect may cause maternal effects to influence offspring performance. For example, those insects that gall vigourous plant parts are often larger and more fecund than those that gall less vigourous plant parts (Price, 1999). Additionally, utilization of a different host species than the mother is likely to influence offspring performance via maternal effects, as the gall environment (including type and abundance of the food source, and ability to protect from predators) is likely to be different between the two host species. . As such, having a galling species that can develop on multiple host species allows an opportunity to study maternal effects beyond studying within host heterogeneity in galling site quality.

Aulacidea pilosellae is a leaf-galling wasp native to central Europe that is being investigated as a potential biocontrol agent of multiple hawkweed species in the genus *Pilosella* (Moffat et al., 2013). Hawkweeds are herbaceous perennial invasive weeds in many parts of the world, especially North America and New Zealand (Makepeace, 1985a; Wilson, 2006; Wilson et al., 1997). Some hawkweeds species are targets for biocontrol, as some are more invasive than others (Beaumont et al., 2009b; Ensing et al., 2013) due to a range of morphological traits between the different species (Moffat et al., 2015). *Aulacidea pilosellae* appears to exhibit host density-dependent attack in its native range, with galls often found on the most abundant hawkweed host in mixed-species patches (Moffat et al., 2013). This suggests a host-switching mechanism is present, though the relative wasp performance on the multiple hawkweed hosts is unknown.

We aimed to identify *A. pilosellae*'s performance on two common European field hosts, meadow and yellowdevil hawkweeds (*P. caespitosa* and *P. glomerata*, respectively), which are both currently present in North America (Wilson, 2006). We predicted that: 1) maternal effects through maternal host species will improve offspring performance when exposed to the same host as the mother; and 2) offspring host effects will be stronger than maternal effects in determining wasp performance, due to evolved host specialization. Understanding the baseline performance of *A. pilosellae* on two commonly utilized host species can provide information on the most effective host to target with a biocontrol programme. Identifying the conditions conducive to maximizing insect performance (i.e. maternal effects) are very important to biocontrol programmes, as establishment in the field and successful control of the target species by the agent can both be improved as a result of improved insect performance.

Materials and Methods

Plants

The plant species used were *P. caespitosa* (original seed from Revelstoke, BC, Canada) and *P. glomerata* (original seed from Nelson, BC, Canada). Seeds were germinated on water-saturated vermiculite in sealed glass Petri dishes. Seedlings were transferred to root trainers containing 1:1:1 Cornell mix: sand: loam (Boodley & Sheldrake, 1977) and allowed to grow in a greenhouse for 4-6 weeks, at day/night temperatures of 22/18°C and natural light supplemented with high-pressure sodium artificial light for 16 hour days. Plants in root trainers were watered as necessary by soaking for several hours before draining. The plants were then transplanted into 15 cm diameter pots containing the same soil mix as the root trainers. Watering was automated using drip irrigation at 60, 80, 100 or 120 mL per day, depending on the size of the plants. The plants were allowed to grow under the same greenhouse conditions for 1-3 weeks prior to experimental use.

Insects

Aulacidea pilosellae wasps induce unilocular galls (i.e., one larva per gall) on the midrib of hawkweed leaves, generally towards the leaf base. Galls are 2-4 mm in length and ellipsoid, with the body length of emerging adults about 1-2 mm. The wasps of this study were univoltine (one generation per year) and sexual, with three larval instars, adult lifespan of 7-10 days, and males that emerge 1-2 days before females. They are believed to emerge with all of their eggs fully developed (proovigenic), which has been noted in other cynipid gall-wasps (Ito & Hijii, 2004). The *A. pilosellae* wasps used for the experiment were originally field-collected from Sosnowka, Poland from *P. caespitosa*, and imported to the Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada from the Centre for Agriculture and Biosciences International in 2012 as third instar larvae within galls.

The wasps were reared for one generation in quarantine on *P. caespitosa* prior to the experiment. Galls were overwintered artificially at 5 °C for five months, and moved to 10/8°C and 16/8hr (day/night) conditions for one week, then to 22/18°C and 16/8hr (day/night) in a mesh-covered container (250 mL) in a rearing room with grow lights, until emergence. Containers were checked daily for adult wasp emergence, with emerged living males placed back into the container to facilitate mating with emerging females during the next day. Females were removed each day, and used in experimental setup or preserved in 95% ethanol for dissections to count eggs as a performance measure. Females from peak emergence were used (starting 1-2 days after initial female emergence) for the experiment, and were placed individually on 0.25 mm Dacron mesh-covered plants immediately (approximate volume of covered plant cage: 1200 cm^3, cone-shaped), with plants covered for two weeks before the mesh was removed.

Experimental design

To determine if there are effects on gall wasp performance as a result of host species used by a maternal generation, a greenhouse experiment was conducted using the gall wasp *A. pilosellae* on two host hawkweed species, *P. caespitosa* and *P. glomerata*. The experiment was performed in two sequential stages; a maternal wasp generation trial (Maternal Trial) and an offspring wasp generation trial (Offspring Trial) (Figure 3.1). The Maternal Trial consisted of 30 potted plants of each of *P. caespitosa* and *P. glomerata*, (seeds germinated simultaneously) and chosen for size uniformity at setup (ground cover, visually identified). The Offspring Trial consisted of 60 plants of each species, seeded and germinated over three weeks, with plants also chosen by size uniformity.

Plants from both trials were split into six equal blocks, with plants within blocks randomly arranged spatiotemporally on greenhouse benches.

Female wasps previously exposed to males for mating were introduced singly to one of either *P. caespitosa* or *P. glomerata* (maternal host), and their offspring were then likewise introduced to one of either *P. caespitosa* or *P. glomerata* (offspring host), resulting in four maternal-by-offspring host treatments (Figure 3.1). Wasps from maternal block 1 were used for offspring block 1 and so on, with the exception of offspring block 6, which used wasps from maternal blocks 4 (maternal *P. glomerata*) and block 5 (maternal *P. caespitosa*) due to a greater magnitude of emergence from these blocks than maternal block 6. All blocks were filled sequentially as insects emerged; the Maternal Trial was set up over 10 days, with Offspring Trial set up over eight days. An additional four plants of each species for the Offspring Trial had a gravid female wasp introduced in order to provide galls for later dissections to monitor larval development.

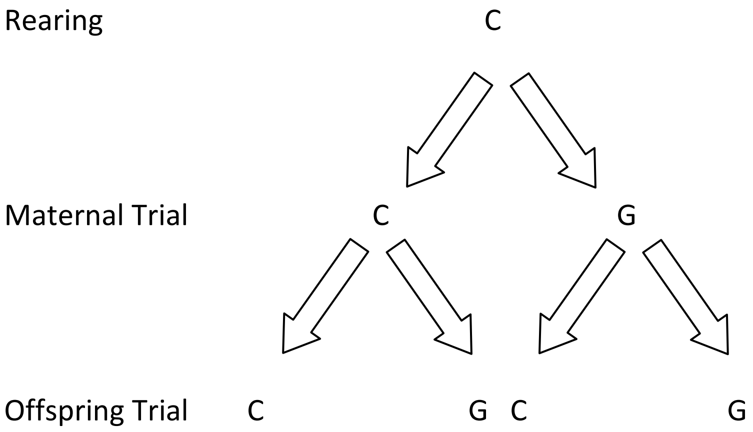

Figure 3.1. Setup of the experiment is shown. *Aulacidea pilosellae* wasps were reared for one generation on *P. caespitosa* (C), and offspring were used to set up the Maternal Trial on both *P. caespitosa* and *P. glomerata* (G). The offspring from the Maternal Trial plants were set up on either *P. caespitosa* or *P. glomerata* for the Offspring Trial, resulting in four maternal-by-offspring treatments of CC, CG, GC and GG.

Measurements

Measurements taken per plant for the Maternal Trial were; total gall counts, adult emergence success (adult's emerged/total galls), sex ratio of emerged adults, and egg counts/female using a 10% subsample of emerged females per plant (n=114 females).

In order to ascertain the number and age of leaves available for ovipositing wasps at the time of initial wasp introduction in the Offspring Trial, leaves were counted and marked for age with India ink. This was accomplished by following the leaf whorl and separating leaves into young (not yet fully unfurled leaves), middle and old aged leaves (split evenly into both categories), with middle leaves marked on the tip. When plants were unwrapped after gall formation, galled leaf age was determined based on relative position of leaves to India ink markings. Measurements taken for the Offspring Trial were; gall and galled leaf counts, and subsamples for individual gall volumes (n=756) and final instar (L3) larva weights (n=1962). All data was collected when plants were harvested at 101-107 days after initial wasp introduction. Galls were measured using calipers on all three axes to 0.01 mm, and their volumes were estimated as ellipsoids ((x*y*z)/8*4/3*π). Larval weights were measuring using a microbalance to 0.001 mg. To standardize gall volumes and larval weights, only individual galls (as opposed to overlapping galls) on the basal 1/3 of leaves were measured, and only third instar larvae from these individual galls were weighed.

Statistical analysis

All data were analyzed using R (R Development Core Team, 2015), with Maternal and Offspring Trials analyzed separately. Generalized linear models (GLM-P) assuming Poisson distribution were used on count data, except for over-dispersed count data, for which negative-binomial GLMs (GLM-NB) were used. Pearson product-moment correlation was used to determine correlation between gall size and larval weight. All other data were analyzed using ANOVA or ANCOVA with covariates. For the Maternal Trial, factors were block and host, and included number of female wasps dissected as a covariate for egg counts only. For the Offspring Trial, factors were block, maternal host, offspring host, maternal and offspring host interaction, and number of leaves at initial wasp introduction as a covariate due to variable plant size at initial wasp introduction. The number of larvae weighed was used as a covariate for larval weights in the Offspring Trial. In the Maternal Trial, some females produced only male offspring (possibly because they had not mated), and thus were not used for statistical analysis. Any plants that did not develop galls were removed from analysis.

Results

Maternal Trial

The total number of galls produced per female wasp was significantly different between host species, with over twice as many galls produced on *P. caespitosa* than on *P. glomerata* (Figure 3.2A, Table 3.1). However, the number of eggs each female contained at emergence (Figure 3.2B), emergence success, and sex ratio of adults on both *P. caespitosa* and *P. glomerata* were not significantly different (Table 3.1).

