

THE IMPACT OF HOST-PLANT STRESS
ON THE PERFORMANCE OF
TWO INSECT
BIOLOGICAL CONTROL AGENTS
OF BROOM (*CYTISUS SCOPARIUS*)

A thesis
submitted in fulfilment
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By
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DEDICATION

This thesis is dedicated to my husband, Peter Jones.

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ABSTRACT

Abstract of a thesis submitted in fulfilment of the
requirements for the degree of Doctor of Philosophy

THE IMPACT OF HOST-PLANT STRESS ON THE PERFORMANCE OF TWO BIOLOGICAL CONTROL AGENTS OF BROOM (*CYTISUS SCOPARIUS*)

by K.E. Galway

Weeds often grow over a wide range of environments, experiencing a number of environmental stresses with varying intensities. As a consequence these weeds will often exhibit differences in morphology and physiology. It has been suggested that these differences in host-plant morphology and physiology will affect insect herbivores. Determining how insect herbivores will be affected is important for weed management programs, so that environments where greatest establishment, population growth and impact on host-plants by weed biological control agents can be identified.

The Plant Stress Hypothesis (PSH), Plant Vigour Hypothesis (PVH), and Insect Performance Hypothesis (IPH) have been used to explain changes in insect herbivore performance, when host-plants are experiencing environmental stress. A review of more than 200 studies on insect performance across five insect feeding-guilds and 12 stress types was conducted. It was found that both insect feeding-guild and stress type were important determinants of predicting insect performance, which were not considered by these three hypotheses.

The performance of *Leucoptera spartifoliella* and *Arytainilla spartiophila*, two biological agents of Scotch broom (*Cytisus scoparius*) were assessed over a range of host-plant soil moistures and shading levels. It was found that overall performance of *L. spartifoliella* and *A. spartiophila* were greatest on broom growing in drier soil conditions. Populations of *L. spartifoliella* were greatest in sunnier environments under laboratory conditions, or in the field when populations were low. However, when populations in the field were medium or high, there were no differences

between shaded and non-shaded environments. The greatest impact to broom might occur in shaded environments, where broom is not growing optimally and is combined with the added pressure of *L. spartifoliella* attack.

As the laboratory experiments did not allow ovipositing females to select between hosts of varying quality, further laboratory experiments were conducted, giving ovipositing females the choice of host-plants. Relationships between oviposition preference and oviposition performance were similar in most cases for *L. spartifoliella* and *A. spartiophila* across a range of host-plant soil moisture and shade gradients. The results give some support to the hypothesis that preference and performance are linked in monophagous insects that have immobile juvenile stages. These positive preference-performance results also increase the strength of predictions made in the earlier laboratory experiments.

In a survey on *L. spartifoliella* pupal populations in New Zealand and in Australia, pupae were found on all ages of broom surveyed, from plants as young as two to 23 years old. No evidence was found to suggest that *L. spartifoliella* pupal populations were influenced by the age of host-plants. However, populations increased as the amount of available feeding resource (one-year-old shoots) increased. The continual feeding damage inflicted by *L. spartifoliella* on plants of a young age may hasten plant senescence and death.

This study showed that environmental stress significantly affects insect herbivore performance, and that both insect feeding-guild and stress type need to be considered when predicting insect performance. Broom management strategies can be enhanced as environments where insect establishment, population levels and impact may be greatest have been identified. Control of broom by these two biological agents will be greatest where broom is growing in dry and shaded environments.

Key words: *Cytisus scoparius*, Scotch broom, *Leucoptera spartifoliella*, *Arytainilla spartiophila*, Lyonetiidae, Psyllidae, insect-plant interactions, herbivory, insect performance, insect preference, biological control, weed management, plant stress

hypothesis, plant vigour hypothesis, insect performance hypothesis, induced defence hypothesis, plant age hypothesis, moisture stress, light stress, plant age.

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Chapter 1

The impact of host-plant stress on biological control strategies for Scotch broom (*Cytisus scoparius*).

1.1 Introduction

An invasive plant will frequently grow over a wide range of environments across which it will experience a variety of different environmental stresses. As a consequence, the plant will exhibit differences in both morphology and physiology over its environmental range. How do these differences in the plant affect the performance of insect herbivores? How does insect performance vary depending on the intensity of the stress the plant is under, or the type of stress encountered? Are the insect responses to host-plant stress the same for all insect herbivore species? These are some of the questions that will be investigated to further understand the interactions between environmental stress, plants, and insect herbivores.

This thesis focuses on the hypothesis that insect herbivore performance and impact is affected by environmental stresses experienced by the host-plant, in addition to environmental stresses acting directly on the insect. Investigating the interactions between the environment, plants, and their insect herbivores may (a) improve our understanding of the way herbivore performance varies with host-plant quality, and (b) aid weed management strategies by identifying environments where the performance and impact of agents may be greatest.

1.2 Biological control of weeds

A weed is any plant growing in a situation where it has a detrimental effect on mankind or conservation (Harley & Forno 1992). The effects of weeds on society are diverse, for example weeds can cause allergies and sickness in humans, poison and injure domestic pets and livestock, reduce production of crops and pastures, harbour feral animals and reduce biodiversity. It has been estimated that weeds cost Australia more than \$4 billion annually (CRC 2003) in lost production and cost of control and

alien weeds are causing more than a third of the estimated US\$350 billion worldwide annual economic damage from all introduced pests (Pimentel 2002, Sheppard *et al.* 2003).

As the majority of weed problems come from exotic plant species, biological control is one method commonly used to suppress weeds (Julien & Griffiths 1998). This method depends on the utilisation of other organisms (e.g. insects) for the regulation of host-plant densities (Myers & Bazely 2003). The most widely known method, classical biological control, involves the introduction of natural enemies of a target weed from their native range into an exotic range, where their host-plant has become a weed. The intention is that the natural enemies will reduce the density of a weed to a level that is acceptable and will maintain the weed density at that level over the long term (Julien 1997, McFadyen 1998, Briese 2000).

Like all control methods, weed biological control has its advantages and disadvantages. It is considered by some to be the most appropriate, as it is thought to be environmentally friendly (reduces chemical usage), relatively cheap and self-sustaining (Julien 1997, Louda *et al.* 2003, Carruthers 2004). However, biological control may take many years to achieve, requires government support, and is unsuitable where fast acting short-term control is required (Julien 1997). In addition, there are concerns over direct and indirect non-target impacts. Direct non-target impacts are when an agent feeds on another host, or worse, switches completely to another host. This is of particular concern when these other hosts are rare, thus increasing the likelihood of displacement and even extinction, or when the host is of economic significance, such as an agricultural crop plant (Louda *et al.* 2003). Indirect non-target impacts result from specific agents achieving high abundance in a habitat and affecting other species indirectly. This is of particular concern when the agent is not suppressing the weed and so the high abundance is not short-term (Pearson & Callaway 2003).

It is getting more difficult to import new agents because of concerns over non-target impacts (Follet & Duan 1999, Fowler *et al.* 2000, Louda *et al.* 2003) and increased regulatory constraints (Fowler *et al.* 2000, Sheppard *et al.* 2003, Hoddle 2004). Therefore, it is increasingly important to improve the effectiveness and efficiency of

weed biological control agents. One way of doing this is to assess the impact environmental stress has on a weed species, and on the performance and impact of the agent. This will enable practitioners to identify where biological control will be most successful, or where other forms of control may be more appropriate.

1.3 Environmental stress on weeds

As weeds frequently grow over a wide range of environments, they experience many forms, and intensities of environmental stress. Environmental stress, defined simply by Price (1991), is a factor that reduces plant performance below that achieved under optimal conditions. There are many types of environmental stress that can affect plants. Heinrichs (1988) has listed several possible factors, dividing them into seven abiotic categories:

- Water (includes deficit and excess)
- Electromagnetic energy (includes light intensity, quality, and photoperiod)
- Physical and chemical properties of the soil (includes salinity and mineral deficiencies and excesses)
- Air pollution (includes ozone, acid rain, sulfur oxides, halogens and heavy metals)
- Mechanical damage (includes fire, wind, lightning, hail, dust, rubbing, bending and pruning)
- Temperature (ranges from chilling and freezing to very high)
- Pesticides / growth regulators (includes insecticides, fungicides and herbicides).

Plants growing under environmental stress may exhibit a variety of morphological and physiological responses (Mattson & Haack 1987a), depending on the plant species and the severity, duration, and type of stress encountered (Bradford & Hsiao 1982, Heinrichs 1988, Grime & Campbell 1991). Examples of morphological changes exhibited by plants growing under moisture deficit stress include; leaf rolling, wilting and shedding and increased root to shoot ratio and leaf-cuticle wax thickness (Bradford & Hsiao 1982, Heinrichs 1988). Examples of physiological changes include; reduced photosynthetic ability, respiration, absorption of inorganic ions,

protein and starch concentrations, and wound healing and increased stomatal closure, amino acids and sugar levels (Mattson & Haack 1987b).

This thesis looks at the impacts these morphological and physiological changes in the host-plant have on insect herbivore performance. By doing so it attempts to predict whether the effectiveness of a biological control agent is increased or decreased if the host-plant is stressed.

1.4 Environmental stress on insects

Environmental conditions may influence the effectiveness of weed biological control agents directly, and indirectly via the host-plant. Agents may be directly affected by factors such as temperature, humidity, wind and photoperiod (Gullan & Cranston 2004). Many of these direct effects can be assessed through laboratory studies, and climate matching (e.g. using computer simulation packages that can predict the best environments for release). Agents may also be affected indirectly, via host-plant responses to environmental conditions.

Numerous studies have shown that insect performance is affected when host-plants encounter environmental stress (Chapter 2, Section 2.8 Appendix references), and several hypotheses have predicted how insect herbivores will respond. Three major plant stress – insect herbivory hypotheses have been developed: the Plant Stress Hypothesis (PSH), the Plant Vigour Hypothesis (PVH), and the Insect Performance Hypothesis (IPH).

The PSH states that physiologically stressed plants become more susceptible to insect herbivores (White 1969). White (1969) suggested that insect populations are generally limited by insufficient nitrogen, and when plants are under stress they have a higher nitrogen content that leads to an increase in insect herbivore populations. White (1978, 1993) suggested this hypothesis held for all groups of insects.

However, The PVH states that some insect herbivores perform better on vigorously growing non-stressed host-plants (Price 1991). Price (1991) suggested that this

hypothesis applies to herbivores closely associated with plant growth processes, such as gall-forming insects. Price (1991, 2003) suggested 'herbivores closely associated' could include insects where the female selects the oviposition site within a few centimetres of where larvae will feed, and that hatching occurs soon after oviposition. This hypothesis was based on observations of insect herbivores preferentially attacking young and vigorously growing plants and plant parts, over older plants or plant parts.

Linking the PSH and PVH, the IPH states that insect performance on stressed or non-stressed plants is related to the insect feeding-guild (Larsson 1989). Larsson (1989) predicted wood-feeding, sap-feeding and mining insects would respond positively to stressed host-plants, while the opposite would be the case for leaf-feeding and gall-forming insects. The rationale for this was based on: a) woody plants under stress have reduced oleoresin flow, making them less resistant to attack by wood-feeders; b) sap-feeding insects generally encounter low nitrogen levels so that when a plant is stressed with resulting higher nitrogen levels insect performance improves; c) miners are able to avoid consuming harmful defensive compounds produced by the plant while taking advantage of the higher nitrogen content of stressed plants; d) leaf-feeders do not separate out the chemical fractions in their food as efficiently as other feeding-guilds that discriminate against defensive compounds in stressed plants, so do better on vigorously growing plants; and e) galling insects prefer large sized buds which are found on vigorously growing plants.

Could these plant stress – insect herbivory hypotheses (PSH, PVH, IPH) be used to predict where the performance of weed biological control agents will be greatest? If it were found that these hypotheses could reliably predict insect performance on host-plants, across a range of environments, it would be of enormous benefit to weed management programs.

A few biological control programs have investigated the effects of environmental stress on a target weed, and in turn how this affects the performance of biological control agents. Clark (1953) found that *Chrysolina quadrigemina* Suffrian (Col: Chrysomelidae), a biological control agent for *Hypericum perforatum* (Asteraceae), does not lay eggs on plants growing in the shade. Steinbauer (1998) found that

Carmenta mimosa Eichlin and Passoa (Lep: Sessidae), a biological control agent for the woody legume *Mimosa pigra* (Mimosaceae), inflicted greater damage on the weed when it was growing in moist soil and under shaded conditions. Hinz and Müller-Schärer (2000) showed that *Rhoplaomyia* n. sp. (Dip: Cecidomyiidae), a biological control agent for *Tripleurospermum perforatum* (Asteraceae), could establish over a wide range of habitats, but establishment and population growth of the insect was enhanced when *T. perforatum* was growing in nutrient-rich, moist habitats. Nowierski and Pemberton (2002) state that of the five *Aphthona* spp. flea beetles established on leafy spurge (*Euphorbia esula* complex) in North America, all perform better on and have greatest impact the host plant in sunny sites, while each species has its own preference for soil moisture from wet though to dry soil conditions (Hansen *et al.* 2004). In all of these studies, environments where biological control success would be greatest, or not suitable, for a particular agent, were identified. This allowed more appropriate use of resources, so that biological control practitioners could concentrate their efforts in environments where agents are more likely to succeed, on seeking agents suitable for specific environmental conditions and recommending other control methods in areas where the agents are not expected to do well.

1.5 Why study the Scotch broom system?

Potentially, these plant stress - insect herbivory hypotheses offer enormous benefits to weed biological control strategies. However, it is clear that the interactions between environment, host-plant, and insect herbivore are not clearly understood. To investigate these interactions, the Scotch broom (*Cytisus scoparius* (L.) Link. Fabaceae, subsequently referred to as broom) biological control system was selected for study for several reasons:

- Broom is classified as a noxious weed in several countries, and is a serious threat to the New Zealand and Australian agricultural economies and native ecosystems. This woody shrub is difficult to manage, and biological control may provide the only cost effective and efficient method of management in many situations. Information that aids biological success would be very beneficial.

- Broom grows in a wide range of habitats and environments, and is exposed to a range of environmental stresses.
- *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) and *Arytainilla spartiophila* Förster (Hem: Psyllidae) are two biological control agents established in New Zealand and Australia. They both feed on different vegetative parts of the plant and they belong to different feeding-guilds. Another agent, *Bruchidius villosus* (F.) (Col: Chrysomellidae) also in New Zealand and Australia was not studied as it feeds on the reproductive plant structures, and is not currently widely established in either country.
- Propagation methods for broom are available, therefore stocks of plants can be grown for experimentation; also broom is readily accessible in the field, thus viable for surveying under natural conditions. Also, rearing protocols for both insects are available, enabling laboratory studies to be conducted. *Leucoptera spartifoliella* is also abundant enough in the field for population surveys.

1.6 Scotch Broom (*Cytisus scoparius*)

1.6.1 Broom as a weed

Broom is found throughout numerous countries. This leguminous, temperate shrub, is native from northern Sweden southwards to Spain and the Azores, and west from Ireland across to central Ukraine (Tutin *et al.* 1968). It is classified as a weed in many temperate areas of New Zealand, Australia, USA, Hawaii, Canada, Chile, Argentina, India, Iran and South Africa (Parsons & Cuthbertson 1992, Fowler *et al.* 1996, D. Simberloff pers. comm. 2005).

Broom is abundant in many parts of New Zealand and Australia. In New Zealand, broom was first recorded in 1872 and was grown as an ornamental. However, it escaped from cultivation, and has since become widespread and abundant (Syrett *et al.* 1999). About 60,000 ha of agricultural land in the South Island are infested, with greater than 40% of infestations occurring in the Canterbury region (Bascand & Jowett 1981). Extensive infestations are also found in disturbed areas, river-beds and native grasslands (Williams 1981, Parsons & Cuthbertson 1992). In Australia, Waterhouse (1988) suggested that the introduction of broom might have occurred

around 1800, as a hop substitute for beer brewing. Since its introduction, broom has infested more than 200,000 ha, invading sub-alpine areas, disturbed bush-land, roadside edges, grassland, woodland/open forest, along water courses and pasturelands in south eastern Australia (Hosking *et al.* 1996). Broom is declared a noxious weed throughout South Australia and Western Australia, and in parts of New South Wales, Victoria, and Tasmania (CRC 2000).

Broom is considered a serious economic and environmental problem in New Zealand and Australia. Estimating economic costs associated with broom is difficult. However, Fowler *et al.* (2000) reported that the total cost of broom to New Zealand was \$NZ 5.8-13.6 million/year, and if broom was controlled completely by biological means the benefit to New Zealand (after subtracting the cost benefits of broom e.g. beekeeping industry) is estimated at \$NZ 4.33-12.13 million/year. The effects on the New Zealand and Australian environments are diverse. Infestations reduce plant species diversity by forming dense monocultures and shading native plants (Gilkey 1957, Waterhouse 1988). A number of plant and animal species, listed as rare or threatened, are found only in broom-infested areas, particularly where broom infests Australian sub-alpine areas (CRC 2000, Heinrich & Dowling 2000). The thick canopy of broom provides cover for feral animals, such as pigs (Parsons & Cuthbertson 1992) and blackbirds (Smith 1994a), which further threaten the survival of many endangered flora and fauna species (Heinrich & Dowling 2000). Broom is considered a nuisance in pastures and cultivated fields (Gilkey 1957), and hinders grazing and forestry regimes (Waterhouse 1988, Hosking *et al.* 1996, Syrett 1996), causing economic losses (Syrett 1996). Infestations can also restrict access to recreational areas (Waterhouse 1988).

1.7.2 Broom and its environment

Broom has many characteristics that aid successful invasion into a wide range of environments, including a deep tap-root, reduced leaf surface area, and photosynthetic stems with sunken stomata and thick epidermal coverings. In addition, it is deciduous in drought, has hard seed with high longevity and a high rate of reproduction. It has strong seed dispersal mechanisms and characters suited for dispersal by man and other animals, rapid growth, is tolerant of frost, and being a legume is able to survive on nitrogen-poor soils (Waterhouse 1988, Bossard 1991, Smith & Harlen 1991, Parsons

& Cuthbertson 1992, Zielke *et al.* 1992 Bossard & Rejmanek 1994, Hosking *et al.* 1998, CRC 2000).

Due to its numerous 'weedy' characteristics, broom has infested diverse geographic regions throughout New Zealand and Australia (Williams 1981, Hosking *et al.* 1996, CRC 2000), but in doing so is exposed to a range of environmental stresses. Broom is found across a range of soil moisture levels, from sites receiving high rainfall to those experiencing drought conditions (Williams 1981, Parsons & Cuthbertson 1992, Hosking *et al.* 1996). Infestations also occur across a range of shading intensities, from open grasslands and river-beds, through to the understorey of open Eucalypt forests, and to heavily shaded *Pinus radiata* (Pinaceae) and *Eucalyptus nitens* (Myrtaceae) plantations (Williams 1981, Parsons & Cuthbertson 1992, Hosking *et al.* 1996, Barnes & Holz 2000). Broom grows in a wide range of soils, from rich alluvial soils, to soils low in organic matter and phosphorus (Williams 1981). The woody shrub is vulnerable to fire, which is sometimes used for control (Clark 2000, CRC 2000, Downey 2000), and becomes temporarily flattened in some areas under heavy snow (Smith 1994b).

Differing levels of soil moisture is one factor that may affect broom survival and growth. Walter (1968) (cited in Williams 1981) stated that broom originated from the western Mediterranean where its southern distribution is limited by drought. In Australia, Parsons and Cuthbertson (1992) reported broom generally grew in areas with moderate to high rainfall, and Hosking *et al.* (1996) suggested that in drier climates broom might be restricted to the edges of watercourses, though a report by CRC (2000) stated it would not grow in swampy areas.

Another factor that may affect the survival and growth of broom is shading. Williams (1981) reported that broom seedlings can tolerate a wide range of light regimes, and Parsons and Cuthbertson (1992) stated that young plants could survive in 90% shade. However, Hosking *et al.* (1996) suggested broom does not grow in heavily shaded areas under natural field conditions, as seedlings usually die if germination occurs beneath parental, or other relatively dense canopy cover. There is also evidence showing that different light intensities affect the morphology of broom. Williams (1981) observed that plants growing in full sun possessed strong lateral shoots,

heavily nodulated root systems, and were more prostrate, whereas plants growing in the shade often possessed a single upright shoot with little or no lateral branching, were taller, and had poorly nodulated root systems.

To investigate the relationship between environmental stress, host-plants and insect herbivores, soil moisture and shading were selected as the environmental factors for study for several reasons.

- There is extensive literature on the morphological and physiological changes that occur in plants exposed to a range of soil moisture and light levels.
- The literature on broom biology suggests its distribution may be limited to some extent by these two stress factors. These two stresses are commonly encountered in both New Zealand and Australia, and can be simulated under experimental conditions.
- Relatively little is known about how the morphological and physiological changes that occur when broom is growing under either of these two stresses may affect broom insect herbivores and their success as broom biological control agents.

1.6.3 Biological control of broom

Broom is often very difficult to control due to the low value and or difficult accessibility of infested land. The use of herbicides, slashing, fire, or a combination of these, has not proven successful, as these methods require access to infestations and a reasonable economic return from the infested land. In addition, these methods require follow-up control every second year for at least 20-25 years (Waterhouse 1988, Smith & Harlen 1991). Sheep and goats will graze young broom plants, suppressing rather than killing plants (Zielke *et al.* 1992, Allan *et al.* 1993, Clark 2000, CRC 2000). However, in New Zealand, the economic downturn in the sheep industry has meant that graziers have reduced flock numbers, and are spending less on herbicides, so that weeds such as broom, are spreading into pastures (P Syrett pers. comm 2002). Biological control has therefore become attractive as a potentially effective and efficient technique for broom management.

A large suite of insect herbivores has been collected from broom in its native range (Syrett & Emberson 1996). More than 35 specialist insect herbivores were recorded in

England, which collectively suppressed broom by reducing growth and reproduction, and increasing mortality (Waloff 1968). In a long-term insect exclusion experiment, Waloff and Richards (1977) showed that plants with insect herbivores grew more slowly, were smaller, had reduced internode lengths, and fewer viable seeds per pod compared with plants without insect herbivores. After 10 years, nearly 50% of the plants with herbivores had died compared with fewer than 25% of plants without herbivores.

Programs for biological control of broom have been initiated in several countries. Programs began in the United States with the importation of the broom twig-miner, *L. spartifoliella*, in 1960 from Europe (Frick 1964). However, one of *L. spartifoliella*'s natural enemies, *Tetrastichus evonmellae* (Bouché) (Hym: Eulophidae), a parasitic wasp, was accidentally introduced (Frick 1964). This has possibly reduced *L. spartifoliella* populations (Syrett & Harman 1995), which in turn may have reduced this agent's impact on broom. While there are now a total of 12 specialist broom feeders established in the United States (Syrett *et al.* 1999), biological control is yet to be considered successful (Hosking *et al.* 1998). Biological control of broom programs commenced in New Zealand in 1981, and in Australia in 1990 (Fowler *et al.* 1996, Syrett *et al.* 1999). *Leucoptera spartifoliella*, and the broom psyllid, *A. spartiophila*, have both established in New Zealand (Harman *et al.* 1996) and Australia (Hosking *et al.* 1996). The broom seed beetle, *B. villosus*, has also established in New Zealand (Syrett *et al.* 1999), but only recently in Australia (A. Sheppard pers. comm. 2005).

Leucoptera spartifoliella possesses a univoltine lifecycle (Parker 1964, Syrett & Harman 1995). Adult moths are small (3–4 mm long) and white (Scheele & Syrett 1987), and have a life span of about two weeks (Agwu 1967). In New Zealand and Australia, the adult moths are found in the field during December and January (Syrett & Harman 1995). Eggs are generally laid on the previous year's plant growth, on the lower branches of broom (Frick 1964) and incubate for about two weeks (Agwu 1967). There are six larval instars, of which five mine in the epidermal cells of stem-twigs (Agwu 1967, 1974). Pupation occurs in October (in New Zealand and Australia), usually on the underside of branches (Syrett & Harman 1995), with a duration of about one month (Parker 1964).

Leucoptera spartifoliella was accidentally introduced into New Zealand (Harman *et al.* 1996), at least as early as 1950 (Scheele & Syrett 1987), and is now common and widespread throughout the country (Scheele & Syrett 1987, Syrett *et al.* 1999). In Australia, *L. spartifoliella* was introduced from New Zealand, and released in 1993 (Wapshire & Hosking 1993). It has established in many areas (Syrett *et al.* 1999). An exclusion experiment conducted by Memmott *et al.* (1997) showed that *L. spartifoliella* significantly retarded broom growth in New Zealand. Also, it has been reported that when infestations are high, twigs may be weakened and die, and whole plants may even succumb (Parker 1964, Scheele & Syrett 1987, Syrett & Harman 1995, Partridge & Harman unpublished).

Arytainilla spartiophila is also univoltine (Watmough 1968). Adult psyllids are small (3 mm long), red-brown, sap-feeders that are present during summer (Syrett 1991). Eggs undergo a winter diapause for 5–6 months (Watmough 1968). Development through the immature stages, consisting of five nymphal instars (Watmough 1968), takes about two months (Syrett 1991).

Arytainilla spartiophila was first released in New Zealand in 1993 and in Australia the following year (Hosking *et al.* 1996, Syrett *et al.* 1999), and has established in both countries (Syrett *et al.* 1999, P Hodge pers. comm 2001). Both adults and nymphs inflict damage to broom by feeding on buds and on tender, actively growing parts of the plant (Watmough 1968). To date, recorded levels of *A. spartiophila* damage are low compared to Europe, with only one major outbreak observed suggesting that it may take several years before an impact is seen (Syrett *et al.* unpublished).

To investigate the relationship between environmental stress, host-plants and insect herbivores, *L. spartifoliella* and *A. spartiophila* were selected as the herbivores for study for several reasons.

- Both insects feed on different vegetative parts of the plants and belong in different feeding-guilds (miner and sap-feeder) and they are also both established in both New Zealand and Australia.

- Rearing protocols for *L. spartifoliella* and *A. spartiophila* are available, enabling populations to be reared in the laboratory for experimentation.
- *Leucoptera spartifoliella* populations in the field are high enough to measure population densities (P Syrett & H Harman pers. comm 2000).

1.7 Predicting environments where biological control of broom will be most successful

Can it be predicted where the performance of *L. spartifoliella* and *A. spartiophila* on broom will be greatest? There is ample evidence to show that broom infests a large, diverse geographic range, and that broom is affected, morphologically and physiologically, by both soil moisture and shade stress. According to the PSH and IPH, both *L. spartifoliella* and *A. spartiophila* will perform better in environments where broom is growing under stress (i.e. where broom growth is reduced), while the PVH predicts the opposite for *L. spartifoliella*. These three-level interactions and hypotheses need further investigation, as conflicting outcomes have been highlighted. To predict those environments where the performance of these agents will be greatest, experiments were designed to assess how *L. spartifoliella* and *A. spartiophila* performance will be affected when broom is growing under various levels of soil moisture or shading. Laboratory experiments often do not allow the insects to select between hosts, even though this happens naturally in the field. Therefore preference-performance relationships were assessed, to increase the reliability of the laboratory performance results. In addition, as laboratory experiments often use plants of similar ages, investigating the effect of host-plant age in the field on insect performance was also used to improve the reliability of the laboratory results.

1.8 Insect preference-performance relationships

One of the main benefits of investigating preference-performance relationships is to link laboratory performance studies to field situations where host-plant choice mechanisms are operating.

Insect preference and performance, though often used interchangeably, are distinctly different measures (Singer 1986). As defined by Singer (1986), preference is a measure of the relative likelihood of accepting plants that are encountered, while performance is a measure of offspring survival, growth and reproduction.

If insects select environments (preference) where offspring will do best (performance), the likelihood of success for biological control in these environments will be enhanced. Several proposals have been made as to which insects will exhibit positive preference-performance relationships. There is a suggestion that this will occur for insect species where the immature insect stage is relatively immobile, that is, the survival of the juvenile relies heavily on the site selection of the ovipositing female (Ives, 1978). Craig *et al.* (1989) suggested that monophagous insects would also show positive relationships between preference and performance. Preszler and Price (1995) also suggested that positive preference-performance relationships would occur if two conditions were met. They suggest preference and performance will be correlated and greater on vigorously growing host-plants when (a) oviposition by the female is associated with juvenile feeding site, and (b) the successful development of juveniles is related to plant characters. For both *L. spartifoliella* and *A. spartiophila*, the juvenile stages rely on host-plant selection by the female, oviposition is associated with juvenile feeding site, and they are monophagous. Also, since the development of juveniles can be related to plant characters (especially for *A. spartiophila* and to a lesser extent for *L. spartifoliella*), one could therefore predict that both insects will show positive relationships between preference and performance.

There are many examples of positive preference-performance relationships. For example, Ives (1978) found that *Pieris rapae* (L.) (Lep: Pieridae) females made choices that benefited their larvae by selecting large, young plants growing at lower light levels, and Bultman and Faeth (1988) showed that *Stigmella* sp. (Lep: Nepticulidae) preferred, and performed better in, shaded habitats. However, Rausher (1979) studied three papilionid species, finding that oviposition was higher in sunnier environments for two of the three species, yet all three species had significantly higher survival rates in shaded environments and Foss and Rieske (2003) found *Lymantria dispar* (L.) (Lep: Lymantriidae) performance was greatest on *Quercus palustris* (Fagaceae), but was least the least preferred out of eight *Quercus* species.

1.9 The effects of host-plant age on insect herbivores

While it has been shown that environmental stresses on host-plants, such as moisture (DeBruyn 1995, Christiansen & Austarå 1996) and shading (Rausher 1979, Collinge & Louda 1988), can affect insect performance, there is also evidence to suggest that the abundance of an insect species may not be evenly distributed across host-plants of different ages. Several authors have found insect herbivores to be more abundant on younger plants (Landwehr & Allen 1982, Price *et al.* 1987, Craig *et al.* 1989, Spiegel & Price 1996). Some studies have shown the opposite (Marrs 1986) and others have found mixed results (Kearsley & Whitham 1989).

Several hypotheses try to explain the relationship between host-plant age and insect abundance: the Plant Age Hypothesis (PAH), the Plant Vigour Hypothesis (PVH) and the Induce Defence Hypothesis (IDH). The PAH is based on evidence that many plant traits change as plants age (e.g. leaf size, resin flow, phloem thickness). Thus as plants age susceptibility to different insects is altered (Kearsley & Whitham 1989 and references within). This will depend on the age-related growth phase of the host-plant and the feeding style or habit of the insect herbivore. The PVH (see Section 1.3.2 page 4) suggests that younger, vigorously growing plants are favoured by insect herbivores. The IDH (Karban 1987) suggests that older host-plants may become less suitable for herbivores, based on the hypothesis that a plant attacked as a seedling/sapling, which has survived the initial attack, is physically, chemically and/or physiologically changed, so that it is subsequently more resistant to attack. These hypotheses have all predicted that insect herbivore population abundance varies with host-plants age.

The effects of host-plant age on broom insect herbivores have not been investigated although Partridge and Harman (unpublished) observed that *L. spartifoliella* mining damage resulted in death to some young plants. Combining this observation with the PVH and IDH predictions, it is likely that *L. spartifoliella* populations will be higher on younger plants.

1.10 Thesis outline

The aim of this thesis is to assess the impact of host-plant stress on the performance of two insect biological control agents of broom. In an attempt to answer this larger question, three smaller questions were addressed:

- How do morphological and physiological differences in the host-plant affect the performance of insect herbivores?
- How does insect performance vary depending on the type and intensity of the host-plant stress encountered?
- Are the insect herbivore responses to host-plant stress the same for all insect herbivore species?

There are six sections in this thesis each written as a separate chapter.

In chapter 2, published studies assessing insect performance, when host-plants were growing under environmental stress were collated and analysed, thereby determining which plant stress – insect herbivory hypothesis gained the most support. This section also provided a theoretical framework for interpreting the subsequent laboratory experiments and field surveys. This chapter was slightly modified for publication:

Galway K, Duncan R, Emberson R, Sheppard A & Syrett P. 2004. Insect performance and host-plant stress: review from a biological control perspective. In, Proceedings of the XI International Biological Control of Weeds Symposium. Cullen JM, Brieseman DT, Kriticos DJ, Lonsdale WM, Morin L & Scott JK (Eds.). pp. 394-399. Canberra, Australia.

Chapters 3 and 4 focus on glasshouse and semi-controlled environment studies. Two different environmental stresses, moisture (chapter 3) and shading (chapter 4), each involving four levels of stress intensity, were assessed to ascertain whether insect performance varied between stress intensity, stress type and insect species. In chapter 4, a field survey on *L. spartifoliella* pupal populations in shaded and non-shaded environments, in New Zealand and Australia was also conducted.

In chapter 5, the relationship between insect oviposition preference and performance was investigated. The same two stress types and intensities were imposed on plants as

in chapters 3 and 4. However, insects were allowed to select plants for oviposition rather than being confined onto a particular plant. If positive preference-performance relationships are demonstrated, this would suggest that predictions from the performance studies (chapters 3 and 4) are more likely to hold in a field situation, where host-plant choice mechanisms are operating.

Chapter 6 reports on a field investigation of *L. spartifoliella* that was conducted in both New Zealand and Australia. Surveys were undertaken across a range of host-plant ages, to assess if host-plant age was a factor influencing *L. spartifoliella* pupal populations.

Chapter 7 is a general discussion on how and why host-plant stress affects the performance of two biological control agents of broom. The implications this study has to plant stress – insect herbivory theory and to broom management programs are also discussed.

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Chapter 2

Insect performance and host-plant stress: a review from a biological control perspective.

