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Title: Biotic Barriers to Colonizing New Hosts by the Cinnabar Moth *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae).

Abstract approved:_____
Peter B. McEvoy

The cinnabar moth (*Tyria jacobaeae* (L.), Lepidoptera: Arctiidae) is an icon in population ecology and biological control that has recently lost its shine based on evidence that (1) it is less effective than alternatives (such as the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae) for controlling ragwort *Senecio jacobaea* L. (Asteraceae), (2) it eats nontarget plant species (including arrowleaf ragwort *Senecio triangularis* Hook. (Asteraceae), a native North American wildflower), and potentially harms the animals that depend on these native plant species, and (3) it carries a disease (caused by a host-specific microsporidian *Nosema tyriae*). This presents us with an opportunity to study whether poor nutrition and disease might constrain colonization of new hosts by this phytophagous insect and thereby mitigate risk of biological control to nontarget plant species.

We evaluated the interactions within a tritrophic system composed of the cinnabar moth (herbivorous insect), its Old and New World Host plant species, and its entomopathogen (*Nosema tyriae*) both at the individual and population levels in a controlled environment. Chapter one concentrates on the two trophic (herbivore-host plant) interactions, addresses the importance of the preference and performance relationship, and the reasons why we might observe a weak relationship between preference and performance. Performance was measured both by vital rates and by population dynamic parameters, and we conclude that the projected population growth rate of the cinnabar

moth population is the best indicator of host suitability. We found a positive correlation between preference and performance in the cinnabar moth (*Tyria jacobaea*) on Old World and New World host plants. The second chapter incorporates the third trophic level, the pathogen *Nosema tyriae*, and measures the individual and interacting effects of pathogen dose and host plant species on the performance of the cinnabar moth. It concludes that all cinnabar moth vital rates (rates of growth, development, survival, and reproduction) decrease with the increasing dose of pathogen (*Nosema*) spores. Vital rates generally were lower on the New World host S. triangularis compared to Old World host *S. jacobaea*. The projected population growth rates of cinnabar moth populations were more sensitive to low infection dose in cinnabar moth populations on the New World host *S. triangularis* compared to the Old World host *S. jacobaea*. At high pathogen doses, the effect of the pathogen was so overwhelming that no effect of host could be expressed. In conclusion, we observed a strong positive correlation between preference and performance of the cinnabar moth on the New World and Old World test plants. In the most successful new host-herbivore association, the cinnabar moth was more vulnerable to the impact of the natural enemy on New compared to Old host plant species.

Biotic Barriers to Colonizing New Hosts by the Cinnabar Moth *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae)

by
Evrim Karacetin

A DISSERTATION

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APPROVED:

Major Professor, representing Environmental Science

Director of the Environmental Sciences Program

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature authorizes release of my thesis to any reader upon request.

Evrim Karacetin, Author

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This thesis wouldn't have come to life if I did not have the support of the friends, family and colleagues. My deepest and most sincere thanks to all of you.

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One day, in the greenhouse, my larvae were all dying. I was deeply grieving as my babies were sick, (of course I was only worried about the larvae, not about my work!). I got contacted Denny Bruck, and he identified the disease that my babies were suffering from. After that event, the whole course of the thesis changed, and Denny was always there, always very supportive. Thank you Denny.

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CONTRIBUTION OF AUTHORS

Peter B. McEvoy helped with experimental design, analysis, interpretation, synthesis and writing. Denny Bruck helped with design and data collection of Chapter 3.

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CHAPTER 1: GENERAL INTRODUCTION

The cinnabar moth, (*Tyria jacobaeae* (L.), Lepidoptera: Arctiidae) was introduced to North America for biological control of tansy ragwort, *Senecio jacobaea*. The insect was first released in California in 1959 (Frick and Holloway 1964) and in Oregon and Washington in 1960 (Isaacson 1973), and later redistributed to Idaho and Montana (Coombs et al. 2004). Although it is an icon in population ecology and biological control, it has recently lost its shine based on evidence that (1) it is less effective than alternatives (such as the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae) for controlling ragwort *Senecio jacobaea* L. (Asteraceae). (2) it eats nontarget plant species (including arrowleaf ragwort *Senecio triangularis* Hook. (Asteraceae), a native North American wildflower), and potentially harms the animals that depend on these native plant species, and (3) it carries a disease (caused by a host-specific microsporidian *Nosema tyriae*). In addition, the collapse of the primary host resource (McEvoy et al. 1991), the accumulation of competitors (McEvoy et al. 1993, McEvoy and Coombs 1999) and natural enemies (Hawkes 1973, Dempster 1975, Myers and Campbell 1976) of this herbivore, have all conspired to reduce cinnabar moth abundance on ragwort in the Pacific Northwest and raise the possibility that life might be better for the cinnabar moth on New World Hosts. Host specificity tests and observations conducted prior to its introduction confirm that the cinnabar moth had a broad fundamental host range including species from four genera including *Senecio*, *Packera*, *Erechtites,* and *Tussilago* (Cameron 1935, Bucher and Harris 1961, Tinney et al. 1998a). The field use of one native North American species *S. triangularis* has already been widely reported (Diehl and McEvoy 1990, McEvoy and Coombs 2000, Pemberton 2000, Fuller et al. 2002). Host specificity tests including all the native *Senecio* species currently exposed to the cinnabar moth have not been conducted. In addition the impact of *Nosema tyriae*, an accidentally introduced (Hawkes 1973) microsporidian pathogen of the cinnabar moth *Tyria jacobaeae* (Canning et al. 1999), on the cinnabar moth has not been investigated. This study system presented us with an opportunity to study whether poor nutrition and disease might constrain

colonization of new hosts by this phytophagous insect and thereby mitigate risk of herbivory by a biological control agent to nontarget plant species.

In this thesis we choose to study the interaction of Oregon populations of the cinnabar moth with its the Old World Hosts *Senecio jacobaea* and *S. cineraria* and the New World hosts (*Senecio triangularis, S. integerrimus, P. flettii, P. bolanderi, P. pseudaurea* and *P. subnuda*) and its most influential enemy, the microsporidian *Nosema tyriae*, to test whether these factors might mediate host shifts by the cinnabar moth.

The first chapter asks whether the preference-performance relationship constrains the colonization of new host plants. First, we measure host acceptability and suitability of the North American native plants (related to ragwort *Senecio jacobaea*) that have been exposed to the cinnabar moth in Oregon. Second, we contrast the preferences of mothers and offspring and identify possible conflicts. Third, we contrast the preference-performance relationship in new and old insect-plant associations. Fourth we test whether qualitative description of the preference-performance relationship varied with operational measures of demographic performance ranging from the vital rates of growth, development, survival, and reproduction to projected population growth rates. Finally, we ask whether preference and performance are related to the nutritional quality of the host plant measured by nitrogen, carbon, water content and carbon to nitrogen ratio.

The objectives of the second chapter are to estimate the independent and interacting effects of two factors, host plant species and pathogen dose, on cinnabar moth demography and population growth rates. We measure the direct influence of the two hosts on the cinnabar moth, and the direct effect of the entomopathogen in cinnabar moth populations feeding on each of the two host plant species. We further estimate the prevalence and severity of disease at natural field populations of the cinnabar moth and discuss if *Nosema* can mitigate the risk of non-target impacts by a weed biological control organism.

The following two chapters deal with fundamental aspects of our understanding of the three trophic interactions from individual to population levels of organization. Finally, chapter four concludes with a recap of the key results from these studies, and a look to the future.

CHAPTER 2: PREFERENCE AND PERFORMANCE OF THE CINNABAR MOTH, *Tyria jacobaeae*, ON OLD AND NEW HOST PLANTS

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ABSTRACT

Theories of foraging behavior predict that parents and offspring should choose resources that optimize the performance of the offspring. Yet empirical studies of insect-plant associations do not always find the expected strong, positive correlation between preference and performance. A weak relationship between preference and performance may be related to the young evolutionary-age of consumer-resource association, conflict between parents and offspring, limited sensory capacity of the consumer, the level of learning by the consumer, variation in the abiotic and biotic environment, or differences among investigations in how preference and performance are measured. A weak relationship between preference and performance may constrain the colonization of new host plant species by phytophagous insects if suitable hosts are not acceptable or acceptable hosts are unsuitable. Here we report results of a laboratory study on the relationship between preference and performance in North American populations of the cinnabar moth (*Tyria jacobaeae,* Lepidoptera: Arctiidae) that are feeding on old hosts native to Europe and new hosts native to North America. The cinnabar moth was introduced to North America from Europe for biological control of tansy ragwort, *Senecio jacobaea* (Asterales: Asteraceae), and feeding observed on native plants related to ragwort may cause environmental harm. To discover whether the preference-performance relationship constrains the colonization of new host plants, we measured the preference and performance of both adults and larvae of the cinnabar moth on six new world test plant species (*Senecio triangularis, S. integerrimus, Packera bolanderi, P. flettii, P. pseudaurea, P. subnuda*) and two old world test plant species (*S. jacobaea, S. cineraria*) in the greenhouse and lab. We estimated suitability of hosts in a Life Table Response Experiment (LTRE), measuring the effect of host plant species on cinnabar moth vital rates and using a matrix model to project how changes in vital rates lead to changes in cinnabar moth population growth.

We found that the cinnabar moth could complete development from egg to pupal stage on all the test plant species. We refined estimates of host suitability by showing that

among the test plants, only four species, *S. jacobaea*, *S. triangularis*, *P. flettii,* and *P. bolanderi,* were suitable for cinnabar moth population growth (the finite rate of increase $\lambda > 1$) and *S. integerrimus*, *P. pseudaurea* and *S. cineraria* were unsuitable, predicting population decline ($\lambda < 1$). We found that host suitability increased with nitrogen concentration and decreased with increasing values of the carbon to nitrogen ratio in the foliage of plant species. We found a positive, rank correlation between preference and performance encompassing both Old World and New World hosts. We conclude that the preference-performance relationship posed little obstacle to colonization of new hosts by the cinnabar moth.

INTRODUCTION

Theories of optimal foraging behavior predict that consumers should chose resources that maximize fitness (Remington 1952, Wiklund 1975, Thompson 1988b, Nylin and Janz 1993, Janz and Nylin 1997, Berdegue et al. 1998, Scheirs et al. 2000, Scheirs and De Bruyn 2002). Yet empirical studies of insect-plant associations reveal a surprising amount of variation in the form and strength of the relationship between preferences (adult oviposition or larval feeding) and the offspring performance (variously measured as growth, development, survival, reproduction, population growth, and fitness) (Remington 1952, Wiklund 1975, Chew 1977, Thompson 1988b, Singer et al. 1994, Mayhew 1997, 2001, Martin et al. 2005). This variation has been related to the evolutionary age of consumer-resource association (old associations may be more finely-tuned than new ones) (Chew 1977, Wiklund 1984, Bernays and Graham 1988b, Singer et al. 1993), conflict between parents and offspring (the choices that optimize parental fitness may differ from those that optimize offspring fitness) (Godfray et al. 1991, Godfray 1995), the sensory capacity of the consumer (specialists may be superior to generalists in using visual, chemical, tactile, and other cues to detect resources) (Wiklund 1984, Bernays and Graham 1988b, Mayhew 1997), the level of consumer learning (Egas and Sabelis 2001), the environment (like other aspects of interaction norms, the relationship between preference and performance may vary with environment) (Cronin et al. 2001, Hellmann 2002, Stacey et al. 2003), or variation in the operational measures of preference and performance that are favored by particular investigators (Thompson 1988b, Stacey et al. 2003). Coordination of consumer preference and performance is required for effective host plant use by insects especially those invading new environments. An insect invading a new area might select unsuitable hosts while ignoring suitable ones. Here we investigate if the preference and performance correlation of the cinnabar moth differs on its new and old world hosts nearly 50 years after its introduction to North America.

Causes of variation in the preference – performance relationship

A strong positive relationship between preference and performance may develop as consumers evolve with their hosts (Wiklund 1975, Chew 1977, Thompson 1988b, Singer et al. 1993). Wicklund (1975) found no positive relationship between preference and performance in new insect-plant associations formed when alien plant species related to the conventional host invaded the environment of the butterfly *Papilio machaon* in the Baltic Region, but he found a strong, positive relationship between preference and performance in old host associations. The more time a consumer spends with its host, the more likely preference patterns converge on performance patterns. Changes in preference can evolve quite quickly. Singer and Thomas et al. (1993) found *Euphydryas editha* evolved increased preference for a relatively abundant alien plant species *Plantago lanceolata* and decreased preference for a relatively rare native host plant species *Colinsia parviflora* over the time span of a decade. Evolution may be required to fine-tune the preference-performance relationship in new associations, but the time scale for evolution can be short.

Host selection mechanisms and the sensory capacity of adult and larval stage might influence preference and performance patterns (Mayhew 1997, 1998, Bernays 2001, Mayhew 2001). A female or a mobile larva must be able to search out and recognize its specific host growing in diverse vegetation (Schoonhoven et al. 1998). Visual cues used by insects to find hosts include leaf morphology (Ladner and Altizer 2005), plant height (Wiklund 1984), leaf thickness, and structure of leaves (Schoonhoven et al. 1998, Speight et al. 1999). The chemical composition of a plant, mainly the secondary metabolites, is a key determinant of host use by insects (Barbosa 1988, Schoonhoven et al. 1998, Hadacek 2002, Honda et al. 2004, Poykko et al. 2005, Wheeler and Ordung 2005, Johnson and Gregory 2006). Secondary metabolites may function as attractants or repellents that guide host choices or as toxins or digestibility reducers in determining performance of insects. Numerous studies show that a female insect has the capacity to select plants of higher nutritional quality, by selecting plants containing higher levels of nitrogen (Barros and Zucoloto 1999; Lower, Kirshenbaum et al. 2003; Kerpel, Soprano et al. 2006). Nutritional

suitability is a necessary, but not a sufficient condition for host use. Preference and performance of insects is reported to be low in some insects despite high levels of nutrients in plant tissues (Dukas and Ellner 1993, Bernays 2001). Insects use variety of sensory mechanisms to detect these visual and chemical cues. For example, olfactory sensilla (odor receptors) that are placed in the antennae (Bernays and Chapman 1994, Hallem et al. 2006), or contact chemoreceptors (taste sensor) located on mouthparts, tarsi and ovipositor (Bernays and Chapman 1994). Number and sensitivity of these receptors might also influence the host selection mechanisms which in turn influence the preference and performance patterns.

The relationship between preference and performance may be influenced by conflicting interests of parents and offspring (Godfray et al. 1991, Roitberg and Mangel 1993, Godfray 1995). The theory of parent-offspring conflict predicts that parents increase the number of progeny by increasing clutch size. However, if resources in the environment (host plant quantity and quality) are not be sufficient for all the eggs laid on the plant, there may be strong competition among offspring (Roitberg and Mangel 1993, Godfray 1995). In insects with sedentary larvae, the larva is confined to plants chosen by its mother (Jermy 1984). In insects with mobile larvae like the cinnabar moth, the larva is free to leave the host plant in search of more suitable hosts and thereby reduce the risk of intraspecific competition (Roitberg and Mangel 1993). Thus choices by both adults and larvae become important for understanding and predicting preference - performance relationships.

Preference and performance are traits of an insect-plant interaction, and the expression of traits generally varies across a range of environments (Thompson 1988a, Thompson 1988b). The closed, homogeneous environment of the laboratory does not match the open, heterogeneous environment of the field. A host of influential factors operating in the field (suboptimal abiotic conditions, low food quality, competitors, natural enemies, etc.) are generally altered or absent in the laboratory (which generally features optimal abiotic conditions, high food quality, absence of competitors and natural enemies). Thus, different environmental and experimental settings might yield to different estimates of the acceptability and suitability of hosts.

The relationship between preference and performance is influenced by how these variables are measured. There has been an extensive debate in the literature on how to measure consumer preferences (Singer and Thomas 1988) and fitness (Ariew and Lewontin 2004). Some studies define preference by using the order in which plants are selected (Singer and Thomas 1988), whereas some others use the number of eggs laid on a plant being compared (Macel et al. 2002). Likewise performance of offspring can be defined in many different ways, including survival of immature stages (egg, larva, pupa), larval growth rate (for all or for later larval stages) and efficiency as indicated by nutritional indices (Scriber and Slansky 1981a), pupal mass, adult fecundity and fertility, and adult longevity (Thompson 1988b). A problem of interpretation arises when qualitative description of the preference and performance relationship varies among different operational measures of performance (Thompson 1988b).

The preference and performance relationship is influenced by consumer learning behavior with experience resulting in improved reproductive success (adaptive learning) (Egas and Sabelis 2001). For example grasshopper nymphs enhance their growth rate by learning (Dukas and Bernays 2000), parasitic wasps enhance offspring survival by learning to avoid superparasitism (Van Alpen and Visser 1990), and an herbivorous mite, *Tetranychus urticae* improves its fitness by learning of host preference (Egas and Sabelis 2001). There might be a lack of correlation between preference and performance when invading herbivores first encounter novel species, yet through adaptive learning a positive or negative correlation might develop.

For these and other reasons, we may fail to find a simple, positive relationship between consumer preference and performance.

Consequences of variation in the preference-performance relationship

The preference-performance relationship is crucial to understanding, predicting, and managing insect-plant interactions. The functional form of this relationship is useful for defining the necessary and sufficient conditions for a host shift and adaptive radiation

of insects on host plants (Futuyma 1983, Diehl and Bush 1984, Bush 1992); conserving native insect species that gain the ability to use alien plants while losing the ability to use the native hosts that managers are working to restore (Singer et al. 1993); controlling pests of crops that acquire new pests as crop area increases (Strong 1979); and for controlling invasive plants species through classical biological control, the use of introduced natural enemies to control alien pests (McEvoy 1996). The preference - performance relationship is an important component of host specificity, the chief criterion scientists and regulators use for assessing the risks of biological control introductions. Host specificity tests measure whether potential agents have the ability to feed, oviposit or develop on plants other than the target weed (Day 1999).

Objectives of this study

Here we report results of a lab study on the relationship between preference and performance in the cinnabar moth. This insect was first introduced to North America from Europe in 1959 for biological control of ragwort, *Senecio jacobaea*, a plant species invading North America from Europe. Since its introduction into North America (NA), the cinnabar moth has formed new associations with North American plant species while retaining old associations originating in Europe (Diehl and McEvoy 1990, McEvoy and Coombs 2000, Fuller et al. 2002, McEvoy unpublished data). First, we measure host acceptability and suitability of the NA native plants (related to ragwort *Senecio jacobaea*) that have been exposed to the cinnabar moth in Oregon. Second, we contrast the preferences of mothers and offspring and identify possible conflicts. Third, we contrast the preference-performance relationship in new vs. old insect-plant associations. A fourth objective was to test whether qualitative description of the preference-performance relationship varied with operational measures of demographic performance ranging from the vital rates of growth, development, survival, and reproduction to projected population growth rates. Finally, we asked whether preference and performance are related to the nutritional quality of the host plant measured by nitrogen, carbon, water content and carbon to nitrogen ratio.

STUDY ORGANISMS

Cinnabar Moth, *Tyria jacobaeae*

The cinnabar moth, *Tyria jacobaeae* was introduced to North America for biological control of tansy ragwort, *Senecio jacobaea*. The insect, was released in California in 1959 (Frick and Holloway 1964) and in Oregon in 1960 (Isaacson 1973), and then redistributed to Idaho and Montana (Coombs et al. 2004).

The cinnabar moth is univoltine. The life cycle includes 8 stages including egg, 5 larval stages, pupa, and adult. Moths emerge from the overwintering pupal stage in spring, mate, and females lay eggs in late spring to midsummer in clusters (mean of 30 to 40 eggs per cluster) (Dempster 1982) on the undersides of basal leaves of rosettes. Eggs hatch about 4 to 20 days depending on the temperature (Rose 1978). Larval stages 1 through 4 each last 4-5 days, while the fifth larval stage lasts 5-9 days before pupation. Larval survival (L1 to P) varies depending on abiotic and biotic environmental conditions, ranging from 67% to 90%. The cinnabar moth larvae occur in clusters and due to its gregarious feeding behavior, food resources can be easily depleted. The cinnabar moth has a highly mobile late instar stage: it has been recorded that fully grown starving larvae are capable of several hundred meters of dispersal when food is in short supply (Dempster 1970b, 1982). Hence, in a host specificity tests for insects like the cinnabar moth, larval as well as adult preference tests are essential. About 50 % of over wintering pupae reach adulthood (Isaacson 1973, van der Meijden 1973, Dempster 1975, Rose 1978).

Host specificity tests and observations conducted prior to its introduction confirm that the cinnabar moth had a broad host range including species from four genera including *Senecio*, *Packera*, *Erechtites,* and *Tussilago* (Cameron 1935, Bucher and Harris 1961, Tinney et al. 1998a). The strong reduction in ragwort abundance achieved by biological control (McEvoy et al. 1991) has led to food limitation in cinnabar moth populations and may increase the likelihood that cinnabar moth will shift from Old World to New World test plant plants.