Offspring Trial

Gall number and leaves galled

Wasps for which the maternal host was *P. caespitosa* produced more galls and galled more leaves on average than those whose maternal host was *P. glomerata* (Figure 3.3, Table 3.2), regardless of offspring host. However, there were no offspring host effects on gall number or leaves galled, nor were there any offspring host interactions with maternal host or number of leaves at setup (Table 3.2). There was a main effect of the covariate, number of leaves per plant at time of setup (Table 3.2), with generally more galls and more leaves galled when more leaves were available at setup (Figure 3.4), but only when the host was *P. caespitosa* ($F_{(1, 56)} = 22.06$, $p < 0.0001$). When *P. glomerata* was utilized as a host, number of leaves at setup did not influence number of galls produced ($F_{(1, 55)} = 3.137$, $p = 0.082$) . All galled leaves were young at time of oviposition and gall initiation as determined by galled leaf positions relative to marked leaves.

Gall volume and third instar larval weights

Larval weights were strongly positively correlated to gall volumes, as detected using Pearson's product-moment correlation ($r = 0.64$, $n = 234$, $p < 0.0001$). The gall volume was strongly affected by the offspring host, with *P. caespitosa* plants containing much larger galls than *P. glomerata* plants, with no maternal host effects detected (Figure 3.4A, Table 3.2). There was an interaction between offspring host and the covariate of leaf number at setup (Table 3.2) on gall volume, with larger galls produced on *P. caespitosa* when more leaves were available at setup ($p < 0.0001$); whereas gall volume on *P. glomerata* was unaffected when more leaves were available ($p > 0.05$) (Figure 3.5). The only detected effect on larval weight was due to offspring host, with heavier larvae from *P. caespitosa* than from *P. glomerata* (Figure 3.4B, Table 3.2). We detected no maternal host effects or maternal host interactions with either gall volume or larval weight in the Offspring Trial.

Table 3.1. Summary table of all statistical tests used for analysis of each response variable and the significance of the independent variables for the maternal generation. Generalized linear models (GLMs) used negative binomial (NB) distributions. Significance codes: p-value = '*' < 0.05, '**' < 0.01, '***' <0.001. Supplemental statistical tables for each test are referenced.

Response variable	Test	Block	Host	Reference
Gall number	GLM-NB	ns	***	Table S3.1
Egg counts	GLM-NB	ns	ns	Table S3.2
Emergence	ANOVA	ns	ns	Table S3.3
Sex ratio	ANOVA	ns	ns	Table S3.4

Table 3.2. summary table of all statistical tests used for analysis of each response variable and the significance of the manipulated variables for the offspring generation. Generalized linear models (GLMs) used negative binomial (NB) and Poisson (P) distributions. All pair-wise interactions were not significant, unless otherwise specified. Significance codes: p-value = '*' < 0.05, '**' < 0.01, '***' <0.001. Supplemental statistical tables for each test are referenced.

Response variable	Test	Block	Maternal Host	Offspring Host	Number of Leaves	Reference
Gall number	GLM-NB	*	*	ns	**	Table S3.5
Galled leaves	GLM-P	***	*	ns	***	Table S3.6
Gall volume[1]	ANOVA	**	ns	***	*	Table S3.7
Larva weight	ANOVA	ns	ns	***	ns	Table S3.8

[1] Also had a significant (**) Offspring x Number of leaves at setup interaction.

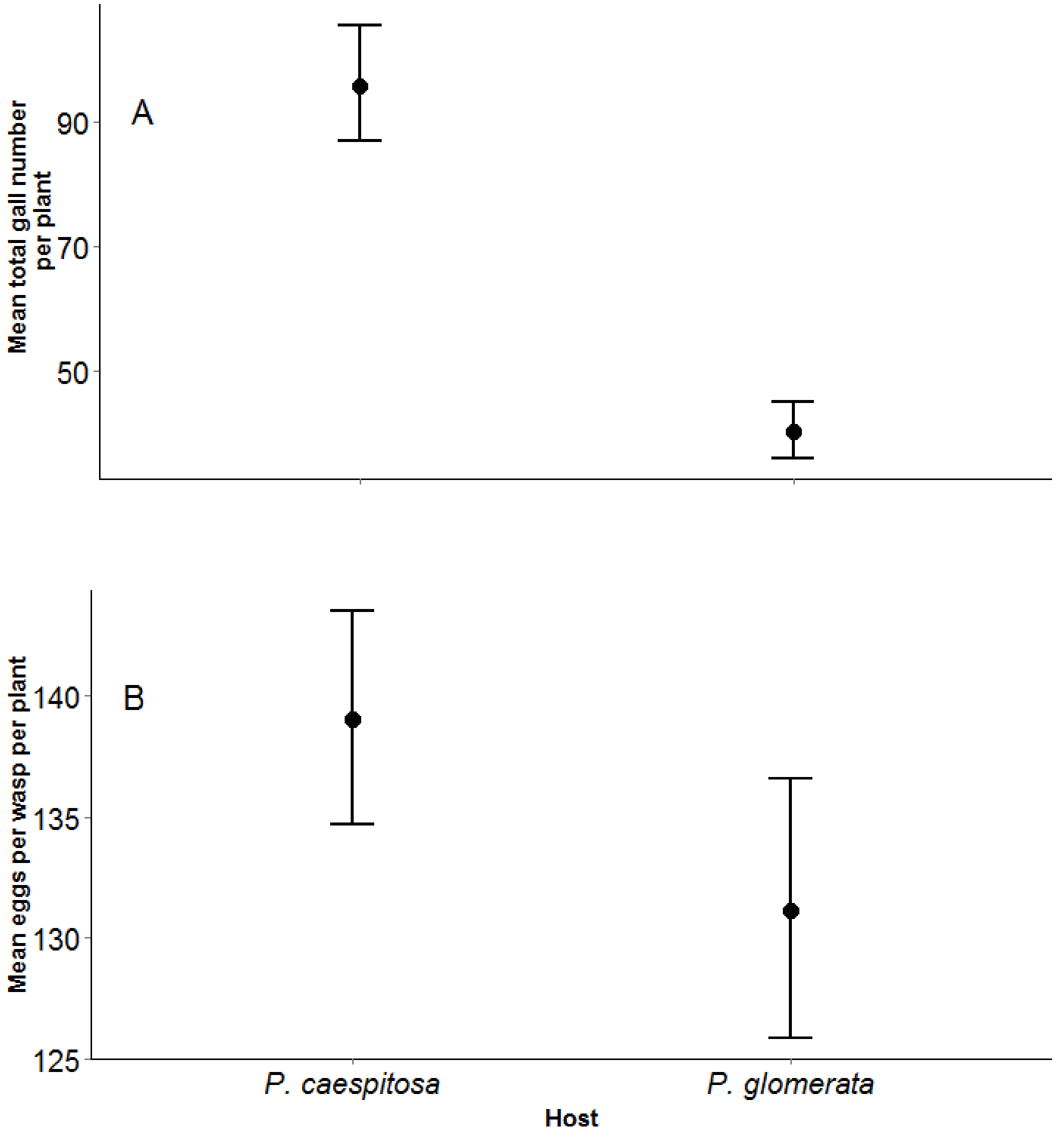

Figure 3.2. The effects of host plant in the maternal generation on; (A) number of galls produced and (B) wasp egg load/wasp/plant, adjusted for number of wasps dissected. Data shown as least squares means ± standard error, with (A) n = 53 (29 *P. caespitosa* and 24 *P. glomerata*) and (B) n = 39 (22 *P. caespitosa* and 17 *P. glomerata*).

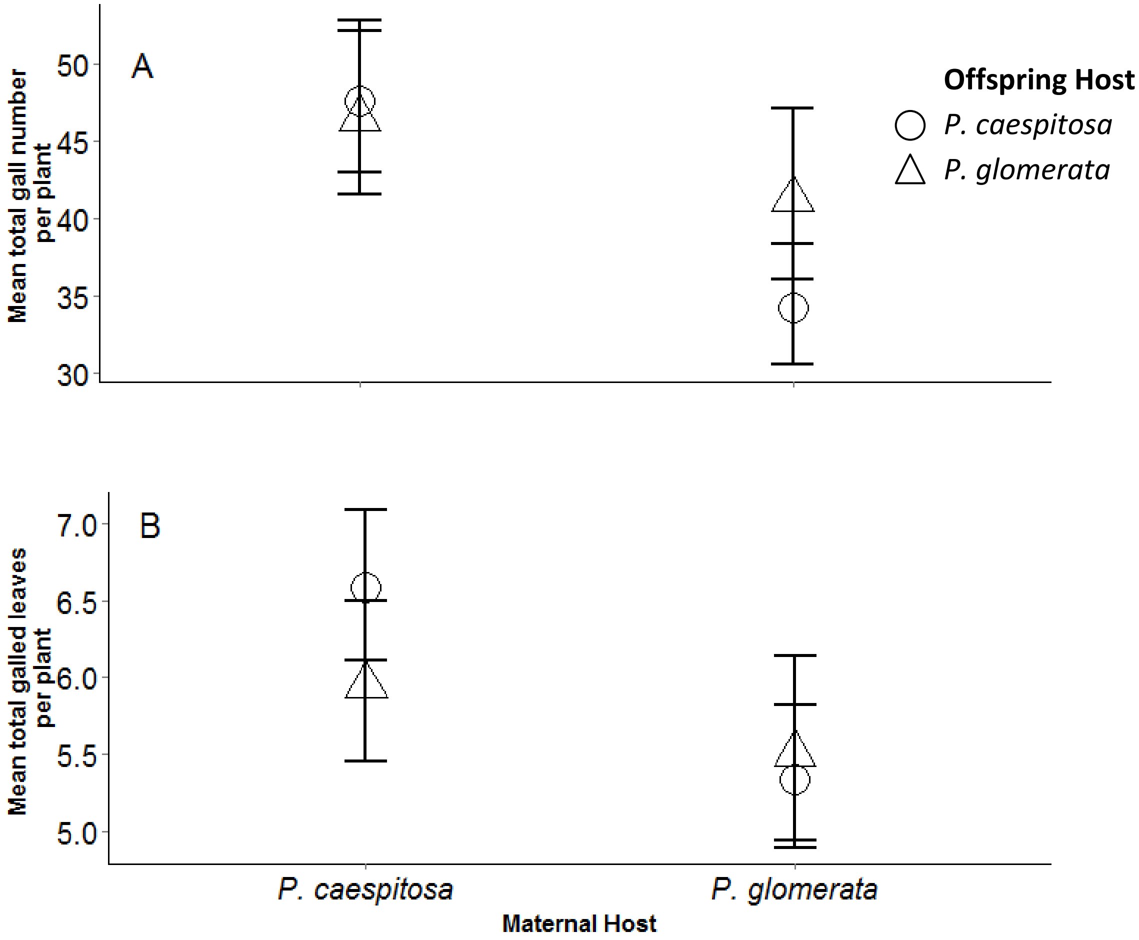

Figure 3. 3. The effects of maternal and offspring hosts in the Offspring Trial on; (A) total gall number and (B) total number of leaves galled, both using least squared means. Data were adjusted for the covariate of leaves at initial wasp introduction. Data shown are means ± standard error, with total (A) n = 115 and (B) n = 115.