2.1 Abstract

Three hypotheses predict how insect herbivores perform on stressed host-plants. The Plant Stress Hypothesis (PSH) predicts improved insect performance on stressed hosts. The Plant Vigour Hypothesis (PVH) predicts that insects closely associated with their host, such as gall-formers, will perform better on vigorously growing non-stressed hosts. The third hypothesis, the Insect Performance Hypothesis (IPH) predicts that wood-feeders, sap-feeders and miners will perform better on stressed hosts, while leaf-feeders and gall-formers will perform better on non-stressed hosts. These hypotheses were developed however, from few studies and did not separate different types of plant stress. The hypotheses were tested across five insect feeding-guilds and twelve host-plant stress types, using data from more than 200 published studies on insect performance. When all host-plant stress types were pooled, the results suggested that wood, sap and leaf-feeders performed better on stressed host-plants, while miners and gall-formers performed better on non-stressed host-plants, thus supporting the PVH. However, when all insect feeding-guilds were pooled, it was found that host-plant stress type also influenced insect performance, which was generally higher when host-plants were growing under reduced moisture, light or CO₂, increased soil-nitrogen, or on younger plants. When host-plant stress type and insect feeding-guild were separated, it was found that insect performance across feeding-guilds varied with the type of host-plant stress encountered suggesting that insects in different feeding-guilds may respond to different physiological and morphological changes in the plant. The results from this review indicate that the current plant stress - insect herbivory hypotheses do not adequately predict insect performance on stressed and non-stressed plants, largely due to inadequately defining hypotheses and ignoring stress type. This review highlights the fact that insect performance is often significantly affected by host-plant stress, but that the direction of the response is variable. There were however, consistent relationships between

some insect-feeding guilds and host-plant stress types that would allow the prediction on whether a specific biological control agent might perform better under a specific host-plant stress.

2.2 Introduction

Environmental stress is a factor that reduces plant performance below that achieved under optimal conditions (Price 1991). All plants encounter stress because optimal conditions are rarely encountered in the field, due to variations or fluctuations in environmental conditions. Several morphological and physiological changes may occur in plants under stress (Mattson & Haack 1987), depending on the plant species, and the severity, duration, and type of stress encountered (Grime & Campbell 1991). Under moisture deficit stress, for example: many plants show reduced leaf water, starch and carbohydrates; and increased leaf nitrogen and soluble sugars (Miles *et al.* 1982, Mattson & Haack 1987, English-Loeb *et al.* 1997). In contrast, low light levels can lead to reduced soluble sugars and increased leaf nitrogen and leaf water (Collinge & Louda 1988, Attridge 1990, Potter 1992).

The morphological and physiological changes that occur in plants under stress may affect the performance of insect herbivores feeding on those plants (Mattson & Haack 1987). Several authors have suggested that changes in insect performance under host-plant stress may be predictable, with three plant stress – insect herbivory hypothesis developed. White (1969, 1993) suggested that plants under stress become more susceptible to insect herbivores, with the increase in insect performance driven by increases in leaf soluble nitrogen (the Plant Stress Hypothesis; PSH). Price (1991, 2003) agreed that some insects would perform better on stressed host-plants but suggested that some other insects would perform better on more vigorously growing (non-stressed) host-plants, particularly herbivores closely associated with their host-plant (the Plant Vigour Hypothesis; PVH). Price suggested ‘herbivores closely associated’ would include insects where the female selects the oviposition site within a few centimetres of where larvae will feed, and that hatching occurs soon after oviposition. This hypothesis was based on observations of insect herbivores preferentially attacking young and vigorously growing plants and plant parts over

older plants or plant parts. Combining elements of both the PSH and the PVH, Larsson (1989) suggested that certain insect feeding-guilds (wood-feeding, sap-feeding and mining insects) perform better on stressed host-plants, whereas other insect feeding-guilds (leaf-feeding and gall-forming insects) perform better on vigorously growing host-plants. This was termed the Insect Performance Hypothesis (IPH). The rationale for this was based on: a) woody plants under stress have reduced oleresin flow, making them less resistant to attack by wood-feeders; b) sap-feeding insects generally encounter low nitrogen levels so that when a plant is stressed with resulting higher nitrogen levels insect performance improves; c) miners are able to avoid consuming harmful defensive compounds produced by the plant while taking advantage of the higher nitrogen content of stressed plants; d) leaf-feeders do not separate out the chemical fractions in their food as efficiently as other feeding-guilds that discriminate against defensive compounds in stressed plants, so do better on vigorously growing plants; and e) galling insects prefer large sized buds which are found on vigorously growing plants.

If insect herbivores perform differently when host-plants are under stress, then this has important implications for the effectiveness of insect herbivores released as biological control agents. Herbivores may be more effective in reducing plant performance over certain parts of a plant species' range, depending on whether they perform better on stressed or vigorously growing plants. For example, knowing that an insect herbivore performs better on vigorously growing non-stressed host-plants, and that the same insect performs poorly on stressed host-plants, may indicate that an additional biological control agent that performs well on stressed plants, or other forms of control, are required in those parts of a plant's range where it is subject to stress.

Huberty and Denno (2004) assessed the affects of moisture stress on the performance of sap-feeders and chewing insects (wood borers, gall-formers, free-living chewing insects and miners) by using vote-counting and meta-anaylsis approaches. It was found that host-plant moisture deficit negatively affected sap-feeders and gall-formers, positively affected borers, while responses for free-living chewing insects and miners were inconsistent. Two other studies have reviewed the evidence for relationships between insect performance over a range of host-plant stresses. Waring

and Cobb (1992) assessed insect performance in relation to host-plant moisture and nutrient stress using a vote-counting approach, reporting that the type of stress was a stronger predictor of insect performance than insect feeding-guild. In contrast, Koricheva *et al.* (1998) argued that the insect feeding-guild (using the same guilds as Larsson 1989) could predict insect performance for host-plants under moisture, light and pollution stresses. They used a meta-analysis to detect a weak but overall significant relationship consistent with the IPH.

The aim of this chapter is to assess both the strength and variability of the relationship between insect performance and host-plant stress by collating the results of published studies that have examined insect performance across insect feeding-guilds and host-plant stress types. If this relationship is to be of predictive use in biological control then it requires strong, consistent relationships between the performance of insect feeding-guilds and host-plant stress.

2.3 Materials and Methods

2.3.1 Selection of insect feeding-guilds and stress types

This review assesses insect performance across five insect feeding-guilds on plants subject to 12 stress types.

I have defined insect feeding guilds as a group of insects that feed in a similar manner, for example, leaf-feeders include any insect that feeds primarily on leaves of plants. In this definition insects may belong in different Orders, for example the leaf-feeders could consist of insects from Coleoptera, Hemiptera, Hymenoptera and Lepidoptera. The insect feeding-guilds selected were wood-feeders, sap-feeders, miners, leaf-feeders and gall-formers. Only these feeding-guilds were selected for study because (a) these are the same guilds considered in the IPH by Larsson (1989) and reviewed by Koricheva *et al.* (1998), (b) the insects in these feeding-guilds attack the vegetative parts of plants, and (c) in latter chapters the performance of *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) and *Arytainilla spartiophila* Förster (Hem: Psyllidae) will be assessed, where one species is a miner, the other a sap-feeder. Predictions on these two feeding-guilds are essential when testing the reliability of

plant stress – insect herbivory hypotheses. Flower and seed feeding guilds were not included as (a) the PSH, PVH and IPH do not mention these feeding-guilds, therefore assessing the reliability of these plant stress-insect herbivory hypotheses is difficult, and (b) they affect the reproductiveness of plants.

Five of the seven abiotic categories listed by Heinrichs (1988) as affecting plant growth were investigated. The two abiotic categories omitted were temperature and pesticides/growth regulators, because these have strong, direct effects on insect performance as well as on host-plant growth. Heinrichs (1988) listed numerous stresses for each abiotic category, and a few of these were highlighted in section 1.3, page 3. The selection of stress types for each category was determined by the abundance of published and accessible studies. For example numerous studies were found on water deficit and water excess, in the category of moisture, so both stress types were incorporated into the review. However in the mechanical damage category, only one stress type was selected, fire. Fire was the most researched stress with three studies fitting the section criteria, whereas no studies matched the criteria for wind, lightning, hail, dust, rubbing, bending and pruning. Categories and stresses selected for this review included: moisture (water deficit and excess), electromagnetic energy (light and ultraviolet-B radiation), physical and chemical properties of the soil (soil-nitrogen, salinity and acidity), air pollution (ozone, carbon dioxide, sulphur dioxide and acid rain), and mechanical damage (fire- where plants had been previously exposed to fire). Plant age was also included as a 'stress' because several authors have shown that insect performance is affected by age (Price *et al.* 1987, Caouette & Price 1989, Craig *et al.* 1989, Roininen *et al.* 1993), and age was a factor considered by Price (1991) in developing the PVH.

2.3.2 Selection of studies

Key word searches in CAB Abstracts were used to source studies. Studies were found by entering a stress type and herbivory (e.g. moisture and herbiv*), and by entering the name of each of the three hypotheses. Additional studies were found by searching the reference lists of the papers collected. Studies were selected if they assessed the performance of individual insect species, where those species belonged to one of the five listed feeding-guilds, and where the host-plant was under one of the 12 listed stresses. Studies measuring insect performance as changes in fecundity, abundance,

growth rate, or generation time in relation to host-plant stress were selected. Studies using only feeding rates or preferences were not included. In addition, only papers written in English that could be obtained in New Zealand were used in the review.

2.3.3 Definition of a 'stressed host-plant'

A stressed host-plant is defined as one with reduced growth relative to that experienced under optimal conditions (Price 1991). Stressed host-plants were therefore found in environments with reduced moisture, light, UV-B, soil-nitrogen, and CO₂; increased salinity, acidity, ozone, SO₂ and acid rain. Plants displayed vigorous growth after burning and when they were younger in age.

2.3.4 Analytical Approach

In other reviews attempting to depict generalised relationships between insect feeding-guilds and host-plant stress types have used meta-analysis (Koricheva *et al.* 1998), vote-counting (Waring & Cobb 1992) or both (Huberty & Denno 2004).

Meta-analysis is frequently advocated as the best approach for combining the results from several studies to provide an overall test of a hypothesis, because it assesses the magnitude of the effect across studies (Gurevitch & Hedges 1993). In this study, however, the primary interest was not in testing for an overall effect, but in examining variability in the outcome of studies assessing the relationship between insect performance and host-plant stress. A weak but significant overall relationship between insect performance and host-plant stress, such as that found by Koricheva *et al.* (1998), may be of little practical significance if the aim is to reliably predict the performance of insect biological control agents on host-plants in different parts of their range.

Vote-counting has been criticised because it relies on the statistical significance reported in individual studies, which varies as a function of the sample sizes employed in those studies (Gurevitch & Hedges 1993). Studies could show a non-significant result but nevertheless show a consistent tendency towards a particular outcome, which would not be detected using a vote-counting approach, but is more likely to be detected using meta-analysis, which considers the reported effect sizes (Gurevitch & Hedges 1993).

The use of vote-counting in this study is justified on three grounds. First, most of the studies examined did not report the outcome of experiments in sufficient detail to be included in a meta-analysis. A vote-counting approach allowed a greater number of studies to be included so that the variability of outcomes across different insect feeding-guilds and host-plant stress types could be better assessed. Second, over three quarters of the studies examined reported a statistically significant result one way or the other, allowing insect performance with regards to host-plant stress to be clearly categorised. Third, Huberty and Denno (2004) assessed their data by using both vote-counting and meta-analysis approaches, with results between the two approaches very similar.

The number of studies that showed a significant positive relationship, a significant negative relationship, or no significant relationship (including studies that showed a non-linear response: that is, insect performance initially increased as stress intensity increased, but subsequently decreased) between insect performance and host-plant stress were tallied. The following additional information from each study was also collated: stress type, insect feeding-guild, arthropod family and species, and plant species.

2.4 Results

Data were collated from 201 studies on insect performance in relation to host-plant stress, from 105 papers published between 1955 and 2000. These 201 studies investigated the performance of 132 arthropod species (from 47 families and 7 orders) on 86 plant species.

When all stress types were pooled, the variability in the response of insect herbivores to host-plant stress was highlighted (Figure 2.1). Of the 153 studies showing significant results, 77 showed that insect performance increased significantly on stressed host-plants, whereas 76 showed that insect performance decreased significantly on stressed host-plants, giving little support to the PSH. This data set supported the PVH ($\chi^2_1=6.8$, $P=0.009$), as it was found that miners and gall-formers

(this review considered those two guilds to be closely associated with the host-plant) were represented in a greater proportion of studies showing a negative relationship between insect performance and host-plant stress, compared with other guilds that tended to show the opposite. The data set did not support the IPH, with only 38% of wood-feeders, sap-feeders and miners performing better on stressed hosts ($\chi^2_1=0.001$, $P=0.971$).

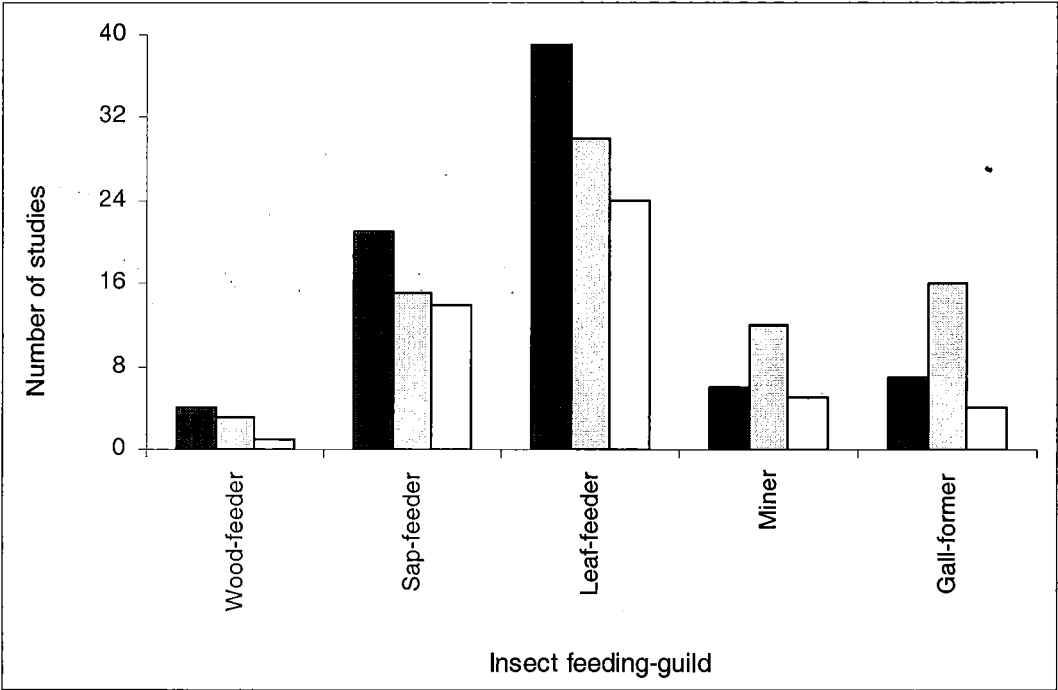


Figure 2.1: The number of studies where insect performance improved on stressed hosts (dark-shaded bars), improved on non-stressed hosts (light-shaded bars), or showed no relationship with host-plant stress (open bars), for five insect feeding-guilds.

When all feeding guilds were pooled, insect performance was higher on host-plants growing under reduced moisture, light or CO₂, increased soil nitrogen, or on younger plants (Figure 2.2).

The data set also suggested that insect performance differs between insect feeding-guilds, depending on the host-plant stress type encountered (Table 2.1). For example, the performance of leaf-feeders improved when host-plants were growing under

reduced moisture, light or CO₂, or increased soil nitrogen. The performance of miners improved when plants were growing under reduced CO₂, or increased moisture or soil nitrogen.

Several studies assessing the performance of leaf-feeders and miners also measured plant physiological responses to the stress being imposed. From this data, the performance of leaf-feeders tended to improve with increased plant nitrogen, while no clear and consistent physiological response was found for miners.

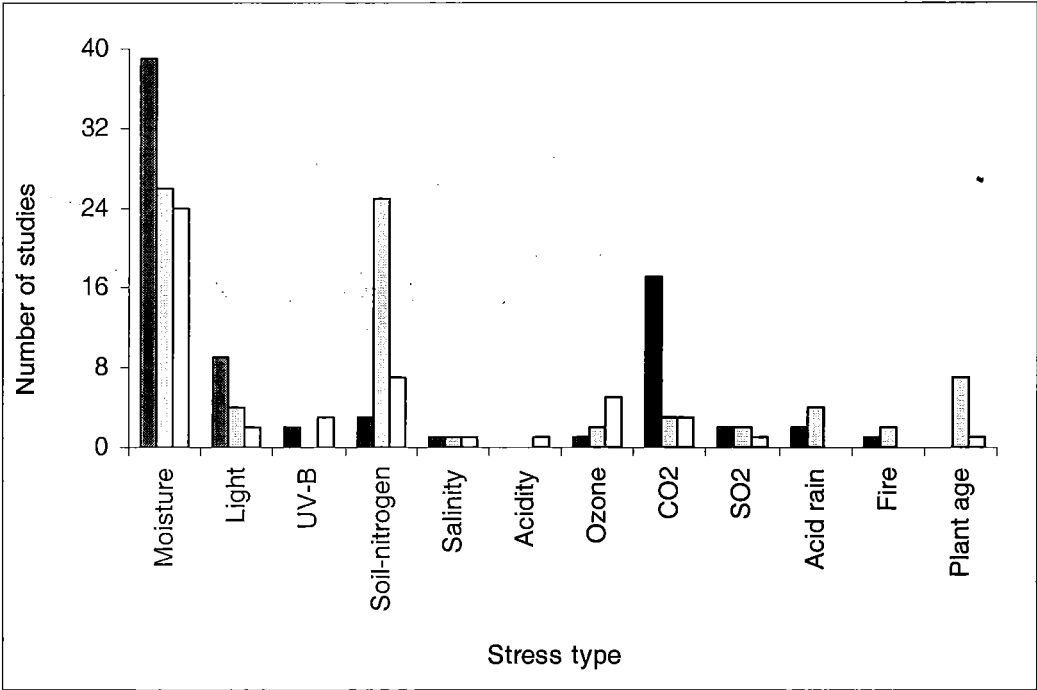


Figure 2.2: The number of studies where insect performance improved on stressed hosts (dark-shaded bars), improved on non-stressed hosts (light-shaded bars), or showed no relationship with host-plant stress (open bars), for twelve stress types.

2.5 Discussion

2.5.1 The effects of host-plant stress on insect herbivores

Hypotheses predicting relationships between insect performance and host-plant stress may allow the identification of parts of a host-plant’s range where insect herbivores released for biological control are likely to be effective and where other control strategies will be required. For this to be of practical use requires consistent

relationships between insect performance and host-plant stress. However, the results of this study suggest that insect response to host-plant stress can vary greatly according to the feeding-guild that the insect belongs to and on the form of stress encountered by the host-plant.

Figure 2.1 highlights the variability in the response of insect herbivores to host-plant stress, where insects in the same feeding-guilds showed both positive and negative performance responses to host-plant stress. Though this data set supported the PVH, it does not provide a reliable basis for predicting the performance of insect feeding-guilds under host-plant stress, as only 56% of mining and gall forming insects showed a positive response to non-stressed host-plants. However, the pooling of different host-plant stress types may have masked the possibility that feeding guilds may have been responding differently to the different stresses (Watt 1994, Koricheva *et al.* 1998).

When assessing insect performance in relation to types of host-plant stress, the data suggested that plant nitrogen levels might play a key role in insect performance. In this review improved insect performance occurred under reduced host-plant moisture, light and CO₂, increased host-plant soil nitrogen, and on younger host-plants (Figure 2.2); all of which have been generally associated with increased plant nitrogen.

The importance of assessing both insect feeding-guild and host-plant stress type when predicting insect performance was examined by Waring and Cobb (1992) and Koricheva *et al.* (1998). Waring and Cobb (1992) reported that stress type, not insect feeding-guild, was the more important determinant of insect performance on stressed and non-stressed hosts, while Koricheva *et al.* (1998) found the opposite. These contrasting results may have arisen because each study reviewed a different range of stress types. Waring and Cobb (1992) focussed on moisture and nutrient stress, while Koricheva *et al.* (1998) reviewed moisture, light and pollution stresses. Results presented here showed that insect performance generally improved when hosts were under moisture deficit, and decreased when experiencing nutrient deficit, as indicated by soil nitrogen and as predicted by Waring and Cobb (1992). Consistent with Koricheva *et al.* (1998) these results also showed improved insect performance when host-plants were under moisture, light and, pollution stresses. If Koricheva *et al.*

(1998) had considered a wider range of stress types, and included nitrogen stress under which insect performance appears to decline, their results may well have showed that stress type is an important determinant of insect performance.

This study indicates that to predict insect performance in relation to host-plant stress, both insect feeding-guild and stress type must be considered. Small sample sizes prevented detailed statistical analysis of insect performance by feeding-guilds with plant stress, though some patterns emerged (Table 2.1). For instance, under moisture stress leaf-feeders performed better on stressed hosts. Waring and Cobb (1992) found that “chewers” (wood-borers, stem-borers, root-feeders, leaf-miners and leaf-chewers) all responded positively to moisture stress. As several feeding-guilds were included in this grouping the results should be used cautiously, especially as our results indicated that miners performed better on non-stressed hosts. The results from this review support those of Waring and Cobb (1992) that leaf-feeders, miners and gall-formers performed better on fertilised (non-stressed) hosts and those of Koricheva *et al.* (1998) in that leaf-feeders performed better on light-stressed hosts. Bezemer and Jones (1998) found that as CO₂ increased, sap-feeders’ performance increased, and that the performance of leaf-feeders and miners decreased. This review supported those findings for leaf-feeders and miners, but insufficient data was collated on sap-feeders to compare findings.

Insects belonging to different insect feeding-guilds may be responding to different mechanisms. In this review leaf-feeders appeared to respond positively to stresses (reduced moisture, light and CO₂ and elevated soil-nitrogen) associated with increased nitrogen, as did gall-formers. However, no clear plant-physiological mechanism was evident for miners suggesting that they may be responding to another factor such as changes in leaf-morphology. Potter (1992) observed that changes in performance of a leaf-miner were related to changes in leaf structure rather than the nutritional quality of leaves. Details of plant physiological and morphological changes that occur under all the different stresses reviewed are beyond the scope of this study.

Table 2.1: Predictions for improved insect performance across five insect feeding-guilds on stressed host-plants or on non-stressed host-plants, and where no consistent relationship with improved insect performance and host-plant stress was found. The number of studies showing improved insect performance on stressed host-plants, on non-stressed host plants, and the number of studies where no differences between stress levels were found are presented in the brackets.

Insect feeding-guild	Improved insect performance on:		
	Stressed host-plants	Non-stressed host-plants	No consistent relationship with host-plant stress
Wood-feeder			Moisture (3:2:1)
Sap-feeder		CO ₂ (Bezemer & Jones)	Moisture (11:9:9)
Leaf-feeder	Moisture (19:5:9) Light (4:0:1) CO ₂ (13:3:2)	Soil nitrogen (0:16:4)	
Miner	CO ₂ (3:0:0)	Moisture (0:4:2) Soil nitrogen (0:4:2)	Light (3:3:1)
Gall-former		Soil nitrogen (0:3:1) Plant age (0:6:0)	Moisture (6:6:3)

The mixed response of sap-feeders to host-plant moisture stress in this study may be related to the application of the moisture stress applied to host-plants. The studies assessed in this review covered both laboratory and field experiments, in which moisture application methods varied. Huberty and Denno (2004) assessed the performance of sap-feeders and chewers in relation to host-plant moisture deficit. They found significant differences in sap-feeder response when host-plants were grown under continuous or intermittent moisture deficit. Continuous moisture deficit is often used in laboratory studies, while intermittent stress is regularly seen in field studies, especially studies focussing on insect outbreaks following periods of droughts (Huberty & Denno 2004). Under continuous deficit plant cells lose turgor, reducing the ability of the insect to tap into the increased plant nitrogen, leading to a decline in herbivore performance. Under intermittent moisture deficit periodic watering of the plants allows cells to regain turgidity, and in turn insects are able to utilise the higher plant nitrogen, leading to an increase in herbivore performance. The importance of plant turgor has been suggested by several authors (Kennedy & Booth 1959, Wearing and vanEmden 1967), however the constant references to links between increased

insect performance and increased plant nitrogen has often overshadowed this important fact.

Huberty and Denno (2004) reported that host-plant moisture deficit positively affected wood-feeders (6 studies with improved insect performance on stressed hosts, 4 on vigorous, and 2 with no difference in performance). No consistent relationship was found in this study between wood-feeders and moisture due to the small sample size, however, more of the studies showed greater performance on stressed hosts (3 studies with improved insect performance on stressed hosts, 2 on vigorous, and 1 with no difference in performance). Changes in oleoresin flow is one factor that has commonly been linked to increased performance on stressed host-plants (Lorio & Hodges 1968, Ferrell 1978, Dunn & Lorio 1993).

Huberty and Denno (2004) reported that host-plant soil moisture deficit negatively affected gallers, however, the response of gallers to host-plant moisture stress in this study was mixed. All of the studies that showed positive responses to moisture deficit stress were based on field surveys, and of these, five of the six insect species were reportedly responding to morphological changes not physiological changes in the plant, even though there were significant differences between stressed and non-stressed plants in tissue water potential, percentage phenolic leaf resin, leaf phenols and plant nitrogen. Therefore, the gallers may have been able to take advantage of the higher plant nitrogen rates but other plant factors (e.g. loss of cell turgidity) may not have been high enough to negatively affect them, leading to higher performance. Studies where galler response was negatively affected by moisture deficit were based on both surveys and experimental approaches.

2.5.2 Flaws in the plant stress –insect herbivory hypotheses

White (1969) initiated the debate on host-plant stress and the effects it has on insect populations by formulating the PSH. Data presented in here did not support the PSH. This is due largely to (a) the PSH being based on only a few studies, and (b) making large assumptions with little supporting evidence. One assumption was that the performance of all insect herbivores would increase when encountering host-plant stress, due to increases in plant soluble nitrogen. However, it was clearly shown that the performance of insects might increase, decrease or remain constant as plant stress

increases (see Appendix). Secondly, when plants are stressed not always is there an increase in plant nitrogen (Specht 1965, Floater 1997) and not always is the increase in plant nitrogen related to improved insect performance (Waring & Price 1990, Estiarte *et al.* 1994). Some studies showed that insect performance could increase even though no increase in nitrogen occurred (Specht 1965, De Bruyn *et al.* 2002). To complicate the nitrogen link further, Huberty and Denno (2004) reported that even though plants under stress may have higher plant nitrogen, insects might not be able to access it if plant turgor is too low. It is recommended that the PSH be re-defined, for example, the performance of insect herbivores should increase when (a) host-plant stress leads to increases in plant soluble nitrogen, (b) that the insect responds to changes in plant nitrogen, and (c) the insect is able to assess the higher nitrogen.

Price (1991) took the next step in the debate, noting that not all insects respond positively to host-plant stress and formulated the PVH. This hypothesis states that some insects, ‘insects closely associated with the host-plant’, will perform better on vigorously growing, or non-stressed, host-plants. The results from this thesis gave most support to the PVH of the three hypotheses assessed. However, the term ‘insects closely associated with the host-plant’ is not well defined, leaving the term open to interpretation by the investigator. Like Price, many authors have used broad feeding guilds to define what insects are ‘closely associated with the host-plant’. For example, galling and mining insects were considered to be ‘closely associated with the host-plant’ (Price 1991, Inbar *et al.* 2001). This brings forward two questions, (a) are all galling and mining insects closely associated with the host plant, and (b) does that mean all insects belonging to the sap-feeder guild for example are not closely associated with the host plant? I question this, as in latter chapters the performance of two biological control agents, *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) and *Arytainilla spartiophila* Förster (Hem: Psyllidae) will be assessed, in relation to host-plant soil moisture and shade stress. *Leucoptera spartifoliella* is a miner (feeds by mining in stem-twigs) and will therefore be placed in this ‘closely associated with the host plant’ grouping, unlike *A. spartiophila* a sap-feeder (feeds on leaf and flower buds). However, under host-plant soil moisture stress for example, *L. spartifoliella* will not lose its feeding resource, but *A. spartiophila* will. So which insect is more closely involved with the host?

Larsson (1989) linked the PSH and PVH, forming another modification to the original idea with the IPH. Larsson predicted which insect groups (defined by feeding-guilds) would do better on stressed and non-stressed host-plants. However, these predictions were made from few studies. In a later study by Koricheva *et al.* (1998), which involved Larsson, the hypothesis was further refined to include stress types. They showed that feeding-guilds were important, not stress type. However, they chose to investigate only three stress types, which gave similar results. If they had increased the number of stress types, or even chosen other types, then I believe their results may have matched those found in chapter 2. The use of these particular guilds may have confounded results, as the guilds were defined in relation to feeding style and did not consider the feeding resource.

2.5.3 Defining of insect feeding-guilds

Many authors do not define guilds adequately (Hawkins & MacMahon 1989, Simberloff & Dayan 1991). This problem is largely due to no explicit criteria being set for defining of guilds. The defining is up to the investigator to decide which species belong in which groups (Hawkins & MacMahon 1989).

Larsson (1989) used guilds that separated feeding styles between the insects, however the feeding resource was not distinguished. This may have masked some trends between the plant stress-insect herbivory relationships. For instance ‘miners’ include insects that mine leaves and insects that mine stems. However, insects that mine leaves may respond to different host-plant changes than insects that mine stems. Especially when leaves are often shed under soil moisture deficit (Bradford & Hsiao 1982, Heinrichs 1988). Therefore, should these feeding-guilds be further broken down, into smaller sub-guilds? When members of a guild are feeding on different resources, then smaller sub-guilds should be constructed, for example miners being divided into insects that mine leaves (leaf-miners) and insects that mine stems-twigs (stem-miners). Huberty and Denno (2004) broke sap-feeders into small several smaller sub-guilds (phloem-feeders, mesophyll-feeders, xylem-feeders).

2.5.4 Why results vary between studies

Not all of the reviews showed similar results, for example, inconsistent responses for leaf-chewers and miners were found by Huberty and Denno (2004), while this study

found leaf-feeders performed better on moisture deficit hosts and leaf-miners performed better on vigorous hosts. These differences are likely related to the variation between the studies assessed (Larsson 1989, Hurberty & Denno 2004).

Larsson and Björkman (1993) reported that the plant stress–insect herbivory hypotheses were based on a limited number of experiments that used different experimental approaches. These experimental approaches varied in stress intensity, type of stress, duration of stress, whether experiments were conducted in the laboratory or in the field, and the age of the host. Huberty and Denno (2004) also discussed the inconsistencies between studies, which confounded insect performance predictions. These included many of the factors mentioned by Larsson and Björkman (1993) and also: lack of evidence showing plants were stressed, pooling of different stress types, different analytical approaches and different stress applications.

2.5.5 Resource Regulation Hypothesis

The three hypotheses that have been investigated have looked at plant stress – insect herbivory interactions. In the larger ecological picture there are two schools of thoughts when it comes to plant-insect interactions. The symmetrical view, believes insects and plants both exert major effects on each other, while the asymmetrical view believes plants have a major effect on insect herbivores, but insect herbivores have little or no effect on plants (McEvoy 2002). The Resource Regulation Hypothesis (RRH) has been used to represent the symmetrical view, while the PVH has been used to represent the asymmetrical view (Dhileepan 2004). The RRH proposes that an herbivore might maintain high quality resources on a plant for the subsequent generations of that herbivore species on that individual plant (Craig *et al.* 1989). Craig *et al.* (1989) found that galling can stimulate plant vigour thereby increasing resources for subsequent generations. Just as there has been mixed support for the PSH, PVH and IPH, there have also been mixed opinions for the RRH (Dhileepan 2004).

2.5.6 Direct effects of environmental stress on insect herbivores

This review focussed on how host-plant stress affects insects, but stress also directly affects insect herbivores. Stress types will affect insects in different ways. For example, in very dry environments (where host-plants are experiencing soil moisture deficit) the water content of the insect herbivores can be reduced, leading to depressed

metabolism and retardation of development (Wigglesworth 1972). Another example is with shading. Before activities like flight can be achieved insects require body temperatures to reach a set level. However, in shaded environments it can take longer for insects to reach the required body temperatures (Wigglesworth 1972).

2.5.7 Effects of host-plant stress on the insect herbivores predators and parasites

According to Teder and Tammaru (2002) variation in plant quality can also affect the predators/parasites of insect herbivores. Teder & Tammaru (2002) showed that the performance (measured as size) of two insect herbivores, *Nonagria typhae* Thunb. (Lep: Noctuidae) and *Archanara sparganii* Esp. (Lep: Noctuidae), was greatest when their host, *Typha latifolia* L. (Typhaceae) was growing vigorously, across varying moisture and shade levels. In addition, positive responses were also found for the parasitoids of these two herbivores. However, it was concluded that the favorable change in the quality of the host would be more positive for insect herbivores than for the predators/parasitoids. This may explain why outbreaks of insect populations occur; when host-plant stress is favorable to insect herbivores, increases in these populations are seen, however, these insect herbivores are not restrained by their predators/parasites as the increases are not proportional.

2.5.8 Conclusion

The results from this review indicate that the current plant stress - insect herbivory hypotheses do not adequately predict insect performance on stressed and non-stressed plants. This is largely due to all three hypotheses being inadequately defined and ignoring stress type. It is clear that the type of stress imposed on the plant, as well as the insect feeding-guild, is important in determining insect performance. There may be underlying associations between plant morphology and physiology and insect performance (e.g. plant cell turgidity, available plant nitrogen, changes in oleoresin concentration) that are affected by the nature of the stress.

The application of these results could provide benefits for weed biological control, particularly when agents belong in the leaf-feeding and mining guilds. For example, it is predicted that a leaf-feeding insect might perform better on host-plants growing under drought-stressed or shaded conditions, or in environments with high soil nitrogen levels, while a miner might perform better in environments where host-plants

are receiving optimal soil moisture or soil nitrogen.