Test Plants

The *Senecio* genus is currently being revised and the genus *Packera* (= aureoid *Senecio* complex) has been elevated from Senecio as a separate monophyletic group (Bain and Jansen 1995, Bain and Walker 1995). There are 70 New World *Senecio* and 62 *Packera* species distributed in North America (USDA 2007). Fuller et al. (2002) evaluated the risk of host use for ten native *Senecio* and ten native *Packera* species occurring in Western Oregon (Table A1.1 for the list of other native *Senecio* and *Packera* species) (Figure 2.1). They concluded that three New World *Senecio* species *(Senecio hyrdophilus, S. integerrimus* and *S. triangularis)* and six New World *Packera* species (*Packera bolanderi, P. cana, P. subnuda, P. flettii, P. macounii,* and *P. pseudaurea*) species have likely been exposed to the cinnabar moth west of the Cascade Mountains in Oregon. They further show that three of these species (*S. triangularis, P. pseudaurea and P. subnuda*) are used in the field; oviposition is observed on *P. pseudaurea* and *S. triangularis*, larval feeding is observed on *P. pseudaurea* and *P. subnuda* and completing the life cycle is observed on *S. triangularis*. The field use of *S. triangularis* has already been widely reported (Diehl and McEvoy 1990, McEvoy and Coombs 2000, Pemberton 2000, Fuller et al. 2002).

In this paper we selected 6 out of these 9 species exposed to the cinnabar moth, excluding three candidate species (*S. hydrophilus, P. cana, P. macounii*) due to difficulties in locating and/or culturing these plants. Our final test plant list included two Old World species (*S. jacobaea*, *S. cineraria*) originally from cinnabar moth's native home in Europe and six New World species (*P. bolanderi, P. subnuda, P. flettii, P. pseudaurea, S. integerrimus and S. triangularis*) native to the cinnabar moth's adopted home in North America (Table 2.1). We obtained test plant material for preference and performance tests either directly from the field or from plants grown in the greenhouse (for the details of the location of each test plant see Table A1.2).

We use the following terms when referring to test plants. “Target” plant refers to *S. jacobaea*, the species targeted for biological control. “Nontarget plant” refers to all other test plants, the plants of ecological or economic value that are potentially at risk from attack by the cinnabar moth. “Old World Test Plants” are *S. jacobaea* and *S. cineraria*; the rest of the test plants are referred as “New World Test Plants”.

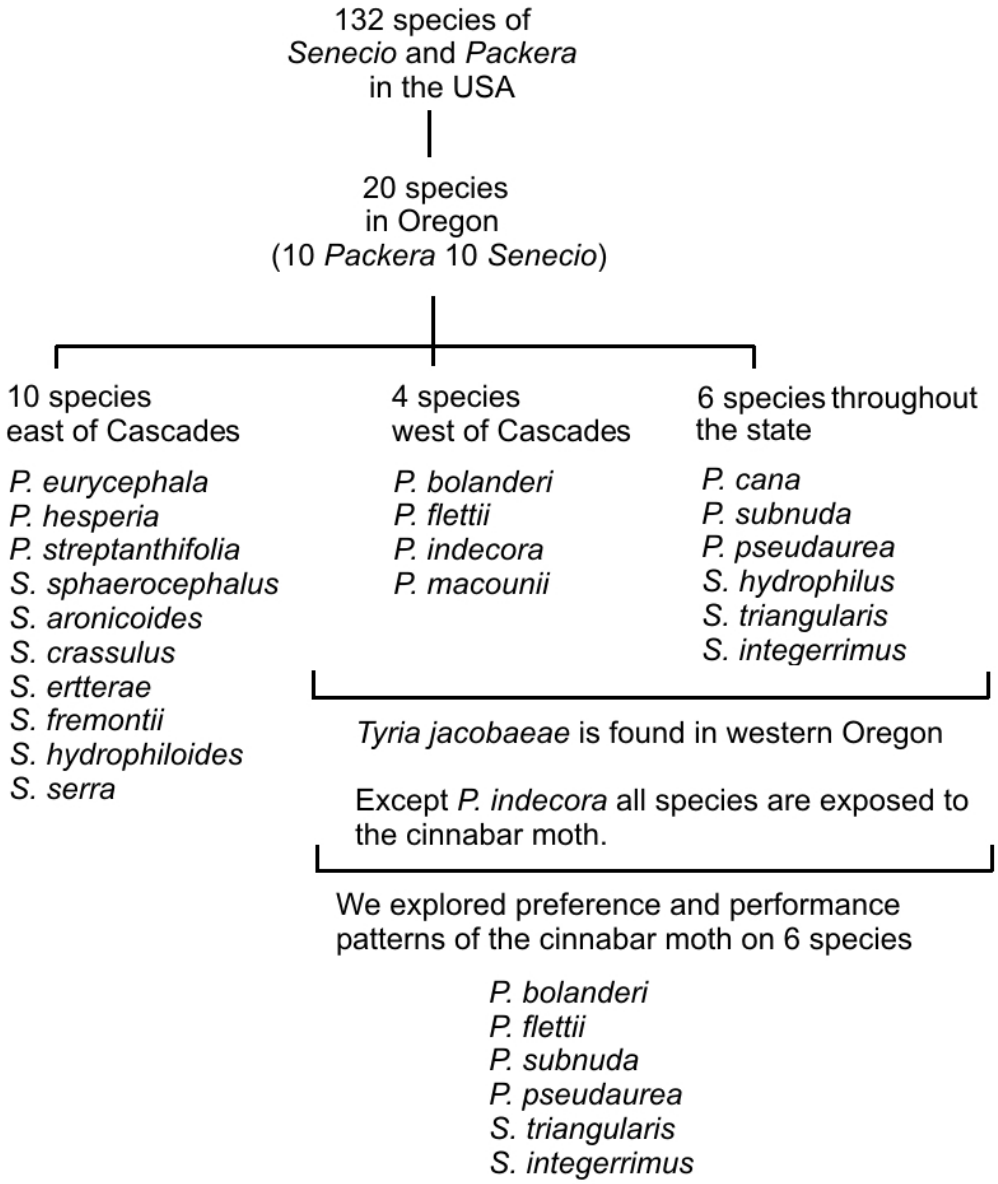


Figure 2.1: A decision tree for selecting candidate study plants. Number of NA *Senecio* and *Packera* species in the US, Oregon, eastern Oregon, western Oregon and both (Fuller et al. 2002).

METHODS

Our study was designed and carried out as a Life Table Response Experiment (LTRE) combining several experiments to estimate treatment effects on cinnabar moth vital rates and a matrix population model to translate changes in vital rates into changes in population growth.

Experimental design and environmental conditions

We tested consumer preference and performance on Old World and New World plants in four sets of experiments referred to as (1) Adult oviposition preference, (2) Larval feeding preference, (3) Larval demographic performance, and (4) Adult demographic performance (Figure 2.2). Our fifth experiment compared nutritional quality of the plants.

We conducted all these experiments in a semi-controlled environment of the greenhouse, or strictly-controlled growth chamber or lab environment (Conditions described in Figure 2.2).

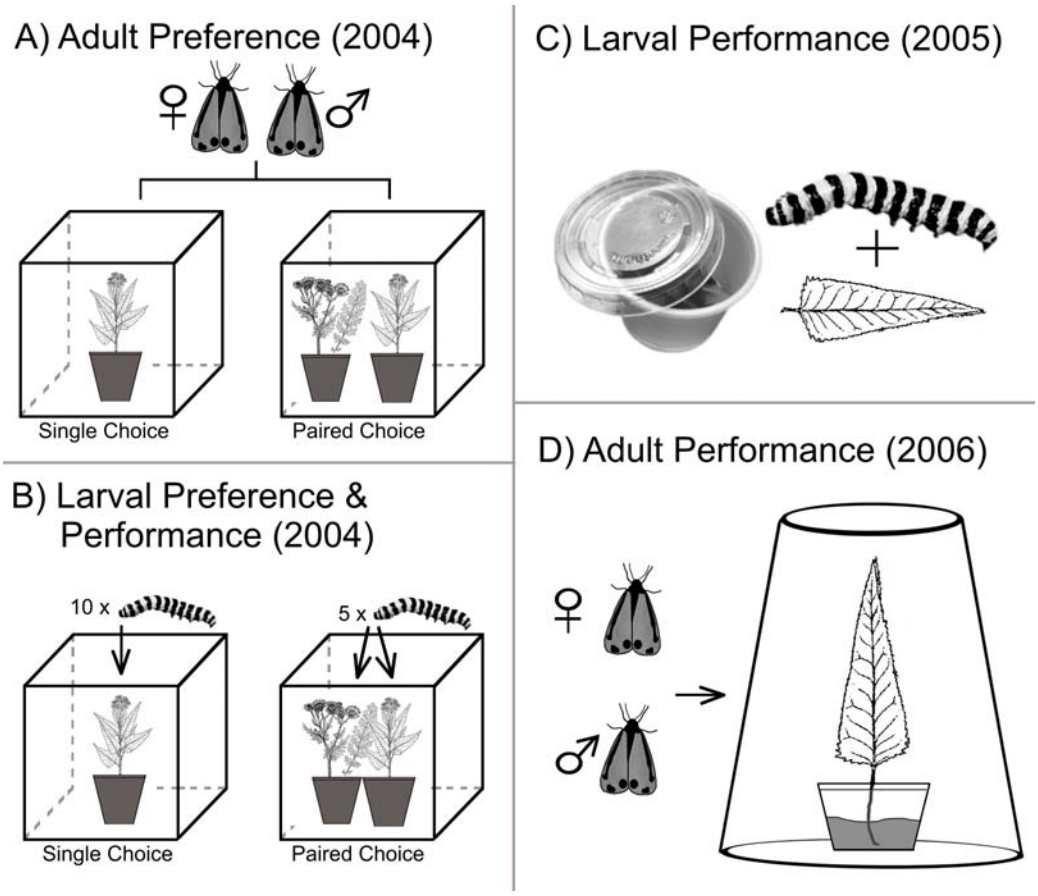


Figure 2.2: Overview of the designs for four experiments. A) 2004 Adult preference experiments were carried out in a semi-natural greenhouse environment (Light 16h ~22°C, Dark 8h 12°C, humidity ~ 70-100%). We used cages as experimental units. The cages were prepared with PVC pipes (0.5 m x 0.5 x 0.5m = 125 m3), covered with standard cage covers (mesh size = 1mm). We placed one male and female into the cages and measured adult preference parameters. B) 2004 Larval Preference and Performance experiments were in a greenhouse as in the adult preference experiments. We placed 10 larvae on test plants, 10 on one in single choice case and 5 on each in paired choice case. We used the same conditions and experimental design as the adult preference experiments. C) 2005 Larval Preference experiments were carried out in a strictly controlled incubator environment (Hoffman Model SG30-110V) (Light 16h 25°C , Dark 8h 15°C, humidity ~ 90%). We reared larvae individually in 1 oz. plastic cups in which we placed test plants and a piece of cotton (to maintain balanced humidity). We changed the plants and the cups daily. D) Adult Performance experiments were carried out in our lab (Light 16h 18°C, Dark 8h 18°C, humidity ~ 90%). We placed one female and one male into 32 oz. transparent containers along with a leaf placed on a 1 oz. water cup.

Table 2.1: The list of the test plant species and their acronyms. How the test plant species were used in each of six experiments is signified by the following acronyms (A_S: Adult oviposition single-choice, A_P: Adult oviposition paired-choice, L_P: Larval Preference, L_{PER04}: Larval Performance 2004, L_{PER05}: Larval Performance 2005, PU_P: Pupal Emergence, Adult Fertility and Fecundity Experiments. 0 = Not Assessed, 1 = Tested..

Test Plants	Symbol	Origin	A_S	A_P	L_P	L_{PER04}	L_{PER05}	PU_P
Senecio jacobaea (L.)	SEJA	Europe (Old World)	1	1	1	1	1	1
S. cineraria (DC.)	SECI	Europe	1	1	1	1	0	0
Senecio triangularis (Hook.)	SETR	North America (New World)	1	1	1	1	1	1
Senecio integerrimus (Nutt.)	SEIN	North America	1	1	1	1	1	0
Packera flettii (Weig.)	PAFL	North America	1	1	1	1	1	0
Packera bolanderi (Gray)	PABO	North America	1	1	1	1	1	0
Packera pseudaurea (Rydb.)	PAPS	North America	1	1	1	1	0	0
Packera subnuda (Buek)	PASU	North America	0	0	0	0	1	0

Experiment 1: Adult oviposition preference

Experimental design

We tested the oviposition preference of female cinnabar moths on seven test plant species offered both alone (single-choice tests) and in combination with the target host *S. jacobaea* (paired-choice test) in the greenhouse in 2004 (Figure 2.2A). We collected moths from the field (Table A1.3), randomly placed one female and at least one male, into each cage, and observed oviposition behaviors.

We predicted that a female would be more likely to lay eggs on a nontarget plant if that were her only choice, less likely to lay on a nontarget plant if the choices included the target host. Single-choice experiments tested the response of the cinnabar moths exposed to only one individual of the non-target test plant species in a single pot per cage (Figure 2.2A). Paired-choice experiments tested the response to a test plant species in the presence of the target host. We offered one pot with a single individual of the target species *S. jacobaea* and one pot with a single individual of a non-target test plant species in each cage (Figure 2.2A).

We observed each individual female daily for the entire oviposition period (5 to 10 days). We inspected the cages each day until the female died, and we collected and photographed leaves with eggs to record oviposition, number of eggs laid, number of egg batches and number of eggs per batch on each plant. Other explanatory variables that we recorded were the age, developmental stage (vegetative or flowering) and stem height of every plant in the experiment, the origin of the cinnabar moths and the collection date of the moths (Table A1.3 for the details on collection date and origin of the moths). We recorded additional explanatory variables signifying foliage quantity and quality: leaf-length, leaf-width, and leaf-surface area of the leaves that received eggs. We collected leaves, and measured leaf length, widest leaf width and later we photographed the leaves with a ruler for scale, and we measured the surface area by using the software Image Pro

(5.0) (2003 Media Cybernetics). These foliage quality variables turned out to be highly correlated, so we used only one leaf size variable (leaf-width) in the analyses.

We stored eggs collected during this experiment in incubator (Light 16h 25°C, Dark 8h 15°C, humidity ~ 90%) before using them in larval preference and performance experiments.

Statistical analyses

In single-choice tests, we compared number of eggs laid, number of egg batches, and number of eggs per batch on each plant using one-way ANOVAs with Tukey's Multiple Adjustment, adding other explanatory variables into the model. We applied a square root transformation to each response variable to meet the normality assumption. We wanted to know which component of oviposition, number of eggs batches or number of eggs per batch, contributed more to variation in number of eggs laid.

In paired-choice tests, we compared three response variables (the number of eggs laid, number of egg batches, and number of eggs per batch) target and on non-target test plants within each cage using a log ratio test (one sided; Ho: log Ratio of response variable on nontarget over the response variable on target = 0). We added a small constant (=1) to calculate the log in cases of zero values. We also ran a nonparametric Wilcoxon rank sum test (one sided; H_o: parameters on nontarget = parameters on target). Both parametric and non-parametric tests yielded the same results (Table A1.4). We sought to estimate how the odds of 'straying off-target' varied among different nontarget test plants paired with the single target host.

We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for statistical analyses.

Experiment 2: Larval preference

Experimental design

We used a paired-choice design to estimate the larval preferences for host plant species (Figure 2.2B). Into each cage we placed one pot of target and one pot of assigned non-target test plant species so that plants touched each other and each larva could migrate from one plant to the other. We placed 10 larvae into each cage, 5 on target and 5 on the randomly assigned nontarget test plant near the point where plants were touching and allowed larvae to redistribute themselves over time. To reduce the possibility that one larva's choice was influenced by the choices of other larvae, we place larvae on plants sequentially one-by-one rather than simultaneously (we waited approximately 10 seconds).

We recorded the number of larvae on each plant at daily intervals until larvae started to pupate. Pupation started earlier in some cages compared to the others (the earliest 18 days, the latest 40 days). To standardize the observation period for comparisons among treatment cages, we used only the first 18 days' data in our analysis.

Controlling for insect disease

In the 2004 preference and performance experiments, larvae contracted a disease in some of cages (for number of cages with and without detectable spores see Table A1.5). Denny Bruck (USDA-ARS Horticultural Crops Research Lab, Corvallis, OR) identified the pathogen *Nosema tyriae* by following the methods of Canning et al. (1999). Since disease might influence cinnabar moth behavior and demography in our experiments, we recorded the disease status of insects in each cage by collecting pupae, storing them in the refrigerator at 4-10ºC under complete darkness, and later dissecting and examining these pupae for *Nosema* spores under 40X magnification using a light microscope (Leica DM1000). In addition to pupae, we also stored dead larvae collected from the cages in the freezer (-80ºC) and examined them for the presence of spores. If we detected spores in a pupa or in a dead larva, we classified the whole population in that cage as 'with detectable

spores' (regardless of the presence of spore-free individuals in the same cage); if we detected no spores in individuals from one cage, we classified that cage as 'without detectable spores'; and finally if none of the individuals from that cage was available for disease testing (no individuals were recovered because all were dead AND lost), we classified that cages as 'not assessed' (N/A). Therefore, we had 3 different disease categories (With spores, Without spores, N/A) for all of the experiments. In the paired-choice preference experiment, we could access the status of each cage (See Table A1.5 for the number of cages under each category). We included disease as an explanatory variable in the statistical analysis, and if we found no detectable effect of disease, we concluded the response of cinnabar moth to host plant species was unaffected by the pathogen. In 2005 we excluded disease by using disease-free insects.

Statistical analysis

We compared the preference of larvae among the plants by the time spent on each plant over the observation period of 18 days, which was the number of days to the first observation of pupation across all cages. We first calculated the daily log ratio of number of larvae on nontarget over target, and we further estimated the mean log ratio value for each cage (by taking the average 18 observations taken over 18 days). We used a one-way ANOVA to test for significant effects of test plant and disease on the mean log ratio of larvae number. We further applied a log ratio test to test whether the number of larvae on each nontarget was higher than the number of larvae on target (one sided H_A: log Ratio >0; Ho: log Ratio = 0). We added a small constant (= 1) to be able to calculate the log values in cases of zero.

We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for all of the statistical analyses.

Experiment 3: Larval demographic performance

We estimated the performance of larvae on different test plant species by measuring survival, developmental time, and pupal mass. These experiments were carried out in 2004 (greenhouse experiments) (Figure 2.2B) and 2005 (incubator experiments) (Figure 2.2C). In the 2004 greenhouse experiments, we tested each plant species alone (single-choice) and in combination with *S. jacobaea* (paired-choice) (Figure 2.2B). In 2005 incubator experiments, we conducted only single-choice experiments (Figure 2.2C).

2004 Greenhouse experiments

Experimental design

In 2004, we introduced 10 first instar larvae into each cage and we fed larvae *ad libitum* (Figure 2.2B). Designating the cage as the experimental unit, we made daily observations on survival (number of individuals surviving per cage) and developmental time (mean number of days to complete development per cage), and we measured pupal mass (mean pupal mass per cage) using a Sartorius balance ($\pm$ 0.001). Other explanatory variables were the origin of larvae and location of the cage in the greenhouse. Sample size was at least 7 except in two cases (*P. bolanderi* N=1 and *P. flettii* N = 2) where the amount of foliage available was not sufficient to complete 7 replicates. Each replicate trial was started at same time and kept under same conditions in the greenhouse.

We used eggs from adult oviposition experiments plus the eggs collected directly from Santiam Pass by Eric Coombs of the Oregon Department of Agriculture (ODA). Three main locations were sources for the eggs: Baskett Slough (44°57′08″N, 121°16′09″W), Neskowin (45° 06' 25"N, 123° 58' 59"W) and Santiam Pass (44° 24' 08"N, 121° 51' 01"W). We added origin of eggs as a factor in the statistical analysis.

The cages in the greenhouse were located randomly, however the greenhouse room was big and some benches were closer to the air conditioner, causing fluctuating temperature at certain benches at certain times of the day. Temperature is a very important

factor influencing an insect's development time (Taylor 1981), so we recorded the bench of each cage added into the statistical analysis as the location of the cage.

Controlling for insect disease

In the 2004 preference and performance experiments, larvae contracted disease in some of the cages (Table A1.5). Both prior and present work indicates that microspora infection influences insect demography (including survival, developmental time and pupal mass) (Phoofolo et al. 2001, Reardon et al. 2004, Hatcher et al. 2005). Our preliminary data analysis indicated strong suppression in vital rates in the infected cages. For example, in our control group (*S. jacobaea*), we observed 100 % mortality in the cages with individuals carrying spores, whereas 10% mortality in the cages with individuals carrying no spores). As a result, unlike larval preference experiments, we excluded 11 cages bearing diseased insects from single choice and 19 cages from paired choice experiments leaving with only two disease categories (uninfected and N/A) (Table A1.5). For developmental time and pupal mass analysis there was only one disease category; N/A category was only present in *S. integerrimus* and *S. cineraria* and no individual could survive in these groups.