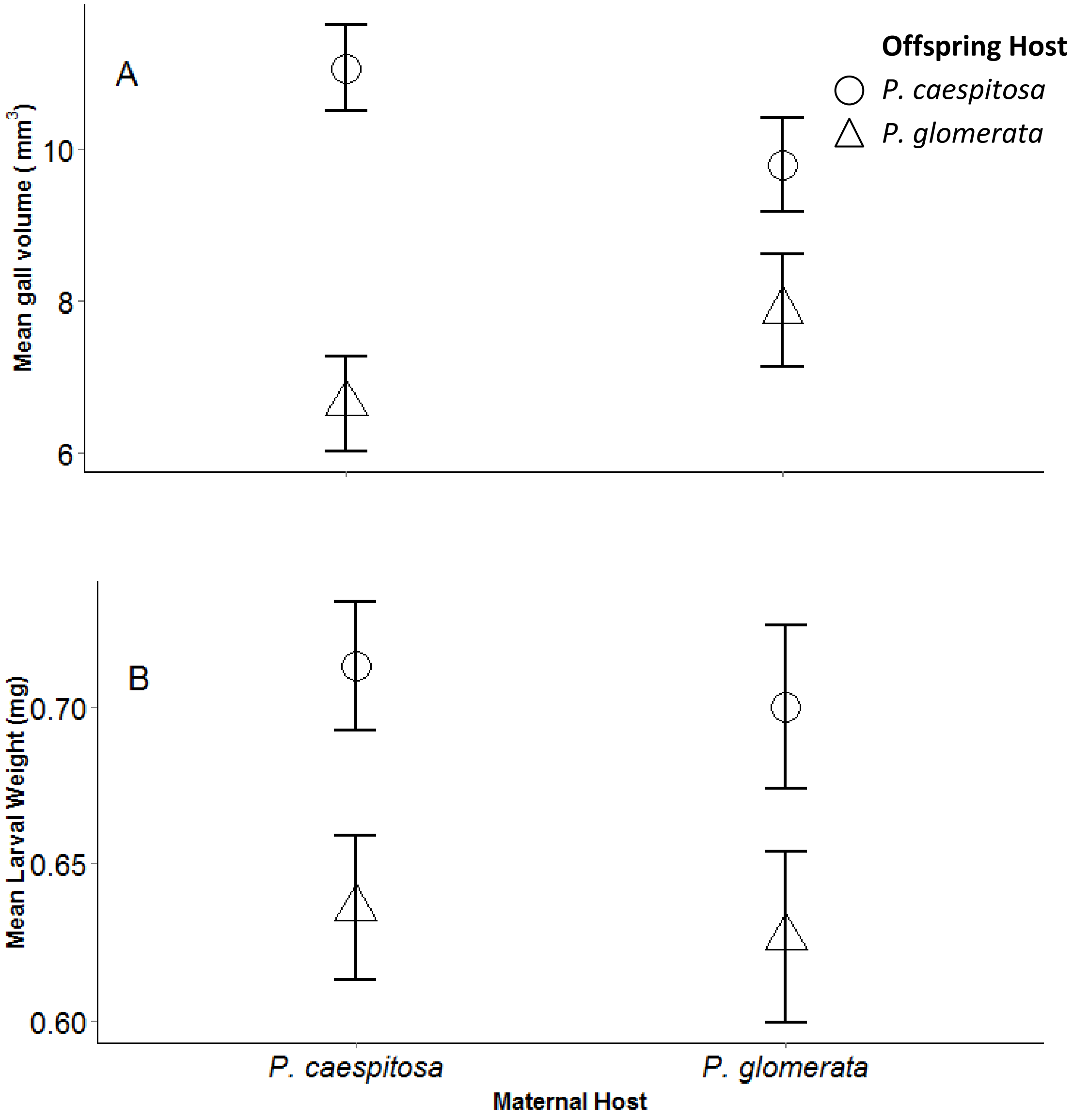

Figure 3.4. The effects of maternal and offspring hosts in the Offspring Trial on; (A) gall volume and (B) larval weight adjusted for number of larvae weighed using least squares means. Data were adjusted for the covariate of leaves at initial wasp introduction. Data shown are means ± standard error, with total (A) n = 115 and (B) n = 115.

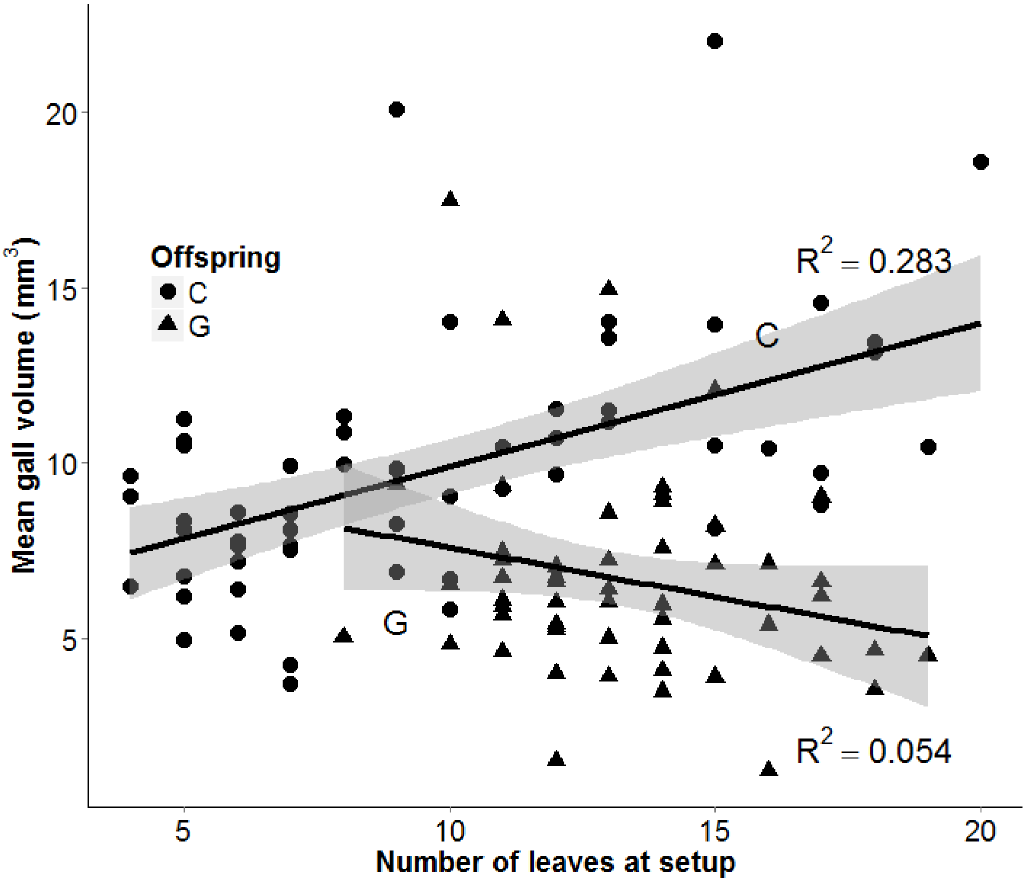

Figure 3.5. Gall volume in the Offspring Trial as a result of the number of leaves at setup and offspring host, with C = *P. caespitosa*, G = *P. glomerata*. Data points are mean gall volume per plant, with n = 58 (*P. caespitosa*) and n = 57 (*P. glomerata*). Lines are linear models with 95% confidence intervals, with R^2 values shown. Slopes of linear models are different from zero with regression p-values for the *P. caespitosa* and *P. glomerata* of 1.756e-05 and 0.0821, respectively.

Discussion

We predicted that maternal effects would promote *A. pilosellae* performance on the same host as the mother; however, our results indicate that *P. caespitosa* is the better host. Females that emerged from *P. caespitosa* produced more galls on both host species than females that emerged from *P. glomerata* in the offspring trial (Figure 3.3). This suggests that those females emerging from *P. caespitosa* may be more fecund than their counterparts that emerged from *P. glomerata*, even though egg loads in wasps that emerged from both species for the maternal generation were not significantly different. The utilization of hosts of different species or quality has previously been shown to influence offspring performance via maternal effects, often giving offspring a boost to performance when they are introduced to a different environment than the mother. For example, Or and Ward (2007) found that maternal effects strongly influenced offspring number but not offspring mass in a bruchid beetle (*Caryedon palaestinicus* Southgate, Coleoptera: Bruchidae), with females grown on healthy *Acacia raddiana* Savi (Leguminosae) trees producing more offspring than those on stressed trees. On the other hand, Spitzer (2004) found that maternal host use of the soft scale insect *Saissetia coffeae* Walker (Hemiptera: Coccidae) did not influence offspring performance on either of the disparate host species. In the large milkweed bug (*Oncopeltus fasciatus*) maternal effects influenced egg size, with larger eggs produced when the offspring diet is different than the maternal diet (Newcombe et al., 2015). However, we did not observe maternal effects increasing performance on the maternal or opposite host; instead these effects only improved performance if *P. caespitosa* was utilized by the mother.

In the Maternal Trial, there were over twice as many galls formed on *P. caespitosa* than on *P. glomerata* (Figure 3.2A), which we speculate may have occurred as a result of oosorption; that is, they reabsorbed their eggs in order to increase their longevity on a sub-optimal food source, *P. glomerata*. This has been observed in other cynipid gall wasps, notably the invasive Asian chestnut gall wasp, *Dryocosmus kuriphilus*, when food is unavailable after emergence (Graziosi & Rieske, 2014). We believe *A. pilosellae* to be pro-ovigenic, emerging as adults with all of their eggs produced, which has been noted in other cynipids (Ito & Hijii, 2004). We discovered that even though there were more galls produced by female *A. pilosellae* from *P. caespitosa* as the maternal host, egg loads were not significantly different between wasps from both *P. caespitosa* and *P. glomerata*. If oosorption was occurring on the suboptimal host of *P. glomerata*, we would expect the wasps to produce fewer galls on *P. glomerata* in the Offspring Trial compared to *P. caespitosa*. However, we did not observe this difference in the number of galls formed in the Offspring Trial; instead there were more galls produced when the maternal host was *P. caespitosa*. As such, there may be host quality based maternal effects, related to the response of the hosts to the galling stimuli. It is possible that mortality of the egg or young larvae was higher on *P. glomerata*, which can help to explain the discrepancy in number of galls formed between the two species in the maternal generation. We cannot conclusively say that oosorption was not occurring, as we did detect an effect of the covariate of number of leaves at introduction, nor have we any more insight into plant caused mortality. Hence, more research into this oosorption phenomenon and host suitability is required.