2.6 References

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2.7 Appendix: Studies assessing insect performance over various stress types and insect feeding guilds. Part a: Improved insect performance on stressed host-plants. Part b: Improved insect performance on non-stressed host-plants. Part c: No relationship between insect performance and stressed host-plants.

Stress type	Feeding guild	Insect Order: family	Insect species	Plant type	Plant species	Author
Part a						
Moisture	Wood-feeder	Col: Scolytidae	<i>Dendroctonus ponderosae</i>	woody	<i>Pinus contorta</i>	Thomson & Shrimpton 1984
Moisture	Wood-feeder	Col: Scolytidae	<i>Dendroctonus frontalis</i>	woody	<i>Pinus taeda</i>	Dunn & Lorio 1993
Moisture	Wood-feeder	Col: Scolytidae	<i>Scolytus ventralis</i>	woody	<i>Abies concolor</i>	Ferrell 1978
N-Fertilizer	Wood-feeder	Col: Scolytidae	<i>Poecilips rhizophorae</i>	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
Moisture	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus cinnabarinus</i>	non-woody	<i>Zea mays</i>	Chandler et al. 1979
Moisture	Sap-feeder	Acari: Tetranychidae	<i>Oligonychus pratensis</i>	non-woody	<i>Zea mays</i>	Chandler et al. 1979
Moisture	Sap-feeder	Araci: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Mentha piperita</i>	Hollingsworth & Berry 1982
Moisture	Sap-feeder	Araci: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Chrysanthemum morifolium</i>	Price et al. 1982
Moisture	Sap-feeder	Hem: Aphididae	<i>Schizaphis graminum</i>	non-woody	<i>Triticum aestivum</i>	Dorschner et al. 1986
Moisture	Sap-feeder	Hem: Aphididae	<i>Myzus persicae</i>	non-woody	<i>Brassica oleracea</i>	Wearing 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Brevicoryne brassicae</i>	non-woody	<i>Brassica oleracea</i>	Wearing 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Schizaphis graminum</i>	non-woody	<i>Sorghum</i> sp.	Kindler & Staples 1981
Moisture	Sap-feeder	Hem: Aphididae	<i>Cinara costata</i>	woody	<i>Picea abies</i>	Bjorkman & Larsson 1999
Moisture	Sap-feeder	Hem: Aphididae	<i>Aphis pomi</i>	woody	<i>Crataegus</i> spp.	Braun & Fluckiger 1984
Moisture	Sap-feeder	Hem: Psyllidae	<i>Cardiaspina</i> spp.	woody	<i>Eucalyptus</i> spp.	White 1969
Light	Sap-feeder	Hem: Psyllidae	<i>Cardiaspina densitexta</i>	woody	<i>Eucalyptus fasciculosa</i>	White 1970
Light	Sap-feeder	Hem: Tingidae	<i>Stephanitis pyrioides</i>	non-woody	<i>Rhododendron</i> sp.	Trumble & Denno 1995
UV-B	Sap-feeder	Hem: Psyllidae	<i>Strophingia ericae</i>	woody	<i>Calluna vulgaris</i>	Salt et al. 1998
N-Fertilizer	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus</i> sp.	woody	<i>Salix sericea</i>	Orians & Fritz 1996
N-Fertilizer	Sap-feeder	Hem: Aphididae	<i>Adelges picae</i>	woody	<i>Abies amabilis</i>	Carrow & Graham 1968
Salinity	Sap-feeder	Hem: Aphididae	<i>Aphis pomi</i>	woody	<i>Crataegus</i> spp.	Braun & Fluckiger 1984
Ozone	Sap-feeder	Hem: Miridae	<i>Lygus rugulipennis</i>	woody	<i>Pinus sylvestris</i>	Manninen et al. 2000
CO ₂	Sap-feeder	Thy: Thripidae	<i>Frankliniella occidentalis</i>	non-woody	<i>Asclepis syriaca</i>	Huges & Bazzaz 1997
SO ₂	Sap-feeder	Hem: Aphididae	<i>Cinara pilicornis</i>	woody	<i>Picea abies</i>	Holopainen et al. 1991
Acid rain	Sap-feeder	Hem: Aphididae	<i>Euceraphis betulae</i>	woody	<i>Betula pendula</i>	Neuvonen & Lindgren 1987
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Nomadacris septemfasciata</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Locustana pardalina</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Locusta migratoria</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Melanoplus sanguinipes</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Schistocera gregaria</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Hem: Acaridadae	<i>Chortoicetes terminifera</i>	non-woody	---	White 1976
Moisture	Leaf-feeder	Lep: Geometridae	<i>Selidosema suavis</i>	woody	<i>Pinus radiata</i>	White 1974
Moisture	Leaf-feeder	Lep: Geometridae	<i>Neocleora herbuloti</i>	woody	---	White 1974
Moisture	Leaf-feeder	Lep: Geometridae	<i>Bupalus piniarius</i>	woody	---	White 1974
Moisture	Leaf-feeder	Lep: Geometridae	<i>Nepytia phantasmaria</i>	woody	---	White 1974

Moisture	Leaf-feeder	Lep: Geometridae	<i>Anacamptodes clivinaria</i>	woody	<i>Cerocarpus ledifolius</i>	White 1974
Moisture	Leaf-feeder	Lep: Geometridae	<i>Choristoneura fumiferana</i>	woody	---	White 1974
Moisture	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	Birch forest	Koltunov & Andreeva 1999
Moisture	Leaf-feeder	Lep: Notodontidae	<i>Phalera bucephala</i>	woody	<i>Betula pendula</i>	Thomas & Hodgkinson 1991
Moisture	Leaf-feeder	Lep: Tortricidae	<i>Choristoneura fumiferana</i>	woody	<i>Picea</i> sp. / <i>Abies</i> sp. forest	Greenbank 1956
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion gillettei</i>	woody	<i>Pinus ponderosa</i>	McMillin & Wagner 1995
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion edulicolis</i>	woody	<i>Pinus edulis</i>	Mopper & Whitham 1992
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion sertifer</i>	woody	<i>Pinus sylvestris</i>	Saikkonen et al. 1995
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion sertifer</i>	woody	<i>Pinus sylvestris</i>	Christiansen & Austara 1996
Light	Leaf-feeder	Lep: Noctuidae	<i>Spodoptera exigua</i>	woody	<i>Copaifera langsdorfii</i>	Feibert & Langenheim 1988
Light	Leaf-feeder	Lep: Papilionidae	<i>Parides montezuma</i>	non-woody	<i>Aristolochia orbicularis</i> , <i>A. micrantha</i>	Rausher 1979
Light	Leaf-feeder	Lep: Papilionidae	<i>Battus philenor</i>	non-woody	<i>Aristolochia orbicularis</i> , <i>A. micrantha</i>	Rausher 1979
Light	Leaf-feeder	Lep: Papilionidae	<i>Battus polydamus</i>	non-woody	<i>Aristolochia orbicularis</i> , <i>A. micrantha</i>	Rausher 1979
UV-B	Leaf-feeder	Lep: Pieridae	<i>Pieria rapae</i>	non-woody	<i>Arabidopsis thaliana</i>	Grant-Petersson & Renwick 1996
CO ₂	Leaf-feeder	Lep: Lasiocampidae	<i>Malacosoma disstria</i>	woody	<i>Populus tremuloides</i>	Lindroth et al. 1993
CO ₂	Leaf-feeder	Lep: Lasiocampidae	<i>Malacosoma disstria</i>	woody	<i>Acer saccharum</i>	Lindroth et al. 1993
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Populus tremuloides</i>	Lindroth et al. 1993
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Quercus alba</i>	Cannon 1993
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Betula allegheniensis</i>	Traw et al. 1996
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Betula populifolia</i>	Traw et al. 1996
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Betula papyrifera</i>	Roth & Lindroth 1994
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Pinus strobus</i>	Roth & Lindroth 1994
CO ₂	Leaf-feeder	Lep: Nymphalidae	<i>Junonia coenia</i>	non-woody	<i>Plantago lanceolata</i>	Fajer 1989, Fajer et al. 1989, Fajer et al. 1991
CO ₂	Leaf-feeder	Lep: Saturniidae	<i>Hyalophora cecropia</i>	woody	<i>Betula papyrifera</i>	Lindroth et al. 1995
CO ₂	Leaf-feeder	Lep: Saturniidae	<i>Actias luna</i>	woody	<i>Betula papyrifera</i>	Lindroth et al. 1995
CO ₂	Leaf-feeder	Lep: Saturniidae	<i>Antheraea polyphemus</i>	woody	<i>Betula papyrifera</i>	Lindroth et al. 1995
CO ₂	Leaf-feeder	Col: Chrysomelidae	<i>Gastrophysa viridula</i>	non-woody	<i>Rumex obtusifolius</i>	Brooks & Whittaker 1998
SO ₂	Leaf-feeder	Col: Coccinellidae	<i>Epilachna varivestis</i>	non-woody	<i>Glycine max</i>	Hughes et al. 1982, Hughes et al. 1983
Acid rain	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Quercus rubra</i>	Cannon 1993
Light	Miner	Lep: Nepticulidae	<i>Stigmella</i> sp.	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1988
Light	Miner	Lep: Cosmopterigidae	<i>Stilbosis juvantis</i>	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1988
Light	Miner	Dip: Drosophilidae	<i>Scaptomyza nigrita</i>	non-woody	<i>Cardamine cordifolia</i>	Collinge & Louda 1988, Collinge & Louda 1989
CO ₂	Miner	Lep: Nepticulidae	<i>Stigmella</i> sp.	woody	<i>Quercus</i> sp.	Stiling et al. 1999
CO ₂	Miner	Lep: Gracillariidae	<i>Cameraria</i> sp.	woody	<i>Quercus</i> sp.	Stiling et al. 1999
CO ₂	Miner	Lep: Cosmopterigidae	<i>Stilbosis</i> sp.	woody	<i>Quercus</i> sp.	Stiling et al. 1999
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. a	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. b	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. c	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. d	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. e	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Chloropidae	<i>Lipara lucens</i>	non-woody	<i>Phragmites australis</i>	DeBruyn 1995
Fire	Gall-former	Dip: Cecidomyiidae	<i>Contarinia</i> sp.	woody	<i>Palicourea rigida</i>	Vieira et al. 1996

Part b

Moisture	Wood-feeder	Lep: Sesiidae	<i>Carmenta mimosa</i>	woody	<i>Mimosa pigra</i>	Steinbauer 1998
Moisture	Wood-feeder	Col: Scolytidae	<i>Dendroctonus frontalis</i>	woody	<i>Pinus taeda</i>	Lorio & Hodges 1968
Light	Wood-feeder	Lep: Sesiidae	<i>Carmenta mimosa</i>	woody	<i>Mimosa pigra</i>	Steinbauer 1998
Moisture	Sap-feeder	Araci: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Glycine max</i>	Mellors et al. 1984
Moisture	Sap-feeder	Acari: Tetranychidae	<i>Panonychus ulmi</i>	woody	<i>Malus</i> sp.	Specht 1965
Moisture	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus bimaculatus</i>	non-woody	<i>Medicago sativa</i>	Butler 1955
Moisture	Sap-feeder	Hem: Aphididae	<i>Brevicoryne brassicae</i>	non-woody	<i>Brassica oleracea</i>	Wearing & van Enden 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Brevicoryne brassicae</i>	non-woody	<i>Calendula officinalis</i>	Wearing & van Enden 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Schizaphis graminum</i>	non-woody	<i>Triticum aestivum</i>	Sumner et al. 1983
Moisture	Sap-feeder	Hem: Aphididae	<i>Aphis fabae</i>	woody	<i>Euonymus europaeus</i>	Kennedy & Booth 1959
Moisture	Sap-feeder	Hem: Aphididae	<i>Aphis fabae</i>	non-woody	<i>Beta vulgaris</i>	Kennedy & Booth 1959
Moisture	Sap-feeder	Hem: Diaspididae	<i>Unaspis euonymi</i>	non-woody	<i>Euonymus fortunei</i>	Cockfield & Potter 1986
N-Fertilizer	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Rhaphanus sativus</i>	Mellors & Propts 1983
N-Fertilizer	Sap-feeder	Hem: Aphididae	<i>Adelges cooleyi</i>	woody	<i>Pseudotsuga menziesii</i>	Mitchell & Paul 1974
SO ₂	Sap-feeder	Hem: Aphididae	<i>Cinara pilicornis</i>	woody	<i>Picea abies</i>	Holopainen et al. 1991
Acid rain	Sap-feeder	Hem: Aphididae	<i>Schizolachnus pineti</i>	woody	<i>Pinus sylvestris</i>	Kidd 1990
Acid rain	Sap-feeder	Hem: Aphididae	<i>Schizolachnus agilis</i>	woody	<i>Pinus sylvestris</i>	Kidd 1990
Acid rain	Sap-feeder	Hem: Aphididae	<i>Cinara pini</i>	woody	<i>Pinus sylvestris</i>	Kidd 1990
Moisture	Leaf-feeder	Lep: Noctuidae	<i>Heliothis zea</i>	non-woody	<i>Gossypium hirsutum</i>	Slosser 1980
Moisture	Leaf-feeder	Lep: Noctuidae	<i>Spodoptera exigua</i>	non-woody	<i>Lycopersicon esculentum</i>	English-Loeb et al. 1997
Moisture	Leaf-feeder	Lep: Thaumetopoeidae	<i>Ochrogaster lunifer</i>	woody	<i>Acacia concurrens</i>	Floater 1997
Moisture	Leaf-feeder	Hym: Symphyta	<i>Gilpinia hercyniae</i>	woody	<i>Picea abies</i>	Bjorkman & Larsson 1999
Moisture	Leaf-feeder	Col: Coccinellidae	<i>Epilachna varivestis</i>	non-woody	<i>Glycine max</i>	McQuate & Connor 1990
N-Fertilizer	Leaf-feeder	Hem: Delphacidae	<i>Prokelisia marginata</i>	non-woody	<i>Spartina alterniflora</i>	Bowdish & Stiling 1998
N-Fertilizer	Leaf-feeder	Lep: Geometridae	<i>Operophtera brumata</i>	woody	<i>Calluna vulgaris</i>	Kerslake et al. 1998
N-Fertilizer	Leaf-feeder	Lep: Pieridae	<i>Pieris rapae</i>	non-woody	<i>Brassica oleracea</i>	Loader & Damman 1991
N-Fertilizer	Leaf-feeder	Lep: Hesperidae	<i>Phocides pigmalion</i>	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
N-Fertilizer	Leaf-feeder	Lep: ?	<i>Alarodia slossoniae</i>	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
N-Fertilizer	Leaf-feeder	Lep: Saturniidae	<i>Automeris</i> sp.	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
N-Fertilizer	Leaf-feeder	Lep: Megalopygidae	<i>Megalopyge opercularis</i>	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
N-Fertilizer	Leaf-feeder	Lep: Pyralidae	<i>Samea multiplicalis</i>	non-woody	<i>Salvinia molesta</i>	Room et al. 1989
N-Fertilizer	Leaf-feeder	Lep: Noctuidae	<i>Helicoverpa armigera</i>	non-woody	<i>Capsicum annuum</i>	Estiarte et al. 1994
N-Fertilizer	Leaf-feeder	Lep: Noctuidae	<i>Heliothis zea</i>	non-woody	<i>Gossypium hirsutum</i>	Slosser 1980
N-Fertilizer	Leaf-feeder	Lep: Notodontidae	<i>Phalera bucephala</i>	woody	<i>Betula pendula</i>	Thomas & Hodkinson 1991
N-Fertilizer	Leaf-feeder	Lep: Sphingidae	<i>Mimas tiliae</i>	woody	<i>Salix cinerea</i>	Thomas & Hodkinson 1991
N-Fertilizer	Leaf-feeder	Lep: Thaumetopoeidae	<i>Ochrogaster lunifer</i>	woody	<i>Acacia concurrens</i>	Floater 1997
N-Fertilizer	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion edulicolis</i>	woody	<i>Pinus edulis</i>	Mopper & Whitham 1992
N-Fertilizer	Leaf-feeder	Col: Curculionidae	<i>Neochetina bruchi</i>	non-woody	<i>Eichhornia crassipes</i>	Heard & Winterton 2000

N-Fertilizer	Leaf-feeder	Col: Curculionidae	<i>Cyrtobagous salviniae</i>	non-woody	<i>Salvinia molesta</i>	Room et al. 1989, Room & Thomas 1985
Salinity	Leaf-feeder	Hem: Delphacidae	<i>Prokelisia marginata</i>	non-woody	<i>Spartina alterniflora</i>	Bowdish & Stiling 1998
Ozone	Leaf-feeder	Hym: Diprionidae	<i>Gilpinia pallida</i>	woody	<i>Pinus sylvestris</i>	Manninen et al. 2000
Ozone	Leaf-feeder	Col: Chrysomelidae	<i>Plagioderma versicolora</i>	woody	<i>Populus deltoides</i>	Coleman & Jones 1988a
CO ₂	Leaf-feeder	Lep: Geometridae	<i>Operophtera brumata</i>	woody	<i>Calluna vulgaris</i>	Kerslake et al. 1998
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Quercus rubra</i>	Cannon 1993
CO ₂	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Quercus rubra</i>	Lindroth et al. 1993
SO ₂	Leaf-feeder	Col: Coccinellidae	<i>Epilachna varivestis</i>	non-woody	<i>Glycine max</i>	Hughes et al. 1982
Acid rain	Leaf-feeder	Lep: Lymantriidae	<i>Lymantria dispar</i>	woody	<i>Quercus alba</i>	Cannon 1993
Fire	Leaf-feeder	Lep: Tortricidae	<i>Choristoneura pinus</i>	woody	<i>Pinus banksiana</i>	McCullough & Kulman 1991
Moisture	Miner	Lep: Gracillariidae	<i>Cameraria</i> sp. a	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1987
Moisture	Miner	Lep: Gracillariidae	<i>Cameraria</i> sp. b	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1987
Moisture	Miner	Lep: Tortricidae	<i>Epinotia tedella</i>	woody	<i>Picea abies</i>	Bjorkman & Larsson 1999
Moisture	Miner	Lep: Tortricidae	<i>Rhyacionia frustrana</i>	woody	<i>Pinus taeda</i>	Ross & Berisford 1990
Light	Miner	Lep: Tischeriidae	<i>Trischeria</i> sp.	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1988
Light	Miner	Lep: Gracillariidae	<i>Cameraria</i> sp.	woody	<i>Quercus emoryi</i>	Bultman & Faeth 1988
Light	Miner	Dip: Agromyzidae	<i>Phytomyza ilicicola</i>	woody	<i>Ilex opaca</i>	Marino et al. 1993
N-Fertilizer	Miner	Lep: Gracillariidae	<i>Phyllocnistis</i> sp.	woody	<i>Salix sericea</i>	Orians & Fritz 1996
N-Fertilizer	Miner	Lep: Tortricidae	<i>Ecdytolopha</i> sp.	woody	<i>Rhizophora mangle</i>	Onuf et al. 1977
N-Fertilizer	Miner	Lep: Tortricidae	<i>Rhyacionia frustrana</i>	woody	<i>Pinus taeda</i>	Ross & Berisford 1990
N-Fertilizer	Miner	Dip: Agromyzidae	<i>Phytomyza ilicicola</i>	woody	<i>Ilex opaca</i>	Marino et al. 1993
Age	Miner	Lep: Tortricidae	<i>Rhyacionia neomexicana</i>	woody	<i>Pinus ponderosa</i>	Spiegel & Price 1996
Moisture	Gall-former	Hym: Tenthredinidae	<i>Euura lasiolepis</i>	woody	<i>Salix lasiolepis</i>	Price & Clancy 1986, Preszler & Price 1988
Moisture	Gall-former	Hym: Tenthredinidae	<i>Euura lasiolepis</i>	woody	<i>Salix lasiolepis</i>	Waring & Price 1988
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. f	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. g	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Rhopalomyia</i> sp.	woody	<i>Tripleurospermum perforatum</i>	Hinz & Muller-Scharer 2000
Moisture	Gall-former	Dip: Tephritidae	<i>Eurosta solidaginis</i>	non-woody	<i>Solidago altissima</i>	Sumerford et al. 2000
N-Fertilizer	Gall-former	Hym: Tenthredinidae	<i>Phyllocolpa nigrita</i>	woody	<i>Salix sericea</i>	Orians & Fritz 1996
N-Fertilizer	Gall-former	Hym: Tenthredinidae	<i>Phyllocolpa terminalis</i>	woody	<i>Salix sericea</i>	Orians & Fritz 1996
N-Fertilizer	Gall-former	Dip: Cecidomyiidae	<i>Rhopalomyia</i> sp.	woody	<i>Tripleurospermum perforatum</i>	Hinz & Muller-Scharer 2000
Fire	Gall-former	Lep: Cosmopterigidae	<i>Periploca ceanothiella</i>	woody	<i>Ceanothus herbaceous</i>	Throop & Fay 1999
Age	Gall-former	Hym: Cynipidae	<i>Diplolepis fusiformans</i>	woody	<i>Rosa arizonica</i>	Caouette & Price 1989
Age	Gall-former	Hym: Cynipidae	<i>Diplolepis spinosa</i>	woody	<i>Rosa arizonica</i>	Caouette & Price 1989
Age	Gall-former	Hym: Tenthredinidae	<i>Euura exiguae</i>	woody	<i>Salix exigua</i>	Price 1989
Age	Gall-former	Hym: Tenthredinidae	<i>Euura mucronata</i>	woody	<i>Salix cinerea</i>	Price et al. 1987
Age	Gall-former	Hym: Tenthredinidae	<i>Euura lasiolepis</i>	woody	<i>Salix lasiolepis</i>	Craig et al. 1989
Age	Gall-former	Hym: Tenthredinidae	<i>Euura amerinae</i>	woody	<i>Salix pentandra</i>	Roininen et al. 1993

Part c

Moisture	Wood-feeder	Col: Scolytidae	<i>Dendroctonus frontalis</i>	woody	---	King 1972
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Moisture	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Phaseolus vulgaris</i>	English-Loeb 1989
Moisture	Sap-feeder	Acari: Tetranychidae	<i>Tetranychus urticae</i>	non-woody	<i>Rhaphanus sativus</i>	Mellors & Propts 1983
Moisture	Sap-feeder	Hem: Aphididae	<i>Myzus persicae</i>	non-woody	<i>Brassica oleracea</i>	Wearing 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Myzus persicae</i>	non-woody	<i>Calendula officinalis</i>	Wearing & van Enden 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Aphis fabae</i>	non-woody	<i>Vicia faba</i>	Wearing & van Enden 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Aphis fabae</i>	non-woody	<i>Calendula officinalis</i>	Wearing & van Enden 1967
Moisture	Sap-feeder	Hem: Aphididae	<i>Cinara costa</i>	woody	<i>Picea abies</i>	Larsson & Bjorkman 1993
Moisture	Sap-feeder	Hem: Psyllidae	<i>Elatobium abietinum</i>	woody	<i>Picea sitchensis</i>	Major 1990
Moisture	Sap-feeder	Hem: Tingidae	<i>Stephanitis pyrioides</i>	non-woody	<i>Rhododendron</i> sp.	Trumble & Denno 1995
Ozone	Sap-feeder	Hem: Aphididae	<i>Schizolachnus pineti</i>	woody	<i>Pinus sylvestris</i>	Manninen et al. 2000
Ozone	Sap-feeder	Hem: Aphididae	<i>Cinara pinea</i>	woody	<i>Pinus sylvestris</i>	Manninen et al. 2000
Ozone	Sap-feeder	Hem: Aphididae	<i>Chaitophorus populicola</i>	woody	<i>Populus deltoides</i>	Coleman & Jones 1988b
Ozone	Sap-feeder	Hem: Aphididae	<i>Schizolachnus pineti</i>	woody	<i>Pinus sylvestris</i>	Kainulainen et al. 1994
CO ₂	Sap-feeder	Hem: Aphididae	<i>Aphis fabae</i>	non-woody	<i>Cardamine pratensis</i>	Salt et al. 1996
Moisture	Leaf-feeder	Lep: Lasiocampidae	<i>Malacosoma californicum</i>	woody	<i>Rosa</i> sp.	Myers 1981
Moisture	Leaf-feeder	Lep: Noctuidae	<i>Panolis flammea</i>	woody	<i>Pinus contorta</i>	Watt 1986
Moisture	Leaf-feeder	Lep: Noctuidae	<i>Heliothis virescens</i>	non-woody	<i>Gossypium hirsutum</i>	Navasero & Ramaswamy 1993
Moisture	Leaf-feeder	Lep: Pieridae	<i>Pieris rapae</i>	non-woody	<i>Brassica napus</i>	Miles et al. 1982
Moisture	Leaf-feeder	Lep: Sphingidae	<i>Smerinthus ocellatus</i>	woody	<i>Salix cinera</i>	Thomas & Hodkinson 1991
Moisture	Leaf-feeder	Col: Chrysomelidae	<i>Paropsis atomaria</i>	woody	<i>Eucalyptus camaldulensis</i>	Miles et al. 1982
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion fulviceps</i>	woody	<i>Pinus ponderosa</i>	Wagner & Frantz 1990
Moisture	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion autumnalis</i>	woody	<i>Pinus ponderosa</i>	Wagner & Frantz 1990
Moisture	Leaf-feeder	Hym: Symphyta	<i>Gilpinia hercyniae</i>	woody	<i>Picea abies</i>	Larsson & Bjorkman 1993, Bjorkman & Larsson 1999
Light	Leaf-feeder	Hym: Tenthredinidae	<i>Strongylogaster lineata</i>	non-woody	<i>Pteridium aquilinum</i>	MacGarvin et al. 1986
UV-B	Leaf-feeder	Lep: Noctuidae	<i>Spodoptera litura</i>	non-woody	<i>Trifolium repens</i>	Lindroth et al. 2000
UV-B	Leaf-feeder	Lep: Noctuidae	<i>Graphania mutans</i>	non-woody	<i>Trifolium repens</i>	Lindroth et al. 2000
UV-B	Leaf-feeder	Lep: Noctuidae	<i>Trichoplusia ni</i>	non-woody	<i>Arabidopsis thaliana</i>	Grant-Petersson & Renwick 1996
N-Fertilizer	Leaf-feeder	Lep: Sphingidae	<i>Smerinthus ocellatus</i>	woody	<i>Salix cinera</i>	Thomas & Hodkinson 1991
N-Fertilizer	Leaf-feeder	Lep: Sphingidae	<i>Mimas tiliae</i>	woody	<i>Betula pendula</i>	Thomas & Hodkinson 1991
N-Fertilizer	Leaf-feeder	Lep: Notodontidae	<i>Phalera bucephala</i>	woody	<i>Betula pendula</i>	Thomas & Hodkinson 1991
N-Fertilizer	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion sertifer</i>	woody	<i>Pinus sylvestris</i>	Bjorkman et al. 1991
Salinity	Leaf-feeder	Hem: Delphacidae	<i>Prokelisia marginata</i>	non-woody	<i>Spartina alterniflora</i>	Bowdish & Stiling 1998
Acidity	Leaf-feeder	Lep: Lycaenidae	<i>Lycaena dispar</i>	non-woody	<i>Rumex hydrolaputhum</i>	Bink 1986
Ozone	Leaf-feeder	Hym: Diprionidae	<i>Neodiprion sertifer</i>	woody	<i>Pinus sylvestris</i>	Manninen et al. 2000
CO ₂	Leaf-feeder	Lep: Geometridae	<i>Operophtera brumata</i>	woody	<i>Calluna vulgaris</i>	Kerslake et al. 1998
CO ₂	Leaf-feeder	Lep: Lasiocampidae	<i>Malacosoma disstria</i>	woody	<i>Quercus rubra</i>	Lindroth et al. 1993
SO ₂	Leaf-feeder	Col: Chrysomelidae	<i>Melasoma lapponica</i>	woody	<i>Salix borealis</i>	Kozlov et al. 1996
Age	Leaf-feeder	Lep: Noctuidae	<i>Heliothis virescens</i>	non-woody	<i>Gossypium hirsutum</i>	Navasero & Ramaswamy 1993
Moisture	Miner	Lep: Gracillariidae	<i>Phyllonorycter</i> sp.	woody	<i>Salix lasiolepis</i>	Preszler & Price 1995
Moisture	Miner	Dip: Agromyzidae	<i>Liriomyza trifolii</i>	non-woody	<i>Chrysanthemum morifolium</i>	Price et al. 1982
Light	Miner	Dip: Agromyzidae	<i>Phytomyza ilicicola</i>	woody	<i>Ilex opaca</i>	Potter 1992

N-Fertilizer	Miner	Dip: Agromyzidae	<i>Phytomyza ilicicola</i>	woody	<i>Ilex opaca</i>	Potter 1992
N-Fertilizer	Miner	Lep: Gracillariidae	<i>Phyllonorycter salicifoliella</i>	woody	<i>Salix sericea</i>	Orians & Fritz 1996
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Asphondylia</i> sp. h	woody	<i>Larrea tridentata</i>	Waring & Price 1990
Moisture	Gall-former	Dip: Cecidomyiidae	<i>Rhopalomyia</i> sp.	woody	<i>Tripleurospermum perforatum</i>	Hinz & Muller-Scharer 2000
Moisture	Gall-former	---	---	woody	---	Blanche 2000
N-Fertilizer	Gall-former	Hym: Tenthredinidae	<i>Euura lasiolepis</i>	woody	<i>Salix lasiolepis</i>	Waring & Price 1988

Chapter 3

The performance of two biological control agents of Scotch broom (*Cytisus scoparius*), *Leucoptera spartifoliella* (Lep: Lyonetiidae) and *Arytainilla spartiophila* (Hem: Psyllidae), on moisture stressed host-plants.

3.1 Abstract

The performance of *Leucoptera spartifoliella* and *Arytainilla spartiophila*, two biological agents of Scotch broom (*Cytisus scoparius*), may vary over a range of host-plant soil moistures. A glasshouse experiment was conducted to test the hypothesis that both agents will perform better on broom that was growing in soil moistures that would promote vigorous growth. Broom was grown under four soil moisture levels, each with or without, *L. spartifoliella* or *A. spartiophila*. Broom grew vigorously in two moderate soil moisture levels. *Leucoptera spartifoliella* oviposition showed a negative linear relationship with increased host-plant soil moisture deficit, while pupal abundance and survival showed the opposite relationship. *Arytainilla spartiophila* oviposition scars, adult abundance and survival all demonstrated significant quadratic relationships with host-plant soil moisture levels, where populations were significantly lower on broom growing in excessively moist soil, peaked on plants growing in one of the moderate conditions and then lowered on plants growing in the lowest soil moisture level. The presence of either agent significantly reduced the number of live shoot-tips and stem diameters, and increased the number of dead shoot-tips. Plants also tended to be smaller. It is predicted that *L. spartifoliella* will perform better on broom that is drought-stressed and *A. spartiophila* will perform better on broom growing in moderate soil moistures where growth is vigorous, while the impact of either agent will be greatest on broom growing in low soil moisture environments and lowest on broom growing in wet habitats.

3.2 Introduction

Scotch broom (*Cytisus scoparius* (L.) Link., a leguminous, temperate shrub, is a serious economic and environmental problem in New Zealand and Australia. Fowler *et al.* (2000) estimated that the total cost of broom to New Zealand is \$NZ 5.8-13.6 million/year. In addition to the high economic cost, the impact this weed is having on the New Zealand and Australian environments is enormous. Broom infestations reduce plant species diversity (Gilkey 1957, Waterhouse 1988), threaten the survival of many plant and animal species (CRC 2000, Heinrich & Dowling 2000), provide cover for feral animals (Parsons & Cuthbertson 1992, Smith 1994), are a nuisance in pastures and cultivated fields (Gilkey 1957), hinder grazing and forestry regimes (Waterhouse 1988, Hosking *et al.* 1996, Syrett 1996), and restrict access to recreational areas (Waterhouse 1988).

Biological control may be the most effective and efficient technique for broom management (Waterhouse 1988), because much of the infested land is of low value and difficult to access making other control measures both costly and time inefficient (Smith & Harlen 1991, Zielke *et al.* 1992, Clark 2000, CRC 2000). Biological control programs for broom have been implemented in New Zealand and Australia (Fowler *et al.* 1996, Syrett *et al.* 1999), with three agents, *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae), *Arytainilla spartiophila* Förster (Hem: Psyllidae) and *Bruchidius villosus* (F.) (Col: Chrysomelidae) released (Harman *et al.* 1996, Hosking *et al.*, 1996, Syrett *et al.* 1999). The first two of these insects damage the vegetative parts of broom, and were therefore selected for study. The larvae of *L. spartifoliella* mine in the epidermal cells of stem-twigs (Agwu 1967, 1974), while the nymphs and adults of *A. spartiophila* feed on buds and on tender, actively growing parts of the plant (Watmough 1968).

The success of biological control is likely to vary over the range of environments infested by broom. Weeds infesting a wide environmental range show morphological and physiological differences within the species, which may then affect the performance of insect herbivores, or more specifically biological control agents (White 1969, Mattson & Haack 1987, Waring & Cobb 1992). Since broom occurs in a wide range of habitats varying in levels of soil moisture (Williams 1981, Parsons &

Cuthbertson 1992, Zielke *et al.* 1992, Hosking *et al.* 1996), determining how *L. spartifoliella* and *A. spartiophila* may be affected is important for broom management programs.