Statistical analyses

We carried out a one-way ANOVA with Tukey's Multiple Comparison Procedure (Ramsey and Schafer 2002) to test if survival (mean number of individuals completing development in each cage), developmental time (mean number of days for individuals in each cage), and pupal mass (mean pupal mass for individuals in each cage) varied due to test plant species or other explanatory variables. Removing experimental units infected with pathogen led to an unbalanced design which in turn had implications for the analysis; cage location, and larval origins had to be included as separate variables (as they were embedded in the block). There were total 59 cages with 14 different food choices in paired-choice and single choice experiments (for the number of infected and uninfected cages, see Table A1.5), 4 cage locations (4 rows of benches indicating the distance from the ventilator), 2 disease conditions (spore-free and N/A), and 3 different larval origins (Coast, Willamette Valley, Cascades). In 17 cages among these 59, no larvae completed development, leaving 42 cages for estimating developmental time and pupal mass. In 2005

experiments many of these explanatory variables were removed and experiments were repeated in a more controlled environment, increasing the power of the statistical analyses.

We also investigated the relationship between paired choice and the performance parameters by comparing estimated vs. measured performance parameters. Measured and estimated developmental time was correlated but we couldn't find such correlation in survival and pupal mass (For all the details see Figure A1.3).

We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for all of the statistical analyses.

2005 Incubator experiments

Experimental design

We repeated the single-choice experiments in a disease-free lab environment with controlled light, temperature, and humidity in 2005. We collected females from the field and used their eggs in these experiments. As soon as larvae emerged from the egg stage, we reared them individually as described in Figure 2.2C and fed them *ad libitum* with the assigned test plant. We followed their development and recorded survival, developmental time and pupal mass. We tested six different food plants (*S. jacobaea*, *S. triangularis*, *S. integerrimus*, *P. flettii*, *P. bolanderi*, and *P. subnuda*), and blocked the experiment by the female moths (three females), i.e. by family, to control for possible effects of maternal genotype or environment. Thirty larvae were tested (N=30) for each plant species.

Statistical analysis

We applied logistic regression to test the effects of test plant and block on survival and applied ANOVA with Tukey's Multiple Comparison Adjustment (Ramsey and Schafer 2002) for testing developmental time and pupal mass. We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for all of the statistical analyses.

Controlling disease

In the 2005 incubator experiments, we used uninfected eggs. We collected females from the Baskett Slough National Wildlife Refuge (44°57′08″N, 12°16′09″W) in Rickreall, Oregon. Three females (block) and 180 larvae from these females were used for the experiments. We determined the disease status of female (and the male if females were mated artificially in the lab) first by examining individual adults under the microscope. We then determined the disease status of 10 eggs and larvae haphazardly sampled from each female. If we detected no spores in the adult eggs or larvae, we used the resulting offspring in the experiments. We were 100% successful in obtaining a spore-free lab colony of cinnabar moths using these procedures.

Experiment 4: Adult demographic performance

We measured the effect of food plant species on the adult performance, which we defined as adult emergence from the pupal stage (pupal survival), fecundity (number of eggs laid) and fertility (number of eggs hatched). We use fecundity to the number of eggs laid; we use fertility to refer to the number of eggs that hatched. We concentrated on two hosts, *S. triangularis* and *S. jacobaea,* as they were the most acceptable and suitable hosts according to the previous experiments.

In parallel experiments in 2006 (reported in Chapter 3), we tested the impact of *Nosema tyriae* on the cinnabar moth on these two host species. In this paper, we concentrate only on the effect of two hosts on cinnabar moth demography in the absence of *N. tyriae*.

Experimental design

We reared larvae on *S. jacobaea* and *S. triangularis* individually (under the same conditions as in 2005 Incubator Experiments) and later stored pupae under conditions shown to optimize diapause development (in a refrigerator from 4-10 ºC for 4 months) (Zoelen et al. 1986). We removed insects from cold storage on the 11 February 2006 and

put each into an 8 oz transparent plastic container, which provided sufficient space for healthy emergence and opportunity for us to keep track of individuals. We sprayed each container with water weekly to maintain humidity. We paired and mated adults as they emerged in the experimental set up described in Figure 2.2D, then recorded the number of eggs laid by each female and the proportion of eggs hatched.

The moths used in these experiments were from four different locations and were blocked by seasonality and origin to protect our inferences about host plant species effects from possible confounding due to differences among moths: Early season Fanno Bog larvae were assigned to Block 1, Middle Season Santiam Pass (44° 24' 08"N, 121° 51' 01"W) and Mary's Peak (44° 30' 16"N, 123° 33' 00"W) individuals were assigned to Block 2 and late season Onion Peak (45° 48' 58"N, 123° 53' 05"W) individuals were grouped under Block 3.

Statistical analysis

We tested whether adults emerged (odds of transition from pupa to adult stage), whether females laid eggs (fecundity: odds of a female laying eggs) and whether a female's eggs hatched (fertility: odds of a female's eggs hatching) by applying logistic regression. We further applied a Kruskal Wallis Rank sum test to determine if the percentage of eggs hatching (fertility) differed between moths fed different test plant species within each block.

We also tested if there was a linear relationship between the pupal mass and fecundity (total number of eggs laid), and pupal mass and fertility (fraction of hatching eggs). There was a weak, positive linear relationship between fertility and pupal mass but no correlation between fecundity and pupal mass (Figure A1.4).

We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for all statistical analyses.

A Matrix Population Model for translating vital rates into population growth rates

We used a matrix model (Caswell 2001) to translate measured changes in vital rates into a projected change in population growth rate, a measure of the overall suitability of each test plant species for cinnabar moth populations. We developed a stage-structured, linear, deterministic model to represent the population dynamics.

A life cycle graph for the cinnabar moth included 8 stages: egg (E), 5 larval stages (L1, L2, L3, L4, L5), pupa (P), and adult (A) (Figure 2.3). Transitions were first calculated as the probability of transition to stage *i* from stage *j*; stages vary in duration (measured in days), so we converted these transitions to daily rates to yield a uniform, 1-day time step for each transition in the matrix and a 1-day projection interval, following the methods described for the medfly (Ebert 1999).

We prepared a transition matrix for each of the cages used in 2004 larval performance experiments. We estimated L1 to L2, L2 to L3, L3 to L4, L4 to L5 and L5 to P stage transitions using the 2004 Larval Performance Experiments that measured survival at each stage and stage duration (in days). We calculated the duration of each larval stage as the mean of the minimum and maximum development times for each stage for insects on each test plant species. Pupal Survival, Fertility and Fecundity and Egg Survival parameters were estimated from the 2006 disease experiments (Chapter 3) (see Adult Performance for details) for *S. jacobaea* and *S. triangularis*.

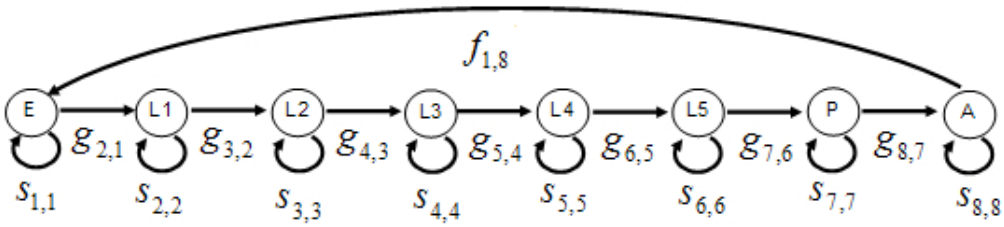
We used *S. triangularis* reproductive parameters (pupal survival, fertility, fecundity and egg survival) to estimate the same parameters for the other nontarget plant species *P. flettii, P. pseudaurea, P. bolanderi, S. cineraria* and *S. integerrimus*. Pupal mass varies with larval diet, and pupal mass is positively correlated with fecundity in this species (Figure A1.3). If pupal mass did not differ among eight suitable nontarget host plants (Figure 2.6), then we assumed that performance parameters for all species in the group can be adequately approximated by the values for *S. triangularis*. In effect, we

assume one nontarget test plant, S. *triangularis*, was representative of all other nontarget test plants with respect to these reproductive parameters of the cinnabar moth.

We used information from previous studies for the missing variable egg duration to complete the life table. We did not measure the duration of the egg stage E, so we substituted a literature value of 5 days. The weighted mean temperature T in our experiments was 21.7 °C, calculated by weighting day and night temperatures by the proportion of time associated with each phase of the photoperiod 16:8 L:D and thermoperiod 25:15 °C (Harman et al. 1989). We substituted the 5 days value of egg duration corresponding to 22 °C (Rose 1978, Harman et al. 1990).

We calculated the finite rate of increase λ as the dominant eigenvalue associated with each matrix and compared the finite rate of increase projected for cinnabar moth populations on each plant species. We also estimated standard statistics including sensitivity, elasticity, damping ratios, stable stage distributions, and reproductive values for each matrix (Caswell 2001).

We used Excel PopTools (Version 2.7.5 released 25th Sep 2006) for analysis of the matrix population model.



$$A = \begin{pmatrix} s_{1,1} & 0 & 0 & 0 & 0 & 0 & 0 & f_{1,8} \\ g_{2,1} & s_{2,2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & g_{3,2} & s_{3,3} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & g_{4,3} & s_{4,4} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & g_{5,4} & s_{5,5} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & g_{6,5} & s_{6,6} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & g_{7,6} & s_{7,7} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & g_{8,7} & s_{8,8} \end{pmatrix}$$

Figure 2.3: The life cycle graph of the cinnabar moth. It illustrates the eight life-cycle stages representing egg (E), five larval stages (L1, L2, L3, L4, L5), pupa (P), and adult (A). The life cycle graph also illustrates the 16 life-cycle transitions in the model with seven representing growth g, eight representing stasis s, and one representing fertility f. The time-step in the model is one day. The life cycle graph can be represented as an 8 x 8 matrix A, which in turn can be used to project the dynamics.

Experiment 5: Nutritional quality of plants

Experimental design

To measure the nutritional quality of each test plant species, we randomly selected 10 individual plants from each of the test plant species (*S. jacobaea, S. triangularis, S. integerrimus, S. cineraria, P. pseudaurea, P. bolanderi, P. flettii*) (Table 2.1), and we analyzed a mixture of foliage and flowers for nitrogen, carbon and water content.

We sampled plants from field or greenhouse populations so the origin of plant material offered to insects matched the origin of plant material analyzed for nutrients.(Table A1.2). *S. cineraria*, *P. pseudaurea*, *P. flettii*, and *P. bolanderi* samples were from greenhouse, whereas *S. jacobaea*, *S .integerrimus* and *S. triangularis* were sampled directly from field populations (Marys Peak and Neskowin). We also tested the differences in nitrogen levels in the greenhouse and field populations of *P. pseudaurea*.

We collected old leaves, flowers and young leaves separately. We report only the foliage results here since we used only foliage during our feeding experiments. We weighed fresh leaves immediately after collecting them, then dried them at 80°C for 2 days, and ground them to a fine powder for Nitrogen Analysis by the Central Analytical Lab in the Oregon State University, the Department of Crop and Soil Science and obtained dry mass Nitrogen, Carbon concentrations using a Carbon, Nitrogen and Sulfur analyzer (CNS2000, LECO Corporation 2003). The analyzer uses a combustion process to break down substances into small compounds which are then measured. This method measures the total inorganic and organic nitrogen, or Total Kjeldahl Nitrogen (TKN), and obtains results similar to those of acid Kjeldahl digestion (Strickland and Parsons 1972).

We calculated water content by simply taking the difference between fresh mass and dry mass. We further calculated Carbon to Nitrogen (C: N) ratio.

Statistical analysis

We compared the Water, Nitrogen and Carbon content of test plants using ANOVA with Tukey's Multiple Comparison adjustment. Other explanatory variables in the test were the origin of the plant (greenhouse vs. field). We further constructed a plant species vs. nutritional quality matrix (including Water, Nitrogen, Carbon and C:N values) and used this matrix in PCA (discussed under Preference vs. Performance heading). We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) and PC-ORD version 4.17 (McCune and Mefford 1999) for all of the statistical analyses.

Relationship between preference and performance

We compared (1) adult preference with larval preference, (2) adult preference with population performance (finite rate of increase), (3) larval preference with population performance, and (4) population performance with nitrogen content of the test plants. We made scatter plots for each pair of variables (Figure 2.9) and used a Spearman Rank Correlation to assess how well an arbitrary monotonic function could describe the relationship between two variables, without making any assumptions about the frequency distribution of the variables (Siegel 1956).

We investigated the relationship between test plants and preference-performance by Principal Component Analysis (PCA), which is a commonly employed and a conceptually simple ordination method summarizing linear multivariate patterns (Legendre and Legendre 1983, Digby and Kempton 1987, McCune and Grace 2002). We combined all preference-performance measures in a matrix composed of 7 test plant species (*S. jacobaea*, *S. triangularis*, *S. cineraria*, *S. integerrimus*, *P. flettii*, *P. bolanderi* and *P. pseudaurea*) and 6 preference/performance parameters (1) adult preference as log number of batches on nontarget/target, (2) larval preference as log (number of larvae on nontarget/ number of larvae on target), (3) fraction of surviving larvae, (4) developmental time, (5) pupal mass, and (6) finite rate of increase (λ). Our secondary matrix included the

nutritional quality measures (nitrogen, water, carbon and carbon to nitrogen ratio) for each test plant (Larval survival, developmental time and pupal mass values for *P. pseudaurea* and *S. cineraria* were taken from 2004 experiments, these values for the rest of the plants were the average of 2004 and 2005 experiments).

Our cross-products matrix contained correlation coefficients among the main parameters. We used PC-ORD version 4.17 (McCune and Mefford 1999) to perform the analysis and to ordinate the test plants in preference-performance space.

RESULTS

Experiment 1: Adult oviposition preference

If given only one choice, females showed little discrimination among most of the test plants. There was no detectable difference among test plants species in egg batches laid on test plants *S. triangularis*, *P. pseudaurea*, *S. cineraria*, *P. bolanderi*, *P. flettii* except on *S. integerrimus* where adults laid significantly fewer egg batches (ANOVA, F = 5.3, d.f. =28, 6, p = 0.001002) and total eggs (ANOVA, F = 9.6, d.f. =28, 6, p = 0.000010), than all other test plant species (Figure 2.4, ANOVA Table; Table A1.7A, to A1.7C). Six out of 7 females deposited their eggs onto cage covers instead of laying them on *S. integerrimus* plants (Figure 2.4A). Two females laid eggs on *S. integerrimus,* but they laid fewer eggs (31 and 36 eggs on *S. integerrimus* compared to 120 to 298 eggs on *S. jacobaea*) (p = 0.00001, F = 9.6, d.f. = 6, 28) (Figure 2.4, Figure A1.1). The total number of eggs varied among test plants primarily due to variation in the number of batches rather than variation in the number of eggs per batch (Figure 2.4). These results suggest a high level of acceptability of non-target test plant species by adults in the absence of the target species *S. jacobaea*.

If given a choice between target and nontarget plants, females became more selective (Figure 2.5A). Females showed no detectable discrimination between the eggs laid on *S. jacobaea* (x) and *S. triangularis* (nontarget = y) (one sided p test, *Ho* log(y / x)= 0, p-value = 0.42) (Figure 2.5A), but discriminated against *P. flettii* (one sided p test, *Ho* log(y / x)= 0, p-value = 0.01), *P. bolanderi* (one sided p test, *Ho* log(y / x)= 0, p-value = 0.05), *P. pseudaurea* (one sided p test, *Ho* log(y / x)= 0, p-value = 0.008), *S. cineraria* (one sided p test, Ho log(y / x)= 0, p-value = 0.01), and *S. integerrimus* (one sided p test, Ho log(y / x)= 0, p-value = 0.0005) in favor of the target host *S. jacobaea*. (Figure 2.5A) The number of eggs per batch was not influenced by the test plant provided in the cage (p = 0.87, F = 0.39, d.f. = 6, 22) (Figure 2.4, Figure A1.1).

The origin of females and the average leaf width of plants had impact on the total number of eggs laid (as leaf size of the plants get bigger, more eggs were laid) (ANOVA Table; Table A1.7A) but not on number of batches (ANOVA Table; Table A1.7B) or on number of eggs per batch (ANOVA Table; Table A1.7C). Female collection date, stem height, age of the plant, and stage of plant did not make a detectable contribution to variation in two other components of oviposition rate, number of batches (ANOVA Table; Table A1.7B) or number of eggs per batch (ANOVA Table; Table A1.7C).

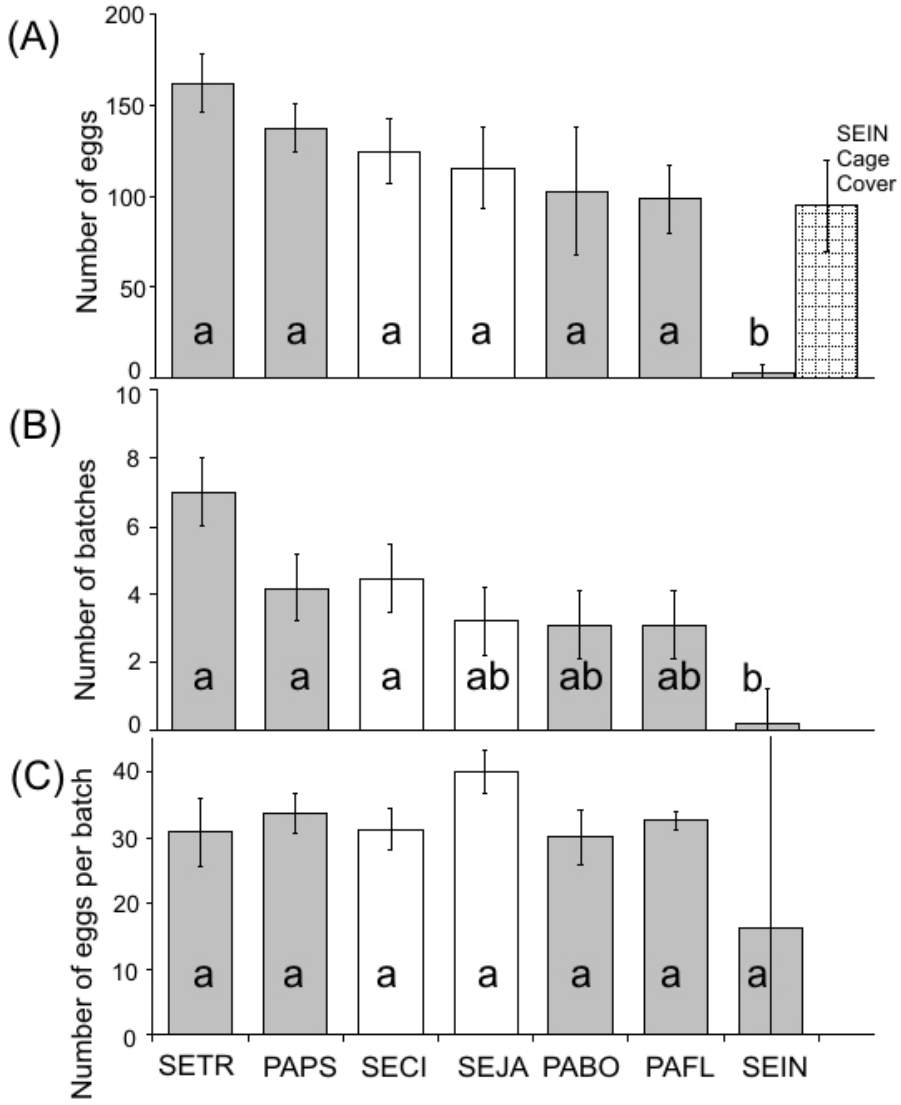


Figure 2.4: The results for the adult oviposition single choice experiments. The pattern of adult oviposition in the single-choice experiment indicated that *S. integerrimus* was not acceptable: (A) Total number of eggs on each plant per cage (per female) varied among test plant species. The last bar shows that females dumped the eggs onto the cage cover in the S. integerrimus cages. (B) Total number of batches per female varied across test plant species. (C) Mean eggs per batch did not vary among test plant species. Letters indicate the groupings after ANOVA test results with Tukey's Multiple Comparison Adjustment. Error bars show 95% confidence interval. White: Old World test plants (SEJA is the target host (Control group). Dark: New World Test Plants.

Experiment 2: Larval preference

An Old World test plant, *S. cineraria* (one sided p test, Ho: (log ratio nontarget / target) =0, $p < 0.0001$) and a New World test plant, *S. integerrimus* (one sided p test, Ho: (log ratio nontarget / target) =0, $p < 0.0001$) were the test plants least preferred by larvae (Figure 2.5B, ANOVA Table; Table A1.7D).