The wasps responded differently to the two host plants in the number of galls formed, depending on the size of the plants as identified by the number of leaves when wasps were introduced. In the Offspring and Maternal Trials, the wasps produced an increased number of galls when more leaves were available on *P. caespitosa*, with number of leaves on *P. glomerata* not having an effect on gall number in either maternal or offspring generation (data not shown). Gall-formers are very closely linked genetically to their host species (Harper et al., 2004; Price et al., 1987), and their performance is often dependent on the growth stage and vigour of their host species (Price, 1991). As *P. glomerata* is a hybrid species of *P. caespitosa* and *P. cymosa* (Krahulcová et al., 2013), it is likely that *P. caespitosa* is the ancestral host of *A. pilosellae*, and as such the gall forming stimulus may be stronger with this host than the relatively recent hybrid host of *P. glomerata*. This close genetic association of gall inducing insects to their host allows them to be very host specific in their ability to initiate gall induction and subsequent tissue manipulation in order to feed and sustain the living insect within (Harper et al., 2004; Schönrogge et al., 2000).

We did not detect maternal host effects on gall size and resulting larval weights in the Offspring Trial, but instead detected strong host effects. The galls that were formed on *P. caespitosa* were much larger than those on *P. glomerata*, and gall size had a strong positive effect on larval weight. This suggests that the offspring host is much more important to individual insect survival and potential fecundity than maternal host. It is possible that the evolutionary relationship between *P. caespitosa* and *A. pilosellae* causes this plant species to respond more strongly to the gall-forming stimulus, causing larger galls to form. Marchosky Jr and Craig (2004) found that gall size strongly influenced larval performance and survival in the gall midge *Asphondylia atriplicis* Townsend (Diptera: Cecidomyiidae). Similarly, Shibata (2002) observed that gall size was strongly correlated to emerged adult size and potential fecundity of the bamboo gall maker, *Aiolomorphus rhopaloides* Walker (Hymenoptera: Eurytomidae) and its inquiline *Diomorus aiolomorphi* Kamijo (Hymenoptera: Torymidae). As such, we propose that females emerging from *P. caespitosa* may be more fit than those emerging from *P. glomerata*, as insect size (and weight) often correlates strongly with insect fitness (Forrest, 1987; Kingsolver & Huey, 2008; Visser, 1994). This is likely why we observed a much greater number of galls formed in the Offspring Trial by wasps emerging from *P. caespitosa* than *P. glomerata* in the Maternal Trial (Figure 3.3); these insects were larger and thus more fecund, even though egg loads were similar, when they utilized the better host of *P. caespitosa*.

Our experiment was constrained to a quarantine greenhouse environment, excluding many factors that may be present under more natural conditions. For example, we know that *A. pilosellae* are susceptible to high parasitism rates in their native range, with anywhere from 17 to 30% of galls parasitized in different regions and years, with up to 90% under certain circumstances (pers. comm. Cortat, G., CABI, Switzerland, Oct 8, 2015). Higher levels of leaf pubescence can negatively influence parasitoid performance (Obrycki et al., 1983), as it restricts them from accessing necessary plant parts. This may contribute to host-switching in *A. pilosellae* towards *P. glomerata*, as this host is quite different in pubescence than *P. caespitosa* (Moffat et al., 2015). *Pilosella caespitosa* has flexible hairs on the upper leaf surface and sparse to many stellate hairs on the lower leaf surface, while *P. glomerata* has stiff hairs and many stellate hairs on the upper and lower leaf surfaces, respectively (Moffat et al., 2015). These differences in pubescence may deter parasitoids from ovipositing within *A. pilosellae* galls on *P. glomerata* but not on *P. caespitosa* in the native range, promoting use of *P. glomerata* as a host. Since escape from enemies is one of the main factors that may facilitate host switching of gall-formers (Abrahamson et al., 1993), we predict that experimenting with *A. pilosellae* under pressure from parasitoids may help explain why *P. glomerata* is a common field host (Moffat et al., 2013). However, this effect may be tempered by the larger galls on *P. caespitosa* than on *P. glomerata*, as larger galls tend to provide greater protection from parasitoids (Fernandes et al., 1999; Ito & Hijii, 2004; Weis et al., 1985), though not all parasitoids are affected by gall size (Waring & Price, 1989). Indeed, there may be a trade-off between the hosts; high parasitism may promote galling on *P. glomerata*, even though this reduces overall performance, with host-switching back to *P. caespitosa* stimulated under low parasitoid presence.

Overall, our results lead us to conclude that host-switching away from *P. caespitosa* to *P. glomerata* may be an ‘ecological trap’, as *A. pilosellae* performance is enhanced when *P. caespitosa* is the maternal and offspring host, at least under greenhouse conditions. These baseline performance data are necessary when determining a target for a biocontrol programme, as there are multiple invasive hawkweed species as potential hosts for *A. pilosellae* that are sympatric in North America (Wilson et al., 2006; Wilson et al., 1997). Accordingly, *A. pilosellae* may be able to easily establish on *P. caespitosa* in the field due to their increased performance on this host, possibly because of the evolutionary relationship between these two species which causes *P. caespitosa* to be more responsive to the gall-forming stimulus of *A. pilosellae*. Early establishment in mixed hawkweed patches could potentially result in some host-switching to *P. glomerata* if *P. caespitosa* densities decreased due to biocontrol efforts, as *A. pilosellae* seems to switch to the most abundant host in the field (Moffat et al., 2013). This type of dynamic biocontrol programme that uses one agent to control multiple target weeds has many benefits, though more research into the likelihood of success of such an endeavour is required.

Chapter 4: Discussion

Nitrogen effects on P. officinarum *performance*

As predicted, increased nitrogen availability for *P. officinarum* resulted in improved plant performance as detected by an increase in number of lateral stolons and above-ground plant dry weight, but nitrogen did not affect main stolon length. Not all plants respond equally to soil nitrogen addition, but generally they increase vegetative growth and biomass (Blackshaw & Brandt, 2008; Blackshaw et al., 2003). In a similar test by Klöppel et al. (2003), increased soil nitrogen enrichment of *P. officinarum* was observed to increase mean main stolon length, number of main and lateral stolons, and biomass. Indeed, *P. officinarum* has been previously detected to respond positively to nitrogen addition (Fan & Harris, 1996; Makepeace, 1985b). The increased number of lateral stolons and dry weight associated with increased soil nitrogen suggests that *P. officinarum* may benefit from increased competitiveness. This may result as their increased number of lateral stolons and resulting daughter rosettes can prevent neighbouring plants from encroaching into hawkweed patches while simultaneously allowing them to invade the space of nearby plants (Makepeace, 1985a; Winkler & Stöcklin, 2002). Additionally, the rate of spread of hawkweed patches may increase when nutrients are readily available, allowing them to opportunistically spread via vegetative growth to any free spaces nearby (Winkler & Stöcklin, 2002). Thus, we conclude that increased nitrogen availability improves *P. officinarum* performance, and potentially their competitiveness, as they may be able to suppress native plants via competition as a result of increased lateral stolon growth.

We accounted for mycorrhizal associations with *P. officinarum* by removing them via soil sterilization, as these associations have previously been observed in the field, and may help this weed species exploit soil nutrient additions more than other plant species (Höpfner et al., 2015). These mycorrhizal associations allow nutrients to be rapidly absorbed, especially when soil nutrients are limiting (Höpfner et al., 2015; Johnson et al., 2010; Smith & Gianinazzi-Pearson, 1988). This change in nutrient availability can alter insect-plant interactions, including through the induction of plant defensive compounds and by reducing insect preference for mycorrhizae-associated plants (Roger et al., 2013). Kempel et al. (2010) found that induction of mycorrhizal associations with several grasses can positively influence plant resistance to herbivory. On the other hand, Koricheva et al. (2009) noted that these mycorrhizal relationships can influence herbivore performance, but the magnitude and direction depends on a number of disparate factors, such as identity of the fungi or feeding mode and diet breadth of the insect herbivore. Since we removed mycorrhizal associations from our experiment, we can conclusively state that the effects detected due to nitrogen additions and *A. pilosellae* are due to the tested factors alone, and not via an interaction with mycorrhizal fungi. However, it is as yet unknown whether the relationship between nitrogen, *A. pilosellae* and *P. officinarum* may be influenced by mycorrhizae in nature.

Addition of nitrogen has previously been used to reduce *P. officinarum* populations, often in the presence of taller, more vigourous, and potentially more competitive species like grasses (Bishop & Davy, 1994). This may occur because *P. officinarum* does not always respond as strongly to nitrogen additions as some other plant species, like grasses; it is instead more responsive to phosphorus (Davis, 1997). In addition to this, *P. officinarum* is not competitive for light as it is a low-growing plant; thus, taller plants have a competitive advantage against this weed (Fan & Harris, 1996). This is offset by *P. officinarum*'s ability to interfere with neighbours through allelopathy with the chemical umbelliferone and ability create an acidic halo around patches, mechanisms which both help to create a bare patch of ground around hawkweed patches, which *P. officinarum* can spread onto with little to no competition (Boswell & Espie, 1998; Makepeace et al., 1985; McIntosh et al., 1995). Controlling *P. officinarum* with nitrogen is especially effective when grazing is reduced or removed, as *P. officinarum* is often too low to the ground to be grazed by livestock, while many other species are competitively inhibited by such activity due to reduced biomass and vegetative growth (Davy & Bishop, 1984; Scott et al., 1990). Increased nitrogen availability has also been shown in other experiments to induce the production of inflorescences in *P. officinarum* (Davy & Bishop, 1984; Lloyd & Pigott, 1967), though this was not observed in our experiment. This is likely because the time required to reach sufficient size for flowering was not achieved, as they commonly require several months to flower after germination (Bishop & Davy, 1994). Producing more inflorescences as a result of increased soil nitrogen may be detrimental to *P. officinarum* populations, as this can increase the decay rate of hawkweed patches as they switch from vegetative to sexual reproductive growth (Davy & Bishop, 1984). Hawkweed patch maintenance is highly dependent upon vegetative growth and daughter rosette production (Davy & Bishop, 1984; Winkler & Stöcklin, 2002). As a result, an increase in soil nutrients may only benefit *P. officinarum* in degraded or low-resource patches, where competition with other plants is relatively low, and space is not a limiting factor.