In chapter 2, predictions of insect herbivore performance covering five insect feeding-guilds and different forms of host-plant stress were made. These predictions were based on a review of three published insect herbivore - plant stress hypotheses; the Plant Stress Hypothesis (White 1969), the Plant Vigour Hypothesis (Price 1991), and the Insect Performance Hypothesis (Larsson 1989). Results from the review predicted that the performance of miners, such as *L. spartifoliella*, would be negatively affected by host-plant soil on moisture stress, but no general trend was found for the performance of sap-feeders, such as *A. spartiophila* (Chapter 2, Table 2.1, page 35). Though the three plant stress-insect herbivore hypothesis did not account for stress type, the Plant Vigour Hypothesis received the most support. This hypothesis suggests that insects 'closely associated' with the host-plant will do better on vigorously growing host-plants. Miners, like *L. spartifoliella*, are often considered to be 'closely associated' with their host (Inbar *et al.* 2001), but *A. spartiophila* could also be considered in this category. The females select oviposition sites within a few centimetres of where juveniles will feed, a criteria set by Price (1991), and *A. spartiophila* feeds on the growth buds of broom (Watmough 1968), with bud production strongly related to host-plant quality or plant viogour.

A few biological control programs have investigated how insect agents perform on host-plants that are moisture stressed. Hosking and Deighton (1980) found that drought stressed *Opuntia aurantiaca* (Cactaceae), when infested with the sap-feeder *Dactylopius austrinus* De Lotto (Hem: Dactylopiidae), died sooner, and therefore predicted that this agent would have greater impact in years of drought. Steinbauer (1998) found that *Carmenta mimosa* Eichlin and Passoa (Lep: Sessidae), a biological control agent for the woody legume *Mimosa pigra* (Mimosaceae), inflicted greater damage on the weed when it was growing in moist soil. Hinz and Müller-Schärer (2000) reported that the gall-forming fly *Rhoplaomyia* n. sp. (Dip: Cecidomyiidae), a biological control agent for *Tripleurospermum perforatum* (Asteraceae), could establish over a wide range of habitats, but establishment and population growth of

the insect was enhanced when the plant was growing in moist habitats.

To test the hypothesis that *L. spartifoliella* and *A. spartiophila* performance would be greater on non-stressed, vigorously growing broom, a glasshouse experiment was conducted. Broom was grown under four levels of soil moisture, with plants in each level having either *L. spartifoliella* or *A. spartiophila*, or no insects. This experiment also aimed to determine the impact these two agents would have on broom that was growing over a range of soil moistures.

3.3 Materials and methods

3.3.1 Plant propagation

In April 2000, a 3-4 year old broom plant growing outdoors at Lincoln, New Zealand (43°38.5'S 172°28.8'E) was used as a propagation source. Cuttings were used, rather than seeds, to minimise plant variation in experiments. Ten, 50 cm long branches were removed (about one-third of the plant). From these branches, 300 cuttings (about 10 cm in length) were taken, with each cutting containing at least three nodes. The cuttings were dipped in a root-promoting compound (Liba 10,000; NuChem™), placed into plastic propagation trays (1/3 peat and 2/3 perlite soil mixture), and a fungicide (Captan™) was applied. Trays were kept in a controlled environment room (20°C), on heat pads (18-20°C), with a photoperiod of 16 hours light. Cuttings were fertilised (Foliar Nitrophoska; BASF™) in May, and two months later were transplanted into plastic 90 mm tubes where they were moved to a heated glasshouse (20°C). In August the cuttings were transplanted into plastic 2.5 litre pots (17 cm diameter) with a half sand and half top-soil (Templeton loam) mixture. This soil mixture was selected as it provided a good soil moisture gradient, suitable for investigating the effects of soil moisture on plants. The taller and shorter plants were discarded, leaving a uniform stock of 160 plants, approximately 12 cm tall.

3.3.2 Experimental design

The experiment was conducted in an enclosed 3 m² glasshouse, which contained steel mesh benches arranged along the walls. In the first week of September 2000, 160 plants were placed on the benches in the glasshouse and randomly assigned to one of

four minimum soil water vapour content (SWVC) treatments. Each SWVC treatment comprised 15 plants for the addition of *L. spartifoliella*, 15 plants for the addition of *A. spartiophila* and 10 plants serving as controls with no insects added. Insects were added to designated plants 12 weeks later, thereby giving plants enough time to adjust morphologically and physiologically to their SWVC treatment. The experiment ended 12 months after insects were added to plants.

3.3.3 Minimum soil water vapour content (SWVC) treatments

Because constant soil moisture levels are difficult to maintain in semi-natural situations (Kramer 1983), intermittent soil moisture stress was applied. Intermittent application also has the advantage that it is a more realistic simulation of field situations, where rain provides periodic relief (Willis *et al.* 1993, Huberty & Denno 2004). The method selected involved probing the soil of all pots daily, to determine soil moisture levels. Plants were watered only when they reached a minimum designated soil moisture stress level. If plants required watering, water was poured into the top of pots, until water flowed freely out of the bottom, indicating that saturation had been reached. Using this method took into account the many changes that plants would undergo during the time-frame of the experiment. Over 15 months the growth of the woody shrub under glasshouse conditions would be substantial and all four climatic seasons would be experienced, thus respiration rates and water uptake by the roots would vary over this time-frame. These rates would be much greater in summer than in winter, with soils reaching their minimum designated soil moisture stress levels faster. Probing the soil daily for soil moisture level overcomes this potential problem.

Four SWVC treatments were chosen ranging from excessive moisture to limited moisture. They were saturation, 25%, 19% and 13% SWVC. In the saturated treatment (approximately 40% SWVC) pots were kept standing in a 20 cm diameter*2.5 cm high saucer that was full of water. The lower end of the scale was selected based on a small pre-trial. When broom plants reached 13% SWVC they wilted, signifying a level of drought stress was being imposed. This experiment required the plants to be stressed, but not so heavily as to result in death, especially when another stress factor (insects) was also to be applied.

The SWVC for each plant in the 25%, 19% and 13% treatments were measured daily using a HydroSense[®] moisture probe. If the soil had reached the designated minimum SWVC for that treatment, the plant was watered until water ran freely from the pot base.

3.3.4 Insects

Leucoptera spartifoliella and *A. spartiophila* were used in this study as these two insects are established biological control agents in both New Zealand and Australia, and are both vegetative feeders but belong to different feeding-guilds. In early November 2000, twigs with *L. spartifoliella* pupae attached were collected from Burnham, New Zealand (43°36'23.7"S 172°42'58.9"E), and taken to the laboratory where they were placed into clear plastic containers. Containers were stored near windows so pupae would receive natural day-length. Newly emerged adults (<24 h old) were sorted into groups that contained five males and five females, and placed onto the designated plants in the glasshouse in late November 2000. *Arytainilla spartiophila* nymphs are more difficult to rear through to the adult stage, as live plant material is required. Adults were collected from the field, however, as the age of these adults could not be determined 10 males and 10 females, rather than five males and five females, were used to account for adults that may have been too old for oviposition. Thus, in late November, *A. spartiophila* adults (age unknown) were collected from plants at Lincoln using a beating tray, sorted into groups that contained 10 males and 10 females, and placed onto the designated plants in the glasshouse. Insects were confined on plants by gauze bags (75*45 cm) that enclosed the entire plant. Control plants were also bagged. Bags and any live insects were removed one week later. In September 2001, plants were again bagged, to confine insects on their plants before they developed into a mobile stage. These bags then were kept on plants for the remainder of the experiment.

3.3.5 Climate recordings

Temperature and humidity levels of the glasshouse were recorded every hour, for the duration of the experiment. This was to ensure no sudden or unexpected changes occurred in the glasshouse that could affect the experiment. These recordings were measured with a data logger (HOBO[®] H8, 2 channelled), which was placed into the

centre of the glasshouse.

3.3.6 Plant morphological measures

The following plant morphological measurements were recorded to assess the effect of minimum SWVC treatment on vegetative growth: total biomass (divided into above and below-ground biomass), height, stem diameter, and the number of live and dead shoot-tips. Heights and stem diameters were recorded in September 2000 when SWVC treatments were imposed, and then every three months until September 2001 to indicate when differences between SWVC treatments began to appear. Height was measured from the tallest stem-tip to the soil line. The diameters of stems were measured at the soil line, using callipers. In November 2001, the number of live (longer than 1 cm) and dead shoot-tips were counted. For each plant, the above-ground biomass was removed, and the below-ground biomass was washed to exclude soil. They were then put separately into brown paper bags (45*25 cm), placed in drying ovens (60°C) for five days, and weighed.

3.3.7 Plant physiological measures

The following plant physiological measurements were recorded to assess the effect of minimum SWVC treatment on broom; stem-water vapour content, percentage total-nitrogen, percentage total-carbon, chloride, bromide, nitrite, phosphate and sulphate contents. Stem-water vapour pressures of three cut shoot-tips (about 6 cm in length) per plant, from six randomly selected insect-free treatment plants from each minimum SWVC treatment were recorded with a pressure chamber (PMS Instruments Co.TM) to confirm that water stress had been imposed on the plants. Such measurements were taken between 11pm and 2am, over three consecutive nights in January and in October 2001. From the dried, above-ground plant material, five samples of shoot-tips per treatment from control plants, from *L. spartifoliella* infested plants, and from *A. spartiophila* infested plants, were randomly selected. From each sample, approximately 30g of shoot-tips were finely ground and analysed for percentage total-nitrogen, percentage total-carbon, chloride, bromide, nitrite, phosphate and sulphate contents using the technique outlined by Walinga *et al.* (1995). Percentage total nitrogen was the primary chemical element of interest as many studies have found a relationship between increased plant nitrogen and improved insect performance.

3.3.8 Insect performance measures

Several measures of insect population size were recorded to determine if the level of host-plant stress affects insect performance. Oviposition rates were recorded in December 2000. Numbers of *L. spartifoliella* eggs, or *A. spartiophila* oviposition scars, per plant were counted using a 10^x magnification hand lens. *Arytainilla spartiophila* oviposition scars were counted, rather than eggs, as females deposit eggs inside the stem. It has therefore been assumed that the number of oviposition scars equates to the number of eggs. Abundance of *L. spartifoliella* pupae or *A. spartiophila* adults were recorded in November 2001. *Leucoptera spartifoliella* pupae were counted by visually searching plants. *Arytainilla spartiophila* adults were counted by placing the gauze covered plants (containing the adults) into a refrigerator for half an hour, then beating the plant over a plastic collecting tray (60 x 40 cm), dislodging the insects so that they could be counted. Survival rates for *L. spartifoliella* (the number of pupae per plant divided by the number of eggs per plant, multiplied by 100) and *A. spartiophila* (the number of adults per plant divided by the number of oviposition scars per plant, multiplied by 100) were calculated.

3.3.9 Analysis

All analyses were performed using SPSS statistical package. To assess the effects of SWVC treatments and time (between January and October) on stem-water vapour pressure a two-way ANOVA was conducted. The data on stem-water vapour pressure was not normally distributed so it was natural log transformed. For each treatment the percentage of plants surviving was calculated. All remaining analyses were based on data from surviving plants. To determine how minimum SWVC treatments and time (3 month intervals) affected height and stem diameter a repeated ANOVA was conducted. To assess the effects of SWVC treatments on plants surviving to November 2001, one-way ANOVA tests followed by post-hoc (LSD) analyses (to determine which treatments varied) were conducted for plant morphology measures (total biomass, above and below-ground biomass, height-September, stem diameter-September, number of live shoot-tips) and plant physiology measures (total nitrogen, total carbon, chloride, bromide, nitrite, phosphate and sulphate levels). A Kruskal-Wallis test was used to assess the effects of the SWVC treatments on the number of dead shoot-tips, as variances were unequal. Linear and quadratic contrasts were

determined for all plant morphological and physiological parameters that showed significant differences between SWVC treatments. If linear contrasts were significant, that would mean the SWVC treatments would show a decrease, or increase, between each treatment, with either end of each treatment (saturation and 13%) significantly different from one another. If a quadratic contrast was significant, then the two middle treatments (25% and 19%) would be significantly different from the treatments at either end (saturation and 13%).

One-way ANOVA tests followed by post-hoc (LSD) analyses were conducted to assess the effects of SWVC treatments on the performance of *L. spartifoliella* (oviposition, pupal abundance and egg to pupal survival) and *A. spartiophila* (oviposition scars, adult abundance and oviposition scars to adult survival). Linear and quadratic contrasts were also determined for each performance measure. Quadratic contrasts become particularly important here since the hypothesis being tested is that insect performance would be greater on vigorously growing broom. Therefore increased performance on plants in the 25% and 19% treatments would be expected.

To determine if the abundance of insects, or insect by SWVC interactions, affected the survival of broom, a logistic regression was conducted. The explanatory variables were SWVC treatments (saturation, 25%, 19% and 13%) and insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*) and the initial height of plants was used as a covariate. To assess the effects of insect presence, or insect by SWVC interactions, on plant morphology (total biomass, above and below-ground biomass, height-September recording, stem diameter-September recording, and the number of live and dead shoot-tips), two-way ANOVA's were conducted. Total biomass (the addition of above and below-ground biomass) was assessed as the primary impact measure. This measure should provide a good indication of the overall effect of insects and insect by SWVC interactions on plant performance. The explanatory variables were SWVC treatments and insect treatments and the initial height of plants was used as a covariate.

3.4 Results

3.4.1 The effects of soil moisture on broom growth

Stem-water vapour pressures, plant morphology and plant physiological measures confirmed that different levels of stress were imposed on broom for all four selected SWVC treatments. Plants in the 13% treatment had significantly higher stem-water vapour pressures (Figure 3.1). As results were above 10 bars, the result implies that plants in the 13% treatment were drought stressed.

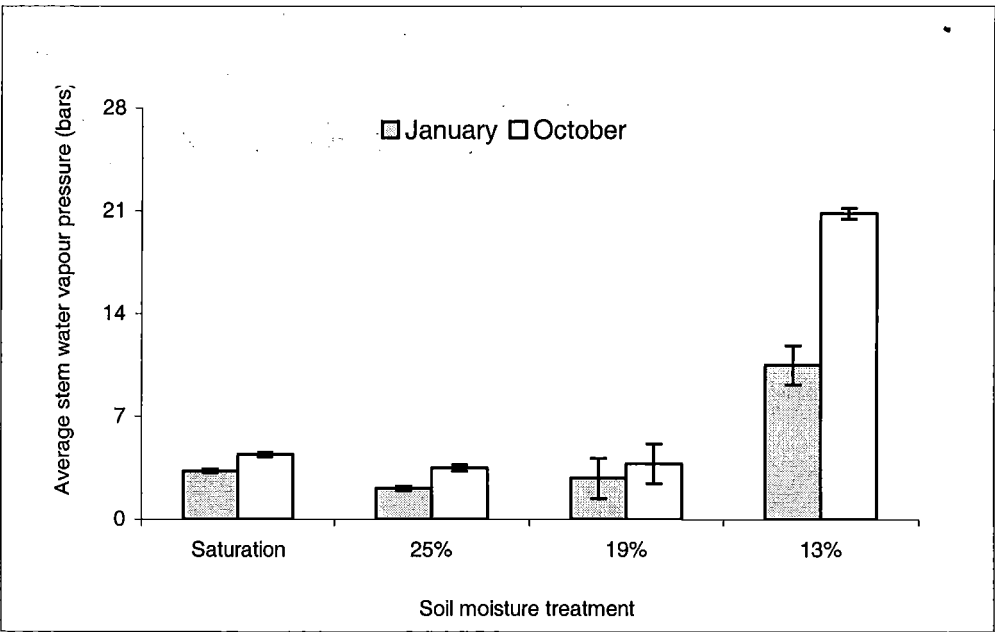
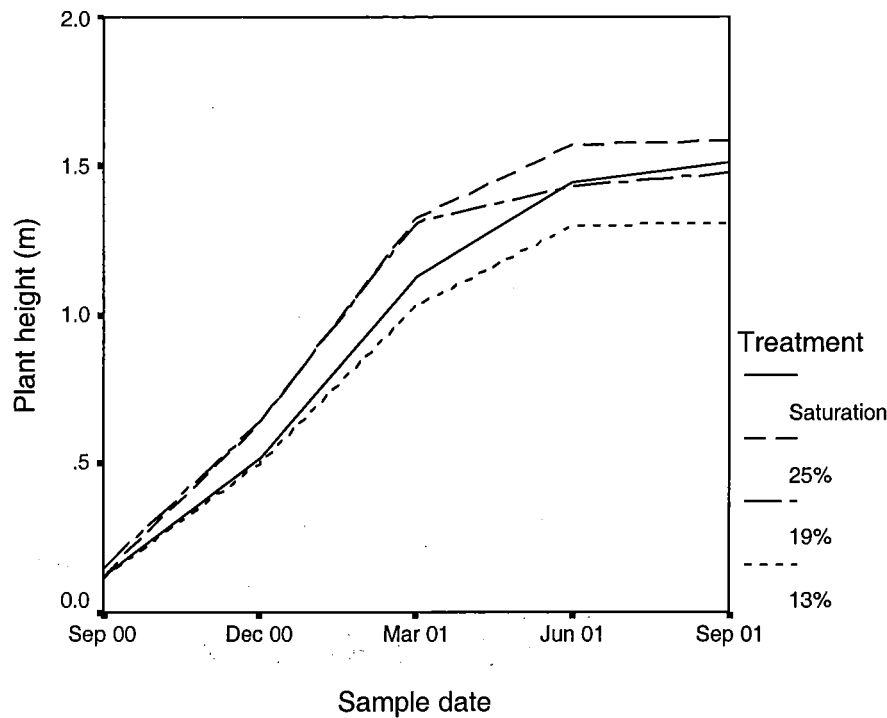


Figure 3.1: Mean and standard errors for stem water vapour pressures taken from broom, in January and October 2001, when plants had been growing under one of four minimum soil water vapour content (SWVC) treatments (saturation \approx 40% SWVC), from September 2000. October readings were significantly higher than those taken in January ($F_1=13.5$, $P=0.001$), and readings were significantly higher in the 13% treatment compared with those in the other treatments ($F_3=38.6$, $P=0.001$).

Significant differences between SWVC treatments were found within the first six months. By January there were significant differences in stem-water vapour pressures (Figure 3.1) and by March significant differences had occurred in plant height and stem diameters (Figure 3.2a and b).

(a)



(b)

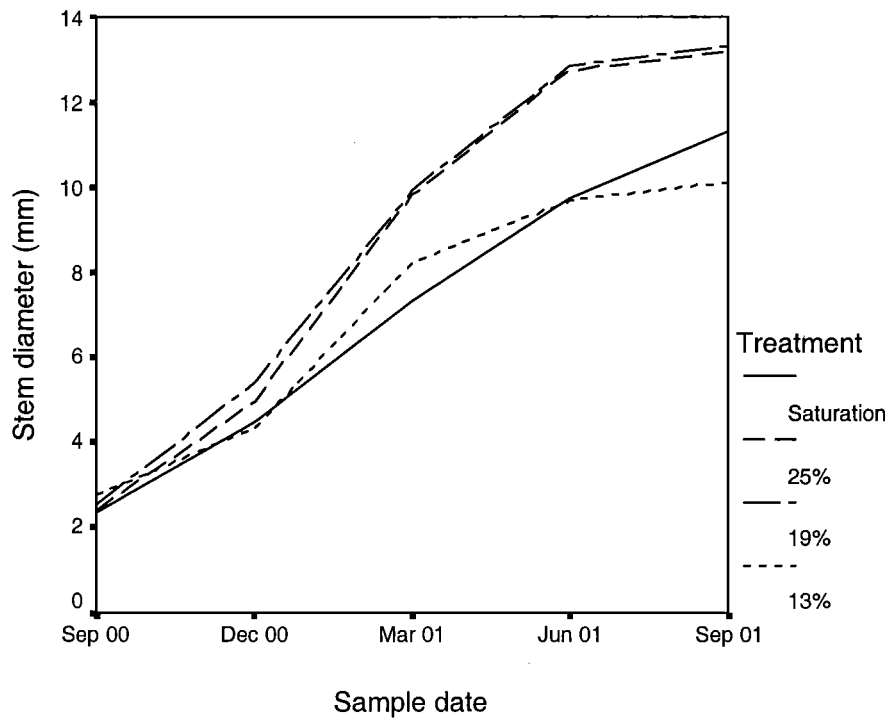


Figure 3.2: Mean (a) heights and (b) stem diameters of broom growing under one of four minimum soil water vapour content (SWVC) treatments (saturation ($\approx 40\%$), 25%, 19% and 13% SWVC) from September 2000 to September 2001.

Plants in the saturated and 13% treatments, compared with the plants in the 25% and 19% treatments, had less total biomass (quadratic contrast $P=0.012$), above-ground biomass (quadratic contrast $P<0.001$), below-ground biomass (quadratic contrast $P<0.001$), were smaller ($P<0.001$) and thinner ($P<0.001$). Though Table 3.1 shows a high mean number of dead shoot-tips on plants in the saturated treatment, no significant differences were found between treatments (Kruskal-Wallis test). The high mean is a result of a few plants that had excessively large numbers of dead shoot-tips. The median was two dead shoot-tips per plant, which was similar to those found in the other treatments.

There were also distinct physiological differences between broom growing under the four SWVC treatments (Table 3.1). Plants in the 13% treatment had significantly higher sulphate levels than plants in the other treatments (quadratic contrast $P=0.006$). Total carbon levels of plants were significantly higher in those plants growing in the 25% treatment (quadratic contrast $P=0.031$). Significantly lower total nitrogen levels were found in plants growing in the saturated treatment (linear contrast $P=0.002$).

Plants growing in the different SWVC treatments also looked different. Broom growing in the saturated treatment looked unhealthy. Although these plants were as tall as those in the 25% and 19% treatments, the stems were thinner, giving a spindly appearance. These plants were also yellowish rather than dark green, and rarely had leaves. Plants growing in the 25% and 19% treatments were tall and bushy, with lots of new growth. These plants were dark green with abundant leaves. Plants growing in the 13% treatment were less vigorous than those in the 25% and 19% treatments, plants were paler in colour, and possessed fewer leaves.

Table 3.1: Mean plant morphological and physiological measurements taken from broom after they had been growing under one of four minimum soil water vapour content (SWVC) treatments (saturation (~40%), 25%, 19% and 13% SWVC), from September 2000 to November 2001. F and P-values are illustrated from one-way ANOVA's.

Plant measurements	SWVC treatments				Analysis	
	Saturation	25%	19%	13%	F	P-value
Morphological measures						
Initial height (m)	0.116	0.122	0.149	0.114	0.458	0.713
Total biomass (g)	63.7	100.4	109.2	53.3	3.45	0.029
Above-ground biomass (g)	53.7	72.0	82.4	38.0	3.26	0.035
Below-ground biomass (g)	10.0	27.8	26.8	9.0	8.02	0.046
Final height (m)*	1.51	1.58	1.47	1.31	5.48	0.001
Final stem diameter (mm)*	11.3	13.2	13.3	10.1	16.1	0.000
Live shoot-tips	271	508	658	383	5.29	0.152
Dead shoot-tips	43	6	5	1	3.47	0.325
Physiological measures						
Sulphate (ppm)	12.2	8.5	11.8	23.6	10.2	0.017
%Total-carbon	47.6	48.3	47.4	47.1	5.19	0.011
%Total-nitrogen	2.2	3.2	3.0	3.4	6.73	0.004
Chloride (ppm)	53.0	49.3	49.4	44.8	0.256	0.856
Bromide (ppm)	0.4	0.2	0.2	0.4	0.127	0.942
Nitrite (ppm)	0.3	0.2	0.2	0.2	0.152	0.927
Phosphate (ppm)	35.3	35.3	35.5	42.7	2.39	0.107
C:N ratio	21.6:1	15.1:1	15.8:1	13.9:1		

*Recording taken in September 2001

**Kruskal-Wallis test was employed rather than a one-way ANOVA

3.4.2 The effects of host-plant soil moisture on insect populations

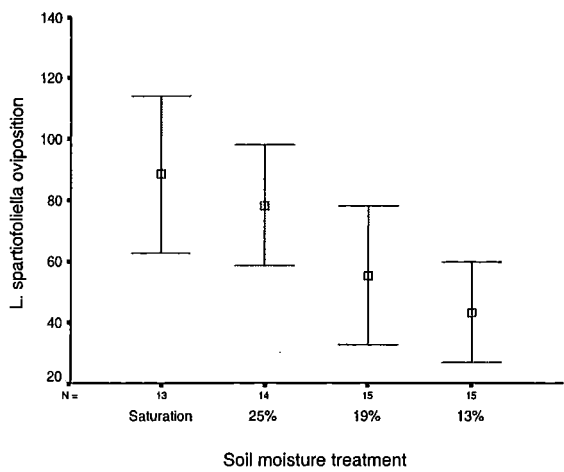
The two insect species responded differently across the SWVC treatments. *Leucoptera spartifoliella* performance measures demonstrated linear relationships with soil moisture deficit, while *A. spartiophila* performance measures fitted quadratic relationships (Table 3.2, Figure 3.3a, b and c, Figure 3.4a, b and c).

Leucoptera spartifoliella oviposition showed a negative linear relationship with increased soil moisture deficit, where oviposition was significantly higher on the plants in the saturated treatment compared with those in the 13% treatment (Table 3.2, Figure 3.3a). However, the opposite trend was found for pupal abundance and survival (Table 3.2, Figure 3.3b and c). In all three measured *A. spartiophila* performance parameters, performance was significantly lower in the saturated treatment and peaked in the 19% treatment (Table 3.2, Figures 3.4a, b and c).

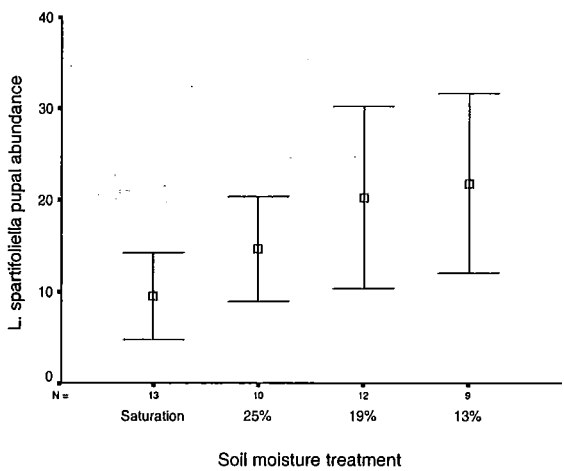
Table 3.2: When broom was grown under four minimum soil water vapour content (SWVC) treatments (saturation (≈40%), 25%, 19% and 13% SWVC), the effects this had on insect performance (measured as oviposition, abundance and survival) was tested by one-way ANOVA's. The performance of two insect species (*L. spartifoliella* and *A. spartiophila*) were examined. F and P-values are shown from ANOVA's, as well as the P-values for linear or quadratic contrasts.

Insect species	Performance measure	ANOVA		P-value for linear and quadratic contrasts	
		F	P	Linear	Quadratic
<i>L. spartifoliella</i>	Oviposition	4.30	0.008	0.001	0.918
	Pupal abundance	2.74	0.056	0.010	0.610
	Survival	5.89	0.002	0.000	0.764
<i>A. spartiophila</i>	Oviposition scars	11.5	0.000	0.000	0.014
	Adult abundance	7.72	0.000	0.007	0.002
	Survival	5.39	0.003	0.030	0.003

(a)



(b)



(c)

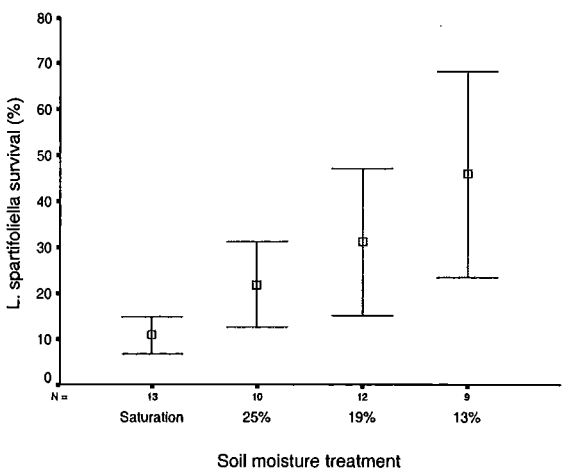
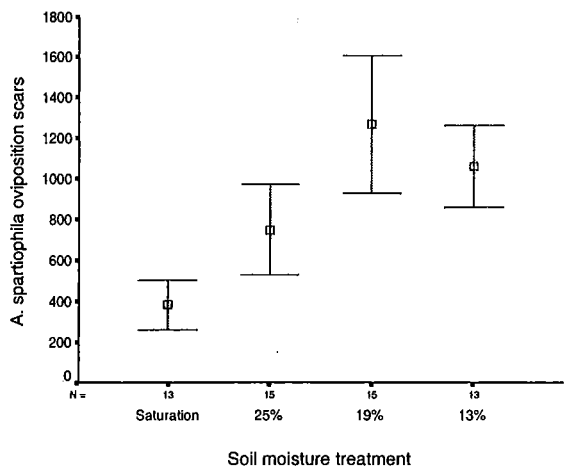
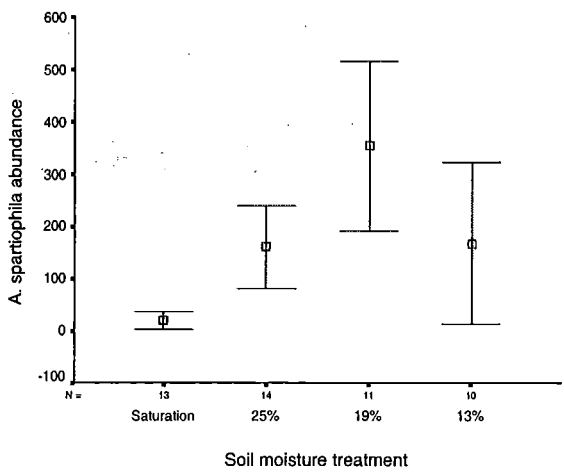


Figure 3.3: The effects of broom minimum soil water vapour content (SWVC) treatments from September 2000 to November 2001, on mean ($\pm 95\%$ CI) *L. spartifoliella* (a) oviposition measured in December 2000 (average oviposition/plant), (b) pupal abundance measured in November 2001 (average pupae/plant), and (c) egg to pupal survival percentage. Saturation $\approx 40\%$ SWVC and N represents the number of plants in each treatment.

(a)



(b)



(c)

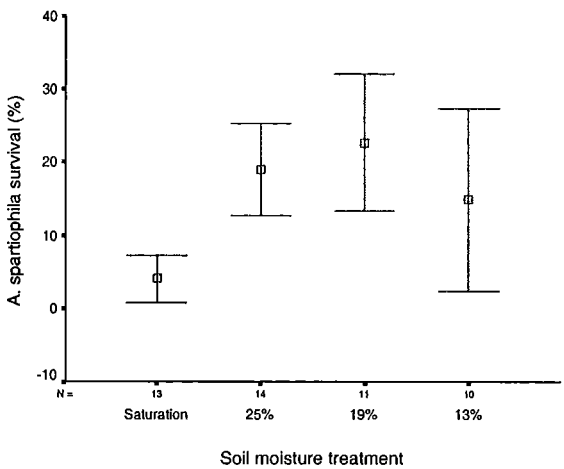


Figure 3.4: The effects of broom minimum soil water vapour content (SWVC) treatments from September 2000 to November 2001, on mean ($\pm 95\%$ CI) *A. spartiophila* (a) oviposition scars measured in December 2000 (average scars/plant), (b) adult abundance measured in November 2001 (average adults/plant), and (c) oviposition scar to adult survival percentage. Saturation $\approx 40\%$ SWVC and N is the number of plants in each treatment.

3.4.3 The effects of soil moisture and insects on broom growth and survival

There was no significant insect by SWVC treatment interaction and no significant main effect of insect treatment on plant survival (Table 3.3). However, the survival of broom was influenced by SWVC treatment, with survival lowest when plants were growing in the saturated treatment (Table 3.3).

Table 3.3: The effects of initial plant height, soil water vapour content (SWVC) treatments (saturation ($\approx 40\%$), 25%, 19% and 13% SWVC), insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*), and SWVC treatment by insect treatment interactions on the survival of broom. Degrees of freedom, ChiSquare and *P*-values from a logistic regression analysis (with the response variable being whether a plant survived or not), as well as the linear contrast are shown.

Analysis	df	ChiSquare	Pr>Chi
Initial height	1	0.151	0.698
SWVC treatment	3	7.98	0.046
Insect treatment	2	1.53	0.465
Insect*SWVC	6	5.40	0.494
Linear contrast- SWVC treatment	1	6.70	0.010

There was no significant insect by SWVC treatment interaction and no significant main effect of insect treatment on total biomass (Table 3.4). The SWVC treatments affected total biomass measures, with biomass of plants in the 25% and 19% treatments greater than plants growing in the saturated or 13% treatments (Table 3.4). Nevertheless, the presence of the insects reduced stem diameters and the number of live shoot-tips and increased the number of dead shoot-tips on plants (Table 3.4, Table 3.5). There was also a tendency for plants to be shorter (Table 3.4, Table 3.5). Though dead shoot-tips showed a significant insect by SWVC interaction, with dead shoot-tips highest on plants growing in the saturated treatment without insects, this result was due primarily to a few plants that bore an excessive number of dead shoot-tips in the control treatment. The SWVC treatments affected above and below-ground biomass, height, stem diameter, and the number of live and dead shoot-tips, as explained in section 3.4.1 on page 69 (Table 3.4, Table 3.5). Plants that were initially taller had thicker stems, more live shoot-tips and greater above and below-ground

biomass (Table 3.4).

Table 3.4: The effects of initial plant height, soil water vapour content (SWVC) treatments (saturation ($\approx 40\%$), 25%, 19% and 13% SWVC), insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*), and interaction of SWVC treatments by insect treatments on the morphology of broom. Degrees of freedom, mean of squares, F and *P*-values are shown from two-way ANOVA's.