When both of the plants were target, there was no detectable difference between number of larvae on the first target and number on the second target (log ratio = 0) as expected (if there is no bias between plants of the same species possibly arising due to location, chance differences between conspecific plants, etc.). Fewer larvae spent time on *S. cineraria* (one sided p test, Ho: (log ratio nontarget / target) =0, $p < 0.0001$) and *S. integerrimus* (one sided p test, Ho: (log ratio nontarget / target =0, $p < 0.0001$) (Figure 2.5B). There were no detectable differences between the number of larvae on *S. triangularis* or *P. pseudaurea* and the number on *S. jacobaea* (Figure 2.5B). The results for the cages *P. flettii* and *P. bolanderi* were inconclusive due to small sample sizes.

Influence of disease on cinnabar moth host selection

We tested whether disease influenced host choice as follows. We included the cages bearing infected and uninfected larvae in the larval preference analysis (even though we excluded infected individuals from larval performance analyses). We had two disease categories (with and without detectable spores) and added these categories in our statistical tests. An ANOVA test indicated that test plant species influenced the number of larvae on each plant (d.f. = 6, 29 $p = 0.00016$, $F = 6.13288$), while disease (df = 1, 29 (diseased and N/A), $p = 0.86$, $F = 0.031$) and origin of larvae (df = 2, 29, $p = 0.11$, $F = 2.32$) had no detectable effect (ANOVA Table; Table A1.7D). We concluded that disease influences demographic performance of the host but has no detectable effect on preference behavior.

Statistical complications

There were only two cages of *P. flettii* (minimal replication) and only one cage of *P. bolanderi* (no replication) *due* to the limited availability of these test plants (For number of test plant species and replications Table A1.5 – Paired Choice Tests). The behavior of larvae on *P. flettii* was variable; and in one cage the larvae highly preferred *S. jacobaea*, while in the other cage larvae preferred *P. flettii* (Figure 2.5B). As a result we couldn't arrive at a conclusion about larvae's choice on *P. flettii* or on *P. bolanderi*.

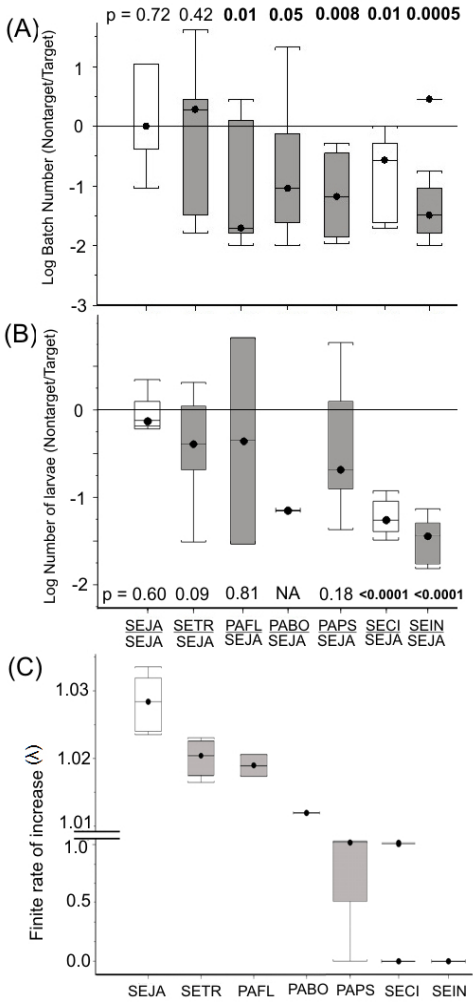


Figure 2.5: An overview for the preference and performance results. (A) Adult oviposition paired choice experiments showed that females became more selective when offered a choice. A log ratio (log ratio of number of batches on nontarget over target for each test plant species) less than zero indicates preference for the target over the nontarget test plant species. (B) Larval preference experiments indicated that larvae preferred *S. jacobaea* over *S. integerrimus* and *S. cineraria*. A log ratio (Log ratio of number of larvae on nontarget over target for each test plant species) less than zero indicates preference for *S. jacobaea* over the test plant species; (C) Finite rate of increase of the cinnabar moth population varied among test plant species. Performance experiments indicated that 4 test plant species were projected to yield cinnabar moth population growth $\lambda > 1$ while 3 test plant species (*P. pseudaurea, S. cineraria, S. integerrimus*) were projected to yield population decline $\lambda < 1$. Box plots show the distribution of data; median, and first-third quantiles were shown, lines with dots outside the boxes show outliers. White: Old World test plants (SEJA is the target host or Control group). Dark: New World Test Plants or the Test Plant Group.

Experiment 3: Larval demographic performance

We contrasted survival (fraction of surviving larvae), developmental time (days to complete larval development), and pupal fresh mass (g) for insects feeding on different test plants. The Old World host *S. jacobaea* was the most suitable test plant species yielding the best insect performance with the highest survival (Figure 2.6A), shortest developmental time (Figure 2.6B), and largest pupae (Figure 2.6C). The New World test plant, *S. triangularis*, was the second most suitable test plant. *S. integerrimus, S. cineraria* and *P. pseudaurea* were the least suitable of all (Figure 2.6). The other plants ranked in between (Figure 2.6, ANOVA Tables; Table A1.6E to Table A1.6J).

Differences among test plants species were robust to variation in environmental conditions in our experiments. We had two years of larval performance experiments; (1) 2004 greenhouse experiments, (2) 2005 Incubator experiments. *S. jacobaea* was the most suitable host in both years (Figure 2.6); in 2004 the 80 % and in 2005 93 % of the individuals on this plant completed their development (Figure 2.6A). The developmental time was the shortest (in 27 days in 2005, 27.1 days in 2004) (Figure 2.6B) and the pupae were the largest (0.20 grams both years) (Figure 2.6C). *S. triangularis* appeared as the second most suitable host after *S. jacobaea*. *P. flettii, and P. bolanderi* were all suitable and ranked after *S. jacobaea* in both years (Figure 2.6). We could test *P. subnuda* only in 2005 incubator experiments; it was a suitable host with 57 % survival, 28.6 days mean developmental time and 0.15 grams mean pupal mass. *S. integerrimus* was the least suitable host in both years (Figure 2.6); in 2004 none of the individuals and in 2005 one out of 30 individuals feeding on *S. integerrimus* survived (egg to pupae). This one individual required the longest time to complete development (33 days) and had the smallest pupal mass (0.10 g).

In 2004 experiments larval survival was low on *P. pseudaurea* (23% survival) *and S. cineraria* (0.05% survival) (Figure 2.6A). The time required to complete development was the longest on *S. cineraria* (46.7 days) and on *P. pseudaurea* (58.5 days) (Figure

2.6B). The pupae were the smallest when larvae were fed with *S. cineraria* (0.055 g) or with *P. pseudaurea* (0.095 g) (Figure 2.6C).

In 2004 greenhouse single choice experiments, we observed that developmental time T changed depending on the origin of moths collected (Figure A1.2). The speed of development 1/T decreased with increasing length of the growing season, measured by Degree Days.

We had 22 cages where the target host was provided with the test plants as a choice (larval performance paired-choice tests) and consequently larvae were free to spend time on the target host, *S. jacobaea*. In these cages, where larvae had the option of feeding on *S. jacobaea*, there was no significant difference (ANOVA with Tukey's Multiple Adjustment) in developmental time, pupal mass and survival among different food choice groups (test plants) (ANOVA Tables; Table A1.7E to Table A1.7G) suggesting the more time spent on *S. jacobaea* in the mixed choice test, the more similar performance would be to *S. jacobaea* in the single choice test. However, when we further investigated the impact of mixed diet on the performance parameters we found paradoxically that time spent on different test plants were not correlated with the performance parameters (Figure A1.3).

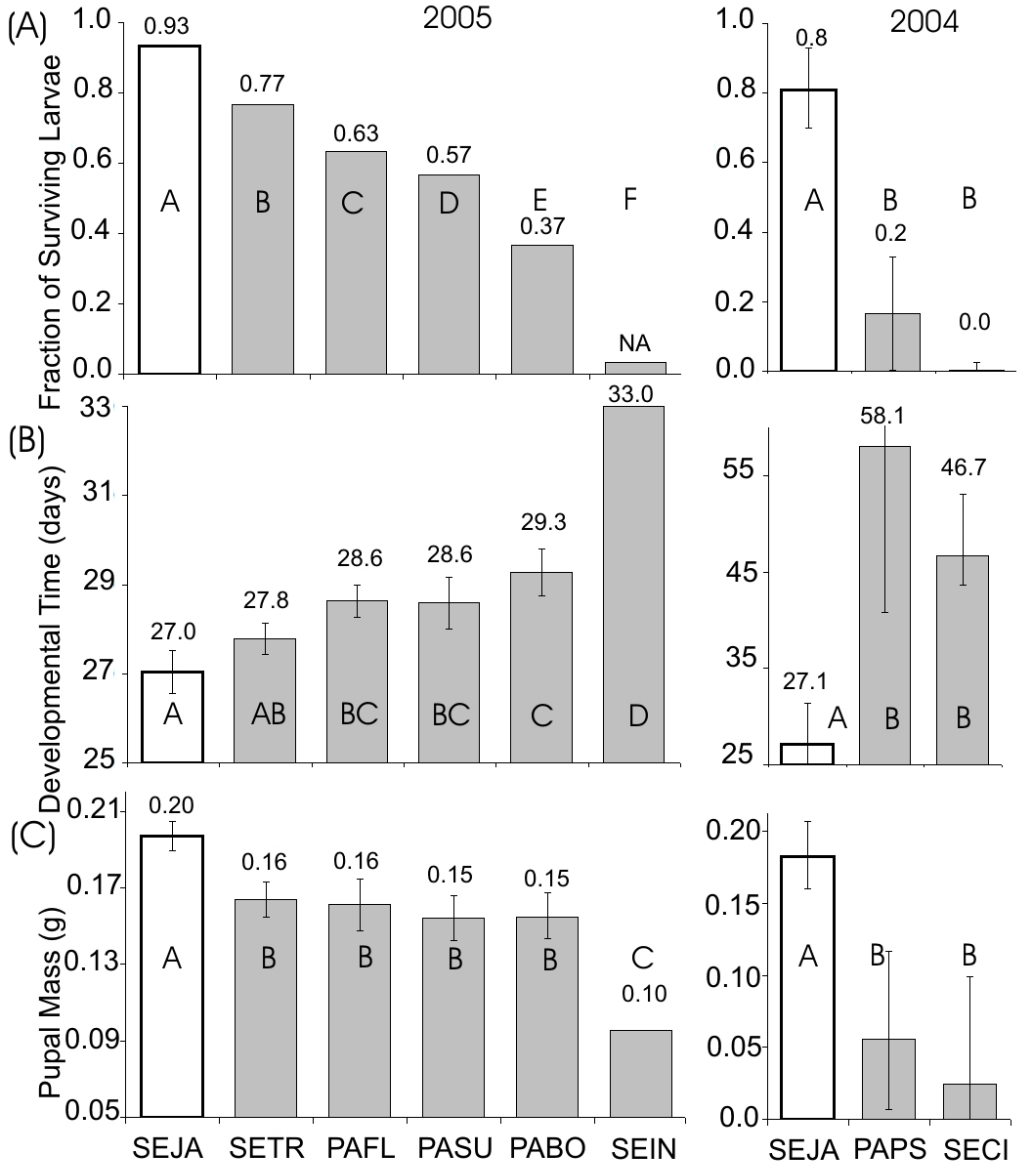


Figure 2.6: The results for the larval performance experiments. In larval performance 2005 (left column) and 2004 (right column) experiments (A) fraction of surviving larvae (B) developmental time in days and (C) pupal mass varied for larvae feeding on different host plant species. Letters indicate the groupings after ANOVA with Tukey's Multiple Adjustment. Error bars indicate the 95% CI. White: Old World test plants (SEJA is the target host (Control group). Dark: New World Test Plants.

Experiment 4: Adult demographic performance

We contrasted adult emergence from the pupal stage and reproductive success on only two test species, *S. jacobaea* and *S. triangularis,* singling out *S. triangularis* for further investigation because it was the most suitable after *S. jacobaea* according to the larval performance tests (Figure 2.7). The odds that an adult emerged from a pupa were higher for pupae reared on *S. jacobaea* (logistic regression, p (χ) = 0.02, residual deviance = 149.4 on 109 d.f.); 54 percent of larvae feeding on target host reached the adult stage whereas only 29 percent of larvae feeding on *S. triangularis* reached adulthood (Figure 2.7A, ANOVA Table; Table A1.7K).

We found no detectable difference between the two test plant species in the probability female laying eggs (logistic regression, p (χ) = 0.20, residual deviance = 21.2 on 20 d.f.) (Figure 2.7B, ANOVA Table; Table A1.7L): 14 out of 17 females reared on *S. jacobaea* laid eggs and 4 of 7 females reared on *S. triangularis* laid eggs.

The odds that a female laid some eggs that hatched did not differ between the two test plant species (p (χ) = 0.06, residual deviance = 8.8 on 14 d.f.): thirteen out of 14 females reared on *S. jacobaea* laid eggs that hatched; 2 out of 4 females reared on *S. triangularis* laid eggs that hatched (ANOVA Table; Table A1.7M). Yet the percentage of eggs hatching per female were approximately 60% higher on females reared on *S. jacobaea* (Kruskal Wallis Rank χ^2= 8.0441, d.f. = 1, p = 0.0046) (Figure 2.7C).

We also investigated the relationship between the pupal mass, fertility and fecundity. We found a weak, positive linear relationship between fertility and pupal mass (linear regression, F-statistic = 24.16, d.f. = 2, 15, p-value = 2.04 x 10-5, R^2 = 0.76); and between fecundity and pupal mass (linear regression, F-statistic = 6.38, d.f. = 2, 21, p-value = 0.019, R^2 = 0.24) (Figure A1.3B).

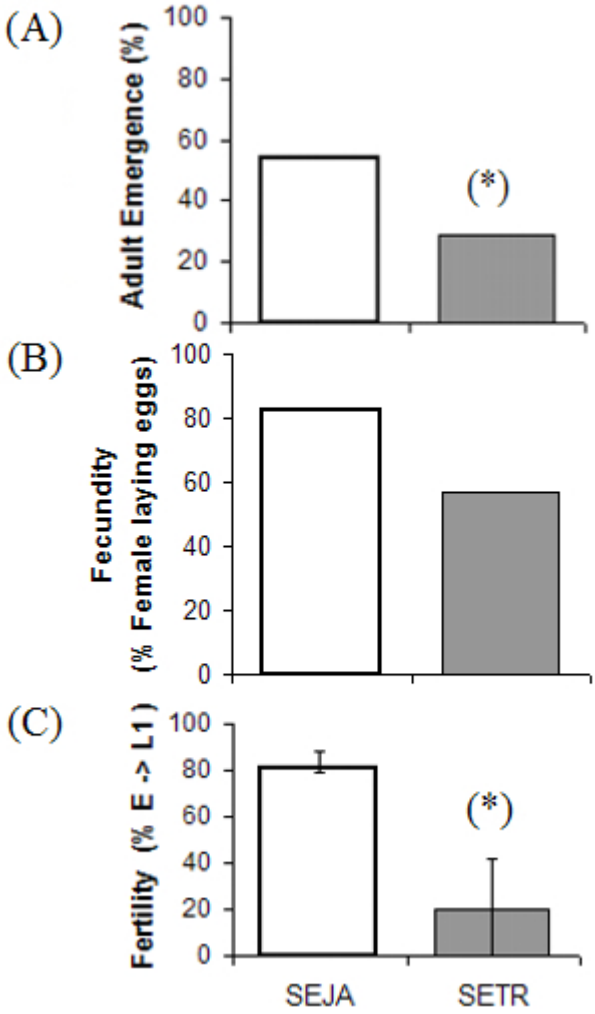


Figure 2.7: The results for the adult performance experiments. Pupal Emergence, Fecundity and Fertility of insects on target (*S. jacobaea*) and nontarget (*S. triangularis*) test plant species (A) The percentage of pupae becoming adult was lower on S. triangularis (logistic regression, p (χ) = 0.02, residual deviance = 149.4 on 109 d.f.); (B) The fecundity (Percent of the females laying eggs) did not differ between two test plant plants (logistic regression, p (χ) = 0.20, residual deviance = 21.2 on 20 d.f.); (C) The fertility of cinnabar moth adults (fraction of eggs hatching to become 1st instar larvae) reared on S. jacobaea was higher compared to *S. triangularis*. * indicates the statistical difference according to the tests. Error bars indicate first and third quantiles. White: Old World test plants (SEJA is the target host (Control group). Dark: New World Test Plants.

Matrix population model

We developed and analyzed a matrix population model to translate variation in vital rates (rates of growth, development, survival and reproduction) into variation in projected population growth rates. *S. jacobaea*, *S. triangularis*, *P. flettii* and *P. bolanderi* were suitable for the cinnabar moth, with projection population growth rates $\lambda > 1$ (Figure 2.5C). The moths reared on *S. jacobaea* had the highest rate of increase ($\lambda = 1.03$ day $^{-1}$). Three of the test plants (*P. pseudaurea*, *S. cineraria*, *and S. integerrimus*) were unsuitable for the cinnabar moth judged by the criterion of projected population growth rates $\lambda < 1$ (Figure 2.5C). In the *S. integerrimus* cages, no individuals could survive ($\lambda = 0$ day $^{-1}$).

Elasticity measures the proportional sensitivity of λ to small perturbations in a life cycle transition $a_{ij.}$ Elasticity analysis showed that the transition from pupal to adult stage accounted for most of the total elasticity in the life cycle (40-50%), while other transitions made smaller contributions, i.e. fecundity (10-16%) and transition from L5 to Pupae (7-15%). The qualitative pattern in elasticities did not vary in cinnabar moth populations on different test plant species (Table A1.8, A1.9 and A1.10).

Experiment 5: Nutritional quality of plants

We compared the nutritional quality of plants by concentrating on Nitrogen, Carbon, Water content and the Carbon to Nitrogen ratio of the test plants.

Mean nitrogen concentration ranged from 1 to 2.8 % across test species. One Way ANOVA and Pair-wise comparison results with Tukey's Adjustment confirmed that (1) *S. triangularis* and *S. jacobaea* were similar in their nitrogen concentration and higher than all other species, (2) *S. cineraria* had the lowest nitrogen levels with of 1 % Nitrogen concentration per mg of dry weight, (3) the other species were similar in their nitrogen levels and ranked in between *S. triangularis* and *S. cineraria* ($p < 0.00001$, $F = 62.1$, d.f. = 6, 66) (ANOVA Table; Table A1.7N).

Origin of plants (field or greenhouse) had significant impact on the nitrogen concentration in the single test case *P. pseudaurea.* The plants from the greenhouse had higher nitrogen levels (1.88%) compared to the field collected plants (1.40 %) ($p = 0.00004$, $F = 19.27$, d.f. = 1, 66) (Figure 2.8A, ANOVA Table; Table A1.7N). This finding serves to justify our taking care to match origin (greenhouse or field) of foliage fed to larvae with origin of foliage analyzed for nutrients.

In similar tests carried out with Carbon and Water content, *S. cineraria* had the lowest water content (73.5 %) ($p < 0.00001$, $F = 30.7$, df = 6, 66) and lowest nitrogen concentration (1.02 %) ($p < 0.00001$, $F = 14.77$, df = 6, 66) and highest carbon to nitrogen ratio (21.8) ($p < 0.00001$, $F = 78.5$, df = 6, 66) (Figure 2.8). There was no detectable difference between *S. triangularis* ([N] =2.60, C:N = 15.4) and *S. jacobaea* ([N] =2.79, C:N = 15.7) in Nitrogen Content and Carbon to Nitrogen ratio but *S. triangularis* had higher Water Content ($SEJA_{[water]} = 75.5$ %, $SETR_{[water]} = 85.9$ %) and less Carbon ($SEJA_{[C]} = 45.2$%, $SETR_{[C]} = 43.2$ %)(Figure 2.8).

S. integerrimus had the second highest nitrogen ([N] = 2.1 %), highest water ([Water] = 87.3 %), lowest carbon ([C] = 42.5 %) concentrations and second highest carbon to nitrogen ratio (21.8) (Figure 2.8). This indicates that our host quality variables cannot explain why *S. integerrimus* was unacceptable or unsuitable for the cinnabar moth larvae and adults as water and nitrogen were among the host plants tested and well above the limits for the survival of insects (Scribe and Slansky 1981).