Nitrogen effects on A. pilosellae *performance*

We did not detect any performance benefits to *A. pilosellae* as a result of *P. officinarum* nitrogen availabilities, though there may be improved performance through successive generations. This wasp population produces one to two generations per year in the field (Moffat et al., 2013) with up to four in the lab, and *A. pilosellae* females were emerging at the time of harvest at seven weeks after final wasp introduction. We observed that *A. pilosellae* galling promoted an increase in leaf nitrogen content in ungalled leaves on galled plants when plant nitrogen availability was improved. This may boost offspring performance on previously galled plants due to increased nutritional quality or increased vigour in these nitrogen-boosted leaves. Tipping et al. (2015) observed that unrestricted herbivory by a defoliating weevil (*Oxyops vitiosa* Pascoe, Coleoptera: Curculionidae) on its host plant *Melaleuca quinquenervia* (Myrtaceae) actually promoted growth of leaves that were more suitable to feeding by the following generation of weevils. Thus, galling and other herbivory can influence plant growth patterns and resource movement in order to promote offspring performance, either through production of new shoots or sequestration of resources to specific plant organs (Craig & Price, 1986; Paulo Inácio & Vieira, 1999). This is known as the “resource regulation hypothesis” (Craig & Price, 1986), in which certain herbivores maintain or increase the production of plant modules which favour further herbivory. Though it must be pointed out that in some gall-wasp systems, increased nitrogen content in the galls can be detrimental to insect survival (Hartley & Lawton, 1992). However, most gall-formers are able to control the amount of nitrogen within their galls, with nitrogen content of galls ranging widely from greater or lesser than other surrounding plant tissues (Hartley, 1998; Hartley & Lawton, 1992; Künkler et al., 2013).

Increased nitrogen availability tends to increase overall plant vigour, as well as vigour and number of plant parts (Leskovšek et al., 2012), and gall-formers prefer vigourously growing tissues (Dhileepan, 2004; Price, 1991). Gall tissues are physiological sinks of nutrients (Castro et al., 2012; Huang et al., 2014; Larson & Whitham, 1991), and as such have the ability to capitalize on increased plant vigour by directing a greater amount of resources to gall tissue and subsequent insect feeding and development (Burstein et al., 1994; Castro et al., 2012; Huang et al., 2014). Most studies of plant vigour and gall insect preference or performance use the overall size of the plant module as a measurement of vigour, though there is evidence that the length of time that the module remains a photoassimilate sink is more reflective of vigour (Höglund, 2014). If nitrogen availability increases the amount of time that young leaves remain nutrient sinks in *P. officinarum*, then we would expect *A. pilosellae* performance to improve when galling these tissues. However, we did not detect any performance benefits for wasps exposed to various nitrogen treated plants. It may be that successive generations may benefit from the increased vigour and number of desired plant parts, as we did detect nitrogen effects that improved lateral stolon (and therefore leaf) production. We suggest a further experiment using nitrogen and wasp presence through multiple generations in order to determine whether nitrogen availability increases *A. pilosellae* performance through plant mediated effects or via galling effects on the suitability of desired plant parts (leaves).

A. pilosellae *effects on* P. officinarum *performance*

Aulacidea pilosellae galls reduced main stolon length under all nitrogen treatments. Similar results were obtained by Klöppel et al. (2003) with *Aulacidea subterminalis*, a congeneric stolon-galling wasp specific to *P. officinarum*, except that these main stolons were reduced in length because the galls terminate stolon growth. The reduction in stolon length is quite important with regards to biocontrol because Winkler and Stöcklin (2002) showed that phenotypic plasticity of stolon length of *P. officinarum* is a pivotal factor for the maintenance of populations with and without competition with other plant species. This occurs because stolons generate daughter rosettes from their nodes, which allows *P. officinarum* to maintain ground cover in the midst of inter- and intraspecific competition and space limitations (Winkler & Stöcklin, 2002). Thus, the shorter stolons as a response to galling suggests a reduction in the competitive ability of *P. officinarum*. The ability to exclude other plants via vegetative growth and spread daughter rosettes in the presence of *A. pilosellae* should be diminished. This may allow regrowth and recolonization by native plants as the hawkweed populations are reduced, as long as native plants are present (Davy & Bishop, 1984; Winkler & Stöcklin, 2002). Additionally, shorter stolon lengths can lead to high interspecific competition due to smaller distances between daughter and mother rosettes. Since the maintenance of *P. officinarum* populations and the principal means of colony growth tends to be clonally through the production of vegetative stolons and thus daughter rosettes (Jacobs & Wiese, 2007; Makepeace, 1985a), we predict that a reduction in stolon length due to *A. pilosellae* should result in reduced *P. officinarum* population sizes.

Aulacidea pilosellae impact on stolon length was first detected at two weeks after wasp introduction, indicating that this effect was primarily due to early gall development and not larval feeding within the developed galls. Galls are known to be nutrient sinks to the host plant at the cost of the plant's own stored and newly created resources to grow and develop these novel insect-induced plant structures (Dsouza & Ravishankar, 2014; Huang et al., 2014). For *A. pilosellae*, galls on *P. officinarum* are at their full size (but not maturity) at 2-4 weeks after oviposition at our greenhouse day temperatures of 22°C, with the enclosed larvae between the first and second instar (pers. obs.). The greatest impact on *P. officinarum* by *A. pilosellae* galling on stolon growth took place within the first two weeks of the experiment, during oviposition and subsequent gall initiation and early gall development. The developing larvae also can continue to redirect newly created plant photoassimilates and absorbed nutrients towards the gall tissues surrounding them and upon which they feed, removing these nutrients from plant growth and reproduction throughout the insects' growth stages (Hartley, 1998).

However, after the initial impact of galling on stolon length, there was no change in stolon length between nitrogen or wasp treatments before the plants switched to lateral stolon production. In their native range, this *A. pilosellae* biotype has one to two generations per year, which in regards to biocontrol can mean that this impact due to gall initiation on *P. officinarum* can be exerted multiple times per year.

Galling reduced the amount of leaf nitrogen in galled leaves on wasp treatment plants compared to ungalled leaves on wasp treatment plants and control plant leaves, under all nitrogen treatments. Various gall-formers can manipulate nitrogen levels within the gall to an optimum level that improves larval growth and development (Gange & Nice, 1997; Hartley & Lawton, 1992; Koyama et al., 2004). Some insects require a minimum amount of leaf nitrogen in order to survive (Guenther et al., 2011); however, survival rates of *A. pilosellae* were not affected by the leaf nitrogen levels under any nitrogen treatments. Gall-former larvae have been shown to manipulate gall nitrogen content to be different than surrounding tissue (Hartley, 1998; Hartley & Lawton, 1992; Künkler et al., 2013), and this effect is often insect and plant species-specific. In fact, gall carbon: nitrogen ratios can also be different between young and mature galls, as developing larvae require different nutrient concentrations (Hartley, 1998; Künkler et al., 2013). In fact, we detected that the leaf nitrogen content was higher at four weeks post wasp introduction (and the first nitrogen treatment) than at seven weeks for all control, ungalled, and galled leaves (data not shown). Therefore, there was manipulation of nitrogen levels within the galled leaves by *A. pilosellae*, which may have to do with the maturity of the leaves. Only mature leaves had galls at week 7, although they were young when they were galled. Most of the ungalled leaves on wasp treatment plants were young to middle aged (with a few older leaves), as all ungalled plant leaves were pooled. As young leaves generally have more nitrogen than mature leaves, due to their active growth and vigour (Field, 1983), this is a possible explanation for our findings.

Interactive effects between nitrogen and A. pilosellae *on* P. officinarum *performance*

We detected strong interactive effects between nitrogen availability and galling on the number of lateral stolons and plant dry weight, with a much stronger effect when nitrogen levels are high, suggesting *P. officinarum* is unable to compensate for the resources lost to galling. Some plants can compensate for insect damage under varying environmental conditions, such as when soil nutrients are high, which can effectively reduce the negative impact of the herbivores (McNaughton, 1983). Klöppel et al. (2003) observed that lateral stolon production in *P. officinarum* was stimulated by *A. subterminalis* galls, as they are stolon-galling wasps that target the apical meristem of these tissues, thus causing the release of lateral bud growth. However, in our experiment, *P. officinarum* plants were unable to compensate for the lost resources due to *A. pilosellae* galling through the production of lateral stolons. This was still true when nitrogen availability was high, as there was an interactive effect that increased the impact of *A. pilosellae* on *P. officinarum* when more nitrogen was available. Meyer and Root (1993) found that spittlebug (*Philaenus spumarius*), beetle (*Trirhabda* sp.), and aphid (*Uroleucon caligatum*) feeding all resulted in a greater impact on goldenrod (*Solidago altissima*) when under high soil fertility versus low soil fertility, supporting the statement that not all plants have the ability to compensate for insect damage when soil nutrients are abundant. Hinz and Müller-Schärer (2000) found that gall midges (*Rhopalomyia* n. sp.,) have a greater impact on their host, scentless chamomile (*Tripleurospermum perforatum*), when plants are exposed to nutrient-rich and moist sites. However, other systems have shown that compensation is possible when nutrients are readily available, as with water hyacinth (*Eichhornia crassipes*) (Bownes et al., 2013b) and rosinweed (*Silphium integrifolium*) (Fay et al., 1996), showing that not all plants respond in the same way to resources or herbivory. Still, site fertility may play a critical role in managing and maintaining top-down control of invasive weeds targeted for biocontrol, either by affecting the impact on the target weed and/or influencing performance of the biocontrol agent (Hovick & Carson, 2015).

Maternal effects of host utilization on A. pilosellae *performance*

There were considerably more galls produced on *P. caespitosa* than *P. glomerata* in the maternal generation by *A. pilosellae* females. However, when we measured the egg load of wasps emerging from both species, there was no significant difference. Adult longevity (reproductive life span) has been proposed to allow a greater prediction of fecundity than egg load, especially when a food source is readily available (Leather, 1988). We believe *A. pilosellae* to be pro-ovigenic, emerging as adults with all of their eggs produced, which has been noted in other cynipids (Ito & Hijii, 2004). A possible explanation for the difference in gall numbers in the maternal generation is that the wasps exposed to *P. glomerata* underwent oosorption; that is, they reabsorbed their eggs in order to increase their longevity on a sub-optimal food source, *P. glomerata*. This has been observed in other cynipid gall wasps, for example the invasive Asian chestnut gall wasp, *Dryocosmus kuriphilus*, when food is unavailable after emergence (Graziosi & Rieske, 2014). However, we did not observe a greater number of galls formed on *P. caespitosa* than *P. glomerata* in the offspring generation. Instead we observed that more galls were formed by wasps that emerged from *P. caespitosa*, regardless of the offspring host species galled. Thus, it seems that female *A. pilosellae* that emerge from *P. caespitosa* are inherently more fecund, forming more galls on both hosts than females emerging from *P. glomerata*. Therefore, we do not believe that oosorption is a contributing factor for our experiment, though further experimentation into this phenomenon is required.