Plant Character	Treatment	Analysis			
		df	MS	F	<i>P</i>
Total biomass	Initial height	1	10123	7.73	0.006
	SWVC treatment	3	30615	23.4	0.000
	Insect treatment	2	2025	1.55	0.217
	Insect*SWVC	6	1179	0.901	0.497
Above-ground biomass	Initial height	1	3328	5.60	0.020
	SWVC treatment	3	13805	23.2	0.000
	Insect treatment	2	947	1.60	0.208
	Insect*SWVC	6	812	1.37	0.235
Below-ground biomass	Initial height	1	1843	7.22	0.004
	SWVC treatment	3	3322	13.1	0.000
	Insect treatment	2	304	1.19	0.308
	Insect*SWVC	6	198	0.776	0.591
Height	Initial height	1	0.155	1.11	0.294
	SWVC treatment	3	0.736	5.30	0.002
	Insect treatment	2	0.350	2.52	0.085
	Insect*SWVC	6	0.162	1.17	0.329
Stem diameter	Initial height	1	39.591	9.67	0.002
	SWVC treatment	3	81.088	19.8	0.000
	Insect treatment	2	25.43	6.21	0.003
	Insect*SWVC	6	7.866	1.92	0.087
Live shoot-tips	Initial height	1	333376	10.2	0.002
	SWVC treatment	3	393364	12.1	0.000
	Insect treatment	2	700164	21.5	0.000
	Insect*SWVC	6	43692	1.34	0.245
Dead shoot-tips	Initial height	1	927	3.45	0.066
	SWVC treatment	3	3096	11.5	0.000
	Insect treatment	2	1511	5.62	0.005
	Insect*SWVC	6	670	2.49	0.026

Table 3.5: Means and standard errors of plant morphological measurements taken from broom after they had been growing under different levels of minimum soil water vapour content (SWVC) treatments, from September 2000 to November 2001. Control without insects (C), with *L. spartifoliella* (L), or with *A. spartiophila* (A).

SWVC treatment	Insect treatment	Plant growth							
		Survival (%)	Total biomass (g)	Above-ground biomass (g)	Below-ground biomass (g)	Height (m)	Stem diameter (mm)	Live shoot-tips	Dead shoot-tips
Saturation ($\approx 40\%$)	C	70	63.7 (± 15.0)	53.7 (± 12.8)	10.0 (± 2.1)	1.51 (± 0.11)	11.3 (± 1.0)	271 (± 49)	43 (± 23.6)
	L	100	30.1 (± 8.2)	25.1 (± 6.8)	5.0 (± 1.3)	0.94 (± 0.13)	8.4 (± 0.5)	92 (± 20)	6 (± 3.4)
	A	83	34.0 (± 8.8)	27.6 (± 7.2)	6.4 (± 1.6)	1.15 (± 0.19)	8.4 (± 0.9)	118 (± 29)	17 (± 5.5)
25%	C	90	100.0 (± 21.3)	72.0 (± 11.7)	27.8 (± 9.6)	1.58 (± 0.14)	13.2 (± 0.9)	508 (± 137)	6 (± 3.1)
	L	67	86.4 (± 17.5)	66.6 (± 10.6)	19.8 (± 6.9)	1.37 (± 0.10)	10.7 (± 0.8)	211 (± 31)	0.7 (± 0.7)
	A	100	97.1 (± 10.2)	76.8 (± 7.0)	20.2 (± 3.2)	1.52 (± 0.08)	12.5 (± 0.4)	366.6 (± 44)	2 (± 0.8)
19%	C	90	109.2 (± 15.9)	82.4 (± 9.6)	26.8 (± 6.3)	1.47 (± 0.09)	13.3 (± 0.6)	658 (± 128)	5 (± 1.8)
	L	83	113.3 (± 11.5)	81.8 (± 4.9)	31.5 (± 6.6)	1.52 (± 0.07)	12.6 (± 0.5)	282 (± 17)	0
	A	83	120.2 (± 12.5)	82.7 (± 4.1)	37.6 (± 8.4)	1.60 (± 0.03)	13.2 (± 0.4)	343 (± 36)	0.5 (± 0.5)
13%	C	90	53.3 (± 5.3)	44.2 (± 3.8)	9.0 (± 1.4)	1.31 (± 0.09)	10.1 (± 0.7)	383 (± 58)	1 (± 0.9)
	L	67	55.0 (± 5.6)	46.6 (± 4.4)	8.4 (± 1.2)	1.23 (± 0.08)	9.8 (± 0.4)	196 (± 20)	0
	A	67	54.4 (± 5.9)	42.3 (± 3.9)	12.1 (± 2.0)	1.31 (± 0.05)	10.5 (± 0.5)	295 (± 57)	5 (± 2.5)

3.5 Discussion

3.5.1 The effects of host-plant soil moisture stress on insect performance

The performance of both *A. spartiophila* and *L. spartifoliella* was affected by host-plant soil moisture levels. The two insects responded differently from each other. *Arytainilla spartiophila* nymphs performed best on the vigorously growing plants at mid-range soil moisture levels, while *L. spartifoliella* larvae did best on drought-stressed plants.

All of the *A. spartiophila* performance parameters demonstrated quadratic relationships in response to host-plant soil moisture levels (Table 3.2, Figure 3.4a, b and c). *Arytainilla spartiophila* was negatively affected by excessive host-plant soil moisture, and this is likely the result of plants in the saturated treatment having greatly reduced feeding resources (lower biomass Table 3.1, Table 3.5, and fewer leaves) and significantly lower total nitrogen levels (Table 3.1). *Arytainilla spartiophila* performance was highest on plants in the 19% treatment, probably as these plants provided abundant feeding resources, and higher plant nitrogen levels than those found in the saturated treatment. *Arytainilla spartiophila* performance may have declined in the 13% treatment as feeding resources were not as abundant as those in the 19% treatment, and the stem water vapour pressure was much higher (Figure 3.1) increasing the difficulty for sap-feeders to draw fluid.

It is not surprising to see *A. spartiophila* performance increase under moderate stress and decrease under severe stress as similar results were found in other studies (Braun & Flückiger 1984, English-loeb 1989, Inbar *et al.* 2001). Braun and Flückiger (1984) observed that populations of the aphid *Aphis pomi* deGreer (Hem: Aphididae) were higher on moderately drought stressed *Crataegus* spp. (Rosaceae) as did English-loeb (1989) with *Tetranychus urticae* Koch (Acari: Tetranychidae) on *Phaseolus vulgaris* (Fabaceae) and Inbar *et al.* (2001) with *Bemisia argentifolii* Bellows & Perring (Hem: Aleyrodidae) on *Lycopersicon esculentum* (Solanaceae).

The pulsed stress hypothesis and the growth differentiation balance hypothesis may explain these changes in insect herbivore response in relation to changes in host-plant chemistry, occurring due to varying intensities of soil moisture deficit. Huberty and

Denno (2004) devised the pulsed stress hypothesis, stating that bouts of moisture deficit stress and the recovery of cell turgor allow sap-feeders to benefit from stress induced increases in plant nitrogen. That is, when plants are under moderate moisture deficit, plant cells lose their turgidity but when plants are re-watered, cell turgidity is regained and sap-feeders are able to tap into the higher plant nitrogen levels, leading to better insect performance. This occurs when plants are under intermittent soil moisture stress. However, when plants are under continuous or very long periods of severe moisture stress, if phloem turgor gets below a certain threshold, phloem tappers are unable to access the stress-induced increases in phloem nitrogen. Often, intermittent moisture stress is seen under field conditions and continuous moisture stress is seen in laboratory studies. However, this study applied intermittent moisture stress to plants. The growth differentiation balance hypothesis (cited in Björkman 2000) suggests that moderate drought stress results in increased concentrations of secondary metabolites (e.g. phenolics), but under severe drought stress a reduction in these secondary concentrations occurs. As few studies have investigated changes in plant chemistry in detail, identifying what compound, secondary metabolite, or plant mechanism the insect is responding to (e.g. plant turgor) is difficult. Björkman (2000) measured many plant chemical changes and found that the survival of *Adelges abietis* L. (Hem: Adelgidae) stem-mothers followed host-plant changes in phenolics. Based on the pulsed stress hypothesis, higher *A. spartiophila* performance in the 19% SWVC treatment was possibly due to the changes in cell turgidity enabling the sap-feeder to take advantage of the higher phloem nitrogen, while performance dropped off in the 13% SWVC treatment due to the long loss of cell turgidity that did not allow the insect to use the extra increase in plant nitrogen.

Leucoptera spartifoliella performance demonstrated linear relationships with host-plant soil moisture for all three measured performance parameters (Table 3.2, Figure 3.3, b and c). Lower pupal abundance and egg-pupal survival in the saturated treatment may have been a result of plants having low total nitrogen levels (Table 3.1) and less feeding resource (lower above-ground biomass, Table 3.1). Pupal abundance and egg-pupal survival peaking in the 13% treatment was unexpected, as plants in the 19% and 25% treatments had more abundant feeding resource. However, the higher total nitrogen levels of plants in the 13% treatment (Table 3.1) may explain the higher

pupal abundance and egg-pupal survival. The PSH suggests, as drought stress increases in plants, nitrogen mobilisation increases, and insect populations consequently increase. But unlike sap-feeders (e.g. *A. spartiophila*), loss of cell turgour may not affect miners, thereby allowing the insect to access the higher plant nitrogen levels (Huberty and Denno 2004). But other studies show no link between increased performance of miners and increased plant nitrogen. For example, De Bruyn *et al.* (2002) showed that *Agromyza nigripes* Meigen (Dip: Agromyzidae) increases on sub-drought stressed *Holcus lanatus* (Poaceae) were not related to any increases in plant nitrogen, suggesting the insect was responding to other plant factors. Chapter 2 also reported that miners might not be responding to changes in plant nitrogen, rather changes in leaf structure.

Based on results from other studies, and the results from chapter 2, it was expected that the performance of *L. spartifoliella* would show quadratic relationships with host-plant soil moisture. For example, Björkman and Larsson (1999) found that higher larval weights of *Epinotia tedella* (Cl.) (Lep: Tortricidae) occurred on vigorously growing *Picea abies* (Pinaceae), Ross and Berisford (1990) reported that higher oviposition and larger numbers of heavier pupae of *Rhyacionia frustrana* (Comstock) (Lep: Tortricidae) were found on vigorously growing *Pinus taeda*, and Inbar *et al.* (2001) reported higher oviposition of *Liriomyza trifolii* (Bergess) (Dip: Agromyzidae) on *Lycopersicon esculentum* (Solanaceae). However, these conflicting results might be due to *E. tedella*, *R. frustrana* and *L. trifolii* feeding in a different manner to *L. spartifoliella* even though they are all considered miners. These other studies reported on leaf miners but this study used a twig-miner, suggesting that this feeding guild may need to be split into two sub-guilds to increase reliability of performance predictions for this type of insect.

Another important issue with the *L. spartifoliella* performance results is that the trend for oviposition was opposite to pupal abundance and egg-pupal survival. It was expected that all three measured performance parameters would show similar trends if ovipositing females were able to select hosts that were most suitable for their progeny. If females cannot distinguish host-plants most suitable for their progeny then no difference between treatments for oviposition would occur.

Contrasting insect performance results have been previously encountered (Bultman & Faeth 1987, Preszler & Price 1995, Björkman 2000, Scheirs & De Bruyn 2005). For example, Björkman (2000) assessed several *Adelges abietis* performance parameters across a range of host-plant soil moisture deficits. Opposing performance responses were found and it was concluded that the response of an early lifestage to environmentally induced changes in the host-plant may be opposite to that of a later life stage, as different lifestages are responding to different compounds and physical conditions, and that neglecting to study one or several life stages could lead to incorrect predictions.

There are a few possible explanations for this contrasting linear relationship. The type of performance measures recorded and compared may have led to these differing responses. Ovipositing females may have been responding to other plant cues, or females may have overcompensated, ovipositing at higher levels on less suitable plants. Increased desiccation of adults as soils moisture deficit increased may have also occurred due to the style of the experiment. Adults were confined on plants that were housed in a glasshouse in summer. The temperatures were over 30°C, which meant fans were on in the glasshouse for most of the day. Adults in the 13% soil moisture treatment were unable to obtain plant moisture, whereas plants in the saturated treatment did have access to water. The timing of these measures is another factor to consider. *Leucoptera spartifoliella* oviposition was recorded in December 2000, but host-plants had been under the stress for only a few months and so may have undergone only minor morphological and physiological changes by this time. Pupal counts and survival rates were measured in November 2001, when host-plants had been under the stress for nearly 15 months and the morphological and physiological changes in the plants would have been much more pronounced. This is supported by stem water vapour readings, which were significantly lower in January than in October (Figure 3.1) and differences between treatments for plant height and stem diameter were found from March (Figure 3.2a and b), three months after oviposition.

English-loeb (1989) and De Bruyn *et al.* (2002) reported that non-linear responses to abiotic stress of plants and their herbivores are probably very common, but as only a

few studies apply multiple stress levels in their experiments, this pattern remains relative undetected. Many of the studies assessed in the chapter 2 review used only a control and one treatment. However, this study used four stress treatments, with one insect showing a non-linear response and the other showing a linear response. Three or more treatments are required to assess if responses will or will not be non-linear, but as this study shows not all insects will necessarily exhibit a non-linear response to varying levels of host-plant stress.

The saturation treatment in this experiment was rather severe, as broom rarely encounters natural conditions of saturation. The closest natural situation to the saturation treatment is with broom infesting the braided river systems in New Zealand (Williams 1981) and in drier climates Hosking *et al.* (1996) suggested that broom can be restricted to the edges of watercourses, where saturation levels are intermittent. The performance of either agent is expected to be lowest in these conditions.

3.5.2 The effects of host-plant moisture stress on insect impact

Though no significant insect by SWVC treatment interaction was found, this result might be anticipated for *A. spartiophila*. The performance of *A. spartiophila* was greatest on plants growing vigorously, however, these plants are most likely able to compensate for insect attack. An impact by *L. spartifoliella* in the 13% treatment might have been expected if the experiment had continued, as broom was not growing optimally and the insect showed highest performance in this treatment.

The additive effects of moisture stress and herbivory on host-plants has been mentioned in a number of studies. Studies have shown that when plant growth is retarded by environmental stress the plant may not have the fitness required to compensate for additional factors, such as insect attack. Fereres *et al.* (1988) found that as moisture deficit increased, wheat harvest was reduced, and harvest was further reduced as the density of the aphid, *Sitobion avenae* (F.) (Hem: Aphididae), increased. Also, Willis *et al.* (1993) found that water shortage resulted in retarded growth in *Hypericum* spp. (Hypericaceae), and growth was further reduced by herbivory from *Aculus hyperici* Liro. (Acarina: Eriophyidae) and/or *Aphis chloris* Koch. (Hem: Aphididae). In this study, there was evidence that the presence of *L. spartifoliella* or

A. spartiophila further retarded the growth of already stressed broom. Though impacts did not affect total biomass, the presence of insects resulted in reduced numbers of live shoot-tips and stem diameters, and a tendency for reduced plant height (Table 3.4). Broom is a woody shrub with a life span up to 20 years, thus it would be unrealistic to expect significant impact in just one year, and especially when the experiment was conducted under ideal growing conditions in a glasshouse. This experiment also spanned only one insect lifecycle, giving *A. spartiophila* very limited feeding time on the plant in which to suppress its growth (approximately 2-3 months in the year). In addition, the insect populations in these laboratory studies were lower than those observed in the field (personal observations). Yet, a significant reduction in the number of live shoot-tips occurred in just one year of insect feeding activity. Therefore, with larger insect populations, and the cumulative level of inflicted damage from multiple generations, larger impacts might be expected.

This study did not assess the impact of *L. spartifoliella* and *A. spartiophila* together on one plant. However, as both insects feed on different vegetative plant parts competition between the two species for feeding resources would be minimal. With both insects showing individual impact on broom, the addition of both agents, I believe, would further increase the impact on broom, especially on plants growing in the drier soil moisture conditions, as these plants were not growing optimally.

3.5.3 Implications for broom management programs

Though insect performance was affected in different ways, both insect species are capable of surviving on broom growing under a range of soil moistures. *Arytainilla spartiophila* establishment and population growth is enhanced when broom is growing vigorously, while *L. spartifoliella* establishment and population growth is enhanced when broom is experiencing drought stress.

As either *A. spartiophila* or *L. spartifoliella* can reduce the fitness of broom in just one year, it is predicted that the impact by either agent will be greater when population levels build up on broom over several years. In particular where broom is growing in environments with lower soil moisture conditions, where the growth of broom is not optimal, and where both agents are present.

3.6 References

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Chapter 4

The effects of shading on Scotch broom (*Cytisus scoparius*) and the performance of two biological control agents, *Leucoptera spartifoliella* (Lep: Lyonetiidae) and *Arytainilla spartiophila* (Hem: Psyllidae).

4.1 Abstract

The performance of two biological control agents, *Leucoptera spartifoliella* and *Arytainilla spartiophila*, of Scotch broom (*Cytisus scoparius*), may not be constant across environments varying in levels of shading. Morphological and physiological changes occur in broom growing under different levels of shading, and it is suggested that these changes might affect the performance of *L. spartifoliella* and *A. spartiophila*. Therefore, the effects of host-plant shading on the performance of these two biological control agents were studied in a controlled experiment. Broom was grown under four shade levels, each with and without *L. spartifoliella* or *A. spartiophila*. In addition, a field survey assessing *L. spartifoliella* pupal populations in New Zealand and Australia, where broom was growing in full sun or shaded by natural vegetation, was undertaken. Populations of *A. spartiophila* were not affected by host-plant shading. *Leucoptera spartifoliella* oviposition, pupal abundance and survival were higher on broom growing in less-shaded environments. When field populations of *L. spartifoliella* were low, pupal densities were also higher on broom growing in the lesser-shaded environments. However, when *L. spartifoliella* populations were high, pupal densities were not affected by the level of host-plant shading. *Leucoptera spartifoliella* and *A. spartiophila* can survive on broom growing across a range of shade levels, but establishment and population growth of *L. spartifoliella* might be greater on broom growing in less-shaded environments. However, when populations of these agents are high the greatest impact might occur on broom growing in shaded environments, as host-plant density is lower and plant growth is not optimal.

4.2 Introduction

In this chapter the effects of host-plant shading on insect performance is investigated. In chapter 3, the performance of two biological control agents, *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) and *Arytainilla spartiophila* Förster (Hem: Psyllidae), of Scotch broom (*Cytisus scoparius* (L.) Link), were studied across a range of host-plant soil moisture levels. In chapter 2, predictions on insect performance across a range of host-plant stresses were made for insects belonging to different feeding-guilds. It was suggested that the performance of miners, like *L. spartifoliella*, would not be affected by levels of host-plant shade stress. Insufficient studies prevented predictions from being made for all host-plant stress types by insect feeding-guild interactions. It was not possible, for example, to predict how sap-feeders might respond to host-plant shade stress. Therefore, to predict how sap-feeders, like *A. spartiophila*, would perform the Plant Vigour Hypothesis was used, as it provided the best-supported predictions out of the three plant stress-insect herbivory hypotheses investigated. This hypothesis predicts that insects closely associated with plant growth processes will perform better on vigorously growing hosts. As *A. spartiophila* feeds on the growing buds of broom it can be defined as an insect closely associated with the host-plant and therefore is expected to perform better on non-stressed vigorously growing host-plants. As plants growing in sunnier environments have been reported to have more vigorous growth (Williams 1981) it is expected that *A. spartiophila* will perform best on broom growing in sunnier environments.

As with host-plant soil moisture, host-plant shading may also affect insect performance, due to changes in host-plant morphology and physiology (White 1970, 1993, Kimmerer & Potter 1987, Bultman & Faeth 1988). For example, plants in shaded environments often have reduced rates of transpiration, respiration and photosynthesis (Attridge 1990), reduced biomass, growth rates, reproduction, and nodulated root systems (Williams 1981, Pierson *et al.* 1990, Lentz & Cipollini 1998). Therefore, reduced plant biomass for example, may negatively affect the performance of leaf and stem feeders.

If insect herbivores are affected by host-plant shading, then the success of weed biological control may vary when weeds are growing in shaded habitats. For example,

Clark (1953) found that *Chrysolina quadrigemina* Suffrian (Col: Chrysomelidae), a biological control agent for *Hypericum perforatum* (Clusiaceae), does not lay eggs on plants growing in the shade. In Australia, it has been suggested that this agent will be successful only where the host-plant is growing in the sun (Shepherd 1985).

Assessing the effects of host-plant shading on broom insect herbivores is important because broom grows across a range shade conditions, and there is evidence to show its growth is affected by shading. Broom is able to infest riverbeds and grasslands that are open to full light, open woodland/Eucalypt forest, and heavily shaded *Pinus radiata* (Pinaceae) and *Eucalyptus nitens* (Myrtaceae) forestry plantations (Williams 1981, Parsons & Cuthbertson 1992, Hosking *et al.* 1996, Barnes & Holz 2000). In a two-month long controlled experiment, Williams (1981) found that broom growing in full sun possessed strong lateral shoots, heavily nodulated root systems, and were more prostrate, whereas broom growing in the shade often possessed a single upright shoot, with little or no lateral branching, plants were taller, and had poorly nodulated root systems. Williams (1981) also reported that broom seedlings could tolerate a wide range of light regimes, and Parsons and Cuthbertson (1992) stated that young plants could survive in 90% shade. However, Hosking *et al.* (1996) and Downey and Smith (2000) noted that broom seedlings usually died if germination occurred beneath dense parental canopies.

To test the hypothesis that the performance of *L. spartifoliella* is not affected by host-plant shade stress and that *A. spartiophila* will perform better on vigorously growing, less-shaded host-plants, a glasshouse experiment was conducted. Broom was grown under four levels of shade, with each level having *L. spartifoliella* or *A. spartiophila*, or no insects. This experiment also aimed to determine how these two agents would impact on broom grown under a range of shade levels. In addition, a field survey of *L. spartifoliella* pupal abundance on broom growing in full sun or shaded by natural vegetation was conducted.

4.3 Materials and Methods

4.3.1 Part A: Laboratory studies

4.3.1.1 Plant propagation

Broom plants were propagated at the same time, and in the same manner, as outlined in chapter 3 (Chapter 3, Section 3.3.1, page 66). In summary, during April 2000, 300 cuttings (10 cm in length) were taken from a 3-4 year old broom plant. Root-promoting compounds, fungicides and fertilisers were applied. Cuttings were kept in heated rooms and regularly re-potted into larger tubes. The only variations from chapter 3 were that in August the cuttings were transplanted into plastic 2.5 litre bags, and a uniform stock of 144, approximately 10 centimetres tall plants was used.

4.3.1.2 Shade treatments and experimental design

In September 2000, 36 plants were randomly assigned to each shade treatment (0%, 30%, 50% and 90% shade). These treatments were selected to represent possible natural field conditions in which broom is readily found. These chosen shade levels were based on recordings taken by a light spectrometer, of the percentage of light reaching broom, when broom was growing under a range of forest canopies. The 0% shade represents riverbeds or grasslands, the 30% and 50% shade levels represent open forested areas such as Eucalypt woodlands, and the 90% shade represents mature *P. radiata* plantations.

A shade shelter was constructed for each treatment. Each shelter consisted of shade cloth (ratings of 30%, 50% and 90% shading) covering a wooden frame structure (1.5 m³), while the non-shaded shelter (0%) was left uncovered.

Each of the four shade shelters contained 14 plants for the addition of *L. spartifoliella*, 14 plants for the addition of *A. spartiophila*, and eight plants without insects. Plants were watered regularly by trickle irrigation.

4.3.1.3 Layout of shade shelters

The site at Landcare Research was chosen for these shelters due to its close proximity to laboratories and irrigation, and because the area was in a secure location. However, this location had limited space in which only four structures would fit, and the shelters

were costly to build. Therefore, one shelter per treatment was constructed. This pseudoreplication is not the best design, as at least two shelters per treatment would have been preferred. However, plants were placed randomly in the shelters and these plants were re-arranged in their shelter every few weeks. The area in which the shelters were built was not shaded from surrounding buildings or trees.

4.3.1.4 Insects

To assess whether insect performance is affected by host-plant shading, broom plants were exposed to the shade treatments two months before insects were added. This gave plants time to adjust morphologically and physiologically to the different levels of shading. This two-month timing was based on the results from Williams (1981), where significant changes in plant structure and in the nodulation of root systems had been observed.

Following this *Leucoptera spartifoliella* and *A. spartiophila* were collected at the same time, in the same manner as previously, and bagged onto plants as outlined in chapter 3 (Section 3.3.4, page 65). *Leucoptera spartifoliella* pupae were collected from the field in October 2000 and reared to adult, emerging from cocoons in late November 2000. *Arytainilla spartiophila* adults were collected directly from the field in late November 2000. Five males and five females of *L. spartifoliella*, or 10 males and 10 females of *A. spartiophila*, were placed on to designated plants in each shade treatment. Insects were confined to plants in gauze bags, and all bags and insects were removed one week later.

4.3.1.5 Plant and insect recordings

Morphological and physiological measurements were recorded from plants, as well as insect performance parameters. Full details of these are outlined in chapter 3 (Sections 3.3.6 - 3.3.8, pages 66-67). In summary, stem water vapour pressures were measured in January and October 2001. Heights and stem diameters were measured every three months from September 2000 to September 2001. Temperature and humidity at the centre of the shade shelters were continually monitored for the duration of the experiment to ensure no sudden or unexpected changes using a data logger (HOBO[®] H8, 2 channelled). When the experiment terminated in November 2001, percent plant

survival, total biomass (divided into above and below-ground biomass), the number of live and dead shoot-tips, total plant nitrogen and carbon, chloride, bromide, nitrite, phosphate and sulphate levels were recorded. *Leucoptera spartifoliella* oviposition and *A. spartiophila* oviposition scars were recorded in December 2000. In November 2001, *L. spartifoliella* pupae and *A. spartiophila* adults per plant were counted. Insect survival rates were also calculated.

4.3.1.6 Repeat of oviposition section

Because it had not been possible to maintain constant water levels across shade treatments, due to varying evaporation rates between the shelters, part of the experiment was repeated the following year. The same shade treatments and protocols for plant propagation and insect collection were used, but the plants in the shelters were kept in trays (4 cm deep), which were kept full of water, rather than watered by trickle irrigation. This saturated level meant broom was then growing in the same moisture level between the four shading treatments. Newly propagated plants were placed into shade shelters in September 2001. Insects were released onto plants in late November 2001 for one week, after which the experiment was terminated. Oviposition rates for both insect species were recorded.

4.3.1.7 Analysis

All analyses were performed using SPSS statistical package. To assess the effects of shade treatments and time (between January and October 2001) on stem-water vapour pressure a two-way ANOVA was conducted. As the data on stem-water vapour pressure were not normally distributed it was natural log transformed. All remaining analyses were based on data from surviving plants at the time of recording.

To assess the effects of shade treatments and time (at three month intervals) on plant height and stem diameter a repeated measures ANOVA was conducted. To assess the effects of shade treatments, on plant morphological measures (total biomass, above and below-ground biomass, height (Sept 2001), stem diameter (Sept 2001), and number of live and dead shoot-tips) and plant physiological measures (total nitrogen, total carbon, chloride, bromide, nitrite, phosphate and sulphate levels), one-way ANOVA tests followed by post-hoc (LSD) analyses were conducted using data from plants surviving to November 2001. However, insufficient plant material was

available for chemical analysis from plants in the 90% shade treatment so only total nitrogen and carbon were assessed from plants in this treatment. LSD analyses were conducted to determine which treatments varied.

To determine differences between 2000 and 2001 oviposition preference a two-way ANOVA was conducted for each insect species. Linear and quadratic contrasts were also conducted to assess if insect preference responses were linearly related to levels of host-plant stress or if both ends of the host-plant stress spectrum were different from the middle two host-plant stress treatments. Plants with no oviposition (by *L. spartifoliella* or *A. spartiophila*) were excluded from insect abundance and survival analyses. One-way ANOVA tests followed by post-hoc (LSD) analyses were conducted to assess the effects of shade treatments on populations of *L. spartifoliella* (pupal abundance and egg to pupal survival) and *A. spartiophila* (adult abundance and oviposition scars to adult survival). Linear and quadratic contrasts were also determined for each performance measure to assess if insect performance is greater on vigorously growing broom. It was expected that plants in the 30% shade treatment would grow most vigorously, as these plants would not be heavily shaded (unlike plants in the 50% and 90% treatments) to suffer reduced vigour, while being protected from wind and frosts (unlike plants in the 0% treatment).

To determine if the presence of insects, or insect*shade interactions affected the survival of broom a logistic regression was conducted. Plants from oviposition treatments where no oviposition occurred (by *L. spartifoliella* or *A. spartiophila*) were excluded from analyses. The explanatory variables were shade treatments (0%, 30%, 50% and 90%) and insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*) and the initial height of plants was used as a covariate. To assess the effects of shading and the presence of either insect species on broom morphology (total biomass, above and below-ground biomass, height (Sept 2001), stem diameter (Sept 2001), and number of live and dead shoot-tips) linear regressions were constructed. Total biomass (the addition of above and below-ground biomass) was assessed as the primary indicator of the overall effect insects and insect*shade interactions would have on plant morphology. The explanatory variables were shade treatments (0%, 30%, 50% and 90% shade) and insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with

A. spartiophila) and the initial height of plants was used as a covariate.

4.3.2 Part B: Field studies

4.3.2.1 Selection of broom infestations

Seven broom infestations from two sites in New Zealand (Hanmer Springs and Hinds) and one site in Australia (Krawarree) were selected for studying broom. Three broom infestations were sampled at Hanmer Springs (42°35'36" S 172°34'56" E, 12 km W of Hanmer Springs): two were in full sun on either side of a *P. radiata* plantation, and the third within the plantation. At Hinds (44°00'55" S 171°32'34" E, 20.4 km S of Ashburton) two infestations were sampled: one was in full sun and the other within an adjacent *P. radiata* plantation. Two infestations were sampled at Krawarree (35°48' S 149°40' E, 110 km SE of Canberra): one was in full sun, the other within an adjacent open eucalyptus forest.

4.3.2.2 Selection of plant age groups

The actual age of broom bushes can only be determined by counting annual growth rings, once the shrub has been cut down. Therefore to ensure a range of ages are selected in a population of living plants, plants can be broadly age-defined in relation to four distinct growth stages. Smith (1994) described four growth stages of broom infesting the Barrington Tops (Australia), and though there may be some differences from other locations, the descriptions can still be used as a guideline. The four growth phases are: -

- The seedling stage: consists of plants generally up to 2 years of age.
- The building stage: generally comprises plants up to 2 m tall, erect with dense canopies, plants often commence flowering in their third year, and plants are commonly between 2 and 4 years old.
- The reproductive stage: generally has the highest density of flowers and seed-pods, plants are about 2–4 m tall with stems starting to develop definite leans, and plants are commonly between 4 and 10 years of age.
- The senescent stage: generally consists of plants that are starting to collapse, with plants often greater than 10 years of age (and that may live up to 20 years or more (Smith & Harlen 1991)).

From each infestation, 10 broom plants from each of the building, reproductive, and senescent growth stages were selected. However, in some infestations fewer than 10 plants were found of a specific growth phase. The seedling stage was omitted, as these plants may not have been present for *L. spartifoliella* oviposition in the previous year.

To identify growth phases, plant stem-bases were examined. Plants in the building stage had thin green, flexible stems, with evidence of some stem-base woodiness; in the reproductive stage stem-bases were thick, woody and with bark; and in the senescent stage stem-bases were split and beginning to rot.

4.3.2.3 Survey timing and design

Field surveys were undertaken during November and December 2001, at which time more than 20 *L. spartifoliella* pupae could be found in less than two minutes of searching in all infestations.

4.3.2.4 Sampling procedure

Several morphological characters were recorded from each sampled plant. Plant height was measured from the longest stem-tip of the plant, following the branch down to the soil level. Stem diameter was recorded at the soil level, with the use of callipers. Plant age was determined by sawing the plant stem at the base, lightly sanding the cut stem to highlight the annual growth rings, and then counting the number of rings. To avoid under-sampling pupae on mature bushes, entire branches rather than stems, were sampled. This was done as *L. spartifoliella* larvae in their final instar undergo a wandering phase to find a place to pupate. On younger plants, larvae wander only over live, green plant material, but on larger plants mature larvae can move to, and pupate on, woody material that is unsuitable for mining. Therefore, one main branch of the plant was randomly removed for processing in the laboratory. The branch was then physically divided and material allocated to six categories: dead material, old growth (woody, older than current year's growth), current year's growth (pliable and green, suitable for larval mining), new growth (soft, flush), flowers, and seed-pods. For each sample, all types of material were individually weighed and the number of *L. spartifoliella* pupae found in each part of the sample was recorded. The length of the current year's growth (linear addition of all pieces) was measured, and the percentage of the sample containing new growth and *L. spartifoliella* density (total

number of pupae from sample divided by the length of the current year's growth) was also calculated.

4.3.2.5 Analysis

One hundred and seventy-nine samples were processed for analysis. Data on total *L. spartifoliella* pupae/branch, plant age (annual growth rings), and length of current year's growth were not evenly distributed, and therefore were natural log transformed before analysis. All analyses were performed using SPSS statistical package.

Linear regressions were constructed to determine if shading affected *L. spartifoliella* pupal populations. Pupal populations were regressed against shading and site, with plant age (annual growth rings) and available mining resource (length of current year's growth) used as covariates.

4.4 Results

4.4.1 The effects of shading on broom growth

Significant differences between shade treatments were found within the first six months. By January there were significant differences in stem-water vapour pressures and by March significant differences were recorded in plant height and stem diameters between treatments (Figure 4.1a and b).

The stem-water vapour pressure measures confirmed that no drought stress was imposed on plants. This is of particular importance for plants in the 0% shaded treatment where plants may have dried out due to wind and high evapotranspiration rates. Though stem-water vapour pressures were significantly higher in the 90% treatment ($F_3=7.64$, $P=0.001$) the pressures recorded were within the expected norm for leguminous plants (D. Moot pers. comm 2002). Pressures were higher in October, than in January ($F_1=35.9$, $P<0.001$).