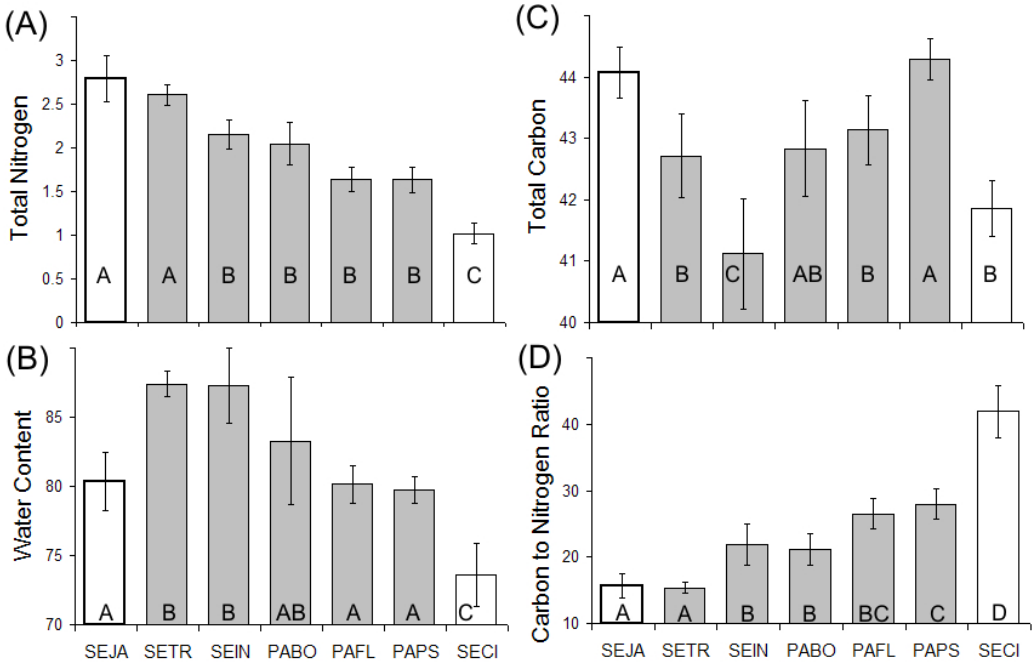


Figure 2.8: The results showing the nutritional quality of plants. (A) Nitrogen Concentration (percentage per mg of dry sample), (B) Water Content (Wet Weight – Dry Weight), (C) Carbon Concentration (percentage per mg of dry sample) and (D) Carbon to Nitrogen Ratio, all vary among test plant species. Letters indicate the groupings according to ANOVA with Tukey's Adjustment. Error bars indicate the 95% CI. White: Old World test plants (SEJA is the target host (Control group). Dark: New World Test Plants.

Relationship between preference and performance

We found positive rank correlations (using Spearman rank correlation tests) among the following preference and performance parameters (1) adult preference vs. larval preference, (2) adult preference vs. population performance, (3) larval preference vs. population performance, and (4) population performance vs. nitrogen concentration. (Figure 2.5, Figure 2.9). We consider the details of these relationships below.

There was a positive rank correlation (symbolized by the Greek small letter rho ρ) between adult preference (egg batches laid on nontarget compared to target plant) and larval preference (number of larvae on nontarget compared to target plant) ($\rho = 0.9464$, for N=7 $\rho > 0.714$ is significant at 0.05 significance level) (Figure 2.5, Figure 2.9A). Adults were more discriminating than larvae; adults discriminated against *P. pseudaurea* whereas larvae did not (Figure 2.5A vs. Figure 2.5B). Adults selected *S. jacobaea* and *S. triangularis,* whereas larvae selected *S. jacobaea*, *S. triangularis* and *P. pseudaurea* when provided with a choice (Figure 2.5A vs. Figure 2.5B).

There was a positive rank correlation between adult preference and performance (projected finite rate of increase of the cinnabar moth population for each test plant species) ($\rho = 0.8214$, for N=7 a value of $\rho > 0.714$ is significant at 0.05 significance level) (Figure 2.5, Figure 2.9B). The Old World Host *S. jacobaea* was the most suitable test plant for cinnabar moth population growth ($\lambda = 1.03$ day^{-1}), second suitable test plants were *S. triangularis* and *P. flettii,* with a projected population growth rate smaller than on *S. jacobaea*. Adults showed no detectable discrimination between *S. jacobaea* and *S. triangularis*, but discriminated against one suitable host *P. flettii* as well as three unsuitable host plants (*S. integerrimus*, *S. cineraria* and *P. pseudaurea*) that were projected to yield declining cinnabar moth populations with $\lambda < 1$.

The rankings of larval preference and performance were positively correlated ($\rho = 0.7321$, for N=7 $\rho > 0.714$ is significant at 0.05 significance level) (Figure 2.9). Larvae

preferred those test plant species (*S. jacobaea, S. triangularis P. flettii and possibly P. bolanderi*) that were projected to yield growing cinnabar moth populations ($\lambda > 1$) . Larvae discriminated against *S. integerrimus* and *S. cineraria,* which were projected to yield declining cinnabar moth populations ($\lambda < 1$). The only discrepancy was *P. pseudaurea*, which *was* relatively acceptable to larvae even though relatively unsuitable for cinnabar moth population growth (Figure 2.5).

There was a positive rank correlation between insect performance and nutritional quality of plants ($\rho = 0.8750$, for N=7 $\rho > 0.714$ is significant at 0.05 significance level) (Figure 2.9D). The most acceptable and suitable plants (Figure 2.5) had the highest N content (*S. jacobaea* at 2.8% and *S. triangularis* at 2.6%) (Figure 2.8A). The least acceptable and least suitable test plant species (Figure 2.5), *S. cineraria*, had the lowest nitrogen content (Figure 2.8A). An exception was *S. integerrimus*, an utterly unacceptable and unsuitable test plant (Figure 2.5) with the second highest nitrogen content (Figure 2.8A).

According to the PCA ordination*, S. jacobaea*, *S. triangularis*, *P. flettii* and *P. bolanderi* were closer to each other and separated from *S. integerrimus*, *S. cineraria* and *P. pseudaurea* in preference and performance space (Figure 2.10). Superimposing the nutritional quality matrix on the ordination space, we infer that the Carbon-Nitrogen ratio was higher, and Nitrogen content was lower, towards the unacceptable and unsuitable species *S. cineraria*, *S. integerrimus* and *P. pseudaurea*. The first axis accounted for 77 % and the second axis 12 % of variation. (Table 2).

Overall the results showed that *S. jacobaea* and *S. triangularis* were the most acceptable and most suitable test plant species, and they had the highest nitrogen concentration. S. *cineraria* and *S. integerrimus* were the least suitable and least acceptable test plants. *S cineraria* had the lowest nitrogen concentration. On the other hand, the utterly unacceptable and unsuitable *S. integerrimus* had relatively high nitrogen levels, suggesting that other factors influence preference and performance on this plant species.

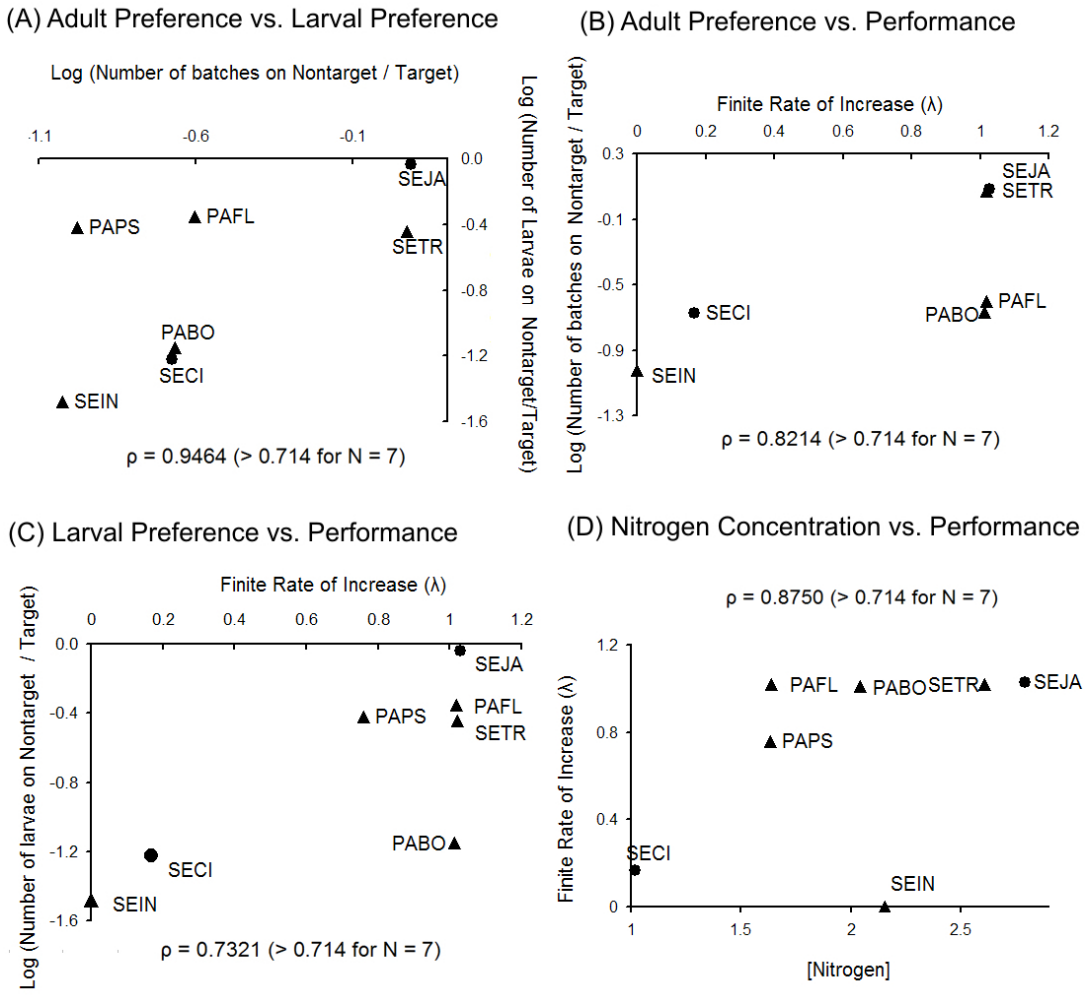


Figure 2.9: Scatter plots showing the relationships among preference, performance and Nitrogen concentration. (A) Adult Preference (log number of batches on Nontarget/Target) showed a positive range correlation with Larval Preference (B) Adult Preference (log number of batches on Nontarget/Target) increased with Performance (finite rate of increase λ) (C) Larval Preference (log number of larvae on Nontarget/Target) increased with Performance (finite rate of increase λ) (D) Nitrogen concentration increased with Performance (finite rate of increase λ). Circles: Old World test plants (SEJA is the target host (Control group). Triangles: Test plants native to Oregon (New World Test Plants). ρ (Greek small letter rho) is the Spearman rank correlation coefficient (values > 0.714 is significant for N=7 at p = 0.05 significance level).

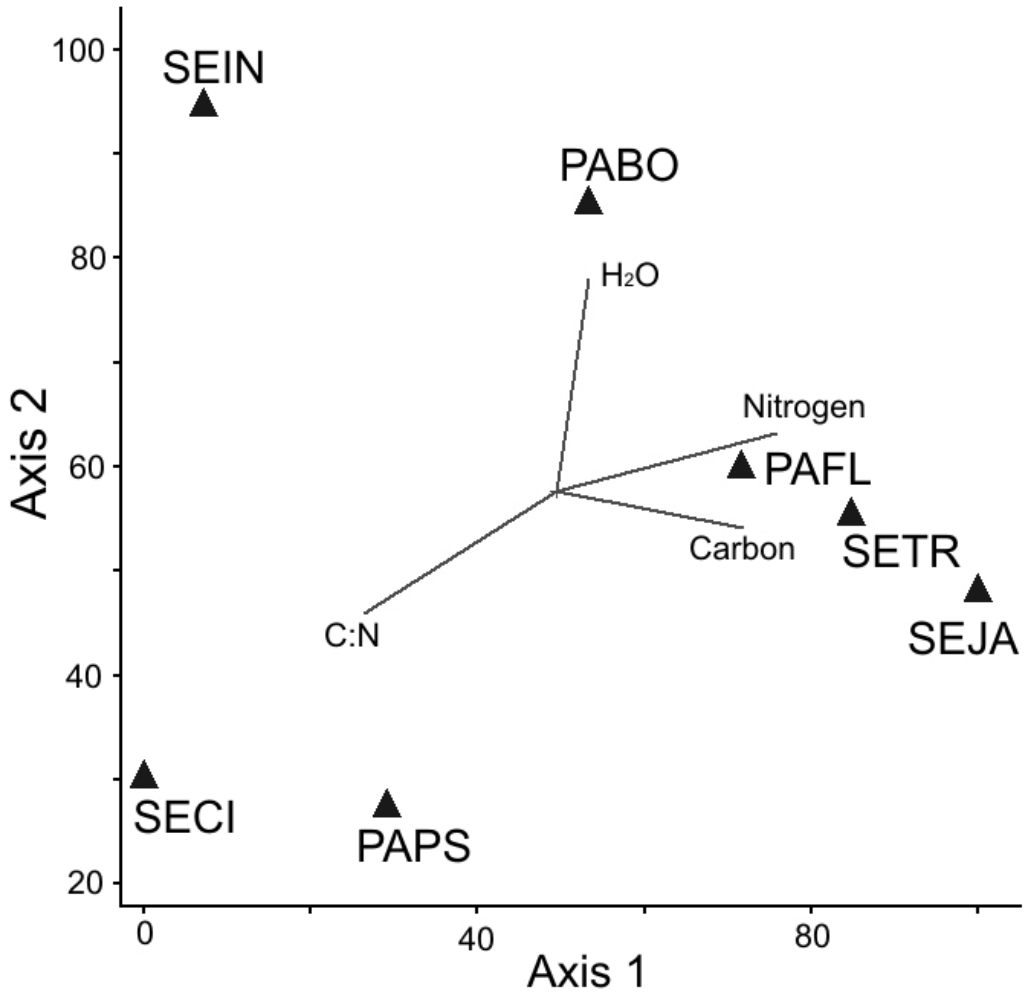


Figure 2.10: Ordination results of the test plant species in preference-performance space. Suitable test plants (*S. jacobaea*, *S. triangularis, P. flettii* and *P. bolanderi*) were separated from relatively unsuitable plants (*P. pseudaurea, S. cineraria* and *S. integerrimus*). Nitrogen content appeared to be distinctively higher and Carbon to Nitrogen ratio appeared to be distinctively lower in suitable compared to unsuitable test plants.

Table 2.2: Principal Component Analysis eigenvalues and the percentage of variance explained by axes. The results show that two axes together explain the 89% of the variation in the data.

AXIS	Eigenvalue	% of Variance	Cum.% of Var.	Broken-stick Eigenvalue
1	4.621	77.018	77.018	2.450
2	0.725	12.077	89.095	1.450
3	0.448	7.459	96.553	0.950
4	0.175	2.922	99.476	0.617
5	0.028	0.470	99.945	0.367
6	0.003	0.055	100.000	0.167

DISCUSSION

Upon moving from Europe to North America, the cinnabar moth encountered 20 novel North American plant species in Oregon alone that are close relatives of its ancestral European host, *S. jacobaea,* , and it readily adopted at least 3 new host species (*S. triangularis*, *P. pseudaurea* and *P. subnuda*) from this group. Six other species (*S. integerrimus, S. hydrophilus, P. flettii, P. bolanderi, P. cana,* and *P.macounii*) have been exposed to the cinnabar moth but are not yet confirmed to be used as hosts in the field (Figure 1) (Fuller et al. 2002). We have refined estimates of host suitability by showing that cinnabar moth populations are projected to grow on some new hosts (*S. triangularis, P. flettii, P. bolanderi*) and decline on others (*P. pseudaurea, S. integerrimus*), even though some individuals of *T. jacobaeae* can complete development at least to the pupal stage on all of these plant species. We found that host suitability increases with nitrogen concentration over the observed range of 1% to 2.8% and decreases with increasing values of the carbon to nitrogen ratio (12.7 to 48.1) in the foliage of plant species. We found a positive, rank correlation between preference and performance including both Old World and New World hosts. Our results suggest that the preference-performance relationship posed little obstacle to colonization of new hosts by cinnabar moths entering new territory in Oregon:.

Host choice by adults and larvae

The preferences expressed by ovipositing females depended on freedom of choice. If given only the test plant species in a one-choice test, adults showed little discrimination among most of the test plants. We found no detectable difference among test plants species in egg batches laid on test plants *S. triangularis*, *P. pseudaurea*, *S. cineraria*, *P. bolanderi*, *P. flettii*, but adults laid significantly fewer eggs on *S. integerrimus*. These results suggest that test plant species are highly acceptable to adults in the absence of *S. jacobaea*. If given the non-target test plant species paired with the target *S. jacobaea* in a two-choice test, adults became more discriminating. Adults showed no detectable discrimination between the two superior hosts *S. jacobaea* and *S. triangularis*, but discriminated against the inferior hosts *P. flettii*, *P. bolanderi, P. pseudaurea, S. cineraria,*

S. integerrimus in favor of the target host *S. jacobaea*. Preference expressed in paired-choice tests is unlikely to protect nontarget plant species in the field. In local populations of the cinnabar moth, adults rarely have a choice between the target (*S. jacobaea*) and nontarget since target and nontarget plants occur in different habitats (target in disturbed environments like roadsides and clearcuts; nontargets in undisturbed, natural areas) at different elevations (target at low, nontarget at high elevations) and do not commonly coexist (Fuller et al. 2002).

Cinnabar moth preferences depended on life history stage. Broadly speaking, mothers and offspring made similar choices. Their likes and dislikes were similar. They shared a preference for S. *jacobaea* and *S. triangularis* and an aversion for *S. integerrimus*. Yet ovipositing females were more discriminating than feeding larvae. Mothers discriminated against *P. pseudaurea* in the presence of *S. jacobaea* but larvae did not. An insect may use variety of senses (smell, taste, vision, and touch) to select a host plant. The observed differences in discrimination by adults and larvae might be attributed to differences in their perception capacity (i.e. adults have more receptors, which are more sensitive and are located on the tarsi, ovipositor and mouthparts in comparison to only on mouthparts in larvae) (Bernays and Chapman 1994, Hallem et al. 2006), life history traits (i.e. adults have higher dispersal capacity, more time available for foraging, greater energy reserves) and conflicting interests (i.e. adults and larvae might prefer different habitats and hosts) (Bernays and Chapman 1994). All these tiny distinctions might be the reasons why adult and larval performance usually matched but adults were more discriminating. A lack of discrimination might help a cinnabar moth larva complete its development when faced with starvation. It is documented that cinnabar moth larvae usually deplete its food sources and wander in search of food (Dempster 1975). When all suitable host plants in the environment are depleted, and only moderately suitable hosts are left, cinnabar moth larvae can accept these hosts, survive on them, and mitigate starvation risk.

Host plant suitability for insect population growth

From life cycles to population dynamics

Host plant suitability directly affects herbivore performance and population dynamics (Preszler and Price 1988, De Bruyn et al. 2002). For example, Preszler and Price (1988) demonstrated that a shoot-galling sawfly *Euura lasiolepis* had higher mortality on water stressed *Salix*. When they constructed a life table, they observed a 27% reduction in galling sawfly populations on stressed compared to unstressed plants. Tammaru and Javoiš (2000) studied the oviposition response of three geometrid moths, and observed a late initiation of oviposition when there was no suitable hosts in the environment. They concluded that female responses to unsuitable hosts were sufficiently strong to have potential significance for population dynamics. As suitable hosts project a population growth on herbivore populations, unsuitable hosts might yield to a population decline. Thus, herbivore performance, especially insect population growth rates, can be useful for diagnosing differences in host plant quality and used as a surrogate measure of host plant suitability. Our estimates of insect population growth rate gives a relative measure of the suitability of different hosts but the actual rates of increase will differ due to abiotic and biotic factors operating in the natural environment (Birch 1948).

We measured the insect vital rates and the population growth rates projected from these vital rates to characterize the response of cinnabar moths to different host plant species. We confirmed cinnabar moth can complete its life cycle on at least two species *S. jacobaea* and *S. triangularis*. All the test plants supported development at least to the pupal stage, and all might thus be considered as part of the fundamental host range of this insect. The ability to complete life cycle development is a commonly used criterion of host suitability, and an insect with the ability to complete life cycle development on a nontarget species of ecological or economic value might be rejected as a candidate for biological control if the likelihood of exposure were high. However, there are important quantitative differences in the demographic performance of the insects on different host plants captured by our analysis. For example three hosts (*S. integerrimus, S. cineraria, and P.*

pseudaurea) showed evidence of inferiority. Only one larva out of 90 could complete its development on *S. integerrimus,* 3 larvae out of 120 on *S. cineraria* and 9 larvae out of 40 on *P. pseudaurea*. Survival was low, development was slow and pupae were small when larvae were reared on these plants. As a result, there were inferences that these plants might not be suitable enough to sustain cinnabar moth populations. Matrix population modeling allowed us to determine if cinnabar moth parameters measured at individual level are likely to have profound impacts at the level of cinnabar moth populations. We found that the plants *S. integerrimus*, *S. cineraria* and *P. pseudaurea* were unsuitable for cinnabar moth population growth ($\lambda < 1$) even though a tiny fraction of larvae were able complete their development. Our matrix model yields estimates of population growth that take account of the many, possibly conflicting changes in vital rates (e.g. there was a detectable difference in survival rates but not on developmental time for some test plants) and provide a more reliable measure of suitability of the test plants.