We had predicted that maternal effects would promote *A. pilosellae* performance on the same host as the mother; however, our results only indicate that *P. caespitosa* is the better host. Females that emerged from *P. caespitosa* produced more galls on both host species than females that emerged from *P. glomerata*. Or and Ward (2007) found that maternal effects strongly influenced offspring number but not offspring mass in a bruchid beetle (*Caryedon palaestinicus*), with females grown on healthy *Acacia raddiana* trees producing more offspring than those on stressed trees. Spitzer (2004) found that maternal host use of the soft scale insect *Saissetia coffeae* did not influence offspring performance on either of the disparate host species, but rather maternal effects presented as an adaptive response to maternal insect density and rates of fungal infection. Many other studies show maternal effects having an influence on egg number

(Fox, 2000; Fox et al., 1997; Heisswolf et al., 2009) or egg size (Bernardo, 1996; Ito, 1997; McIntyre & Gooding, 2000), both of which can improve potential fecundity and population growth rates of the offspring. In the large milkweed bug (*Oncopeltus fasciatus*, maternal effects influenced egg size, with larger eggs produced when the offspring diet was different than the maternal diet (Newcombe et al., 2015). In our experiment, it seems that maternal effects positively influence population growth rates when the maternal host is *P. caespitosa* via increased galls produced, regardless of the offspring host galled.

Offspring host effects on A. pilosellae *performance*

We did not detect maternal host effects on gall size and resulting larval weights, but detected strong host effects. The galls that were formed on *P. caespitosa* were much larger than those on *P. glomerata*, and gall size had a very strong positive effect on larval weight. This suggests that the offspring host is much more important to individual insect survival and potential fecundity than maternal host. This is because the maternal host influenced the number of galls produced, but the offspring host influenced gall size and larval weight, which is more directly related to insect fitness. Marchosky Jr and Craig (2004) found that gall size strongly influenced larval performance and survival in the gall midge *Asphondylia atriplicis*. Similarly, Shibata (2002) observed that gall size was strongly correlated to emerged adult size and potential fecundity of the bamboo gall maker, *Aiolomorphus rhopaloides* and its inquiline, *Diomorus aiolomorphi*. Gall size also influences susceptibility to parasitoids, with larger and thick-walled galls reducing the probability that parasitoids will be able to oviposit within the galls (Fernandes et al., 1999; Marchosky Jr & Craig, 2004; Shibata, 2002). The larger larvae from galls on *P. caespitosa* may explain the detected maternal effects; larger larvae from *P. caespitosa* could produce larger adults, which would have greater realized fecundity, leading to the higher number of galls produced in the following generation. Steiger (2013) found that larger mothers of the burying beetle (*Nicrophorus vespilloides* Herbst, Coleoptera: Silphidae) produced larger eggs and larger offspring than their smaller counterparts. As a result, host effects for *A. pilosellae* and *P. caespitosa* contribute to increases in individual fecundity, producing larger and possibly more protective galls and heavier emerging insects. Thus, it is likely that through successive generations, those *A. pilosellae* populations which utilize *P. caespitosa* consecutively will have larger and relatively fit offspring than those populations which utilize *P. glomerata*.

The size of the plants as identified by the number of leaves when wasps were introduced influenced the number of galls *A. pilosellae* formed on each host species. In the offspring and maternal trials, the wasps produced an increased number of galls when more leaves were available on *P. caespitosa* than on *P. glomerata*; however there were more leaves available in the maternal trial than the offspring trial for both species. The combined data from both trials showed that as the number of leaves available at introduction increased, more galls were formed, but only with *P. caespitosa*. Phenology is critically important for gall-formers, as they require plant tissues that are physiological sinks (undifferentiated meristematic tissues) that can be further exploited to create the gall and maintain a supply of nutrients for insect development (Aoyama et al., 2012; Dreger-

Jauffret & Shorthouse, 1992; Kehl & Rambold, 2011; Yukawa et al., 2013). All leaves galled were young, not yet fully unfurled leaves. It appears that for *P. glomerata,* the number of leaves at initial wasp introduction has very little effect on the number of galls produced; one *A. pilosellae* female will only produce an average of 50 galls/plant on this host (compared to an average of 90/plant on *P. caespitosa*), at least with regards to the young plants that were used in this experiment. We therefore conclude that *P. caespitosa* is more responsive to galling, and can produce the greatest number of galls when the plants are of sufficient size and with ample young leaves available for *A. pilosellae*, and also that the wasp can exploit the phenology of *P. caespitosa* more successfully than they can *P. glomerata*.

The galls that were formed on *P. caespitosa* were much larger than those on *P. glomerata*, and gall size had a very strong positive effect on larval weight. Only young leaves were chosen for oviposition, suggesting that more nutrients were relocated towards galls as young leaves are more vigourous in their growth (Höglund, 2014; Morrison & Quiring, 2009). Gall-formers often follow the plant vigour hypothesis, meaning that they prefer and perform better on more vigourously growing plant parts (Price, 1991). These vigourous plant parts often give rise to larger galls and larger larvae (and thus pupae and adults); hence, these are often used as a measure of insect performance (Gratton & Welter, 1998; Joshi & Vrieling, 2005; Nylin & Janz, 1993). Still, Höglund (2014) found that it is not the final size of the plant part galled per se, but rather the length of time that it remains a sink of photoassimilates that determines the fecundity of the insect. As all of the leaves galled were young, not yet fully unfurled leaves, this suggests that the leaves chosen by *A. pilosellae* remained physiological sinks for the longest amount of time. At the same time, Rehill and Schultz (2001) found that gall volume of *Hormaphis* aphids was the best predictor of fecundity, with little or no influence due to leaf position or leaf choice. Regardless of whether we use gall volume or larval weight, the fact that the largest galls and heaviest larvae in the offspring trial were collected from *P. caespitosa* could give us some insight as to why this host species was also the best maternal host. If heavier larvae emerge from *P. caespitosa*, and larval weight is correlated with fecundity, then we would expect the female wasps that emerged from *P. caespitosa* to produce more galls, regardless of the next host utilized; which is exactly what we observed in our experiment in the Offspring Trial. However, due to the high variability of plant size in the Offspring Trial, and the significant differences in galls produced between the Maternal and Offspring Trial, this experiment should be repeated in order to obtain more robust results.

Another explanation for the difference in number of galls formed between *P. caespitosa* and *P. glomerata* in the maternal generation is that *P. caespitosa* is more responsive to the gall-forming stimulus than *P. glomerata*. As *P. glomerata* is a hybrid species of *P. caespitosa* and *P. cymosa* (Krahulcová et al., 2013), there is likely a closer evolutionary relationship between *A. pilosellae* and the host *P. caespitosa* than with *P. glomerata* as gall wasps are very closely linked genetically to their host species (Harper et al., 2004; Price et al., 1987). This close association of gall-inducing insects with their host allows them to be very host specific in their ability to induce galls and cause the subsequent tissue manipulation in order to feed and sustain the living insect within (Harper et al., 2004; Schönrogge et al., 2000). As for why we detect *A. pilosellae* on both hosts in their native range when utilizing *P. caespitosa* as a host clearly gives an increased wasp performance, further research must be conducted.

Future research questions

Our experiments were constrained to a quarantine environment, excluding many factors that may be present under more natural conditions. For example, we know that *A. pilosellae* are susceptible to high parasitism rates in their native range, with anywhere from 17 to 30% of galls infested with parasitoids in different regions and years, with up to 90% under certain circumstances (pers. comm. Cortat, G., CABI, Switzerland, Oct 8, 2015). Leaf pubescence may be a factor contributing to host-switching with *A. pilosellae*, as *P. glomerata* has stiff hairs and many stellate hairs on the upper and lower leaf surfaces, respectively, while *P. caespitosa* has soft hairs on the upper leaf surface and sparse to many stellate hairs on the lower leaf surface (Moffat et al., 2015). Other systems have shown that insects that gall plant species with higher levels of leaf pubescence have reduced mortality due to predators and parasitoids (Obrycki et al., 1983), as high leaf pubescence restricts parasitoids from accessing the prey galls. We speculate that differences in pubescence may deter parasitoids from ovipositing within *A. pilosellae* galls on *P. glomerata* but not on *P. caespitosa* in the native range. Since escape from enemies is one of the main factors that may facilitate host switching of gall-formers (Abrahamson et al., 1993), we predict that identifying mortality of *A. pilosellae* under pressure from parasitoids may help explain why *P. glomerata* is a common field host (Moffat et al., 2013). In general, gall-formers are attacked by very few parasitoids compared to exposed surface-feeding insects (Price & Pschorn-Walcher, 1988). With respect to released biocontrol agents, escape from natural enemies is common, with fewer and less specific enemies acquired within non-native regions (Prior & Hellmann, 2013; Torchin et al., 2003). As such, we predict that it is unlikely that parasitoids will induce the same mortality rates on *A. pilosellae* in non-native regions as in their native range (Colautti et al., 2004; Torchin et al., 2003). Additionally, attack by parasitoids may be affected by the larger galls on *P. caespitosa* than *P. glomerata*, as larger galls tend to provide greater protection from parasitoids (Ito & Hijii, 2004; Price & Clancy, 1986; Weis et al., 1985). However, many parasitoids are not affected by gall size (Waring & Price, 1989), but may be affected by gall morphology (Cornell, 1983; Stone & Schönrogge, 2003), defensive secondary chemicals (Cornell, 1983), or simply require specific instar larvae (early vs late) to complete their life cycle (Clancy & Price, 1986). As such, further experimentation is required to identify the parasitoid-induced mortality effects on *A. pilosellae*.

There may also be density-dependent effects of galling on *A. pilosellae* performance. Rehill and Schultz (2001) have shown that when more galls are present together on one plant module, the sink strength is stronger, benefiting all gall insects present by increasing their fecundity. Our maternal effects experiment used one gravid female *A. pilosellae* per host plant, which may not be the density that is naturally occurring in their native range. It is possible that with more wasps per plant, more galls may be produced on *P. glomerata* leading to increased sink strength, resulting in improved

A. pilosellae performance through larger galls or heavier larvae. In contrast, a greater density of *A. pilosellae* on *P. caespitosa* could result in reduced wasp performance due to limited host nutrient supply or reduced wasp egg survival as a result of intra-specific competition, which has been noted in other gall-formers (Imai & Ohsaki, 2007; Ozaki, 1993). Either effect could decrease the relative difference in wasp performance from *P. caespitosa* and *P. glomerata* detected in our experiment, possibly allowing the performance of *A. pilosellae* to be similar on both hosts. Additionally, *A. pilosellae* may follow an ideal free distribution model, such that all wasps are distributed such that there is no advantage for any wasps (Stephens & Myers, 2012). Indeed, if this speculation were to be true, host-switching away from the 'better' host (as identified by our experiment) would not be an ecological dead end; instead, it would be equal utilization of available food sources. We suggest using different *A. pilosellae* densities per plant to determine if there are strong Allee effects (when individual fitness is correlated with population size or density), as these may influence host-switching mechanisms.