Many of the plant morphological and physiological measures suggested that different levels of shade stress were imposed on broom between shade treatments (Table 4.1, Figure 4.1a and b). Total biomass, above-ground and below-ground biomass, stem

diameter, the number of live and dead shoot-tips all decreased as shade level increased (linear contrast ($P < 0.001$)). Plant height (quadratic contrast $P = 0.003$) and total nitrogen content ($P < 0.001$) demonstrated quadratic relationships with shading, with plants in the 30% and 50% treatments taller and having greater total nitrogen than plants in the 0% and 90% treatments (Table 4.1).

Visual observations also indicated that broom growth was affected by shading. Broom growing in the 0% shade treatment had few leaves, the stems were very tough, non-pliable, many suffered die-back, and the plants were yellowish rather than green. Plants in the 30% and 50% shade treatments had vigorous growth, with many new shoot-tips and leaves, and were dark green. Broom growing in the 90% shade treatment had larger leaves, fewer stems, and stems that were very pliable.

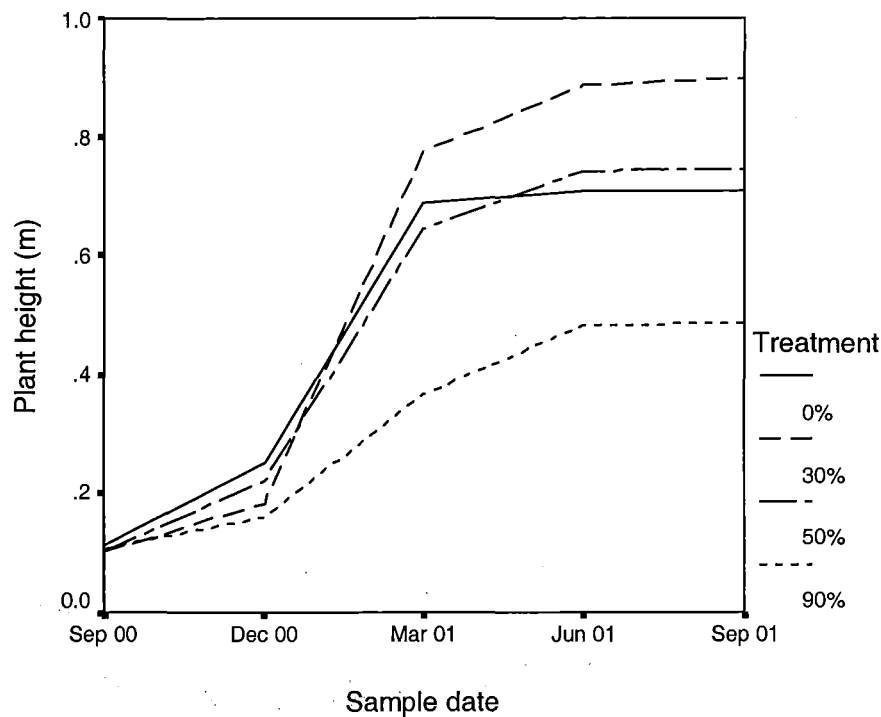
Table 4.1: Mean plant morphological and physiological measurements taken from broom growing under one of four shade levels (0%, 30%, 50% and 90% shade) from September 2000 to November 2001. F and P-values are illustrated from one-way ANOVA's.

Plant recordings	Shade treatments				Analysis	
	0%	30%	50%	90%	F	P-value
Morphological measures						
Initial height (m)	0.11	0.10	0.10	0.11	0.551	0.651
Total biomass (g)	50.3	49.3	33.1	5.6	9.53	0.000
Above-ground biomass (g)	37.8	33.5	22.3	3.8	19.6	0.000
Below-ground biomass (g)	13.4	15.8	10.9	1.3	17.5	0.001
Final height (m)*	0.71	0.90	0.75	0.56	13.2	0.000
Final stem diameter (mm)*	10.3	9.8	7.6	4.7	20.2	0.004
Live shoot-tips	174.8	145.5	146.3	40.6	12.6	0.005
Dead shoot-tips	39.5	1.4	0.9	1.0	13.1	0.004
Physiological measures						
%Total-nitrogen	3.0	3.2	3.2	2.8	4.02	0.026
%Total-carbon	46.6	46.8	46.6	46.3	0.816	0.503
Chloride (ppm)	63.8	39.1	42.9	-----	0.704	0.514
Bromide (ppm)	1.4	0.2	0.6	-----	5.72	0.057
Nitrite (ppm)	0.2	0.2	0.1	-----	1.01	0.400
Phosphate (ppm)	58.3	54.6	54.8	-----	0.183	0.835
Sulphate (ppm)	22.2	26.2	17.7	-----	1.03	0.388
C:N ratio	15.5:1	14.6:1	14.6:1	16.5:1		

* Recording taken in September 2001

----- Insufficient plant material available for chemical analysis

a)



b)

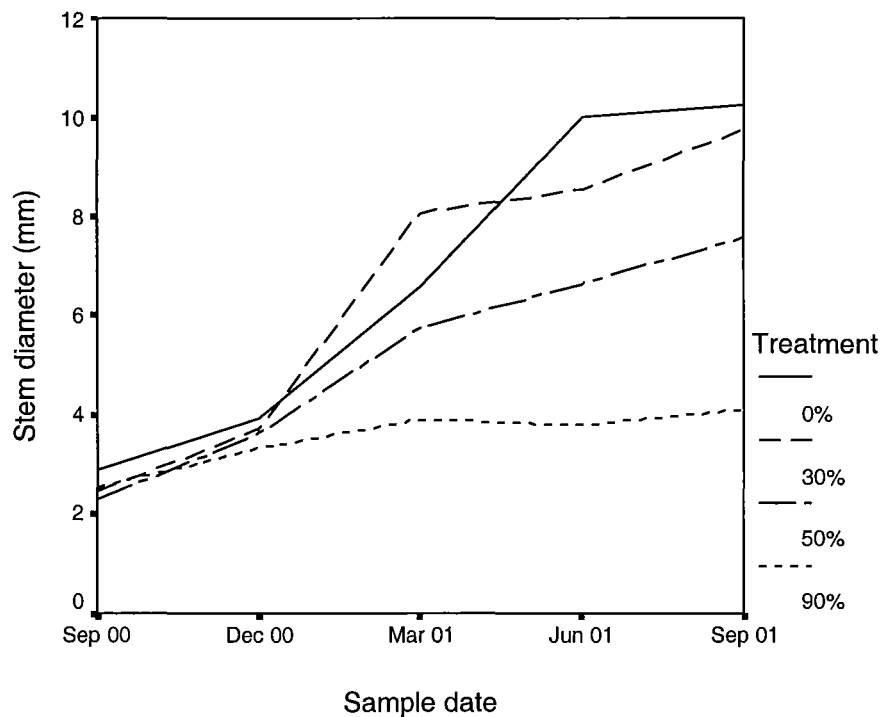


Figure 4.1: Mean (a) heights and (b) stem diameters of broom growing under one of four shade levels (0%, 30%, 50% and 90% shade) from September 2000 to September 2001.

4.4.2 The effects of host-plant shading on insect performance

Leucoptera spartifoliella oviposition, pupal abundance and insect survival were greatest on broom growing in less-shaded environments, and significantly lower on broom growing in heavily shaded environments (Table 4.2, Figure 4.2a, b, c, and d). *Leucoptera spartifoliella* oviposition did not differ between 2000 or 2001 (Table 4.2), suggesting that the different water applications were not affecting *L. spartifoliella* response.

Arytainilla spartiophila oviposition, adult abundance or survival were not affected by host-plant shading (Table 4.3, Figure 4.3a, b, c and d). *Arytainilla spartiophila* oviposition rates were different between 2000 and 2001, suggesting that water application affected performance. *Arytainilla spartiophila* oviposition for 2000 was highest in the 0% treatment, but this treatment also had lower soil moisture. When the soil moisture levels were kept constant between treatments *A. spartiophila* oviposition rates in 2001 were not significantly different between shade treatments.

Table 4.2: The effects of four shade treatments (0%, 30%, 50% and 90% shade) on broom for three insect performance parameters (oviposition for 2000 and 2001, abundance and survival) of *L. spartifoliella*. F and P-values are given from ANOVA's, as well as the P-values for linear and quadratic contrasts.

Performance measure	ANOVA		P-value for linear and quadratic contrasts	
	F	P	Linear	Quadratic
Oviposition-2000	7.20	0.001	0.011	0.916
Oviposition-2001	7.07	0.000	0.000	0.475
Oviposition-comparision				
Years	1.62	0.206	----	----
Treatments	10.6	0.000	0.000	0.567
Years*Treatments	7.78	0.000	----	----
Pupal abundance	14.7	0.000	0.000	0.754
% Survival (egg – pupae)	3.74	0.019	0.003	0.703

Table 4.3: The effects of four shade treatments (0%, 30%, 50% and 90% shade) on broom for three insect performance parameters (oviposition for 2000 and 2001, abundance and survival) of *A. spartiophila*. F and P-values are given from ANOVA's, as well as the P-values for linear and quadratic contrasts.

Performance measure	ANOVA		P-value for linear and quadratic contrasts	
	F	P	Linear	Quadratic
Oviposition scars-2000	5.84	0.002	0.001	0.614
Oviposition scars-2001	1.61	0.202	----	----
Oviposition-comparision				
Years	6.82	0.011	----	----
Treatments	2.33	0.080	----	----
Years*Treatments	3.03	0.033	----	----
Adult abundance	0.501	0.686	----	----
% Survival (oviposition scars – adult)	0.490	0.695	----	----

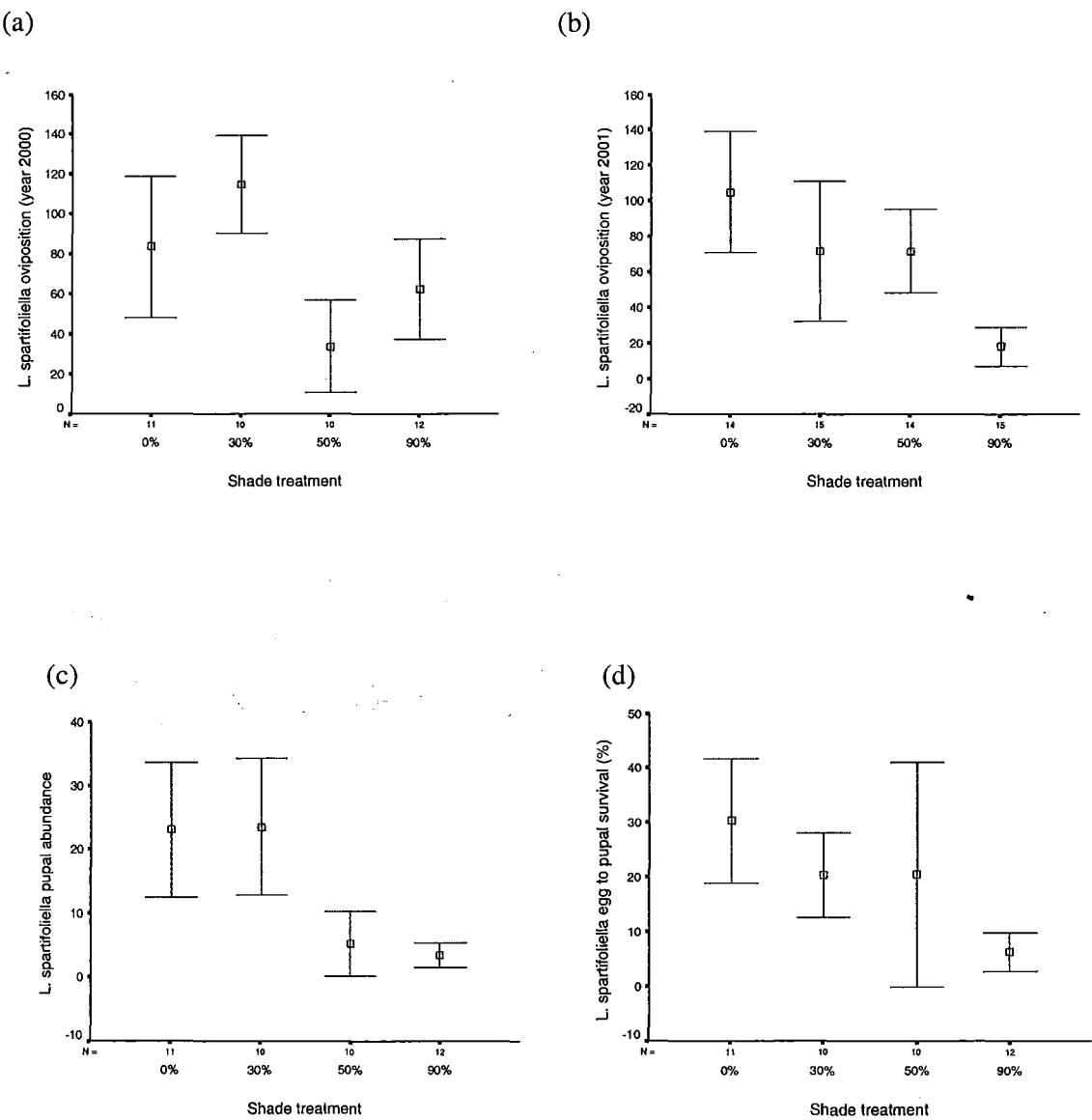


Figure 4.2: The effects of host-plant shading (means \pm 95% CI, N = sample size) per plant on *L. spartifoliella* (a) oviposition (average eggs/plant) measured in December 2000 (b) oviposition (average eggs/plant) measured in December 2001, (c) pupal abundance (average pupae/plant) measured in November 2001, and (d) percentage insect survival.

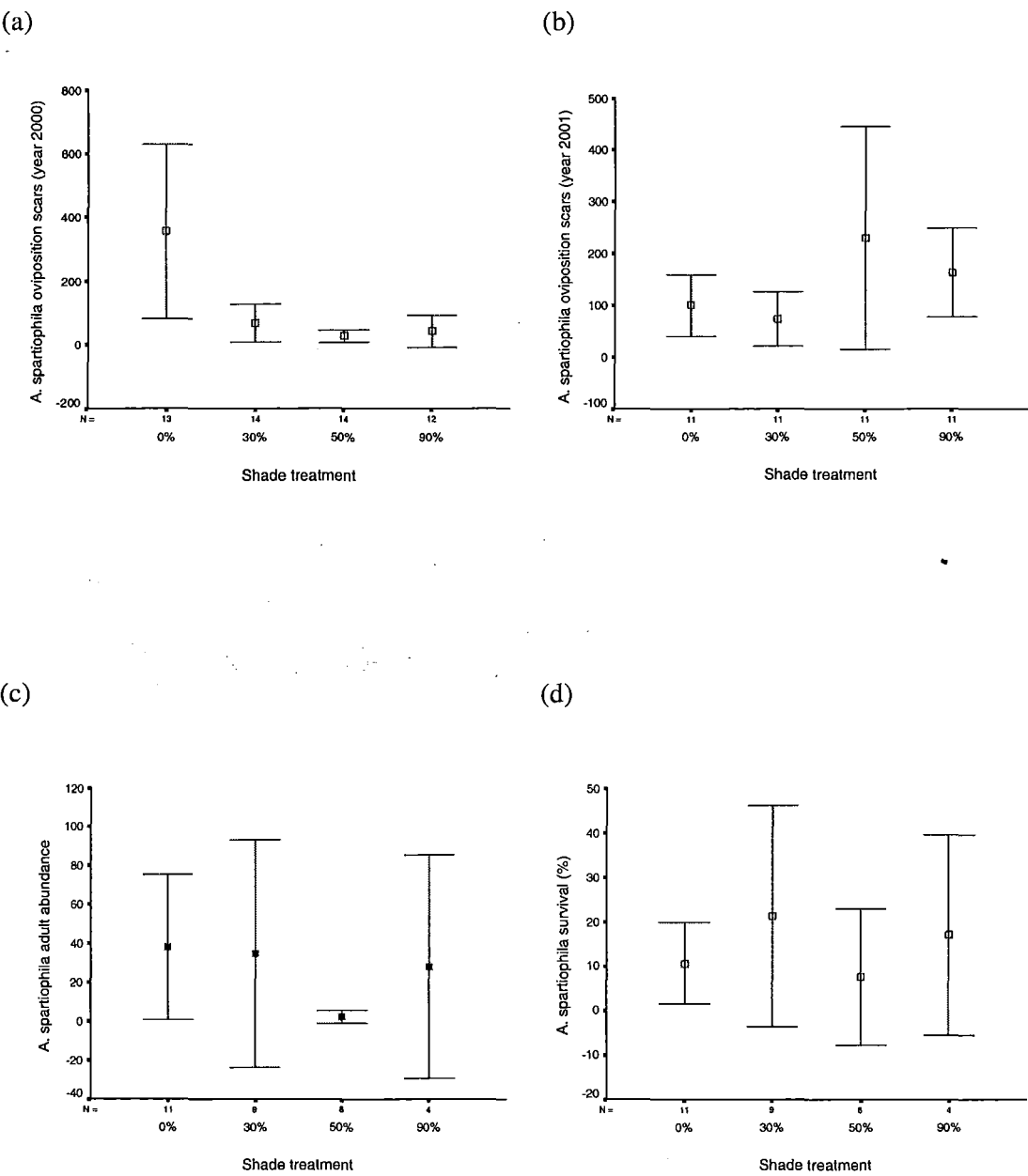


Figure 4.3: The effects of host-plant shading per plant (means \pm 95% CI, and N = sample size) on *A. spartiophila* (a) oviposition scars (average scars/plant) measured in December 2000, (b) oviposition scars (average scars/plant) measured in December 2001, (c) adult abundance (average adults/plant) measured in November 2001, and (d) percentage insect survival.

4.4.3 The impact of insect herbivores in relation to host-plant shading

There was no significant main effect of shade treatment or insect treatment on plant survival (Table 4.4). Very few plants died in the experiment, and therefore no shade*insect interaction could be detected.

Table 4.4: The effects of shade treatments (0%, 30%, 50% and 90% shade) and insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*), on the survival of broom. Initial plant height was used as a co-variate. Degrees of freedom, ChiSquare and Chi figures are given from a logistic regression.

Analysis	df	ChiSquare	Pr>Chi
Initial height	1	0.372	0.542
Shade treatment	3	1.14	0.767
Insect treatment	2	2.01	0.367

The only shade by insect interaction was in the number of dead shoot-tips (Table 4.5). Plants in the 0% treatment had high numbers of dead shoot-tips compared with the other shade treatments. This is most likely to be a result of wind and frost damage. However, the presence of *A. spartiophila* reduced the number of dead shoot-tips in this particular shade treatment, while in all other treatments dead-shoot-tip numbers were increased by the presence of *A. spartiophila*.

The presence of insects significantly affected broom growth, with *A. spartiophila* promoting growth and *L. spartifoliella* showing signs of retarding growth (Table 4.5, Table 4.6). The presence of *A. spartiophila* increased plant total biomass, the number of live and dead shoot-tips were higher, and there was a tendency for plants to be taller. The presence of *L. spartifoliella* on broom showed a tendency to reduce total biomass, above and below-ground biomass, height and the number of live shoot-tips. *Leucoptera spartifoliella* impact was greatest in the 0% and 50% treatments (Table 4.6)

Table 4.5: The effects of shade treatments (0%, 30%, 50% and 90% shade), insect treatments (plants with no insects, plants with *L. spartifoliella*, and plants with *A. spartiophila*), and interaction of shade and insect treatments on the morphology of broom. Initial plant height was used as a co-variate. Degrees of freedom, mean of squares, F and P-values are given from a two-way ANOVA.

Plant Character	Treatment	Analysis			
		df	MS	F	P
Total biomass	Initial height	1	35.6	0.190	0.664
	Shade treatment	3	12837	68.5	0.000
	Insect treatment	2	638	3.40	0.036
	Shade*Insect	6	243	1.30	0.265
Above-ground biomass	Initial height	1	53.3	0.628	0.430
	Shade treatment	3	6221	73.4	0.000
	Insect treatment	2	278	3.28	0.041
	Shade*Insect	6	141	1.66	0.138
Below-ground biomass	Initial height	1	1.78	0.067	0.796
	Shade treatment	3	1199	45.1	0.000
	Insect treatment	2	74.0	2.78	0.066
	Shade*Insect	6	33.9	1.27	0.275
Height	Initial height	1	0.056	2.24	0.137
	Shade treatment	3	0.499	19.9	0.000
	Insect treatment	2	0.075	3.01	0.053
	Shade*Insect	6	0.034	1.35	0.239
Stem diameter	Initial height	1	3.37	1.50	0.224
	Shade treatment	3	154	68.4	0.000
	Insect treatment	2	0.026	0.012	0.988
	Shade*Insect	6	1.39	0.616	0.717
Live shoot-tips	Initial height	1	209	0.066	0.798
	Shade treatment	3	84005	26.4	0.000
	Insect treatment	2	20812	6.53	0.002
	Shade*Insect	6	4088	1.28	0.270
Dead shoot-tips	Initial height	1	185	1.77	0.185
	Shade treatment	3	5233	50.3	0.000
	Insect treatment	2	856	8.22	0.000
	Shade*Insect	6	942	9.05	0.000

4.4.4 *L. spartifoliella* pupal populations in the field

Host-plant shading did affect *L. spartifoliella* pupal populations (Table 4.7, Figure 4.4), as did site and available feeding resource (length of current year’s growth). *Leucoptera spartifoliella* pupal populations were higher in the sun at Krawarree. Pupal populations at Krawarree were significantly lower than those recorded at Hinds, which were significantly lower than those at Hanmer Springs. In addition, *L. spartifoliella* pupal populations increased as the amount of larval feeding resource (length of current year’s growth) increased.

Table 4.6: Means and stand errors for plant morphological measurements of broom plants after they had been growing under different levels of shade, from September 2000 to November 2001. Control without insects (C), with *L. spartifoliella* (L), or with *A. spartiophila* (A).

%Shade treatment	Insect treatment	Plant growth parameters							
		Survival (%)	Total biomass (g)	Above-ground biomass (g)	Below-ground biomass (g)	Height (m)	Stem diameter (mm)	Live shoot-tips	Dead shoot-tips
0	C	100	49.5 (± 8.9)	37.8 (± 6.9)	13.4 (± 0.5)	0.71 (± 0.07)	10.3 (± 0.1)	175 (± 15.2)	39.5 (± 9.5)
	L	86	43.9 (± 4.4)	29.7 (± 2.8)	14.3 (± 1.8)	0.65 (± 0.04)	9.7 (± 0.4)	156 (± 8.8)	36.2 (± 6.5)
	A	93	62.2 (± 6.5)	44.0 (± 4.8)	21.7 (± 2.7)	0.82 (± 0.06)	9.7 (± 0.6)	243 (± 38.3)	8.3 (± 3.2)
30	C	100	49.3 (± 6.0)	33.5 (± 3.2)	15.8 (± 2.9)	0.90 (± 0.03)	9.8 (± 0.4)	146 (± 14.9)	1.4 (± 0.8)
	L	86	53.6 (± 3.9)	39.1 (± 2.7)	14.4 (± 1.4)	0.90 (± 0.04)	10.0 (± 0.4)	153 (± 12.0)	1.2 (± 0.7)
	A	100	56.1 (± 3.8)	44.3 (± 1.9)	18.7 (± 2.0)	0.93 (± 0.02)	10.4 (± 0.4)	199 (± 15.0)	2.8 (± 1.6)
50	C	100	33.1 (± 1.9)	22.3 (± 1.0)	10.9 (± 1.2)	0.75 (± 0.04)	7.6 (± 0.8)	146 (± 13.0)	0.9 (± 0.4)
	L	93	26.3 (± 2.2)	18.7 (± 1.5)	7.6 (± 0.8)	0.73 (± 0.04)	7.0 (± 0.4)	113 (± 9.5)	4.5 (± 1.6)
	A	93	30.8 (± 3.0)	22.6 (± 1.3)	10.2 (± 1.3)	0.78 (± 0.03)	7.5 (± 0.4)	140 (± 15.7)	2.6 (± 0.9)
90	C	100	4.7 (± 1.7)	3.8 (± 1.3)	1.3 (± 0.5)	0.56 (± 0.08)	4.7 (± 0.5)	41 (± 18.2)	1.0 (± 0.9)
	L	93	7.5 (± 0.9)	5.3 (± 0.7)	1.6 (± 0.2)	0.65 (± 0.04)	5.6 (± 0.2)	52 (± 7.3)	0
	A	93	8.9 (± 2.0)	7.7 (± 1.7)	1.8 (± 0.4)	0.72 (± 0.04)	6.2 (± 0.6)	148 (± 21.0)	2.3 (± 0.6)

Table 4.7: The effects of site (Krawarree, Hinds and Hanmer Springs), shade treatments (full sun or shaded), and interaction of site and shade on *L. spartifoliella* pupal density. Plant age (annual growth rings) and available feeding resource (length of current years' growth, natural log transformed) were used as co-variates. Degrees of freedom, mean squares, F and P-values are illustrated from a two-way ANOVA.

	Analysis			
	df	MS	F	P
Site	2	11.2	95.1	0.000
Shading	1	0.672	5.69	0.018
Site*Shading	2	1.19	10.1	0.000
Plant age	1	0.214	1.81	0.180
Available feeding resource	1	9.88	83.6	0.000

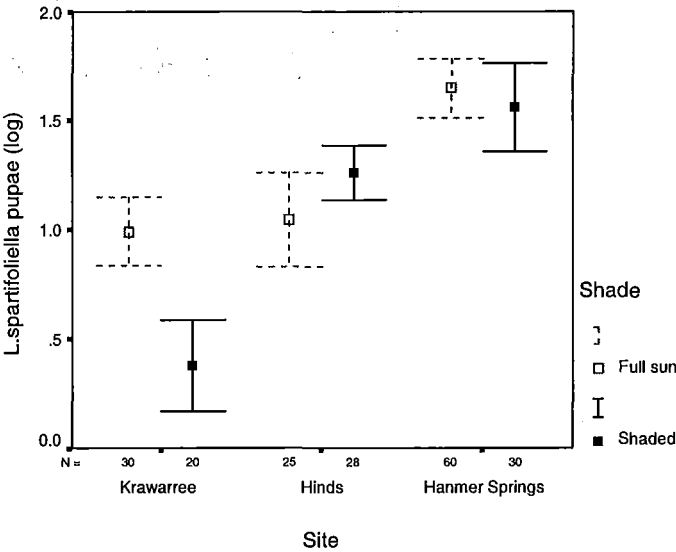


Figure 4.4: The effect of site and shading on *L. spartifoliella* pupal densities natural log transformed.

4.5 Discussion

4.5.1 The effects of host-plant shading on insect performance

The performance of *A. spartiophila* was not affected by host-plant shading, however, *L. spartifoliella* performance decreased as host-plant shade levels increased.

It was expected that *A. spartiophila* performance would be higher on broom growing in the less-shaded environments, as these plants had greater feeding resources, based on the assumption that plants with higher above-ground biomass will have greater numbers of leaf and flower buds. It was also expected that performance would be reduced on plants in the 90% shade treatment, as these plants had much lower above-ground biomass and lower total plant nitrogen levels. However, *A. spartiophila* overall performance (oviposition for the year 2001, adult abundance and survival) was not affected host-plant shading.

Arytainilla spartiophila performance may be more affected by host-plant soil moisture levels than shade levels. In this shade study, oviposition was highest in the 0% shade treatment for the year 2000 (Figure 4.3a). However, plants in this 0% treatment had lower soil moisture levels due to higher evapotranspiration rates, most likely due to wind. In the other three shade treatments evapotranspiration rates were much lower due to the shade cloth coverings blocking out a lot of wind. Though oviposition was higher in the 0% shade treatment, shade levels did not affect adult abundance and survival (Figure 4.3c and d). A section of the experiment was repeated to determine whether the lower soil moisture levels (due to the openness of the 0% shade treatment) or the 0% shade treatment, was the factor for the higher oviposition in the year 2000. This time all plants were kept at the same soil moisture level, saturation; as this was the only moisture level that ensured uniformity across all treatments. But, in chapter 3, populations were significantly lower on plants growing under saturated soil conditions compared with the three other soil moisture treatments. Thus it was not surprising to see *A. spartiophila* oviposition for 2001 low across all shade treatments (Figure 4.3b). *Arytainilla spartiophila* is clearly affected by soil moisture levels (chapter 3), but when plants are under the same moisture level, and only host-plant shading is varied, no differences in *A. spartiophila* performance across shade treatments were found.

It was predicted that *L. spartifoliella* would not be affected by host-plant shade stress. This prediction was based on a review in Chapter 2. But only a small number of studies have been published on the effects of host-plant shade stress on the performance of miners, and the majority of these are leaf-miners, not twig-miners like *L. spartifoliella*. The performance results for this study showed that *L. spartifoliella* was in fact affected by host-plant shade stress. This highlights that plant stress–insect herbivory hypotheses need further refinement before they can be used as reliable predictive tools. It is suggested that better defining insect feeding guilds or breaking the group of miners into two sub-guilds, leaf-miners and stem/twig-miners would increase the reliability of plant stress – insect herbivory hypotheses.

Leucoptera spartifoliella oviposition and pupal abundance were greater on less-shaded broom and survival was significantly lower on heavily shaded broom. Higher performance on plants in less-shaded environments might be related to the greater amount of available feeding resource, as above-ground biomass declined as shading increased (Table 4.1). It is not surprising to see lower performance in the 90% treatment as available feeding resource and total nitrogen levels were significantly lower (Table 4.1). Similar responses were found by Kimmerer and Potter (1987) and Bultman and Faeth (1988). Kimmerer and Potter (1987) observed greater abundance and survival of *Phytomyza ilicicola* Loew (Dip: Agromyzidae) on leaves of plants in the sun, compared to the shade, while Bultman and Faeth (1988) found that the density and survival of *Tischeria* sp. (Lep: Tischeriidae) were higher on trees in the sun.

Host-plant shading affected the performance of *L. spartifoliella* under laboratory conditions, but may only be important under natural field conditions when populations are low. Laboratory populations of *L. spartifoliella* were low, as were those recorded in the field at Krawarree, compared with Hinds and Hanmer Springs populations (personal observations). At Krawarree, pupal densities were higher on broom growing in full sun, compared with the shade. However, *L. spartifoliella* was first released in 1993 (Wapshire & Hosking 1993), only eight years prior to this survey. In addition, *L. spartifoliella* was released on plants growing in full sun (A. Sheppard pers. comm 2001) and adults may not yet have dispersed into the nearby shaded environments. Ovipositing *L. spartifoliella* may initially select broom that is

growing in full sun, but when population levels increase competition for oviposition sites might encourage *L. spartifoliella* to migrate into shaded environments. *Leucoptera spartifoliella* has been in New Zealand for at least 50 years (Scheele & Syrett 1987), giving adults more time to disperse and populations to build. The site at Hanmer Springs has a long record of broom infestation so it is not surprising to see larger populations of *L. spartifoliella* here. At Hinds, broom has infested this particular site only in the last 20 years, allowing less time for agents to reach high numbers, which could explain the lower *L. spartifoliella* densities at Hinds compared with Hanmer Springs (Figure 4.4).

4.5.2 The impact of agents on shaded broom

The presence of *A. spartiophila* did promote broom growth, however, having a few insects on the plant might not have been enough to damage plants, but may have stimulated the plant to combat the attack (Crawley 1997). Alternatively, the Resource Regulation Hypothesis (RRH) might explain this growth promotion. The RRH proposes that an herbivore might maintain high quality resources on a plant for the subsequent generations of the herbivore species on that individual plant (Craig *et al.* 1989). Craig *et al.* (1989) found that galling insects can stimulate plant vigour thereby increasing resources for subsequent generations. Promoting the growth of broom would lead to greater leaf and flower buds, resources required by *A. spartiophila* nymphs.

No significant insect*shade interaction was found for *L. spartifoliella*. However, if the experiment had continued, it is likely that the impact of *L. spartifoliella* on broom would have been more pronounced, especially on plants growing in full sun (0% shade treatment) and plants growing in medium shade (50% shade treatment). Broom growing in full sun may provide a good haven for *L. spartifoliella* to establish and populations to build. However, impact on broom long-term, may be greatest in medium shaded habitats (e.g. Eucalyptus woodlands) as broom growth is not optimal (Tables 4.1 and 4.6) and population densities of the plant are lower (Downey & Smith 2000).

The additive effect of stress and herbivory on host-plants was mentioned in chapter 3 (Section 3.5.2, page 83). Several studies have shown that when plant growth is

retarded by encountering stress, the plant may not have the fitness required to compensate for additional stress factors imposed by the environment, such as insect attack (Ferreles *et al.* 1988, Willis *et al.* 1993). In this study, there was evidence that the presence of *L. spartifoliella* further retarded the growth of already stressed broom, with tendencies to reduce total biomass, above and below-ground biomass, height and the numbers of live shoot-tips (Table 4.5). This is promising for biological control considering that the shrub has a life span up to 20 years, the experiment was conducted under ideal growing conditions in a shadehouse, only one insect generation was assessed and insect populations used were lower than those observed in the field (personal observations). Therefore, successive generations of *L. spartifoliella* attack should show greater impacts in the field, than demonstrated in the laboratory.

As in chapter 3, this study did not assess the impact of *L. spartifoliella* and *A. spartiophila* together on one plant. Competition between the two species for feeding resources would be minimal, as both insect species feed on different vegetative plant parts. Therefore, with the two insect species attacking different plant parts, greater impacts on broom could result, especially if field populations of the agents were higher than those used in this laboratory experiment. In addition, impact might be greatest on plants growing in medium level shade (e.g. Eucalypt woodlands), as plant growth is not optimal.

4.5.3 Implications for broom management

Leucoptera spartifoliella and *A. spartiophila* are able to survive on broom that is growing under a range of shade levels. Establishment of *L. spartifoliella* might be greatest where broom is growing in full sun, but once a strong population has established performance on broom should be similar across host-plant shade levels.