A more reliable criterion for defining the host range

We propose the insect population growth rates projected for a host plant species is a reliable criterion for host suitability. To our knowledge, ours is the first study to compare projected population growth rates of a weed biological control organism on a range of target and nontarget host plant species. Our estimates of population growth rates on different hosts in artificial conditions should be regarded for now as relative not absolute measure of host suitability.

We demonstrated that fundamental host range of the cinnabar moth was narrower than expected. *S. integerrimus* was unsuitable, *P. pseudaurea,* and *S. cineraria* were relatively unsuitable compared to *S. jacobaea, S. triangularis, P. bolanderi* and *P. flettii*. Parallel studies by Diehl and McEvoy (1990) and Fuller et al. (2002) help estimate and explain the realized host range expressed in the field as a small subset of the fundamental host range revealed in laboratory tests. The realized host range revealed in the three years of intensive surveys in Oregon was narrowed than the fundamental host range: Fuller et al. (2002) found that *S. triangularis* and *S. jacobaea* are extensively used in field but there was no evidence in these surveys that *S. integerrimus, P. flettii* and *P. bolanderi* are

attacked. Cinnabar moth larvae have been commonly observed on ornamental *S. cineraria* plants grown in urban gardens (Coombs et al. 2004). Near Mt. Hood, cinnabar moth populations lay eggs on *P. pseudaurea* and early and late instars feed on this plant (Fuller et al. 2002, Coombs et al. 2004). Phytophagous insects often select the less suitable host in nature (Bernays and Chapman 1994). Host selection by phytophagous insects in the field may be influenced by the ecology of host plant, including abundance, distribution and dispersion of individual plants. For example a very abundant host may be chosen when alternative, perhaps more acceptable and suitable hosts are rare (Bernays and Chapman 1994). It is known that after the introduction of three biological control agents, *S. jacobaea* declined to <1% of its former abundance (McEvoy et al. 1991); near Mt. Hood, *S. jacobaea* is sparsely distributed and fairly rare. This rarity might explain why *P. pseudaurea* is chosen by Mt. Hood cinnabar moth populations (spillover impact). Thus we could conclude that the risk of the cinnabar moth on the three nontarget test plants, *S. integerrimus*, *S. cineraria* and *P. pseudaurea* were relatively negligible because according to our results, moth populations feeding solely on these plants faced a population sink. This result might suggest the following condition; instead of cinnabar moth posing a danger for *P. pseudaurea*, the only unsuitable plant consumed in the field, *P. pseudaurea* might provide an ecological trap for the cinnabar moth populations (since cinnabar moth larvae preferentially feeds on this plant but not perform well on it). Further research is required to understand the influence of *P. pseudaurea* on the metapopulation dynamics of the cinnabar moth populations. In novel environments of the cinnabar moth, *P. flettii* or *P. bolanderi* have never been attacked (Fuller et al. 2002). Adult's strong preference toward *S. jacobaea* in the presence of these test plants (Figure4A) and many other ecological factors including habitat quality, phenology of the plant, and host quality in field might be responsible from this outcome.

Positive correlations between preference and performance parameters

Coordination of consumer preference and performance is required for effective host plant use by insects. Otherwise, suitable hosts may be unacceptable, or acceptable hosts may be unsuitable. A weak link between preference and performance may act as a

filter, a barrier to insects colonizing new hosts and new areas. We found a strong, positive linkage between preference and performance. Both mothers and offspring in the cinnabar moth preferred the plant species that yielded the highest projected population growth under the conditions of our study. We found positive rank correlations between adult preference vs. performance and larval preference vs. performance. The most suitable test plant was the Old World Host *S. jacobaea* with the highest population growth ($\lambda = 1.03$ day^{-1}), and the second most suitable hosts were *S. triangularis* and *P. flettii* with projected population growth rates smaller than for moths on *S. jacobaea*. Adults showed no detectable discrimination between *S. jacobaea* and *S. triangularis*, but discriminated against *P. flettii* in addition to the test plants on which cinnabar moth populations were projected to decline. In addition, larvae discriminated against *S. integerrimus* and *S. cineraria* which projected a population decline ($\lambda < 1$) and showed no detectable discrimination against the *S. jacobaea, S. triangularis* and *P. flettii* which projected a population growth $\lambda>1$. The exception in laboratory tests was *P. pseudaurea,* which was acceptable but unsuitable for population growth ($\lambda = 0.67$ day^{-1}) (Figure 4). Overall, suitable new hosts appear to be acceptable, and acceptable new hosts appear to be suitable. Our results suggest that the preference-performance relationship posed little obstacle to colonization of new hosts by cinnabar moths entering new territory in Oregon.

A common intuition is that host plant preference and performance should be positively correlated (Remington 1952, Wiklund 1975, Thompson 1988b, Nylin and Janz 1993, Janz and Nylin 1997, Berdegue et al. 1998, Scheirs et al. 2000, Scheirs and De Bruyn 2002). On the other hand, differences in performance on different hosts in relation to preference can maintain an insect's ability to try new host species especially in novel environments and thus adapt in case the most acceptable and suitable hosts are unavailable (Mitter and Futuyma 1983). This flexibility might be particular adaptive for an invading species. We found a strong correlation between preference and performance regardless of the novelty of the test plant; insects showed a high preference toward the new, suitable test plant *S. triangularis* and low preference towards the new, unsuitable test plant *S. integerrimus* even though these new associations are at most 50 years old. Yet, many contradicting examples can be given from the literature. For example *Pieris napi* females

in Colorado oviposit on seven crucifer species, two of which are introduced species that have glucosinolate profiles similar to the indigenous hosts but the two introduced hosts are fatal to larvae (Chew 1977). Wicklund (1975) found no positive relationship between preference and performance in new insect-plant associations formed when alien plant species (*Bifora radians*, *Levisticum officinale*) related to the natural hosts from Umbelifera family invaded the environment of the butterfly *Papilio machaon* in the Baltic Region, but he found a strong, positive relationship between preference and performance in old host associations. Fine-tuning preference and performance correlation occur on evolutionary time scales: the more time a consumer spends with its host, the more likely preference patterns evolve to converge on performance patterns. Changes in preference can evolve quite quickly. For example Singer and Thomas et al. (1993) found that *Euphydryas editha* and its old host *Colinsia parviflora* and the new introduced host *Plantago lanceolata*; Euphydryas *editha* decreased preference for a relatively rare native host plant species *Colinsia parviflora* from 60% to 10% over the short time span of a decade. Some cases of poor correspondence of oviposition preference and larval performance may reflect simply the lack of time needed to modify preference or performance (Thompson 1988b). Preference may evolve faster than performance, and the rate of evolution in preference and performance may differ between species (Thompson 1988b). Further research is required to understand the evolutionary changes in preference and performance patterns of the cinnabar moth on Old World and New World hosts.

Preference and performance is key component of an insect-plant interaction, and many aspects of insect-plant interaction vary across a range of environments (Thompson 1988a, Thompson 1988b). A norm of reaction describes the pattern of phenotypic expression of a single genotype across a sequence of environments (Gupta and Lewontin 1982). For every genotype, phenotypic trait, and environmental variable, a different norm of reaction can exist; in other words, an enormous complexity can exist in the interrelationships between genetic and environmental factors in determining traits. As a result, different experimental settings might yield to different conclusions. Controlled experiments allow researchers to eliminate confounding of variables characterizing the biotic and biotic environment (Bernays and Chapman 1994), and isolate the variables of

interest. In strictly controlled environments, mostly environmental variables are set to the optimal levels, which do not faithfully represent real environments. Even if a researcher wants to eliminate the impact of biotic factors, abiotic environment always changes in nature; temperature is never constant, humidity always fluctuates, photoperiod changes everyday. In this experiment, we incrementally increased realism from strictly controlled laboratory conditions to semi-controlled greenhouse conditions. As a result, we observed higher variation in greenhouse experiments but the mean values for each parameter were approximately the same (Table A1.6). For example cinnabar moth larvae reared on *S. jacobaea* needed 27 ($\pm$ 0.93) days to complete its development in the growth chamber (Conditions Figure 1C), but needed 27.1 (25.1, 29.3) days in the greenhouse (Conditions Figure 1B) (For other test species and other parameters see Table A1.6). We can confidentially expect a much higher variation under natural conditions with biotic and abiotic factors acting together.

Nutrients as a component of host plant quality

The chemical composition of a plant, mainly the secondary metabolites, plays a key role in plant-herbivore interactions (Schoonhoven et al. 1998). Even herbivores that are apparently adapted to particular host-plant families have been shown to be affected by subtle variations in host-plant chemistry (Agrawal 2000). *Tyria jacobaea* feeds on *Senecio* plants in field and sequesters these pyrrolizidine alkaloids in eggs, larvae, pupae, and adults (Aplin et al. 1968, Aplin and Rothschild 1972, Rothschild et al. 1979, Ehmke et al. 1990, Nickisch Rosenegk et al. 1993). It has been argued that it also locates its hosts by chemical cues provided by these alkaloids (Rothschild et al. 1979). However, prior evidence suggests that neither the diversity (Vrieling and Boer 1999, Macel et al. 2002, Macel and Vrieling 2003) nor the concentration of pyrrolizidine alkaloids (van der Meijden et al. 1984, van der Meijden et al. 1989, Vrieling 2006) affects the host selection or cinnabar moth performance within the genus *Senecio*. There is a growing consensus that variation in pyrrolizidine alkaloid content does not explain variation in preference and performance of cinnabar moth within and between host plant species.

Our results are consistent with prior evidence on nitrogen as a measure of host quality. There is ample reason to believe nutritional quality influences insect preference and performance on host plants (Feeny 1968, Haukioja and Pekka 1979, Awmack and Leather 2002). Prior studies investigating the relationship between the nutritional quality of host plants and its correlation to preference and performance in the cinnabar moth found that nitrogen is a limiting element in cinnabar moth diet (Dempster 1970a, 1971, 1975, 1979, Pajutee 1981) and it might be the factor determining host selection (Myers and Post 1981, van der Meijden et al. 1984, van der Meijden et al. 1989, Tinney et al. 1998b), as adult cinnabar moths selected the high-nitrogen plants. For example, Meijden et al. (1989) showed that female cinnabar moths selected plants with high concentration of organic nitrogen and sugars; they laid more batches on these plants. We found similar results; there was a positive rank correlation between the preference-performance patterns and nitrogen content The lone exception to the positive relationships between acceptability and suitability was *S. integerrimus*, which was unacceptable and unsuitable as a host plant (Figure 4) but had the second highest nitrogen level (Figure 7A). While relatively high nitrogen concentration may contribute to host quality, there appear to be other factors (e.g. tissue toughness, surface chemicals) (Soldaat et al. 1996) operating in the case of *S. integerrimus*. The influence of host plant chemistry may often be subtle and in addition to nutritional quality many other factors might contribute into preference and performance patterns (Agrawal 2000, Awmack and Leather 2002).

Summary

We re-evaluated the host range of the cinnabar moth nearly 50 years after its introduction to North America. We used Insect Population Growth Rates as our criterion for diagnosing differences in host plant suitability. Our results refine estimates of the cinnabar moth's host range. For the cinnabar moth, fundamental host range was smaller than expected, as a colonizing species, cinnabar moth may have benefited from a positive preference-performance linkage. Since colonizing North America, the insect has added new hosts that are acceptable and suitable. Although the potential exists for Parent-Offspring Conflict in host choice in this system where adults were more discriminating

than larvae, the actual conflicts appear to be minor. This conflict might actually allow cinnabar moth larvae to complete its development since there is only a limited menu of alternative host species for the starving larvae wandering in search of food. We found consistent results with the previous studies that nitrogen is a physiological component of host quality and an important factor influencing selection, but there are doubtless other physiological and ecological components. These experiments conducted in controlled environments should be followed by field studies to understand (1) how cinnabar moth population dynamics varies with host plant species under natural conditions, (2) how populations of nontarget plant species are affected by feeding by cinnabar moth larvae.

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CHAPTER 3: EFFECT OF HOST PLANT SPECIES AND MICROSPORIDIAN INFECTION ON CINNABAR MOTH POPULATIONS ON OLD WORLD AND NEW WORLD HOST PLANTS IN NORTH AMERICA

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ABSTRACT

All terrestrial communities are composed of at least three interacting trophic levels: plants, herbivores and natural enemies of herbivores. Herbivores, squeezed in between plants and natural enemies in these tritrophic interactions, are influenced by a variety of direct and indirect, independent and interacting, behavioral, ecological, and physiological effects of natural enemies and plants. Host shifts can be expected if herbivore fitness is higher on new compared to conventional host species, perhaps because the advantage of reduced effectiveness of herbivore natural enemies outweighs the disadvantage of herbivore malnutrition. After its introduction as a biological control agent for the ragwort, *Senecio jacobaea*, the cinnabar moth *Tyria jacobaeae* has acquired new host plants in Oregon. *Nosema tyriae*, a host-specific microsporidian, may be an important factor regulating the cinnabar moth populations. It is possible that the host shift of the cinnabar moth might have occurred because the new host provides a higher fitness in the presence and absence of the natural enemy both at individual and population levels. We used a Life Table Response Experiment combining a factorial experiment and a matrix model to estimate the independent and interacting effects of malnutrition and disease on an herbivore in a tritrophic plant-herbivore-entomopathogen system consisting of the microsporidian *Nosema tyriae*; a phytophagous insect the cinnabar moth *Tyria jacobaeae* (introduced from Europe to North America for biological control of tansy ragwort, *Senecio jacobaea*); and two host plant species, one native to Europe *Senecio jacobaea* (target plant) and one native to North America *Senecio triangularis* (non-target host plant). The factorial experiment estimated the effects of five doses of the pathogen combined with two host species on the demographic performance of the cinnabar moth. All cinnabar moth vital rates (rates of growth, development, survival, and reproduction) decreased with the increasing dose of *N. tyria* spores. Vital rates generally were lower on the New World host *S. triangularis* compared to Old World host *S. jacobaea*. At the individual level, pathogen x host interaction was significant on survival at higher doses, but we didn't observe any effect of the interaction term on other performance parameters (development, growth, fertility, fecundity). At the population level, the effect of one factor depended on the level of the other factor at lower doses of the pathogen. The projected population growth rates

of cinnabar moths were more sensitive to low dosages on the New World host (*S. triangularis*) compared to the Old World host (*S. jacobaea*). At high spore dosages, the effects of the pathogen were so overwhelming that no effect of host species could be expressed. Field prevalence (proportion of infected individuals) of *N. tyria*e declined with elevation and at elevations where two hosts overlapped, prevalence was higher at *S. jacobaea* sites compared to *S. triangularis* sites. The presence of disease might constrain hosts shifts by the cinnabar and provide protection for the non-target plant species against adverse effects of this biological control organism.

INTRODUCTION

"Plant feeding insects live in a world of dominated on the one hand by their natural enemies and on the other by a sea of food plant that, at best, is often nutritionally inadequate and at worst is simply poisonous" (Lawton and McNeill 1979).

All terrestrial communities are composed of at least three interacting trophic levels: plants, herbivores and natural enemies of herbivores (Price et al. 1980). Herbivores, squeezed in between plants and natural enemies in these tritrophic interactions, are influenced a variety of direct and indirect, independent and interacting effects of natural enemies and plants (Price et al. 1980, Awmack and Leather 2002). Herbivore-plant interactions can be mediated by the third trophic level and enemy-insect interactions can be mediated by different plant species. Even host shifts are expected if herbivore fitness is higher on one plant compared to the other, perhaps because the advantage of reduced effectiveness of herbivore natural enemies outweighs the disadvantage of herbivore malnutrition. Host shifts are important to ecology, evolution, and applied fields such as biological control. From ecological perspective, the third trophic level might or might not be influential on the two trophic levels below (Dwyer et al. 2005). In the evolutionary context, the interaction of the enemies and host quality might be fundamental to colonization and radiation of the insects on plants (Price et al. 1980). From biological control point of view, the third trophic level might mediate interactions between species at the first and second trophic levels with implications for the effects of the control organism on both target and nontarget host species. After its introduction as a biological control agent for the ragwort, (*Senecio jacobaea* L.) in North America, the cinnabar moth (*Tyria jacobaeae* L.) has acquired a number of new host plants species (Diehl and McEvoy 1990, Pemberton 2000, Coombs et al. 2004). *Nosema tyriae* is a microsporidian pathogen that is very prevalent in cinnabar moth populations in the field (Hawkes 1973). Here we investigate the tritrophic system composed of the cinnabar moth (herbivore), its new (*Senecio triangularis* Hook.) and old (*Senecio jacobaea*) host plants and its natural enemy (*Nosema tyriae*) using a factorial experiment. We aim to reveal the independent and

interacting effects of the plant and the natural enemy on the cinnabar moth to discover whether the new host plant species provides a higher fitness in the presence and absence of the natural enemy both at individual and population levels.

Population growth rates as a measure of host plant suitability

Host plant quality directly affects herbivore performance and population dynamics (Preszler and Price 1988, De Bruyn et al. 2002). For example, Preszler and Price (1988) demonstrated that a shoot-galling sawfly *Euura lasiolepis* had higher mortality on water stressed *Salix*. From a life table analysis, they concluded that there was a 27% reduction in galling sawfly populations on stressed compared to unstressed plants. Tammaru and Javoiš (2000) studied the oviposition response of three geometrid moths, and observed a late initiation of oviposition when there was no suitable hosts in the environment. They concluded that female responses to unsuitable hosts were sufficiently strong to have potential significance for population dynamics. As suitable hosts lead to growth of herbivore populations, unsuitable hosts might yield to a population decline. In our previous study (Chapter 2) we demonstrated a positive correlation between the population growth rate of the cinnabar moth and the nutritional quality of the test plant species. Population growth rates projected for insect populations on a particular host species are a useful measure of host suitability.

Host plant quality as a component of host suitability

Host plant quality has been defined as the physical or chemical attributes of plants that affect the preference and performance of herbivores. Physical attributes can include plant height, leaf thickness, plant structure, toughness (Wiklund 1984, Schoonhoven et al. 1998, Ladner and Altizer 2005) while influential chemical attributes of plants include nitrogen, carbon, vitamins, inorganic ions, secondary metabolites (Schoonhoven et al. 1998) that affect herbivore preference and performance (Awmack and Leather 2002, Scheirs et al. 2003). An essential nutrient is one that is needed for normal growth,

development and reproduction of the insect. A general deficiency or the improper proportion of nutritional substances, such as proteins, amino acids, fats, carbohydrates, vitamins, inorganic ions, etc. might result in deviations from the basic growth development and reproduction (Tanada and Kaya 1993). Although chemical composition and nutritional quality are essential for the survival, development and reproduction, insects sometimes prefer plants that provide suboptimal performance in the field (Bernays and Graham 1988a, Singer and Thomas 1988, Denno and Roderick 1990, Berdegue et al. 1998), indicating that food quality alone may not explain the mechanisms of host selection. Abiotic (e.g. unfavorable temperatures, humidity, photoperiod) and biotic (e.g. competitors, natural enemies, plant environment interactions) factors may hinder the establishment of new interactions between insects and plants (Morris et al. 2004, Singer and Stireman 2005). In our previous study (Chapter 2) we demonstrated the positive correlation between the performance of the cinnabar moth and nitrogen content of the plants. We also showed that Insect Population Growth Rates can be useful for diagnosing differences in host plant quality.

Pathogens as natural enemies

In microbial diseases, pathogenic microorganisms generally invade and multiply in an insect and spread to infect other insects. These pathogenic microorganisms can broadly be categorized as bacteria, viruses (DNA or RNA), fungi, and protozoa (Apicomplexa, Microspora). Microspora, a pathogen group which form the majority of the protozoa pathogenic to insects, can cause economically serious diseases in beneficial and pest insects and may play an important role in regulating insect populations (Tanada and Kaya 1993). Pathogens like other natural enemies (i.e. predators, parasitoids) can be significant sources of mortality for herbivorous insects and therefore important agents of natural selection (Price et al. 1980, Bernays and Graham 1988a, Berdegue et al. 1996, Stamp 2001). Pathogens, like other natural enemies can change the behavior and host selection of an herbivorous insects. In some cases, selection due to natural enemies might favor herbivores that escape from their natural enemies into ‘enemy-reduced space’ when they

move from old to new host plants species (Berdegue et al. 1996). For example, larval survival of Alaskan swallowtail butterfly, *Papilio machaon aliaska*, is greater on novel hosts in the presence of predators but in the absence of predators survival and growth are greater on the ancestral host (Murphy 2004). Ballabeni, Wlodarczyk et al. (2001) studied another tritrophic system including the leaf beetle (*Oreina elongate*), its two hosts *Adenostyles alliariae* (Asteraceae), *Cirsium spinosissimum* (Asteraceae) and egg predators. They found out that the less suitable host, *C. spinosissimum* was more favored and this host provided the eggs of *O. elongate* with better protection from natural enemies (Ballabeni et al. 2001). Little is known about the impacts of pathogens on insect behavior and host selection mechanisms.