Overall, our results lead us to conclude that host-switching away from *P. caespitosa* to *P. glomerata* would not be beneficial for *A. pilosellae* as their performance is enhanced when *P. caespitosa* is the only host, at least under greenhouse conditions. These baseline performance data are necessary when determining a target for a biocontrol programme, as there are multiple invasive hawkweed species as potential hosts for *A. pilosellae* that are sympatric in North America (Wilson et al., 2006; Wilson et al., 1997). Accordingly, *A. pilosellae* may be able to easily establish on *P. caespitosa* in the field due to their increased performance on this host. Establishment of biocontrol agents can be very difficult (up to 59% successfully established for weed biocontrol agents (Julien et al., 1984)) but is of utmost importance; numbers of release sites, number of agents released per site, and the type of sites the agents are released in are all factors that contribute to the success of establishment (Grevstad, 1999; Shea & Possingham, 2000). Early establishment of *A. pilosellae* on *P. caespitosa* due to increased performance on this host could strongly improve biocontrol success and reduce wasted resources on release attempts. Successful establishment of *A. pilosellae* in mixed hawkweed patches could potentially result in some host-switching to *P. glomerata* if *P. caespitosa* densities decreased due to biocontrol efforts, as *A. pilosellae* seems to switch to the most abundant host in the field (Moffat et al., 2013). This type of dynamic biocontrol programme that uses one agent to control multiple target weeds has many benefits, though more research into the likelihood of success of such an endeavour is required, as the impact of *A. pilosellae* galling on both *P. caespitosa* and *P. glomerata* has yet to be determined. As of 2015, 2.9 million dollars has been spent on controlling *P. officinarum* in New Zealand alone, with a total of five agents released (Paynter et al., 2015). Thus, if one effective agent can be used for multiple target species simultaneously, the time and cost required for a biocontrol programme per target can be reduced. As hawkweed ranges expand at about 16% per year (Wilson & Callihan, 1999), their presence is only going to increase in the near future (Frid et al., 2013), until an effective control option is achieved. We believe that biocontrol can be an effective control option, and this project is one step towards that goal.

The interactions between abiotic factors, insects and plants can be very complex. We tried to clarify some of these interactions with a host-specific gall-former and an invasive weed species, as this model is particularly useful with regards to new biocontrol programmes. We have shown that underlying site fertility may have an impact on not only the invasiveness of hawkweeds, but also the effectiveness of a specific gall-wasp that may be used to control hawkweed populations. Additionally, we show that these evolved relationships between gall-formers and their specific hosts contribute to insect performance, but may be exploited to improve a biocontrol programme. Knowing that the most effective host for this wasp biotype is *P. caespitosa* aids in rearing and choosing release sites, especially considering that maternal effects can help promote performance on both *P. caespitosa* and *P. glomerata*. Indeed, it would be very economic and effective to have one biocontrol agent that can specifically target multiple related invasive weeds. Add to this that in their native range, *A. pilosellae* appear to undergo host density-dependent host switching, and we may have a very useful biocontrol agent. Establishment of a new agent is one of the most difficult endeavours of a biocontrol programme (Grevstad, 1999), but if we can manipulate insect rearing methods or choose optimal release sites to improve agent establishment, the chances of an effective biocontrol programme improve substantially.

In the future we would like to identify how *P. officinarum* is impacted when exposed to *A. pilosellae* and competition from native plants, as this can give us an indication of how well *A. pilosellae* will reduce *P. officinarum* populations in invaded regions. Klöppel et al. (2003) found that under competition, *P. officinarum* had reduced overall plant biomass and number of stolons and lateral stolons. Winkler and Stöcklin (2002) showed that increased competition effectively reduces the number of *P. officinarum* seedlings recruited, thus reducing their spread into new areas. They also found that under high intra-specific competition, mean stolon lengths increased, indicating a greater investment in vegetative growth. If intra- and interspecific competition are high, we believe that *A. pilosellae* galling should quickly reduce *P. officinarum* patch sizes as galling significantly reduced stolon lengths and lateral stolon production, which have been known to be related to competitive ability. As such, we propose that further experimentation should be conducted using *A. pilosellae* and *P. officinarum* under competition from species that are common in invaded ranges.

Implications for biological control of Pilosella *hawkweeds using* A. pilosellae

The interaction between nitrogen availability and *A. pilosellae* on *P. officinarum* performance can have important implications for biocontrol, as wasp galling reduces stolon length and lateral stolon production, and therefore potential ground cover under various soil fertilities. *Pilosella officinarum* is a superior competitor in low fertility soils (Díaz-Barradas et al., 2015), and thus can outcompete native plants in invaded ranges when nutrients are scarce (Fan & Harris, 1996; Makepeace, 1985b). However, if *A. pilosellae* can be released on *P. officinarum* in higher nutrient sites, its impact and the probability of successful control of this weed in these locations is much greater than in low fertility areas (Steer & Norton, 2013; Syrett et al., 2012), as wasp impact is increased in these locations. There is also evidence that *P. officinarum* populations can be reduced when there is competition for resources; because stoloniferous growth is a strong contributor to competition and maintenance of patches (Winkler & Stöcklin, 2002), reduced stolon growth by *A. pilosellae* should reduce *P. officinarum*'s ability to compete. Additionally, increased soil fertility may also improve *A. pilosellae* performance, which can lead to larger population levels and subsequent impact on *P. officinarum.* Indeed, soil fertility has been shown to be highly influential on biocontrol agent success at suppressing invasive weeds (Hovick & Carson, 2015).

Additionally, *P. officinarum* has been identified as a 'sleeper weed' (Groves, 2006) in New Zealand (Day & Buckley, 2011), which is when a weed becomes invasive after already being naturalized in an area for many years. Altered nutrient regimes and environmental degradation may cause some of these 'sleeper weeds' to awaken, resulting in an even greater threat to biodiversity (Groves, 2006). As such, *P. officinarum* may become invasive in new environments in the future. Understanding how invasive weeds will respond to new nutrient availabilities and natural enemies will better enable us to predict their spread and control them before they become a greater threat.

We have observed that this *A. pilosellae* biotype can have up to four generations per year in the lab, though they generally have one to two generations in the field per year (Moffat et al., 2013). With regards to biocontrol, the impact due to galling on *P. officinarum* can be exerted multiple times per year. Galls are known to be nutrient sinks that are formed by the host plant at the cost of the plant's resources (Dsouza & Ravishankar, 2014; Huang et al., 2014). The developing larvae also can continue to redirect newly created plant resources towards the gall tissues surrounding them and upon which they feed, thus removing these nutrients from plant growth and reproduction throughout the insects' growth stages (Hartley, 1998). We have shown that the main effect of *A. pilosellae* galling on *P. officinarum* is a reduction in vegetative growth, including mean stolon length and number of lateral stolons. These effects may be able to reduce *P. officinarum*'s competitive ability and lead to successful control of this weed through reduced populations. Additionally, we show that *A. pilosellae* can successfully attack multiple host species, with differences in performance as a result of maternal and offspring host utilized. Maximizing the wasp performance and reproductive success and therefore the effective population size will give the greatest chance for the agent to establish, which has been known to be one of the most difficult aspects of a biological control programme (Shea & Possingham, 2000). Using this information, we can increase the likelihood of *A. pilosellae* establishment for release sites, which increases the probability of successful control of the target *Pilosella* hawkweed species. Thus, we believe that with further experimentation with *A. pilosellae* on impact on various hosts and wasp density-dependent performance, we may be able to better ascertain whether this wasp can be an effective agent for numerous *Pilosella* hawkweed species.

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Supplementary tables

Table S2.1. GLM-P table for mean number of main stolons of *P. officinarum* harvested at week 7.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			117	9.9799	
Block	4	0.66532	113	9.3146	0.9555
Nitrogen	2	0.33349	111	8.9811	0.8464
Wasp	1	0.80413	110	8.177	0.3699
Nitrogen*Wasp	2	0.30393	108	7.873	0.859

Table S2.2. GLM-NB table for mean number of lateral stolons on each *P. officinarum* plant harvested at week 7.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			117	424.15	
Block	4	18.694	113	405.46	9.03E-04
Nitrogen	2	241.218	111	154.24	2.20E-16
Wasp	1	4.004	110	150.24	0.0453826
Nitrogen*Wasp	2	6.564	108	143.67	0.0375599

Table S2.3. ANOVA table for randomly selected main stolon length of harvested *P. officinarum* at week 7.

Source	Df	SS	MS	F	P
Block	4	15956	3989	1.192	0.318609
Nitrogen	2	6401	3201	0.956	0.387564
Wasp	1	46578	46578	13.916	3.07E-04
Nitrogen*Wasp	2	294	147	0.044	0.95707
Residuals	108	361486	3347		

Table S2.4. ANOVA table for mean stolon lengths of all main stolons harvested per *P. officinarum* plant at week 7.

Source	Df	SS	MS	F	P
Block	4	2642	661	0.868	0.486
Nitrogen	2	356	178	0.234	0.792
Wasp	1	28126	28126	36.961	1.85E-08
Nitrogen*Wasp	2	1524	762	1.001	0.371
Residuals	108	82185	761		

Table S2.5. GLM-P table for mean number of reproductive stems of *P. officinarum* harvested at week 7.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			117	44.06	
Block	4	2.0515	113	42.009	0.7263
Nitrogen	2	4.3358	111	37.673	0.1144
Wasp	1	0.0272	110	37.646	0.8691
Nitrogen*Wasp	2	0.0112	108	37.635	0.9944

Table S2.6. ANOVA table for mean above ground dry weights of harvested *P. officinarum* at week 7.

Source	Df	SS	MS	F	P
Block	4	18.52	4.63	3.852	0.00577
Nitrogen	2	84.68	42.34	35.228	1.67E-12
Wasp	1	22.3	22.3	18.551	3.66E-05
Nitrogen*Wasp	2	10.81	5.41	4.497	0.0133
Residuals	108	129.8	1.2		

Table S2.7. GLM-NB table for total number of senesced leaves of *P. officinarum* accumulated at week 7.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			117	325.41	
Block	4	21.7135	113	303.7	0.0002285
Nitrogen	2	1.0272	111	302.67	0.5983355
Wasp	1	12.9644	110	289.71	0.0003175
Nitrogen*Wasp	2	1.626	108	288.08	0.4435195

Table S2.8. ANOVA table for leaf nitrogen content at week 7 post initial wasp introduction, galled leaves excluded.