Downey and Smith (2000) reported that broom growing in shaded environments were of lower densities as seedlings largely died through suppression of the heavily shaded plants, with recruitment occurring mainly when a light space appeared (senescence of broom plants or those trees creating the shaded environment, e.g. Eucalyptus trees). Broom densities in these shaded environments may be reduced even more if agents could (1) reduce broom biomass already there, and (2) attack the young seedlings as they emerge (Downey & Smith 2000). *Arytainilla spartiophila* feeds on the buds of

broom and *L. spartifoliella* feeds inside shoot-twigs, therefore they should reduce the density of broom already present, especially if both species were in higher populations than used in this experiment. Seedlings were used in this experiment and both agents clearly attack this age bracket. The addition of other agents is also an option, and based on predictions in chapter 2, leaf-feeders and gallers could reduce broom plant biomass even further in medium-shaded areas (such as Eucalypt woodlands), as their performance will be enhanced in these environments. Syrett *et al.* (1999) listed two leaf-feeders *Gonioctena olivacea* (Forster) (Col: Chrysomelidae) and *Agonopterix assimilella* (Treitschke) (Lep: Oecophoridae) and three gallers *Aceria genistae* (Nalepa) (Acarina: Eriophyidae), *Asphondylia sarothamni* H.Loew (Dip: Cecidomyiidae) and *Hexomyza sarothamni* Hendel (Dip: Agromyzidae) as potential biological control agents for broom that require further study. If these insects are brought into New Zealand or Australian quarantine facilities for further study, assessing their performance in relation to host-plant shading is recommended and well as the affect of competition for resources with *A. spartiophila* and *L. spartifoliella*. The question of releasing other agents are discussed in more detail in chapter 7.

4.6 References

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4.7 Appendix

Table 4.8: The effects of site (Krawarree, Hinds and Hanmer Springs), shade level (broom growing in full sun and broom growing under a forest canopy), and interaction of site and shade on broom morphology. Degrees of freedom, mean of squares, F and *P*-values are illustrated from two-way ANOVAs.

Plant Character	Treatment	Analysis			
		df	MS	F	<i>P</i>
Height	Site	2	49679	1.54	0.216
	Shade	1	62587	1.95	0.165
	Site*Shade	2	26971	0.838	0.434
Stem diameter (log)	Site	2	0.126	0.220	0.803
	Shade	1	4.91	8.59	0.004
	Site*Shade	2	1.40	2.45	0.089
Dead weight (log)	Site	2	33.3	36.6	0.000
	Shade	1	0.110	0.121	0.728
	Site*Shade	2	1.21	1.33	0.267
Old weight (log)	Site	2	1.50	2.41	0.093
	Shade	1	5.78	9.29	0.003
	Site*Shade	2	1.26	2.02	0.135
Length of current years' growth (log)	Site	2	3.68	7.01	0.001
	Shade	1	0.024	0.045	0.831
	Site*Shade	2	4.01	7.62	0.001
New weight (log)	Site	2	20.9	17.2	0.000
	Shade	1	0.003	0.002	0.962
	Site*Shade	2	6.10	5.02	0.008

Chapter 5

Relationships between oviposition preference and performance of two biological control agents of Scotch broom, *Leucopetra spartifoliella* (Lep: Lyonetiidae) and *Arytainilla spartiophila* (Hem: Psyllidae).

5.1 Abstract

It has been hypothesized that insects may show positive oviposition preference-offspring performance relationships when (a) the immature stage of an insect is relatively immobile, (b) the insect is monophagous, (c) oviposition by the female is associated with the juvenile feeding site, and (d) the successful development of juveniles is dependent on transient plant parts. Experiments assessing host-plant performance, for two biological control agents, *Leucopetra spartifoliella* (Lep: Lyonetiidae) and *Arytainilla spartiophila* (Hem: Psyllidae), of Scotch broom (*Cytisus scoparius*), across a range of soil moisture or shading levels were conducted in chapters 3 and 4. However, these studies did not give ovipositing females the chance to select host-plants (oviposition preference), which were exposed to varying levels of host-plant soil moistures or shading. As both *L. spartifoliella* and *A. spartiophila* fit all four preference-performance criteria it is predicted that both insect species should display positive preference-performance relationships. Host-plant preference experiments were carried out in a glasshouse where broom was grown under four soil moisture or four shade levels. The preference results were compared with the results obtained in the performance studies (chapters 3 and 4). *Leucopetra spartifoliella* oviposition preference increased as host-plant soil moisture and host-plant shade levels decreased. *Arytainilla spartiophila* oviposition was lowest on plants growing in saturated soil conditions and no preference for host-plant shading levels were demonstrated. In all cases, the oviposition preference results matched the offspring performance results, showing positive preference-performance relationships. These results support the hypothesis that oviposition preference and offspring performance are linked in monophagous insects that have immobile juvenile stages. These positive

preference-performance results also increase the strength of predictions made in chapters 3 and 4, where it was suggested that *L. spartifoliella* populations on broom would be initially higher in drier environments that are open to full sun, like grasslands, and *A. spartiophila* populations would be higher on vigorously growing that encounters optimal soil moisture levels and not affected by levels of host-plant shading.

5.2 Introduction

Biological control may be the most effective and efficient method to control Scotch broom (*Cytisus scoparius* (L.) Link. Fabaceae), a woody, noxious weed in New Zealand and Australia (Waterhouse 1988). The performance of two biological control agents of broom, *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) and *Arytainilla spartiophila* Förster (Hem: Psyllidae) has been assessed in relation to a range of host-plant soil moisture and shade levels (Chapters 3 and 4). In these performance studies, ovipositing females were confined on plants grown under a particular level of soil moisture or shading. However, in natural field situations, ovipositing females are able to select host-plants growing over a range of environmental conditions. To add strength to the predictions made in chapters 3 and 4, determining the preferences of these agents towards host-plants exposed to a range of soil moisture or shading levels is important.

Many studies have used the terms insect 'preference' and 'performance' interchangeably, but they are quite different measures (Singer 1986). Insect preference is a measure of the relative likelihood of female insects accepting plants that are encountered. For example, to assess oviposition preference, oviposition is measured on different plants presented simultaneously. Insect performance is a measure of the fate, or success, of offspring. For example, juveniles may survive with different probabilities depending on the suitability of the oviposition site.

An insect may prefer a particular host-plant, but this may, or may not correlate with increased performance. For instance, Myers *et al.* (1981) found that *Cactoblastis cactorum* (Berg) (Lep: Pyralidae) chose plants of better quality, even when

overcrowded. It has also been found that *Pieris rapae* (L.) (Lep: Pieridae) females made choices that benefited their larvae by selecting large, young plants growing at lower light levels (Ives 1978). Bultman and Faeth (1988) showed that *Stigmella* sp. (Lep: Nepticulidae) preferred, and performed better in, shaded habitats, and Scheirs and De Bruyn (2005) reported that *Chromatomyia milii* (Kalenbach) (Dip: Agromyzidae) preferred (oviposition) and performed (survival) better on vigorously growing *Holcus lanatus* (Poaceae). However, positive preference-performance relationships are not always found. Rausher (1979) studied three papilionid species, finding that oviposition was higher in sunnier environments for two of the three species, yet all three species had significantly higher survival rates in shaded environments. Similarly, Bultman and Faeth (1988) found that *Cameraria* sp. (Lep: Gracillariidae) preferred sunnier environments, though higher survival rates were found in shaded habitats. Fritz *et al.* (2003) reported that *Phyllocolpa leavitti* (Rohwer) (Hym: Tenthredinidae) showed oviposition preference for vigorously growing *Salix discolor* (Salicaceae), but performance not affected by plant vigour. *Lymantria dispar* (L.) (Lep: Lymantriidae) performance was greatest on *Quercus palustris* (Fagaceae), but was least the least preferred out of eight *Quercus* species (Foss & Rieske 2003). These examples not only highlight the importance of using correct terminology but also raise the question: can positive preference-performance relationships be predicted?

A number of authors have made suggestions as to when a positive preference-performance relationship might occur. Ives (1978) suggested that it would occur for an insect with relatively immobile immature insect stages, where the host-plant that an ovipositing female selects directly affects the survival of her progeny. Craig *et al.* (1989) reported that more studies have shown preference and performance are more strongly correlated for monophagous than polyphagous species. Preszler and Price (1995) suggested positive preference-performance relationships would occur if two conditions were met. They suggested preference and performance would be correlated, and greater, on vigorously growing host-plants when (a) oviposition by the female is associated with juvenile feeding site, and (b) the successful development of juveniles is dependent on short-lived plant parts (e.g. leaves, seeds).

Positive preference-performance relationships should therefore be expected for *L. spartifoliella* and *A. spartiophila*. *Leucoptera spartifoliella* fits three of the four criteria and *A. spartiophila* fits all four criteria suggested by Ives 1978, Craig *et al.* (1989) and Preszler and Price (1995). There is a lack of mobility of the immature stages of both species (Ives 1978), and the site the ovipositing female selects directly affects the survival of the progeny (Preszler & Price 1995). When *L. spartifoliella* larvae emerge from their eggs they burrow directly into the stems and when *A. spartiophila* nymphs emerge they make their way to the nearest growing bud. The second criterion suggested by Preszler and Price (1995), that the successful development of juveniles is dependent on temporary plant parts is true for *A. spartiophila*, as later instars feed actively on the leaf and flower buds. Leaf shedding is a common response for broom especially in summer drought or cold winters (Williams 1981, Hosking *et al.* 1996), and bud-formation is seasonal. This criterion is not met by *L. spartifoliella*, which always has access to shoots for its mining larvae to feed on. Finally, both *L. spartifoliella* and *A. spartiophila* are monophagous.

In chapters 3 and 4, the performance of *L. spartifoliella* across a range of host-plant soil moisture levels (Chapter 3, Figures 3.3a, b and c, page 74) and shade levels (Chapter 4, Figures 4.2a, b, c and d, page 104), and *A. spartiophila* across a range of host-plant soil moistures (Chapter 3, Figures 3.4a, b and c, page 75) and shade levels (Chapter 4, Figures 4.3a, b, c and d, page 105) were assessed. From the results obtained it was predicted that *L. spartifoliella* establishment and populations would be greater in drier, minimally shaded environments, and that *A. spartiophila* establishment and populations would be lower in extremely moist environments and not affected by levels of shade.

Glasshouse experiments were designed to test if *L. spartifoliella* and *A. spartiophila* oviposition preferences on broom growing under a range of soil moisture and shading levels matched the results obtained in the performance studies (Chapters 3 and 4).

5.3 Materials and Methods

5.3.1 Plant propagation

Broom was propagated from both seed and cuttings. Seeds were placed into propagation trays (1/3 peat and 2/3 perlite soil mixture) in March 2001. At the same time, cuttings were taken from two 3-4 year old plants growing outdoors at Lincoln, New Zealand (43°38.5'S 172°28.7'E). Full details on the protocols for the propagation and maintenance of cuttings are given in chapter 3 (Chapter 3, Section 3.3.1, page 63). In summary, 10 cm length cuttings were taken, dipped into a root-promoting compound and placed in propagation trays. For both seedlings and cuttings, fungicides and fertilisers were applied, trays were kept in warm conditions, and plants were regularly re-potted into larger tubes or pots.

Broom cuttings were used in the performance studies (Chapters 3 and 4) but, as insufficient numbers were available for this entire study, the soil moisture component of this study used both cuttings and seedlings. This allowed an assessment of how plant source (cuttings verse seedlings) affects insect preference. The shade component in this study used entirely seedling material.

5.3.2 Soil moisture and shade treatments

Eighty seedlings and 64 cuttings were randomly assigned to one of four soil water vapour content (SVWC) treatments, which were imposed in late August 2001. Full details of the SWVC treatments are outlined in chapter 3 (Section 3.3.3, page 64). In brief, four treatments were selected: saturated ($\approx 40\%$), 25%, 19%, and 13% SWVC. The soil moisture level in each pot was measured daily, and if the soil had reached the designated SWVC, the pot was watered until water flowed freely from the pot base.

One hundred and twenty seedlings were randomly assigned to one of four shade treatments in late August 2001. Full descriptions on the shade treatments are given in chapter 4 (Section 4.3.1.2, page 92). In brief, four treatments were selected: 0%, 30%, 50% and 90% shading. Plants were placed under wooden frames covered with shade cloth and watered regularly.

5.3.3 Insect collecting

Different collection methods were used to gather the two insect species. In early November 2001, broom twigs with *L. spartifoliella* pupae attached were collected from Burnham, New Zealand (43°36'23.7"S 172°42'58.9"E), and taken to the laboratory where they were placed into clear plastic containers. Containers were stored near windows to receive natural daylight. Newly emerged (<24 h) *L. spartifoliella* adults (50 males and 50 females) were collected from the containers, on the first morning of each experiment, and released into the centre of the glasshouse. *Arytainilla spartiophila* adults were collected from a broom field plot at Lincoln, using a beating tray, in the morning that each experiment began. Releases of 100 male and 100 female *A. spartiophila* adults were made into the centre of the glasshouse at the start of each experiment. The age of *L. spartifoliella* adults were known, however, the number of *A. spartiophila* adults released were higher to compensate for the uncertainty of age, as some adults would have been at the end of their lifespan when collected.

5.3.4 Experimental design

Six experiments were conducted in an enclosed 3m² glasshouse, which contained steel mesh benches (2.9*1 m, 0.8 m high) aligned along the walls, at Lincoln.

The first experiment tested the oviposition response of *L. spartifoliella* to plants from seedlings or cuttings from the four host-plant SWVC treatments. Plants (10 seedlings and 8 cuttings from each soil moisture treatment) were randomly placed onto benches in the glasshouse and *L. spartifoliella* adults were released from the centre of the glasshouse. After one week all plants and insects were removed.

Experiment two tested oviposition responses to plants presented under one light level, but previously grown under different levels of shading. Fifteen seedlings were taken from each of the four outdoor shade shelters and randomly placed into the glasshouse so all were under the same light level, ensuring only prior shade treatment of host-plants was assessed. *Leucoptera spartifoliella* adults were released in the glasshouse, and all plants and insects were removed one week later.

Experiment three tested oviposition responses to plants previously grown under

different light levels and presented for oviposition in those levels of shade. Two shade shelters per shade treatment were randomly positioned in the glasshouse and fifteen seedlings were taken from each of the four outdoor shade shelters and placed into the glasshouse shelters of the appropriate shade level (shelters were approximately 60 cm³, each containing 7 or 8 seedlings). The 0% shade shelters were covered with clear plastic, while the other shelters were covered with shade-cloth at the designated ratings (30%, 50% and 90% shade). Two ventilation/access slits (5 cm wide) were cut into the plastic/shade-cloth on all sides, for all shelters, thus allowing insects to fly into the shelters and onto plants to oviposit. *Leucoptera spartifoliella* adults were released in the glasshouse, and all plants and insects were removed one week later.

The fourth experiment was a repeat of experiment one, however, oviposition responses of *A. spartiophila* to plants from seedlings or cuttings from four host-plant SWVC treatments were tested on a new set of plants.

Experiment five was a repeat of experiment two, however, oviposition responses of *A. spartiophila* were tested on a new set of plants were used. Experiment six was a repeat of experiment three, but *A. spartiophila* oviposition was recorded using another set of plants.

5.3.5 Measurements

Total oviposition was recorded for the preference tests. For each plant, the number of *L. spartifoliella* eggs or *A. spartiophila* oviposition scars was recorded for all four experiments using a 10* magnification hand lens. As in the performance studies conducted in Chapters 3 and 4, *A. spartiophila* oviposition scars, rather than eggs, were counted.

5.3.6 Analysis

All analyses were based on data from surviving plants at the time of recording. Data were natural log transformed, as they were not normally distributed. A two-way ANOVA was used to assess *L. spartifoliella* oviposition (experiment 1), or *A. spartiophila* oviposition scars (experiment 4), against soil moisture treatment (saturation, 25%, 19% and 13% SWVC) and plant stock (seedlings or cuttings) (experiments 1 and 4). To measure the effects of plant variation due to prior shading

(0%, 30%, 50% and 90% shade) on *L. spartifoliella* oviposition (experiment 2) or *A. spartiophila* oviposition (experiment 5), a one-way ANOVA was conducted. Two-way ANOVA was used to assess *L. spartifoliella* oviposition (experiment 3) or *A. spartiophila* oviposition (experiment 6) against entering shelters of different light levels (0%, 30%, 50% and 90% shade) and between shade shelter replicates (two replicate shelters per shade treatment). All ANOVA's were followed by post-hoc (LSD) analyses to determine which treatments varied from one another. Linear and quadratic contrasts were also used to assess whether insect preference responses were linearly related to levels of host-plant stress, or whether both ends of the host-plant stress spectrum were different from the middle two host-plant stress treatments.

5.4 Results

As host-plant drought-stress increased, *L. spartifoliella* oviposition preference increased (experiment 1, Table 5.1, Figure 5.1a), while performance showed the opposite trend (Figure 5.1b).

When plants had been growing under shade, as prior shading increased, *L. spartifoliella* oviposition preference decreased (experiment 2, Table 5.1, Figure 5.2a). However, when plants were still growing under shade, *L. spartifoliella* oviposition preference demonstrated a weak quadratic relationship with host-plant shading, with oviposition preference highest under the 0% treatment, reduced under the 30% and reduced even further in the 50% treatment, and increased in the 90% treatment (experiment 3, Table 5.1, Figure 5.2b). *Leucoptera spartifoliella* performance was higher in the less-shaded treatments (Figure 5.3c).

Arytainilla spartiophila oviposition preference was significantly lower for host-plants grown under saturated soil conditions, and showed preference for plants grown from seed (experiment 4, Table 5.1, Figure 5.3a). *Arytainilla spartiophila* performance was significantly lower in the saturated treatment (Figure 5.3b).

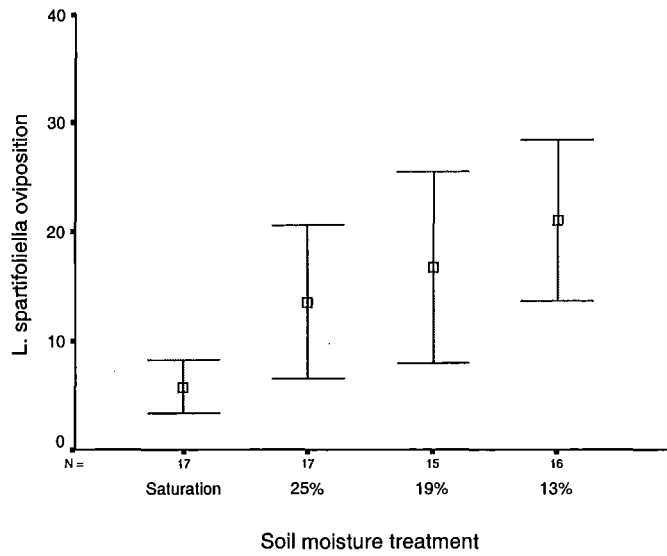
Arytainilla spartiophila did not show any oviposition preference for plants that had been growing under different levels of shading, nor was preference shown for plants

that were still under a particular shade level (experiment 5 and 6, Table 5.1, Figure 5.4a and b). *Arytainilla spartiophila* oviposition performance was not affected by levels of host-plant shading (Figure 5.4c).

Table 5.1: In experiments 1 and 4, the effects of soil water vapour content (SWVC) treatments (saturation ($\approx 40\%$), 25%, 19% and 13% SWVC), and whether plants from seed or cuttings, on insect oviposition preference was assessed. In experiments 2 and 5 the effects of shade pre-treatments (0%, 30%, 50% and 90%) on oviposition preference was assessed. In experiments 3 and 6 the effects of entering shade treatments (0%, 30%, 50% and 90%) and shade shelter replicates on oviposition preference was assessed. Degrees of freedom, F and P-values are given from ANOVA's, as well as the P-values for linear and quadratic contrasts.

Experiment no. and Insect species		ANOVA			P-value for linear and quadratic contrasts	
		df	F	P	Linear	Quadratic
Experiment 1	Seed/Cutting	1	1.81	0.173	----	----
<i>L. spartifoliella</i>	SWVC treatment	3	2.75	0.052	0.013	0.249
	Seed/Cutting*SWVC	3	1.26	0.293	----	----
Experiment 2						
<i>L. spartifoliella</i>	Shade pre-treatment	3	3.70	0.018	0.029	0.294
Experiment 3						
	Shade treatment	3	2.92	0.045	0.063	0.029
<i>L. spartifoliella</i>	Shelter replications	1	1.17	0.285	----	----
	Shade*Replication	3	4.77	0.006	----	----
Experiment 4						
	Seed/Cutting	1	4.22	0.045	----	----
<i>A. spartiophila</i>	SWVC treatment	3	3.56	0.020	0.004	0.206
	Seed/Cutting*SWVC	3	0.819	0.489	----	----
Experiment 5						
<i>A. spartiophila</i>	Shade pre-treatment	3	1.48	0.230	----	----
Experiment 6						
	Shade treatment	3	0.315	0.814	----	----
<i>A. spartiophila</i>	Shelter replications	1	0.004	0.952	----	----
	Shade*Replication	3	0.324	0.808	----	----

(a)



(b)

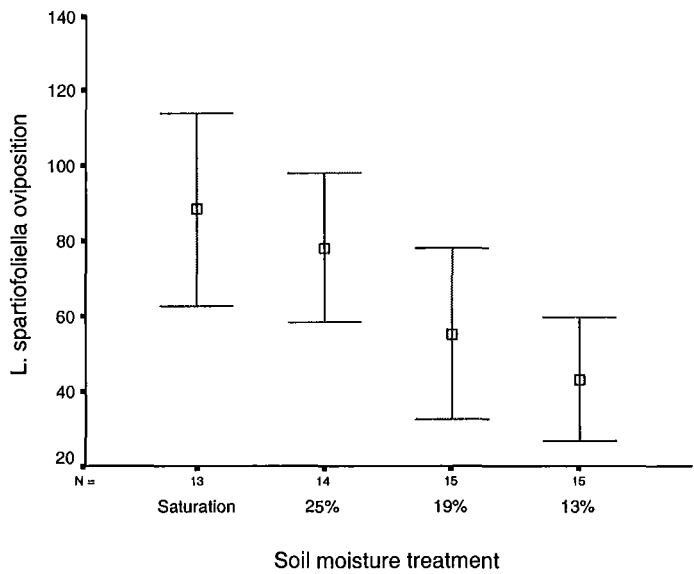
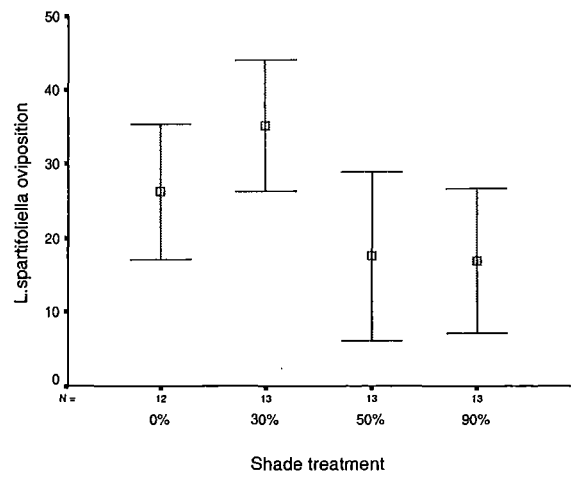
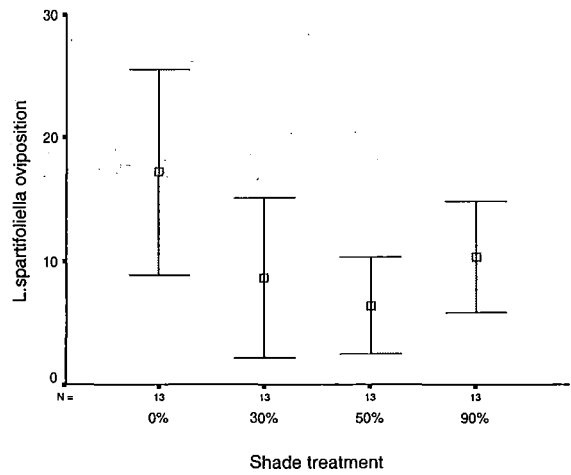


Figure 5.1: Mean ($\pm 95\%$ CI) *L. spartifoliella* oviposition per plant (a) preference for plants growing under four soil moisture treatments, where ovipositing females were allowed to select host-plants from any treatment, and (b) performance on plants under four soil moisture treatments, where ovipositing females were confined onto plants of a particular treatment. N is the number of plants used in the analyses, and saturation $\approx 40\%$ SWVC.

(a)



(b)



(c)

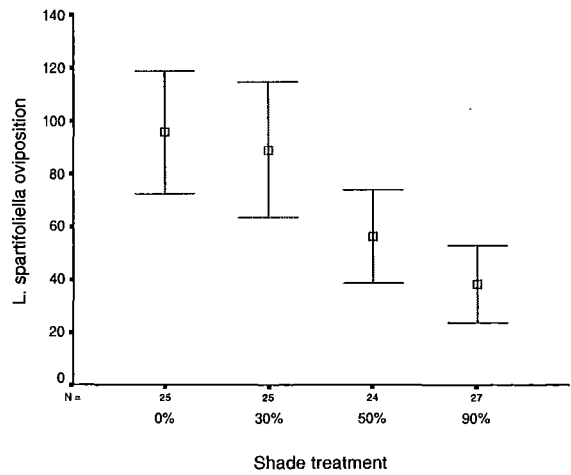
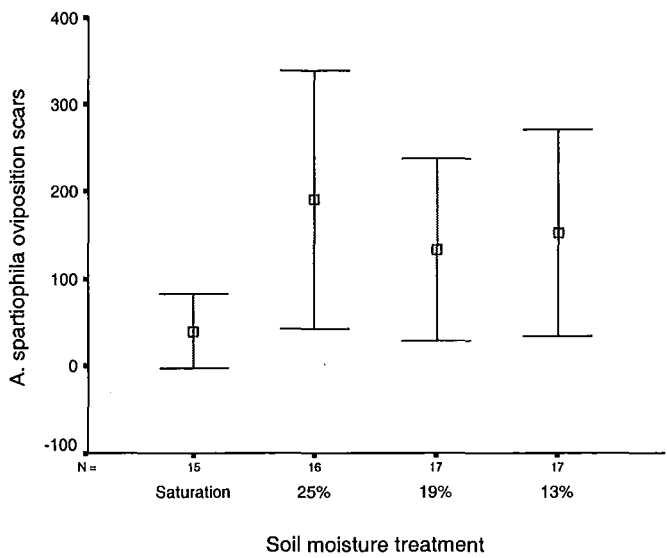


Figure 5.2: Mean ($\pm 95\%$ CI) *L. spartifoliella* oviposition per plant when ovipositing females were (a) given the choice of broom that had been grown under four shade levels before being placed into an open glasshouse ($R^2=0.05$), (b) given the choice of broom that had been grown under four shade levels and that were placed into an open glasshouse still under shade shelters ($R^2=0.09$), (c) confined onto plants under one of the four shade shelter treatments to assess performance ($R^2=0.06$). N is the number of plants used in the analyses.

(a)



(b)

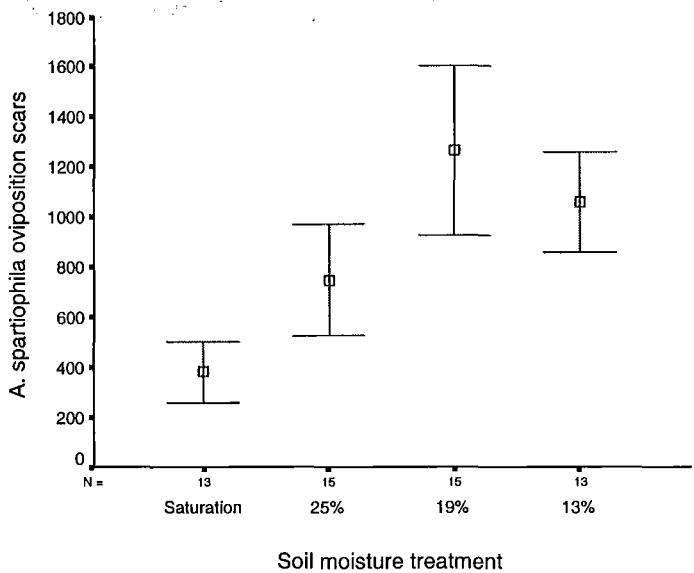


Figure 5.3: Mean ($\pm 95\%$ CI) number of *A. spartiophila* oviposition scars per plant (a) preference for plants growing under four soil moisture treatments, where ovipositing females were allowed to select host-plants from any treatment, and (b) performance on plants under four soil moisture treatments, where ovipositing females were confined onto plants of a particular treatment. N is the number of plants used in the analyses, and saturation $\approx 40\%$ SWVC.

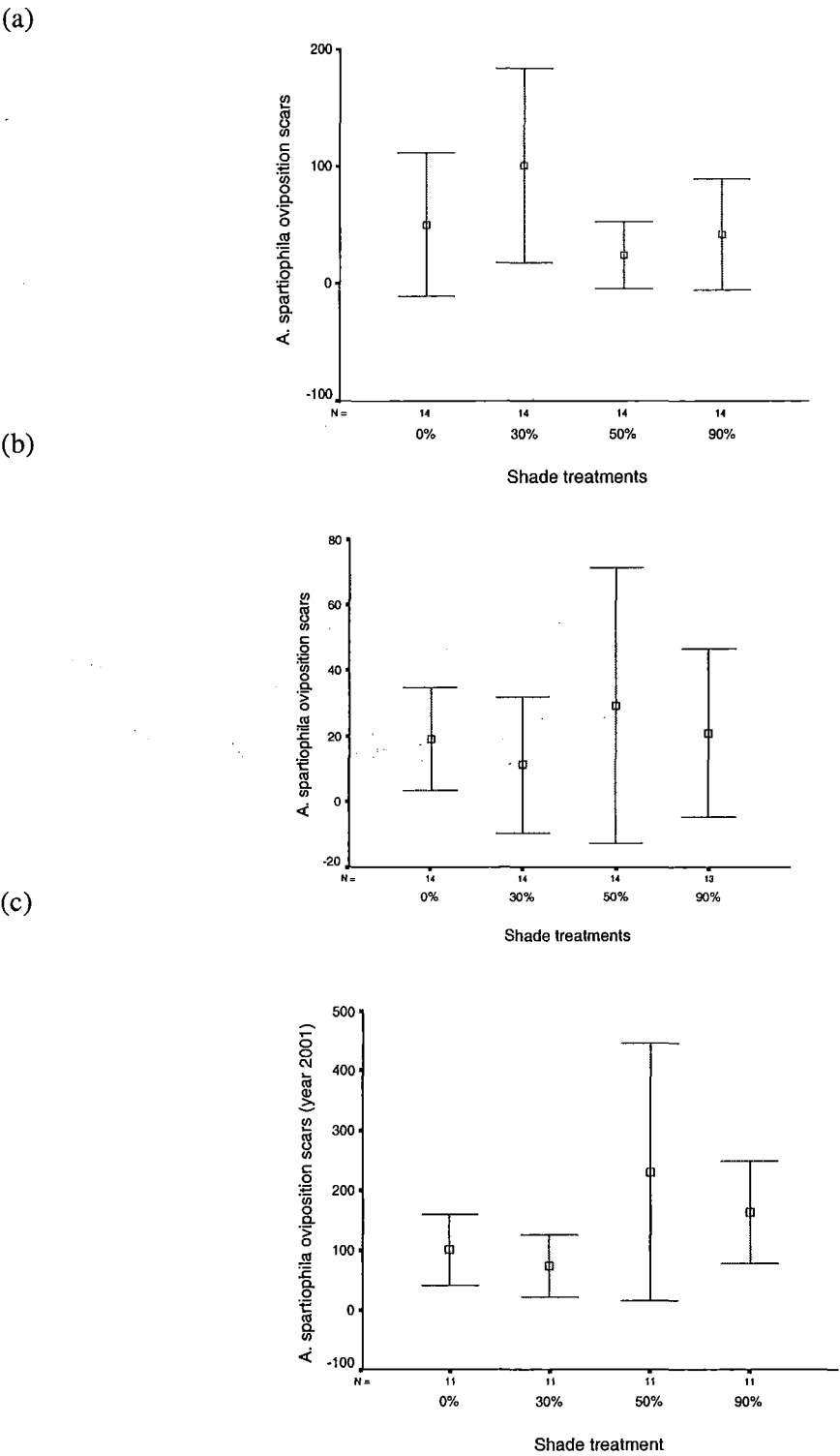


Figure 5.4: Mean ($\pm 95\%$ CI) *A. spartiophila* oviposition per plant when ovipositing females were (a) given the choice of broom that had been grown under four shade levels before being placed into an open glasshouse, (b) given the choice of broom that had been grown under four shade levels and that were placed into an open glasshouse still under shade shelters, (c) confined onto plants under one of the four shade shelter treatments to assess performance. N is the number of plants used in the analyses.

5.5 Discussion

5.5.1 Preference – performance relationships

The preference-performance hypothesis predicts oviposition (preference) should correspond to host-plant suitability for offspring development (performance) (Craig *et al.* 1989, Gonzáles & Gianoli 2003) if four criteria are met (a) the immature stage of an insect is relatively immobile, (b) the insect is monophagous, (c) oviposition by the female is associated with the juvenile feeding site, and (d) the successful development of juveniles is dependent on transient plant parts. Positive preference-performance relationships were found for both insect species over a range of host-plant moisture and shade gradients, suggesting that these four criteria are good predictive factors that need to be considered.