Interaction between host plant quality and natural enemies

Separating the influences of "bottom up" factors like host quality and "top down" factors like pathogens in trophic webs can be difficult because two forces interact in complex ways to influence populations of phytophagous insects (Raymond et al. 2002, Denno et al. 2003). Plants might influence natural enemies directly and indirectly and thus have substantial effect on the survival of herbivores (Price et al. 1980). Plants can directly decrease or increase the fitness of the natural enemies of herbivores via chemical or physical composition. The most direct way that a plant influences an entomopathogen (such as baculoviruses) is through the leaf surface (phylloplane). For example, some plants produce alkaline exudates containing basic ions that can inactivate baculoviruses (Cory and Hoover 2006). Plants can also have antimicrobial compounds (terpenoids, phenols, flavonoids, including catechin and tannins) can suppress the development of certain unfavorable microorganisms in the digestive tract and thus inhibit the growth of microorganisms (Tanada and Kaya 1993). Smirnoff (1967) found that certain plants affected the susceptibility of the ugly-nest caterpillar, *Archips cerasivoranus*, to microsporidian infections. Plants can indirectly affect the fitness of the natural enemies of herbivores through alteration of the susceptibility and/or the behavior of the insect host (Cory and Hoover 2006). Many phytochemicals, especially allelochemicals and nutrients, can modify the physiology and growth of the insect host, affecting its susceptibility to

infection. A healthy insect is generally more resistant to infection than a sick one. Malnutrition caused by plants may result in infection by potential pathogens or by the activation of a chronic to an acute infection (Tanada and Kaya 1993, Cory and Hoover 2006). In spruce budworm *Choristoneura fumiferana* infected with *Nosema fumiferanae*, diseased larvae reared on 2.5% nitrogen had significantly higher mortality than those reared on 4.5 % nitrogen diet (Bauer and Nordin 1988). The first step of revealing these dynamics involves testing the effect of enemies and plants, together and separately.

Objectives of this study

We chose to study the interaction of Oregon populations of the cinnabar moth with its two major hosts (the Old World Host *Senecio jacobaea* and the New World Host *Senecio triangularis*) and its most influential enemy, the microsporidian pathogen *Nosema tyriae,* to understand the factors that might mediate host shifts by the cinnabar moth (Figure 3.1). The cinnabar moth, *Tyria jacobaeae*, was released in Oregon in 1960 to control tansy ragwort, *Senecio jacobaea* (Old World Host) (Isaacson 1973) and started to feed on the native relative of *Senecio; S. triangularis* (New World Host) (Diehl and McEvoy 1990, Fuller et al. 2002, Coombs et al. 2004). *Nosema tyriae* is a microsporidian pathogen of the cinnabar moth *Tyria jacobaeae* (Canning et al. 1999); the pathogen was accidentally introduced along with the insect and became an important factor regulating the cinnabar moth populations in the field (Hawkes 1973). Our experiments were motivated in part by observations that cinnabar moth abundance has declined on ragwort since the insect first arrived in North America in 1959. The collapse of the primary host resource (McEvoy et al. 1991), the accumulation of competitors (McEvoy et al. 1993, McEvoy and Coombs 1999) and natural enemies (Hawkes 1973, Dempster 1975, Myers and Campbell 1976) of this herbivore, have all conspired to reduce cinnabar moth abundance on ragwort in the Pacific Northwest and raise the possibility that life might be better for the cinnabar moth on a New World Host. We hypothesized that an immigrant seeking a better life in the New World might find the quality of life better on a New World host plant species than on an Old World host plant species. To test the hypothesis, we examined the independent and

interacting effects of malnutrition and disease in insects on Old and New host plant species in the laboratory.

Our objectives were to estimate the independent and interacting effects of to two factors, host plant species and pathogen dose, on cinnabar moth demography and population growth rates. We measure the direct influence of the two hosts on the cinnabar moth, and the direct effect of the entomopathogen in cinnabar moth populations feeding on two host plant species. We further test the prevalence and severity of disease at natural field populations of the cinnabar moth and discuss if *Nosema* can mitigate the risk of non-target impacts by a weed biological control organism.

STUDY SYSTEM

We studied interspecific interactions within a tritrophic system consisting of a host-specific pathogen, the microsporidian *Nosema tyriae*; an insect the cinnabar moth *Tyria jacobaeae*; and two host plants species of the cinnabar moth, one the Old World host *Senecio jacobaea* and the New World host *Senecio triangularis* (Figure 3.1).

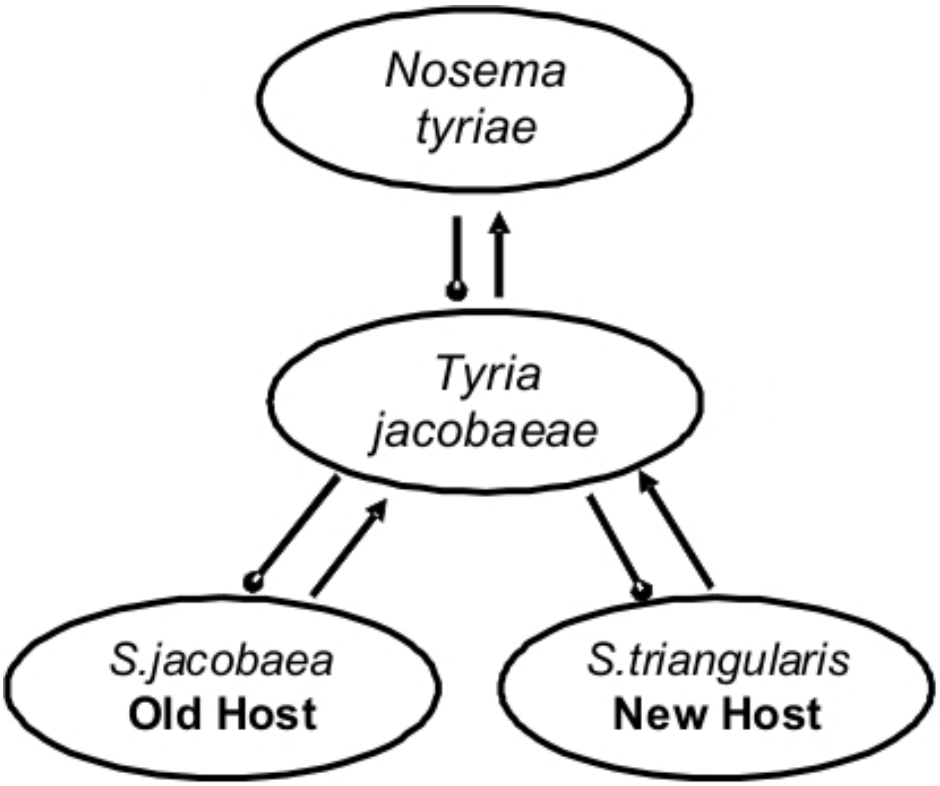


Figure 3.1: A signed diagraph for the tritrophic entomopathogen-herbivore-plant interaction. The pathogen *Nosema tyriae* is the host specific microsporidian attacking the cinnabar moth, *Tyria jacobaea*. *Senecio jacobaea* is the Old World host and *S. triangularis* is a nontarget New World host of the cinnabar moth. Lines indicate an effect from one organism to another, with an arrow head indicating a positive effect and a closed circle indicating a negative effect.

Pathogen: *Nosema tyriae*

Microsporidia are the members of a phylum of protozoa (lately considered to be extremely reduced fungi) (Keeling and Fast 2002). They are highly specialized obligatory intracellular parasites (Solter and Maddox 1998a) and they possess uninucleate or binucleate spores which are protected by a layered wall including proteins and chitin. Microsporidian spores appear to be relatively resistant under environmental conditions (Didiera et al. 2004). Spores are present in feces, the silk or are liberated when an infected host dies. During infection, the polar tube penetrates the host cell and the contents of the spore are pumped through it. Transmission occurs both horizontally (oral ingestion by insects - within the same generation) and vertically (mother to progeny – between generations) (Keeling and Fast 2002). The initial or primary site of infection typically occurs in the epithelial cells lining the gastrointestinal tracts. After germination and infection of the host cells, the organisms multiply within the host by merogony (an asexual replication process used by some Protozoan parasites) and by sporogony (a type of sexual or asexual reproduction by multiple fission of a spore or zygote differentiate into spores) (Didiera et al. 2004).

Nosema is the most common genus of the Microsporidia observed among invertebrates. Studies on other *Nosema* species show that *Nosema* infection causes reduced adult mating success, reduced longevity and fecundity, prolonged larval development and failure in pupation was well as pupal deformities. Degree of these impacts gets elevated especially in the presence of other causes of morbidity and mortality (e.g. weather extremes, malnutrition, in the presence of insecticides) (Solter and Maddox 1998b, Inglis et al. 2003).

Nosema tyriae is a microsporidian parasite of the cinnabar moth *Tyria jacobaeae* (Canning et al. 1999). It is prevalent among introduced cinnabar moth populations in the states of California, Oregon and Washington in the USA of (Hawkes 1973), in native cinnabar moth populations in England (Canning et al. 1999). The pathogen was reported

from laboratory populations of the cinnabar moth during the host specificity tests carried out prior to releasing the insect in Canada (Bucher and Harris 1961). Pathogenicity is reported to be low (Canning et al. 1999) but mortality occurs when the pathogen invades and impairs the function of host tissues (mostly fat body, gut wall, silk glands, malphigian tubules) (Bucher and Harris 1961). Bucher and Harris (1961) state that the progress of the disease is slow, lightly infected larvae may produce normal pupae, and heavily infected larvae die before pupation. Transmission occurs readily between individual larvae reared in groups if any member is infected. The effects of *Nosema tyriae* on cinnabar moth demography and population dynamics are unknown.

Insect: Cinnabar Moth, *Tyria jacobaeae*

The cinnabar moth, *Tyria jacobaeae* was introduced to North America to control tansy ragwort, *Senecio jacobaea*. The insect, was released in California in 1959 (Frick and Holloway 1964) and in Oregon in 1960 (Isaacson 1973), and then redistributed to Idaho and Montana (Coombs et al. 2004).

The cinnabar moth is univoltine. The cinnabar moth life cycle includes 8 stages including egg, 5 larval stages, pupa, and adult. Moths emerge from the overwintering pupal stage in spring, mate, and females lay eggs in late spring to midsummer in clusters (30 to 40 eggs per cluster) (Dempster 1982) on the undersides of basal leaves of rosettes. Eggs hatch about 4 to 20 days depending on the temperature (Rose 1978). Larval stages 1 through 4 each last 4-5 days, while the fifth larval stage lasts 5-9 days before pupation. Larval survival (L1 to P) varies depending on abiotic and biotic environmental conditions, ranging from 67% to 90%. The cinnabar moth larvae occur in clusters and due to their gregarious feeding behavior, food resources can be easily depleted. The cinnabar moth has a highly mobile late instar stage: it has been recorded that fully grown starving larvae are capable of several hundred meters of dispersal when food is in short supply (Dempster 1970b, 1982). Hence, in a host specificity tests for insects like the cinnabar moth, larval as well as adult preference tests are essential. About 50 % of over wintering pupae reach adulthood (Isaacson 1973, van der Meijden 1973, Dempster 1975, Rose 1978).

Host specificity tests and observations conducted prior to its introduction confirm that the cinnabar moth had a broad fundamental host range including species from four genera including *Senecio*, *Packera*, *Erechtites,* and *Tussilago* (Cameron 1935, Bucher and Harris 1961, Tinney et al. 1998a). Our previous study refined estimates of the fundamental host range by showing that cinnabar moth populations are projected to increase on relatively suitable host plant species *S. triangularis*, *P. bolanderi* and *P. flettii* (Chapter 2), while cinnabar moth populations are projected to decline on relatively unsuitable hosts (*S. integerrimus* and *P. pseudaurea*). The strong reduction in ragwort abundance achieved by biological control (McEvoy et al. 1991) may increase the likelihood that cinnabar moth will shift from Old World to New World test plant plants. Yet the pathogen, *Nosema tyriae,* might serve as a mitigating factor on the nontarget impacts of the cinnabar moth.

Host Plants: Old World host, *Senecio jacobaea* and New World Host, *S. triangularis*

The *Senecio* genus is currently being revised and the genus *Packera* (= aureoid *Senecio* complex) has been elevated from Senecio as a separate monophyletic group (Bain and Jansen 1995, Bain and Walker 1995). There are 70 *Senecio* and 62 *Packera* species native to North America (USDA 2007). Fuller et al. (2002) evaluated the risk of host use for ten native *Senecio* and ten native *Packera* species occurring in Western Oregon (Table Appendix01 for the list of other native *Senecio* and *Packera* species) (Chapter 2, Figure 2.1). They concluded that three New World *Senecio* species *(Senecio hyrdophilus, S. integerrimus* and *S. triangularis)* and six New World *Packera* species (*Packera bolanderi, P. cana, P. subnuda, P. flettii, P. macounii,* and *P. pseudaurea*) species have likely been exposed to the cinnabar moth west of the Cascade Mountains in Oregon. They further show that three of these species (*S. triangularis, P. pseudaurea and P. subnuda*) are used in the field. The field use of *S. triangularis* has already been widely reported (Diehl and McEvoy 1990, McEvoy and Coombs 2000, Pemberton 2000, Fuller et al. 2002). In our previous experiment we tested 6 of these plants out of these 9 species excluding test species (*S. hydrophilus, P. cana, P. macounii*) and concluded that *S. jacobaea,* Old World

ancestral host, and *S. triangularis*, the New World species, were the most suitable host plants for the cinnabar moth. In this paper we concentrate on these two host plants to test the impact of the pathogen on New (*S. triangularis*) and Old (*S. jacobaea*) World host plants.

METHODS

We designed laboratory experiments to estimate the independent and interacting effects of host plant species (the Old World Host, *S. jacobaea* and the New World Host, *S. triangularis*) and pathogen dose on cinnabar moth vital rates and projected population growth rates. We used field observations conducted from 2005 to 2006 across 10 field populations of the cinnabar moth to estimate the prevalence and severity of the disease under natural conditions.

Laboratory experiments

The experiment was designed and carried out as an ANOVA with two main effects, host plant species and pathogen dose, and an additional term representing the interaction of the two main effects. We followed these five steps: (1) we collected moths from the field and harvested their eggs; (2) when eggs hatched, we reared larvae individually either on *S. jacobaea* or on *S. triangularis* in laboratory; (3) we infected the larvae with different doses of pathogen (0, 10^1, 10^2, 10^3, 10^4 spores per individual); (4) we followed the development of each larva and recorded developmental time, survival, pupal mass, pupal survival, fecundity and fertility; (5) we ran statistical analyses to test the effect of infection dose, host plant species, and host x dose interaction on the cinnabar moth vital rates and population growth rates (Figure 3.2).

We carried out experiments from April 2005 to July 2006 in the USDA ARS Horticulture Crop Research Laboratory in Corvallis, Oregon. We reared cinnabar moths under optimal conditions for growth and development in a growth chamber (Hoffman model SG30-110V) (Day 16h 25°C, Night 8h 15°C, humidity ~ 90%) (Rose 1978, Diehl and McEvoy 1990, Harman et al. 1990).

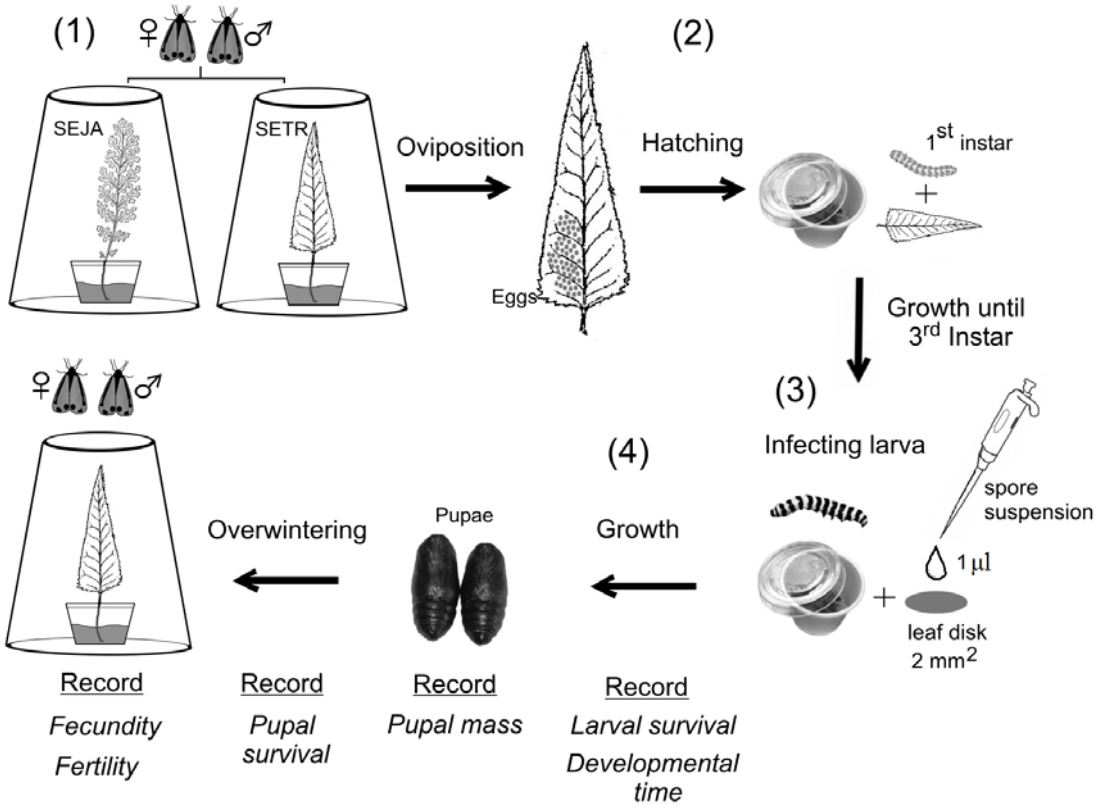


Figure 3.2: Diagram of the experiment. (1) We collected moths from the field and harvested their eggs by placing them in 32 oz. cups along and letting them oviposit; (2) when eggs hatched, we reared larvae individually either on *S. jacobaea* or on *S. triangularis* in laboratory; (3) we infected the larvae with different doses of pathogen (0, 101, 102, 103, 104 spores per individual); (4) we followed the development of each larva and recorded developmental time, survival, pupal mass, pupal survival, fecundity and fertility. After these steps we ran statistical analyses to reveal the effect of infection dose, host plant species, and host x dose interaction on the cinnabar moth vital rates and population growth rates.

Collecting eggs

We sought cinnabar moth populations that were large, disease free, and accessible from our home base in Corvallis; we conducted experiments using insects from four populations matching these criteria: Fanno Bog, Santiam Pass, Mary's Peak and Onion Peak (Table 3.1). We blocked the experiment by cinnabar moth population: We collected moths from different cinnabar moth populations; (1) Fanno Bog (early season, Block 1; (2) Santiam Pass and Mary's Peak individuals (Middle Season, Block 2); and (3) Onion Peak (Late Season, Block 3). We used the moths from the other locations for the field prevalence and severity experiments.

We started a lab colony of cinnabar moths by collecting both sexes of moths using an insect net and placed them into 1 oz. plastic containers individually and cooled down by putting them in cooling box with blue-ice (Freez-Pak, Liofam®) until we arrived at laboratory (~ 2h).

We mated moths by placing a pair (one male and one female cinnabar moth) into 32 oz transparent cups. We also placed a petiole of each leaf (either *S. triangularis* or *S. jacobaea*) in a makeshift 'water pic', a 1 oz container full of water, to prevent desiccation of the leaf (Figure 3.2) and a piece of cotton to remove excess moisture. We harvested the eggs after the oviposition finished. Some of the females laid eggs on the sides of the container as well as leaves. We favored the batches that were laid on the leaves, and that had more than 36 eggs (Prior research indicates that individuals in large batches have higher fitness) (Dempster 1979).

We cleared insects of the pathogen by applying the following protocol. First, we dissected both parents (if available) and checked for spores. If either of the parents had any spores, we discarded the eggs. If both parents (and field-mated female) had no detectable spores, we selected five eggs haphazardly and looked for spores. If the eggs had spores, we discarded the eggs. If there were no detectable spores, we haphazardly selected five first

instars to examine the *N. tyriae* spores. If there were spores, we discarded all larvae; if not, we reserved them for the experiment.