Source	Df	SS	MS	F	P
Block	4	0.507	0.127	8.705	6.26E-04
Nitrogen	1	4.65	4.65	319.501	5.37E-12
Wasp	1	0.064	0.064	4.414	0.052
Nitrogen*Wasp	1	0.14	0.14	9.631	0.007
Residuals	16	0.233	0.015		

Table S2.9. GLM-NB table for total number of galls formed per plant.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			58	65.612	
Block	4	5.0395	54	60.573	0.2833
Nitrogen	2	0.127	52	60.446	0.9385

Table S2.10. Repeated measures ANOVA on adult *A. pilosellae* emergence immediately after *P. officinarum* plant harvest at week 7. Errors were accounted for from Block within each day of emergence.

Error: Block	Df	SS	MS	F	P
Nitrogen	2	258.05	129.03		
Days	2	63.25	31.63		
Error: Day/Block	Df	SS	MS	F	P
Nitrogen	2	769	384.5	5.467	0.0717
Days	16	14577	911	12.953	0.0118
Nitrogen*Days	7	386	55.2	0.784	0.6354
Residuals	4	281	70.3		
Error: Within	Df	SS	MS	F	P
Nitrogen	2	101	50.44	0.564	0.57
Nitrogen*Days	29	1249	43.08	0.482	0.99
Residuals	254	22716	89.43		

Table S2.11. GLM-NB table for total number of *A. pilosellae* emerged per plant.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			58	66.256	
Block	4	4.2886	54	61.967	0.3684
Nitrogen	2	0.0768	52	61.89	0.9623

Table S2.12. GLM-NB table for total number of *A. pilosellae* remaining at 3rd instar after emergence per plant.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			58	71.279	
Block	4	10.0137	54	61.266	0.0402
Nitrogen	2	0.9003	52	60.365	0.6375

Table S2.13. GLM-NB table for total number of *A. pilosellae* eggs per female.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			149	214.88	
Block	4	13.7493	145	201.13	0.00814
Nitrogen	2	0.5007	143	200.63	0.77854

Table S2.14. Repeated measures ANOVA on randomly selected main stolon length, weeks 2, 4, 6, and 7. Errors were accounted for from Block within each Week.

Error: Block	Df	SS	MS	F	P
Wasp	1	3324	3324	0.136	0.748
Nitrogen	1	143	143	0.006	0.946
Residuals	2	48878	24439		
Error: Block/Week	Df	SS	MS	F	P
Week	1	68118	68118	148.484	0.00667
Week*Wasp	1	31	31	0.068	0.81865
Week*Nitrogen	1	266	266	0.58	0.52594
Residuals	2	918	459		
Error: Within	Df	SS	MS	F	P
Wasp	1	172119	172119	63.774	1.17E-14
Nitrogen	2	35636	17818	6.602	0.00149
Week*Wasp	1	526	526	0.195	0.65901
Week*Nitrogen	2	134	67	0.025	0.97556
Wasp*Nitrogen	2	551	276	0.102	0.90295
Week*Wasp*Nitrogen	2	102	51	0.019	0.9813
Residuals	452	1219895	2699		

Table S2.15. Repeated measures ANOVA on total number of lateral stolons, weeks 0, 2, 4, 6, and 7. Errors were accounted for from Block within each Week.

Error: Block	Df	SS	MS	F	P
Wasp	1	595.2	595.2	1.002	0.422
Nitrogen	1	108.7	108.7	0.183	0.711
Residuals	2	118.5	594.2		
Error: Block/Week	Df	SS	MS	F	P
Week	1	25565	25565	59.471	0.0164
Week*Wasp	1	427	427	0.994	0.4238
Week*Nitrogen	1	120	120	0.278	0.6504
Residuals	2	860	430		
Error: Within	Df	SS	MS	F	P
Wasp	1	1547	1547	28.52	1.34E-07
Nitrogen	2	11529	5794	106.24	2.00E-16
Week*Wasp	1	1182	1182	21.78	3.82E-06
Week*Nitrogen	2	10859	5430	100.07	2.00E-16
Wasp*Nitrogen	2	1545	773	14.24	9.23E-07
Week*Wasp*Nitrogen	2	1172	586	10.8	2.50E-05
Residuals	570	30927	54		

Table S16. Repeated measures ANOVA on total number of senesced leaves, weeks 2, 4, 6, and 7. Errors were accounted for from Block within each Week.

Error: Block	Df	SS	MS	F	P
Wasp	1	228	228	13.432	0.067
Nitrogen	1	27.06	27.06	1.594	0.334
Residuals	2	33.95	16.98		
Error: Block/Week	Df	SS	MS	F	P
Week	1	4358	4358	207.996	0.00477
Week*Wasp	1	137	137	6.543	0.12485
Week*Nitrogen	1	13	13	0.602	0.51898
Residuals	2	42	21		
Error: Within	Df	SS	MS	F	P
Wasp	1	74	74.12	9.247	0.00247
Nitrogen	2	0	0.07	0.008	0.99172
Week*Wasp	1	72	72.09	8.994	0.00283
Week*Nitrogen	2	0	0.13	0.017	0.98341
Wasp*Nitrogen	2	3	1.4	0.175	0.83933
Week*Wasp*Nitrogen	2	3	1.53	0.19	0.82735
Residuals	570	4569	8.02		

Table S3.1. Poisson GLM table for total number of *A. pilosellae* galls formed in the maternal generation.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			52	94.406	
Block	5	2.03	47	92.376	0.845
Host	1	33.74	46	58.636	6.30E-09

Table S3.2. Negative binomial GLM table with Poisson distribution for total number of *A. pilosellae* eggs/females in the maternal generation. Wasp dissections were conducted on a 10% subsample of all wasps emerged.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			38	48.061	
Block	4	5.5151	34	42.546	0.2384
Host	1	2.1855	33	40.361	0.1393
Number of Wasps	1	0.0186	32	40.342	0.8916
Host*Number of Wasps	1	0.9787	31	39.363	0.3225

Table S3.3. ANOVA table for emergence success (percent emerged) of *A. pilosellae* from both *P. caespitosa/glomerata* host plants from the maternal generation. Plants that had only males emerge were not used for analysis.

Source	Df	SS	MS	F value	P
Block	5	0.5376	0.10751	1.689	0.162
Host	1	0.0464	0.0464	0.729	0.399
Residuals	36	2.2909	0.06364		

Table S3.4. ANOVA table for percent emergence of *A. pilosellae* males from both *P. caespitosa/glomerata* host plants in the maternal generation. Plants that had only males emerge were not used for analysis.

Source	Df	SS	MS	F value	P
Block	5	0.1217	0.02433	0.772	0.5765
Host	1	0.0921	0.09208	2.92	0.0961
Residuals	36	1.1352	0.03153		

Table S3.5. Negative binomial GLM table for total number of *A. pilosellae* galls in the offspring generation, using number of leaves at initial wasp introduction as a covariate.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			114	150.99	
Block	5	11.6707	109	139.32	0.039589
Maternal	1	6.023	108	133.3	0.01412
Immediate	1	2.5724	107	130.73	0.108743
Number of Leaves	1	7.1379	106	123.59	0.007547
Maternal*Immediate	1	0.3326	105	123.26	0.564148
Maternal*Number of Leaves	1	2.1266	104	121.13	0.144758
Immediate*Number of Leaves	1	1.7098	103	119.42	0.191014
Maternal*Immediate*Number of Leaves	1	0.1401	102	119.28	0.708223

Table S3.6. Poisson GLM table for total number of pilosella leaves galled by *A. pilosellae* in the offspring generation, using number of leaves at initial wasp introduction as a covariate.

Source	Df	Deviance	Residual Df	Residual Deviance	P
NULL			114	189.69	
Block	5	24.4717	109	165.22	0.0001762
Maternal	1	5.3608	108	159.86	0.0205943
Immediate	1	0.3632	107	159.5	0.5467491
Number of Leaves	1	20.0355	106	139.46	7.60E-06
Maternal*Immediate	1	0.2724	105	139.17	0.6016996
Maternal*Number of Leaves	1	2.0791	104	137.11	0.1493239
Immediate*Number of Leaves	1	1.6854	103	135.43	0.1942067
Maternal*Immediate*Number of Leaves	1	0.0312	102	135.39	0.859889

Table S3.7. ANOVA table for mean gall volume of *A. pilosellae* galls on *Pilosella* plants in the offspring generation, using number of leaves at initial wasp introduction as a covariate.

Source	Df	SS	MS	F value	P
Block	5	156.2	31.24	3.68	0.00421
Maternal	1	6.8	6.82	0.803	0.37227
Immediate	1	272.4	272.41	32.085	1.37E-07
Number of Leaves	1	34.2	34.18	4.025	0.04747
Maternal*Immediate	1	33.2	33.21	3.911	0.05066
Maternal*Number of Leaves	1	0.4	0.37	0.044	0.83511
Immediate*Number of Leaves	1	80.6	80.64	9.498	0.00265
Maternal*Immediate*Number of Leaves	1	11.1	11.14	1.312	0.25469
Residuals	102	866	8.49		

Table S3.8. ANOVA table for mean larva weight of *A. pilosellae* on *Pilosella* plants in the offspring generation, using number of leaves at initial wasp introduction as a covariate.

Source	Df	SS	MS	F value	P
Block	5	0.0674	0.01348	1.215	0.308025
Maternal	1	0.003	0.00299	0.269	0.604961
Immediate	1	0.1679	0.1679	15.135	1.87E-04
Number of Leaves	1	0.0003	0.0026	0.024	0.878302
Number of larvae weighed	1	0.1873	0.18764	16.888	8.47E-05
Maternal*Immediate	1	0.0087	0.00869	0.783	0.378428
Maternal*Number of Leaves	1	0.0007	0.00071	0.064	0.800247
Immediate*Number of Leaves	1	0.0216	0.02156	1.944	0.166565
Maternal*Number of larvae weighed	1	0.0101	0.01007	0.908	0.343122
Immediate*Number of larvae weighed	1	0.0146	0.0146	1.316	0.254162
Number of Leaves*Number of larvae weighed	1	0.0042	0.00424	0.382	0.538074
Maternal*Immediate*Number of Leaves	1	0.0105	0.01055	0.951	0.33206
Maternal*Immediate*Number of larvae weighed	1	0.0263	0.02635	2.375	0.126654
Maternal*Number of Leaves*Number of larvae weighed	1	0.0325	0.03251	2.93	0.090218
Immediate*Number of Leaves*Number of larvae weighed	1	0.0021	0.00206	0.185	0.667768
Maternal*Immediate*Number of Leaves*Number of larvae weighed	1	0.052	0.05201	4.689	0.032892
Residuals	94	1.0428	0.01109		