Under host-plant soil moisture levels, *L. spartifoliella* pupal abundance and insect survival in the performance study (Chapter 3, Section 3.4.2 page 73, Figure 3.3b and c page 74) showed the same trend as in oviposition preference, where performance and preference were higher towards plants that were growing in drier soil moistures. Evidently, *L. spartifoliella* oviposition performance was higher on broom growing in saturated conditions, displaying the opposite trend. This oviposition performance result was not as predicted, however, a similar result was found in another study by Björkman (2000), where it was concluded that the response of an early lifestage to environmentally induced changes in the host-plant may be opposite to that of a later life stage, as different lifestages are responding to different compounds. The opposing result might also be related to overcompensation by the female, laying more eggs on plants where progeny performance was lower, or even desiccation (Chapter 4, Section 4.5.1 page 110). This part of the study highlights the importance of comparing the right insect measures when determining preference-performance relationships, that is, oviposition preference with the offspring performance, as suggested by (Gonzáles & Gianoli 2003).

Under host-plant shading, a positive preference-performance relationship was found for *L. spartifoliella*. *Leucoptera spartifoliella* showed a preference for plants that had been growing in less-shaded environments (0% and 30% shade treatments). This matched the results from the performance study where no-choice oviposition and

subsequent abundance were significantly higher in the 0% and 30% shade treatments than in the 50% and 90% shade treatments, and larval survival was significantly lower under heavily shaded conditions (Chapter 4, Section 4.4.2 page 102). These less-shaded plants were growing vigorously, although plants in the 0% shade treatment were more wind damaged (Chapter 4, Section 4.4.1 page 98). A similar trend was expected for *L. spartifoliella* entering different shade levels to oviposit on plants. There was a clear preference for zero shading. However, preference for plants in the 90% shade treatment were higher than for those in the 50% shade treatment. This may have been an effect of differences in air movement caused by cooling fans. As the experiment was conducted in summer, where the glasshouse would easily reach temperatures that were too hot for *L. spartifoliella* to survive in (Gatehouse unpublished), cooling fans were required for most of the day. The 90% shade shelters were made of tightly woven mesh and may have greatly reduced the air-flow over plants compared with the less tightly woven mesh of the 50% and 30% shade shelters. Protection from this air-flow may have encouraged females to enter the protected 90% shade shelters.

Under host-plant soil moisture levels, *A. spartiophila* oviposition preference was significantly lower on host-plants growing under saturated soil conditions. This matched the performance results for oviposition, adult abundance and insect survival obtained in chapter 3 (Section 3.4.2 page 73, Figures 3.4a, b and c page 75). From the performance study it was suggested that *A. spartiophila* performance was higher on plants with greater feeding resources. This is further supported by *A. spartiophila* showing preference for seedlings over cuttings. The plants grown from seed had more abundant buds (personal observations) so presumably provided a greater food resource. Preference and performance were probably low on plants in the saturated treatments due to greatly reduced feeding resources and low total nitrogen levels.

Arytainilla spartiophila oviposition preference was not affected by the level of host-plant shading, and there were no difference in adult abundance or insect survival between shade treatments for this insect in the performance study (Chapter 4, Section 4.4.2 page 102).

The positive preference-performance relationships found in this study strengthen the predictions made in the no-choice performance studies in chapters 3 and 4 for *L. spartifoliella* and *A. spartiophila* populations under natural field conditions. Establishment and population growth of *L. spartifoliella* will be greater where broom is growing in drier environments and in the initial stage better on broom growing in full sun. *Arytainilla spartiophila* establishment and population growth will be enhanced on broom that is growing vigorously, in optimal soil moisture levels, and is not affected by levels of host-plant shading.

5.5.2 Oviposition on poor hosts

Preference for a particular host-plant quality and enhanced performance on these plants was clearly demonstrated in this study. However, oviposition and survival still occurred on less suitable plants. Several authors have given reasons why insects oviposit and survive on poor quality hosts (Ives 1978, Myers *et al.* 1981, Larsson & Ekbom 1995). If insects were selecting plants only of a particular quality, there would be an increase in cannibalism, competition for food, and possible local increase in the density of predators and parasites (Ives 1978). Myers *et al.* (1981) suggested oviposition and survival on poorer hosts might be a survival strategy for insects, preventing the extinction of the insect species, which would occur if they were too good at selecting only high quality host-plants. Larsson and Ekbom (1995) discussed the Novel Association Hypothesis and the Confusion Hypotheses, two different ecological views on why some insects lay eggs on host-plants unsuitable for their offspring. According to the Novel Association Hypothesis, females evolve a discriminative behaviour, which will enable them to avoid the detrimental plant. That is, this occurs when association between the insect and plant is new. The Confusion hypothesis suggests that non-suitable hosts may be retained as a host-plant, or the insect may even shift to a completely different plant type and delete the first from its diet. However, Larsson and Ekbom (1995) re-iterated that insects, which can discriminate, have little advantage over the insects that cannot discriminate when there are an abundance of poor quality hosts and time for oviposition is short.

5.5.3 Implications to biological control

Environments where both of these agents will prefer and perform better in have been identified. However, this may not correspond with environments where impact by

these agents will be greatest. Populations of the agent, density and growth of the host-plant all need to be considered when predicting impact. The establishment and population growth of *L. spartifoliella* will be greater where broom is growing in drier environments. Drier environments in this case should correspond with greatest impact as populations of *L. spartifoliella* will be highest, broom density will be lower and broom growth is not optimal. However, initially establishment and population growth of *L. spartifoliella* will be greater on broom that is growing in sunnier environments. But greatest impact is predicted in medium shaded habitats (e.g. Eucalyptus woodlands); as populations of *L. spartifoliella* should not be affected by host-plant shading levels once the population has reached a good size, densities of the plant are lower in semi-shaded environments (Downey & Smith 2000) and broom growth is not optimal. Impact by *A. spartiophila* might not be as great as *L. spartifoliella*, as the establishment and population growth will be enhanced on broom that is growing vigorously, in optimal soil moisture levels, and tolerance of herbivory is enhanced when light, water and nutrients are more abundant (Crawley 1983).

5.6 References

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Chapter 6

Does age matter? The relationship between a biological control agent, *Leucoptera spartifoliella*, and the age of its host-plant, *Cytisus scoparius* (Scotch broom).

6.1 Abstract

According to the Plant Age, Induced Defence, and Plant Vigour hypotheses, as plants age, insect populations change. The Plant Age Hypothesis predicts that because many plant growth characters change as plants age, insect populations may respond through preference or performance to associated variation with host plant age. The Induced Defence Hypothesis predicts that older plants are less suitable than younger plants for insect herbivores and the Plant Vigour Hypothesis predicts that some insect herbivores will prefer and perform better on vigorously growing, younger plants. Therefore, the abundance and impact of weed biological control agents may not be uniformly distributed across plant-stands of different ages. To determine whether host-plant age is a factor influencing *Leucoptera spartifoliella* pupal populations, broom (Scotch broom, *Cytisus scoparius*) infestations were surveyed in New Zealand and Australia. *Leucoptera spartifoliella* pupae were found on all ages of broom surveyed, from plants as young as two to 23 years old. No evidence was found to suggest that *L. spartifoliella* pupal populations were influenced directly by the age of host-plants. However, populations increased as the amount of available food resource (one-year-old shoots) increased in association with plant age, giving support to the Plant Age Hypothesis. As *Leucoptera spartifoliella* is found on all ages of broom the insect should be a valuable agent in broom management programs and the continual feeding damage inflicted by *L. spartifoliella* on plants from an early age may hasten plant senescence and death.

6.2 Introduction

Populations of insects may not be evenly distributed across host-plants of varying ages (Gimingham 1985, Karban 1987, Kearsley & Whitham 1989, Price 1991). The Plant Age Hypothesis is based on evidence that many plant traits change as plants age (e.g. leaf size, resin flow, phloem thickness), altering the susceptibility to different insect herbivores (Kearsley & Whitham 1989 and references within). Gimingham (1985) also reported that as plants age, different niches in the plant change in their suitability for different types of insects. For example, the building stage (plants of a young age) of *Calluna vulgaris* (Ericaceae) possesses the greatest amount of un lignified shoots, and thus there are likely to be greater numbers of certain psyllids and foliage-feeding lepidoptera on this particular stage of the plant (Gimingham 1985). According to this hypothesis insect populations vary depending on the age-related growth phase of the host-plant as it relates to the feeding style or habit of the insect herbivore. The Insect Defence Hypothesis predicts that plants attacked by insect herbivores early in their life will subsequently become more resistant to those herbivores (Karbon 1987), implying that older host-plants are often less suitable for insect herbivores. This will result in lower insect population densities on older plants. The Plant Vigour Hypothesis proposes that younger, vigorously growing plants are more favourable to certain kinds of insect herbivores, in particular those insects that are closely associated with the host-plant, such as gall-forming insects (Price 1991). For example, Price *et al.* (1987) found that younger, vigorously growing *Salix cinerea* (Salicaceae) plants produced longer shoots than older plants, and that these longer shoots had higher numbers of *Euura mucronata* (Hartig) Man. (Churchill) (Hym: Tenthredinidae). Even though these hypotheses are different and not mutually exclusive, all are consistent in suggesting that insect herbivore populations will be affected by host-plant age.

If host-plant age affects insect herbivores this may have consequences for the establishment and population growth of newly released biological control agents, or for the timing of control methods applied to weed infestations. For example, it is not uncommon to find weeds that have mass-germinated after widespread disturbance such as flooding or fire (Downey 2000), resulting in a weed infestation where plants are all of about the same age. If these young plants are not suited to a particular

biological control agent, then other control methods, such as chemical application, could be preferable. If, however, some of these plants survive such treatments, weed managers could then leave these plants until they reach an age more suitable for the biological control agents.

Biological control programs for Scotch broom (*Cytisus scoparius* (L.) Link., Fabaceae) have been implemented in several countries as this noxious weed poses a serious threat to agricultural economies and native ecosystems (Hosking *et al.* 1996). In New Zealand and Australia, *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) is the most widely established agent (Syrett *et al.* 1999). This insect possesses a univoltine lifespan (Parker 1964), with most of the lifecycle spent in the larval stage. The larvae inflict damage on broom by mining the one-year-old shoots, which can lead to stem weakening, and even plant death (Frick 1964, Scheele & Syrett 1987, Partridge & Harman unpublished).

Based on two of the three plant-age hypotheses (PVH, IDH), and from field observations, *L. spartifoliella* populations should be higher on younger plants. *Leucoptera spartifoliella* may be more abundant on younger plants as suggested by the PVH as (i) broom grows fastest and therefore produces its longest and most vigorous shoots when the plant is young and (ii) *L. spartifoliella* fits Price's (1991) definition of an insect closely associated with its host-plant, with females ovipositing near where the larvae feed, and eclosion occurring soon after oviposition. According to the IDH, as *L. spartifoliella* feeds on the young shoots of plants, this may then cause induced defensive compounds to accumulate as plants age, resulting in lower populations of *L. spartifoliella* on older attacked plants. Field workers looking for establishment of *L. spartifoliella* following releases have noted that mines seem to be more common on younger plants (P Hodge pers. comm), and Partridge and Harman (unpublished) have observed that *L. spartifoliella* mining damage can result in death of some young plants.

To determine if *L. spartifoliella* pupal populations might be higher on younger plants a field survey was conducted. *Leucoptera spartifoliella* pupal populations were measured in New Zealand and in Australia, from plants as young as two years to as old as 23 years.

6.3 Materials and Methods

6.3.1 Selection of broom infestations

Nine broom infestations from five sites were selected for study. Four sites were in New Zealand and one in Australia (Table 6.1). At Hanmer Springs three broom infestations were sampled, two in full sun on either side of a *Pinus radiata* (Pinaceae) plantation and the third within the plantation. At Hinds, two infestations were sampled, one in full sun and the other within an adjacent *P. radiata* plantation. At Burnham and the Port Hills infestations sampled were in full sun. At Krawarree (Australia) two infestations were surveyed, one in full sun, the other adjacent to it where broom was growing under a eucalyptus canopy.

Table 6.1: Locations of sites selected for sampling broom infestations.

Site Name	Longitude/Latitude	Location
Krawarree	35°48'S 149°40'E	110 km SE of Canberra, Australia
Burnham	43°36'24" S 172°42'59" E	29 km S of Christchurch, New Zealand
Hanmer Springs	42°35'36" S 172°34'56" E	12 km W of the Hanmer Springs turnoff, New Zealand
Hinds	44°00'56" S 171°32'35" E	20 km S of Ashburton, New Zealand
Port Hills	43°43'21" S 172°42'59" E	Cavendish Scenic Reserve, Christchurch, New Zealand

6.3.2 Selection of plant age groups

Plant age groups were selected in the same manner as outlined in chapter 4 (Section 4.3.2.2, page 96). Plants were broadly age-defined by selecting ten plants from each of the building, reproductive, and senescent growth stages (as described by Smith 1994). Plants were then cut down so that age could be more accurately recorded by counting annual growth rings.

6.3.3 Timing of sampling

Surveys were conducted during November and December 2001, at which time more than 20 *L. spartifoliella* pupae could be found in less than two minutes of searching in all infestations.

6.3.4 Sampling procedure

Several morphological characters were recorded from each sampled plant as outlined in chapter 4 (Section 4.3.2.4, page 97). On plants measured in the field these included height, stem diameter and age (annual growth rings). A branch was taken back to the laboratory from each plant and divided into categories: dead material, old growth (woody and non-pliable), one-year-old shoot growth (pliable and green, suitable for larval mining), new growth (soft, flush, leafy material), flowers, and seed-pods. Material from each category for each sample was weighed and the number of *L. spartifoliella* pupae recorded. The percentage of material that was grouped as dead, old growth, one-year-old shoot growth and new growth was calculated for each branch sample. The total length of all pieces of the one-year-old shoot growth (feeding resource available to *L. spartifoliella*) was measured and used to calculate *L. spartifoliella* density per unit length one-year-old shoot growth (total of pupae from whole branch divided by the length of the one-year-old shoot growth) for each branch sample.

6.3.5 Analysis

At some infestations not all 10 plants for each growth stage could be found so a total of 240 plant samples (out of a possible 270) were used for analysis. Data for *L. spartifoliella* total numbers of pupae per branch, plant age (annual growth rings) and total length of one-year-old shoot growth were not normally distributed, and therefore were natural log transformed before analysis.

Multiple linear regression, using backward selection, was used to assess whether *L. spartifoliella* pupal populations (total pupae per branch) are affected by the age of host-plants (annual growth rings) and/or the total length of one-year-old shoot growth (available feeding resource). Site and shading were used as covariates. Smith (1994) described how several broom morphological characters changed with age. Therefore, the amount of available feeding resource was regressed against plant age (annual growth rings), with site and shading used as covariates.

6.4 Results

The plant data collected during this study across the five sites, with and without shading, and divided into the three growth stages sampled are presented in Table 6.2. Height and stem diameter increased, and the percentage of new growth decreased as growth stage increased. The percentage of one-year-old shoot growth tended to be higher in the reproductive stage and the percentage of dead material tended to be higher in the senescent stage. The percentage of old growth did not differ between the three growth stages.

Table 6.2: Mean plant height, stem diameter and percentage of dead, old, one-year-old shoot and new growth recorded from three growth stages of broom from nine infestations.

Site	Growth stage	Height (cm)	Stem diameter (mm)	% Dead material	% Old growth	% 1-yr-old growth	% New growth
Krawarree (sun)	Building	120	11.8	2.1	38.4	45.4	14.1
	Reproductive	238	45.9	1.1	46.2	49.9	2.3
	Senescent	335	56.5	4.2	52.4	41.2	2.3
Krawarree (shade)	Building	98	9.7	1.7	37.1	47.4	13.8
	Reproductive	211	21.7	2.2	47.7	44.7	5.4
	Senescent	---	---	---	---	---	---
Hinds (sun)	Building	107	10.3	1.7	30.2	14.8	53.4
	Reproductive	277	43.8	3.8	39.4	38.9	17.9
	Senescent	362	82.0	30.5	42.9	20.8	5.8
Hinds (shade)	Building	132	11.4	5.6	36.2	39.0	19.2
	Reproductive	164	23.7	8.0	39.8	38.8	13.5
	Senescent	226	46.9	37.3	36.6	22.0	4.1
Burnham (sun)	Building	139	11.4	12.9	37.2	22.1	27.8
	Reproductive	223	30.5	11.0	37.5	22.9	28.6
	Senescent	310	73.7	34.0	37.0	15.6	13.4
Hanmer Springs (sun1)	Building	77	9.5	6.0	48.1	35.7	10.1
	Reproductive	211	30.7	9.1	35.4	49.5	5.9
	Senescent	280	44.9	13.3	47.8	37.8	1.0
Hanmer Springs (shade)	Building	143	9.7	12.1	54.0	24.1	9.9
	Reproductive	322	26.6	14.9	47.0	27.3	10.8
	Senescent	260	25.6	49.4	35.8	10.6	4.2
Hanmer Springs (sun2)	Building	116	7.0	12.8	45.0	29.7	12.5
	Reproductive	233	30.4	8.6	51.7	33.7	6.1
	Senescent	318	45.5	18.7	47.4	28.5	5.4
Port Hills (sun)	Building	96	11.5	0.0	32.2	20.6	47.2
	Reproductive	154	40.5	0.6	40.7	47.1	11.6
	Senescent	177	55.8	6.2	40.6	34.8	18.5

--- No plants in the senescent stage could be found in the infestation at Krawarree in the shade.

Leucoptera spartifoliella pupal populations were not affected by host-plant age, although populations were affected by the total length of one-year-old shoot growth (available feeding resource) and by site (Table 6.3). Pupal populations were higher on plants with greater one-year-old shoot growth (Figure 6.1). Populations were significantly higher at Burnham than at the other sites, significantly lower at Krawarree than at the other sites, and populations at Hinds were significantly lower than those recorded at Hanmer Springs (Figure 6.2).

The amount of one-year-old shoot growth was affected by plant age, site, shade and site x shade interactions (Table 6.4). The amount of one-year-old shoot growth peaked when plants were about 10 years old (Figure 6.3). The amount of one-year-old shoot growth was significantly lower at Hinds than at the other sites. At Krawarree, the amount of one-year-old shoot growth was significantly lower on plants in the shade than on plants growing in the sun. The opposite trend occurred at Hinds.

Table 6.3: The effects of one-year-old shoot growth (available feeding resource), plant age, site, shading, and one-year-old shoot growth by site interaction on *L. spartifoliella* pupal populations. Degrees of freedom, mean square, F and P-values are from a multiple linear regression.

	df	Analysis		
		MS	F	P
One-year-old shoot growth	1	43.5	46.8	0.000
Plant age	1	2.50	2.69	0.102
Site	4	6.50	6.99	0.000
Shading	1	0.125	0.134	0.715
Site*1-yr-old shoot growth	4	2.43	2.62	0.036

Table 6.4: The effects of plant age, site, shading, and site by shade interactions on the amount of one-year-old shoot growth (available feeding resource). Degrees of freedom, mean square, F and P-values are from a multiple linear regression.

	df	Analysis		
		MS	F	P
Plant age	1	58.7	115	0.000
Site	4	2.70	5.27	0.000
Shading	1	5.15	10.0	0.002
Site*Shade	2	4.53	8.84	0.000

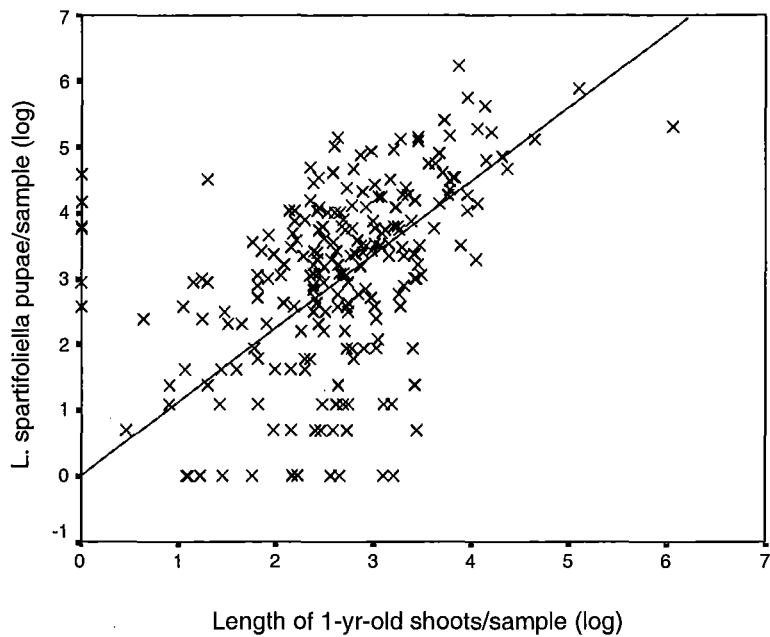


Figure 6.1: The relationship between *L. spartifoliella* pupal populations and one-year-old shoot growth (the amount of available feeding resource), $R^2=0.852$. Both *L. spartifoliella* pupal populations and one-year-old shoot growth were natural log transformed.

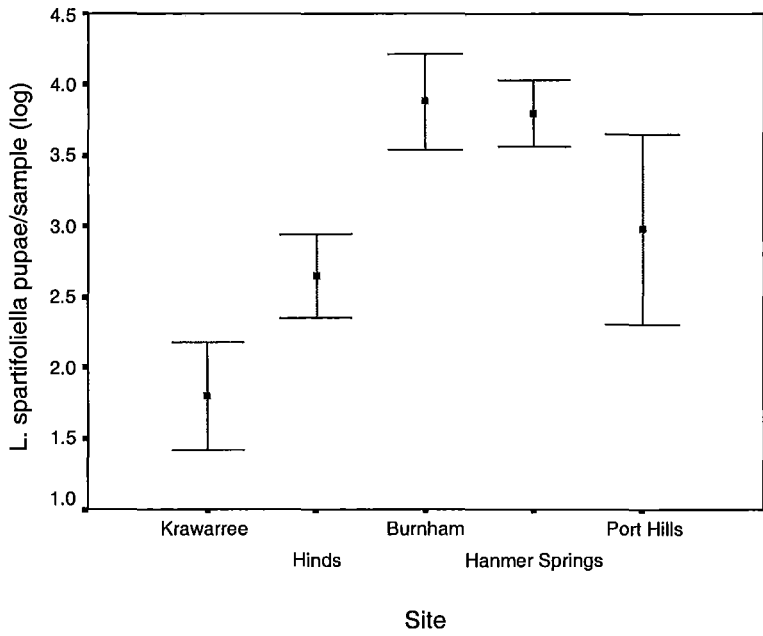


Figure 6.2: Mean total *L. spartifoliella* pupae per branch (natural log transformed $\pm 95\%$ CI's), across five sites.

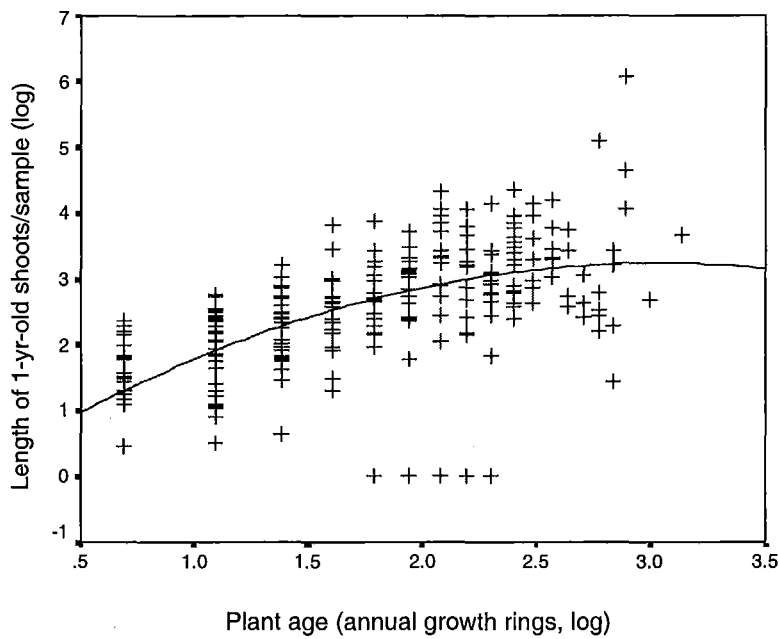


Figure 6.3: The \log_e - \log_e relationship between one-year-old shoot growth (the amount of available feeding resource) and plant age, $R^2=0.921$.

6.5 Discussion

Leucoptera spartifoliella pupal populations were not affected directly by the age of host-plants. Populations were influenced by the total length of one-year-old shoots (amount of available feeding resource) and site. Populations increased as the amount of available feeding resource increased. The amount of available feeding resource was however, affected by the age of plants, peaking when plants were about 10 years old, lending support to the Plant Age Hypothesis.

Zanuncio *et al.* (2001) found a weak effect of plant age for *Stenalcidia grosica* Schaus (Lep: Geometridae) on *Eucalyptus grandis* (Myrtaceae), with abundance of the leaf-feeder higher on mid-aged plants. However, Zanuncio *et al.* (2001) found no significant correlations between plant age and insect abundance were found for several other leaf-feeding Lepidopterans.

Significant variations in *L. spartifoliella* pupal populations were found within and between sites (Figure 6.2), which may be attributed to outbreaks, the time frame for agent establishment and the size of broom infestations. Variation between sites may be related to *L. spartifoliella* outbreaks, as Partridge and Harman (unpublished) found that these occurred at approximately 5-year intervals, were synchronous within stands, but not between locations. Variation between sites may also be related to the time since agent release or establishment. The Krawarree site showed significantly lower *L. spartifoliella* pupal populations compared with the other sites. *Leucoptera spartifoliella* was released at the first Krawarree site in 1993, only 8 years before this survey was conducted (Wapshere & Hosking 1993). Some of the New Zealand sites may have been infested with *L. spartifoliella* for up to 50 years or more (Syrett *et al.* 1999). The size of broom infestations may also explain the variation between sites. In New Zealand, higher *L. spartifoliella* pupal densities were found at Hanmer Springs and Burnham than at Hinds and the Port Hills. At Hinds, infestations were small, with only a few other small infestations in the vicinity. In contrast, the Hanmer Springs region is heavily infested with broom.

Laboratory studies described in chapters 3 and 4 confirmed that eggs are laid on plants as young as six months old, and that these eggs can develop into adult moths. The seedling stage was omitted from these field surveys to avoid misinterpretation of results. If seedlings (approximately 0-2 years) had been surveyed there would have been a high probability of zero pupal recordings on some plants that were too young for oviposition 10 months earlier. By selecting plants greater than two years all plants had been available for *L. spartifoliella* oviposition and subsequent development to pupae.

Though the age of broom does not affect *L. spartifoliella* pupal populations directly, populations are affected via the amount of feeding resource, which peaks on plants at about 10 years of age. Therefore the establishment and population growth of *L. spartifoliella* should be greatest where there is an abundance of feeding resources, on plants that are about 10 years of age. As *L. spartifoliella* is found on all ages of broom, the continual pressure from the mining of stems may lead to an increase in plant senescence and death.

6.6 References

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Chapter 7

Research conclusions: the impact of host-plant stress on the performance of two insect biological control agents of broom (*Cytisus scoparius*).

It was stated at the beginning of this thesis that ‘investigating the interactions between the environment, plants and insect herbivores should (a) improve understanding of the way herbivore behaviour and performance varies with host-plant quality, and (b) aid weed management strategies by identifying environments where the performance and impact of agents may be greatest’. In chapter 2, from more than 200 studies assessed it was shown that environmental stress can affect the performance of insect herbivores. Predictions were made regarding insect performance across five feeding-guilds, in relation to host-plant stress (Table 2, page 35). It was predicted that in the broom system populations of *Leucoptera spartifoliella* Hübner (Lep: Lyonetiidae) will be greatest on broom that is growing under drought-stress or in full sun and that *Arytainilla spartiophila* Förster (Hem: Psyllidae) populations will be greatest on broom growing under optimal moisture levels (Chapters 3, 4 and 5).

Three questions were posed at the beginning of the thesis. The first was ‘How do morphological and physiological differences in the host-plant affect insect performance?’ In chapters 3 and 4 it was shown that when broom was grown under varying levels of soil moisture or shade stress, many growth parameters and chemical concentrations of nutrients in broom were altered. For example, reduced growth depletes the availability of feeding resource, and reduction in plant nitrogen affects nutrition of phytophagous insects, thus influencing the performance of the two test insects, *L. spartifoliella* and *A. spartiophila*. This finding is supported by Mattson and Haack 1987 who stated that plants growing under environmental stress may exhibit a variety of morphological and physiological changes such as increased plant nitrogen which then affects the performance of insect herbivores. The second question was ‘How does insect performance vary depending on the intensity of the stress the host-plant is under, or the type of stress encountered?’ Chapters 3, 4 and 5 demonstrated the importance of host-plant stress intensity and stress type on insect performance and

preference. For example, in chapter 3 numbers of *A. spartiophila* oviposition scars, abundance and survival of the psyllid were not linearly related to soil moisture. Performance was lowest in the wettest soil treatment, highest in one of the middle treatments, and lower again in the driest soil treatment. In addition, it was shown in chapter 2 that the type of stress was important. This finding supports that of Waring and Cobb 1992 who found that insects responded differently to moisture stress and nutrient stress. The third question was 'Are the insect responses to host-plant stress the same for all insect herbivore species?' It was clear from this study that not all insects respond to host-plant stress in the same manner. While *L. spartifoliella* displayed a linear relationship with increased soil moisture deficit, *A. spartiophila* displayed a quadratic relationship.

In chapter 2, a literature review was conducted, involving more than 200 studies that covered 12 stress types and five insect feeding-guilds to test three hypotheses: the Plant Stress Hypothesis (PSH), the Plant Vigour (PSH) and the Insect Performance Hypothesis (IPH). It was found that they were too generalised in their current state and this limited their ability to predict insect response towards host-plant stress. However, their contribution to insect performance – plant stress theory has been crucial. These hypotheses could be modified by more rigorously defining the insect feeding-guilds used, and including stress types so as to improve the level of predicability.

In order to clearly demonstrate the relationships between insect performance and plant stress a number of recommendations can be made based on experience gained through this study. It is recommended that future studies should (a) include at least three levels of stress intensity, (b) measure plant morphological and physiological changes between treatments, (c) impose stress for a sufficiently long period and demonstrate that plants have been stressed, (d) conduct trials in both laboratory and field environments, and (e) consider the effects of different stress application methods. These ideas have been supported by Larsson and Björkman 1993 and Huberty and Denno 1994. Larsson and Björkman (1993) stated that plant stress – insect herbivory hypotheses were based on limited number of studies that used different experimental approaches. Huberty and Denno (1994) found that the application of moisture stress, continuous or intermittent, affected how plants and insect herbivores responded.

Paynter *et al.* (2003) proposed that biological control agents for broom should be sought that both reduce plant fecundity and therefore seedling density, and that defoliate established plants. With reduced competition, other more desirable plant species should establish in the gaps beneath broom stands. The three biological control agents released for broom in New Zealand and Australia are *L. spartifoliella*, *A. spartiophila* and *Bruchidius villosus* (F.) (Col: Chrysomelidae) and between them they should result in reduced broom fecundity and density and increased competitiveness of other more desirable plant species. *Bruchidius villosus* is a seed-feeder, so this agent should reduce plant fecundity directly. *Leucoptera spartifoliella* causes twig weakening and twig death (Syrett & Harman 1995) and *A. spartiophila* feeds on flower and leaf buds (Syrett 1991), so should reduce plant biomass. Damage to stems is particularly important on broom as the plant relies heavily on its green stems for photosynthesis. In another woody, leguminous system, Hoffman and Moran (1998) reported on the success of using three agents in reducing the density of the tree *Sesbania punicea* (Fabaceae). The three agents were *Trichapion lativentre* (Bèguin-Billecocq) (Col: Apionidae) a flower-feeder, *Rhyssomatus marginatus* Fahraeus (Col: Curculionidae) a seed-feeder, and *Neodiplogrammus quadrivittatus* (Olivier) (Col: Curculionidae) a stem-borer. This example highlights how three insects feeding on different parts of the plant can lead to increased pressure on the host-plant, yet competition between the insect species is negligible. This is very promising for the broom system, as three agents that belong in different feeding-guilds have been released in New Zealand and Australia.

The performance of the three agents introduced for biological control of broom will vary across the environments that broom occupies. It is predicted that the greatest impact to broom might occur in drier and shaded habitats. Sheppard *et al.* (2002) reported that the biological control of broom is likely to be most effective at sites where broom regeneration is slow. In this study it was found that broom growth was slower in drier and shaded conditions (Chapters 3 and 4). Populations of *L. spartifoliella* and *A. spartiophila* were also higher in drier environments (Chapter 3). Although the laboratory-based shade studies found higher populations of *L. spartifoliella* in the sun, in the field when populations were high there were no differences between shaded and non-shaded environments (Chapter 4).

Though host-plant quality affects insect herbivore performance, as shown here with *L. spartifoliella* and *A. spartiophila* (Chapters 3, 4, and 5) the performance of their parasitoids and predators may not be affected similarly. Teder and Tammaru (2002) showed that the numerical response of insect herbivore populations to a change in plant quality should exceed the response of the parasitoids, so that in the short term, a favorable change in the quality of the host should lead to increased population densities of the herbivores and lower total parasitism rates. No specialist parasitoids of *L. spartifoliella* and *A. spartiophila* have been recorded in New Zealand or Australia. However, one of *L. spartifoliella*'s natural enemies, the parasitic wasp *Tetrastichus evonmellae* (Bouché) (Hym: Eulophidae), was accidentally introduced into the USA (Frick 1964), explaining the possible poorer performance of *L. spartifoliella* there. *Leucoptera spartifoliella* and *A. spartiophila* will encounter generalist predators and parasitoids in their introduced range, but their populations may not be severely impacted if Teder & Tammaru's (2002) conclusions are applicable here.

In conclusion, environmental stress clearly affects plant morphology and physiology and in turn the performance of insect herbivores. How insect herbivores are affected depends on the type of stress encountered and the feed-guild of the insect herbivore. It is predicted that the greatest impact to broom might occur in drier and shaded habitats.

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