Table 3.1: Coordinates, habitat/elevational quality, and host plants species in the sites visited for the cinnabar moth disease assessment experiments. Insects collected from bold colored locations were used in laboratory experiments.

Site Name	**Habitat Type**	**Elevation (m)**	***Senecio* sp**	**Year**	**Sample Size**	**Lat/Long (NAD27)**
Fanno Bog	Mountain	873	*S. triangularis*	2005	13	44° 52' 44"N, 123° 37' 52"W
Timothy Lake (Mt. Hood)	Mountain	985	*S. jacobaea* *S. triangularis* *P. pseudaurea*	2005 2006	3 25	45° 06' 51"N, 121° 48' 20"W
Little Crater Lake (Mt. Hood)	Mountain	986	*S. jacobaea* *S. triangularis* *P. pseudaurea*	2005 2006	4 13	45° 08' 59"N, 121° 44' 59"W
Mary's Peak	Mountain	1194	*S. triangularis* *S. jacobaea*	2005 2006	17 11	44° 30' 16"N, 123° 33' 00"W
Santiam Pass	Mountain	1474	*S. triangularis*	2005 2006	21 13	44° 24' 08"N, 121° 51' 01"W
Neskowin	Coastal	36	*S. jacobaea*	2005 2006	68 14	45° 06' 25"N, 123° 58' 59"W
Willamette	Valley	400	*S. jacobaea*	2005	10	44° 33' 59"N, 123° 17' 58"W
Horse Rock Ridge	Mountain	830	*S. jacobaea*	2006	17	44° 18' 05"N, 122° 53' 03"W
Onion Peak	Mountain	738	*S. jacobaea*	2005	26	45° 48' 58"N, 123° 53' 05"W
Fairview Meadow	Mountain	1197	*S. jacobaea*	2005 2006	9 17	42° 21' 51"N, 124° 10' 14"W

Rearing larvae

As eggs hatched, we placed larvae individually into 1 oz plastic cups with a piece of wet cotton (to provide humidity) and a piece of plant leaf from the randomly assigned test plant (*S. triangularis* or *S. jacobaea*).

We collected *S. triangularis* leaves form Mary's Peak. We grew *S. jacobaea* in our greenhouse. We sterilized the leaves by cleaning with 2% bleach solution and rinsing with cold water at least three times. Successful rearing in 2004 suggested that development, survival, and fecundity were not influenced by the bleach treatment. We carried out all procedures under semi-sterile laboratory conditions to keep the contamination at minimum levels: we changed cups and food plants daily, cleaned the benches with 80% alcohol, and minimized the physical contact with larvae.

Infecting larvae with a known quantity of spores

We collected diseased larvae from the field, isolated spores from these larvae, prepared different dosages of spore suspensions, and used these suspensions to infect our disease free moth colony.

The first step before infecting the insects is isolating the spores. We first collected infected larvae from field, and reared them in incubator altogether, to allow spore concentration to accumulate. After a week, we homogenized sick larvae individually in a glass tissue grinder, and filtered the suspensions of infected larvae. Then, we purified the spores in the filtrate by centrifugation in a 60% Ludox gradient (Undeen and Alger 1971, Undeen and Avery 1983, Undeen and Becnel 1992). We washed the spores that accumulated at the interface of the Ludox concentration twice in distilled water, and resuspended spores in distilled water at concentrations of 0 (no spores), 10^1, 10^2, 10^3, and 10^4 spores/µl and stored at 5 ± 2°C for at most two months.

Larvae were infected by allowing them to feed on leaf discs dosed with *N. tyriae*. We cut 2 mm^2 leaf disks, and dropped 1 µl of spore suspensions (for control group we used dH_20 instead of spore suspension), waited until the drop dried. We placed these leaf disks (with spores) into the cups of starving newly molted third-instar cinnabar moth larvae and checked the cups 24 hours later. We discarded the larvae which did not consume the whole leaf disk within the first 24 hours.

Measuring responses to treatments

The performance parameters measured were developmental time, larval survival (from egg to pupae), pupal mass, pupal survival, fertility and fecundity.

After inoculating larvae, we recorded daily survival and measured developmental time. One week after the larvae completed their development, we measured pupal mass using a Sartorius Balance (Error $\pm$ 0.005).

We stored pupae in the laboratory (~18ºC) until 10 October 20005 and then transferred pupae to a refrigerator (~ 4-10ºC) for a 4 month chilling period (Bornemissza 1961, Zoelen and Kusters 1986). After 4 months, we placed pupae individually in 9 oz. transparent plastic cups held in the laboratory environment at constant temperature (~18ºC), moistened them at weekly intervals, and recorded adult emergence (pupal survival).

When adults emerged, we mated males and females within each diet and disease treatment group. We followed each mated female, recording how many of the females laid eggs, how many eggs were laid per female (fecundity), how many of the fecund female's eggs hatched, and the proportion of the eggs hatched (fertility).

After all the experiments finished we checked the carcasses of moths and unhatched pupae to confirm that insects were actually infected. In the control group (dose 0), 6 individuals out of 123 (5%) were contaminated, we removed these individuals from the data set prior to statistical analyses.

Statistical design and analysis

The experimental design consisted of five spore doses (0, 10^1, 10^2, 10^3, 10^4 spores per insect), 2 host species (the Old World host *S. jacobaeae* and the New World host *S. triangularis*) and 3 blocks (blocking by geographic origin of insects) (2 Host Species x 5 infection dosage in 3 blocks ~ 600 individuals (minimum 150 individual in each block)).

We tested whether larval survival, developmental time, pupal mass, pupal survival, fertility and fecundity were influenced by the dose of infection, host plant species and by the pathogen x host plant interaction (interaction of plant and infection). We applied logistic regression for larval survival, pupal survival, and fertility (eggs hatched or not) (blocked by origin). We tested the impact of infection dose, host plant and block on developmental time and pupal mass using linear regression.

To assess reproduction we recorded (1) whether a female laid eggs or not, (2) if it did, how many eggs it laid. We tested the first condition using logistic regression. After removing the data of females not laying eggs, we tested if the number of eggs laid was correlated with the dose of infection, host plant or pathogen x plant interaction (interaction of plant and infection) (linear regression). We applied square root transformation on the number of eggs per female to meet the normality assumption.

We used S-PLUS 6.1 for Windows Professional Edition (2002 Insightful Corp.) for statistical analyses.

Life table response experiment

We designed and carried out a Life Table Response Experiment using a matrix model (Caswell 2001) to translate measured changes in vital rates into a projected change in population growth rate, a measure of the overall impact of each test plant species and disease dose on cinnabar moth populations. We developed a stage-structured, linear, deterministic model to represent the population dynamics.

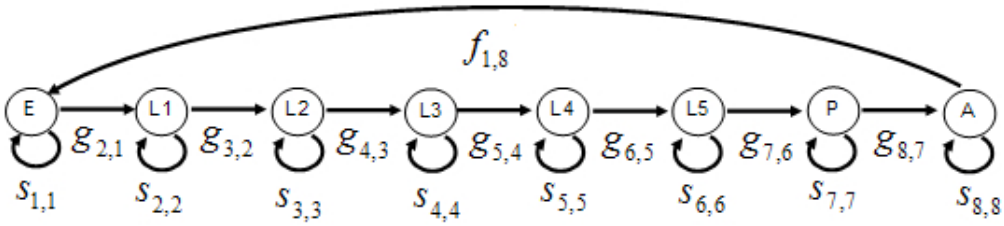
A life cycle graph for the cinnabar moth included 8 stages: egg (E), 5 larval stages (L1, L2, L3, L4, L5), pupa (P), and adult (A) (Figure 3.3). Transitions were first calculated as the probability of transition to stage *i* from stage *j*; stages vary in duration (measured in days), so we converted these transitions to daily rates to yield a uniform, 1-day time step for each transition in the matrix and a 1-day projection interval, following the methods described for the medfly (Ebert 1999).

The factorial experiment yielded parameter estimates for 20 matrices, one matrix (horizontal vs. vertical transmission) for each of 10 treatment combinations (2 Diets X 5 Pathogen Levels). We measured the stage duration (in days) and survival values of L1 to L2, L2 to L3, L3 to L4, L4 to L5, L5 to P, P to Adult stage transitions. We also added Pupal Survival, Fertility and Fecundity and Egg Survival parameters obtained from same experiments. For horizontal transmission we infected the moths with a pulse of pathogen transmission during the third instar (Figure 3.2, step 3); we estimated egg survival values from the control (uninfected) group. For vertical transmission, we used egg survival parameters from the infected group.

We used information from previous studies for the missing variable egg duration to complete the life table. We did not measure the duration of the egg stage E, so we substituted a literature value of 5 days. The weighted mean temperature T in our experiments was 21.7 °C, calculated by weighting day and night temperatures by the proportion of time associated with each phase of the photoperiod 16:8 L:D and thermoperiod 25:15 °C (Harman et al. 1989). We substituted the 5 days value of egg duration corresponding to the 22 °C (Rose 1978, Harman et al. 1990).

We calculated the finite rate of increase λ as the dominant eigenvalue associated with each matrix and compared the finite rate of increase projected for cinnabar moth populations on each plant species. We also estimated standard statistics including sensitivity, elasticity, damping ratios, stable stage distributions, and reproductive values for

each matrix (Caswell 2001). We used Excel PopTools (Version 2.7.5 released 25th Sep 2006) for analysis of the matrix population model.



$$A = \begin{pmatrix} s_{1,1} & 0 & 0 & 0 & 0 & 0 & 0 & f_{1,8} \\ g_{2,1} & s_{2,2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & g_{3,2} & s_{3,3} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & g_{4,3} & s_{4,4} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & g_{5,4} & s_{5,5} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & g_{6,5} & s_{6,6} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & g_{7,6} & s_{7,7} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & g_{8,7} & s_{8,8} \end{pmatrix}$$

Figure 3.3: The life cycle graph of the cinnabar moth. The graph illustrates the eight life-cycle stages representing egg (E), five larval stages (L1, L2, L3, L4, L5), pupa (P), and adult (A). The life cycle graph also illustrates the 16 life-cycle transitions in the model with seven representing growth g, eight representing stasis s, and one representing fertility f. The time-step in the model is one day. The life cycle graph can be represented as an 8 x 8 matrix A, which in turn can be used to project the dynamics.

Field observations

We estimated the prevalence and severity of the disease in field populations of the cinnabar moth on an Old World (*S. jacobaea*) and New World (*S. triangularis*) host-plant species from observations made at 10 locations from 2004 to 2006 (Table 3.1).

We visited many populations of cinnabar moths in Oregon from 2004 to 2006, and collected adults, larvae and eggs and inspected them for the presence of disease. The sites covered variety of habitats and elevations (Oregon coast, Willamette valley and Cascade mountain habitats) and included a variety hosts ranging from the native *Senecio* species (*S. triangularis, Packera pseudaurea, P. subnuda*) to the ancestral host of the Cinnabar moth, *S. jacobaea* (Table 3.1). We recorded location of the collection, elevation, and year as the main explanatory variables.

Collecting moths

Juvenile stages of the cinnabar moth are mostly clumped in nature and most of the time individuals on the same plants are from the same cohort. We took one larva from each plant and assumed that each plant had different cohorts. We put the larvae individually into 1 oz. plastic containers, transported them in an insulated container with blue-ice (Freez-Pak, Liofam®) (~ 2h), and upon reaching the laboratory, we stored them at -80°C until accessed for infection.

Counting spores

We removed the frozen larvae from the freezer, weighed, divided the weight into 30 (dilution factor) and added distilled water, ground larvae and homogenized the solution, counted the spores using hematocytometer, and calculated the number of spores in the whole body of the larva. We measured two main parameters prevalence (the frequency of infected individuals in the population) and the severity (the number of pathogen particles per individual) of disease. Prior research indicates these two variables are highly correlated in populations of the cinnabar moth in California, Oregon, and Washington, USA (Hawkes 1973).

Statistical analysis

We tested whether prevalence and severity of *Nosema* infection were influenced by host plant (2 host species, one Old World Host and one New World Host), elevation (continuous variable). We also added location of the moth collections (10 locations) and year (two years; 2005 and 2006) as explanatory variables. The number of individuals varied among 10 locations (min, max, mean). We applied logistic regression for testing prevalence (N = 338 individual insects). We did not run analysis on severity because the sample size was very small to test the hypotheses (N = 47). Instead, we tested the correlation between prevalence and severity.

RESULTS

Laboratory experiments

Influence of spore dose on larval mortality

The odds of larval survival decreased with the spore dose ingested by the 3rd instar larva (Figure 3.5A). Survival was lower on *S. triangularis* compared to the ancestral host *S. jacobaea* at all infection doses (Figure 3.5A, Table A2.1A). Larvae feeding on *S. triangularis* were disproportionately more vulnerable to disease-related mortality at the highest dose: We ran the analysis separately on high (10^3 and 10^4 spores per individual) and low doses (10^0, 10^1, 10^2 10^3 spores per individual) to investigate the host-plant by pathogen-dose interaction. Results suggested that host-plant x pathogen-dose interaction was not significant in low doses (0, 10^1, 10^2, 10^3 spores), however as the infection dose increased (10^3, 10^4 spore), a dose-plant interaction stood out as significant (Figure 3.5A, Table A2.1A). The block (season and origin of the larvae) was not significant; indicating that resistance/susceptibility of cinnabar moth to *Nosema* did not vary with the origin of larvae (Table A2.1A).

Survival curves showed that larvae feeding on *S. triangularis* started to die earlier compared to the other groups (Figure 3.4). Mortality occurred mostly during the 5th instars accompanied by a prolonged 5th instar (Figure 3). The first individual pupated 26 days (*S. jacobaea*, Dose 0) and the last pupated 44 days (*S. triangularis*, Dose 4) after the eggs hatched.

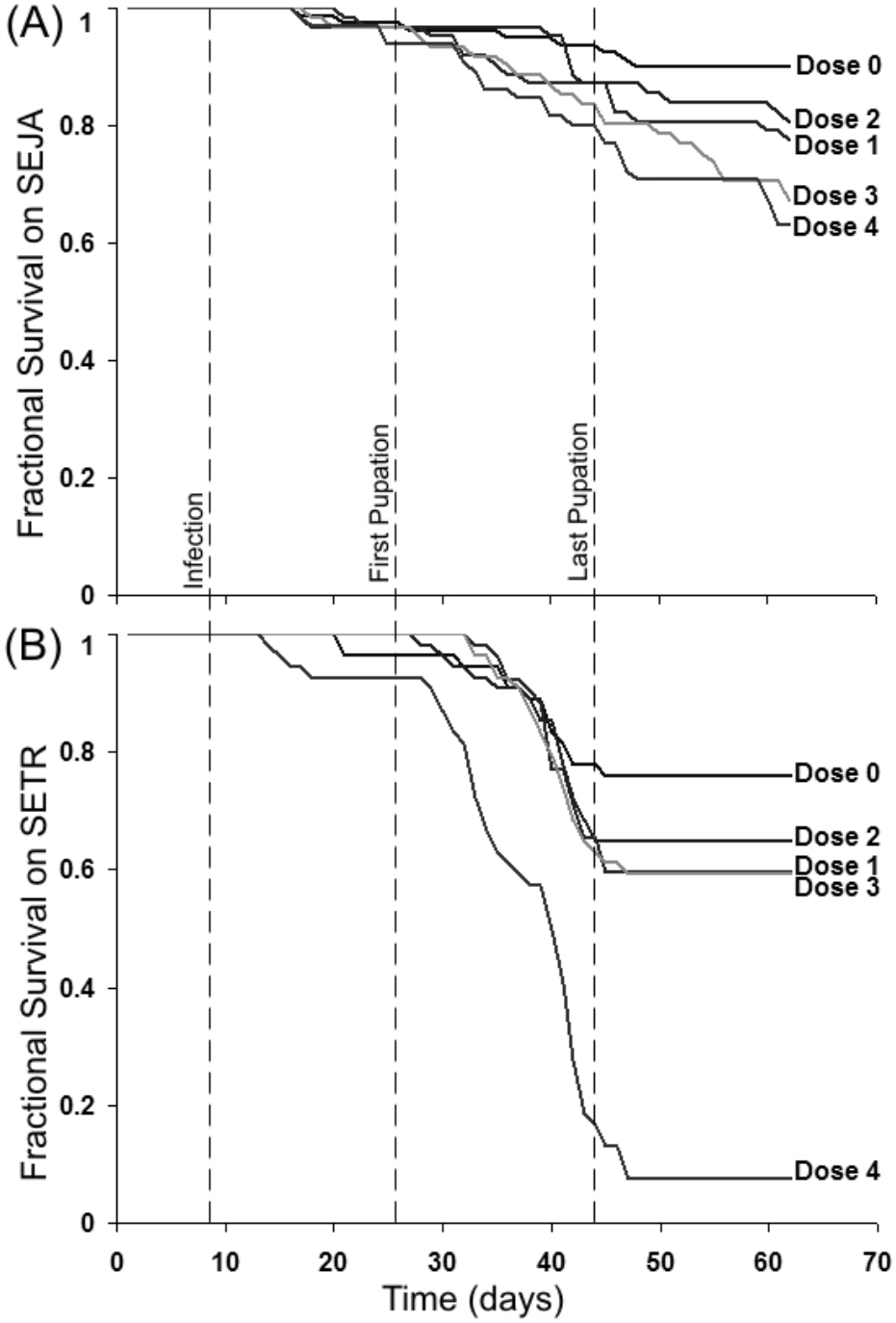


Figure 3.4: Survival curves of the cinnabar moth. The insects feeding on A) *S. jacobaea* (SEJA) and B) *S. triangularis* (SETR). The insects were infected right after the 2nd eclosion (~8 days), the first individual pupated on the 26th day (SEJA Dose 0) and the last individual pupated on the 44th day (SETR Dose 4).

Larval development

Developmental time increased with increasing spore dose, approximately 2.4 % for each log increment in inoculation dose (Figure 3.5C, Table A2.1B). Plant species did not influence developmental time (Figure 3.5C, Table A2.1B). Parameters for the Linear Regression Model were estimated as Developmental Time = 29.7 + 0.71Dose – 0.15Block1 – 0.15Block2. The plant-dose interaction term was not significant (linear regression, F = 0.81; d.f. = 389, 1; p = 0.37; R^2 = 0.19) (Table A2.1B).

Pupal mass

Pupal mass decreased with increasing spore dose, approximately 2.9 % for each log increment in inoculation dose (Figure 3.5D, Table A2.1C). Larvae reared on *S. triangularis* were 7.2 % smaller than on *S. jacobaea* (Figure 3.5D, Table A2.1C). Parameters for the Linear Regression Model were estimated as Pupal Mass = 0.1839 - 0.0053Dose - 0.0132Plant Species. Block (linear regression; F = 1.72; d.f. = 386, 2; p = 0.18; R^2 = 0.24) and plant-dose interaction terms (linear regression; F = 1.20; d.f. = 386, 1; p = 0.27; R^2 = 0.24) did not have a detectable impact on pupal mass (Table A2.1C).

Pupal survival

The odds of an adult emerging from the pupa decreased with the spore dose ingested by the 3rd instar (Figure 3.5B, Table A2.1D). Pupal survival was lower on *S. triangularis* compared to the ancestral host *S. jacobaea* at all infection doses (Figure 3.5B, Table A2.1D). Probability of Pupal Survival = -0.63Dose + 0.40Plant. Block and plant-dose interaction Block did not have significant impact on pupal survival (Table A2.1D).

Fecundity & Fertility

The odds of a female laying eggs did not depend on the inoculation dose (Figure 3.5E, Table A2.1E). However, it was higher on females reared on *S. jacobaea* compared to *S. triangularis* (Table A2.1E). Sample sizes became smaller and more uneven with increasing dose due to mortality experienced throughout the life cycle. Among the females

feeding on *S. jacobaea,* 14 out of 17 (Control), 6 out of 8 (10 spores), 2 out of 6 (10^2 spores) and 2 out of 3 (10^3 spores) laid eggs; there was only one female on 10^4 spore dose, and she didn't lay any eggs. Among the females feeding on *S. triangularis*, 4 out of 7 (Control), none of the females (out of 2) laid eggs at a dose of 10^1 spores; 1 out of 1 laid eggs at a dose of 10^2 spores ; there were no females on doses 10^3 or 10^4 (Table 3.2). However the number of eggs per female (given that she laid eggs at all) was strongly influenced by test plants and inoculation dose (linear regression, $F = 2.785$; d.f = 5, 24, $p = 0.04029$; $R^2 = 0.37$) (Table A2.1G, Figure 3.5E). The females reared on *S. triangularis* laid 26 % fewer eggs. Each increment of increase in inoculation dose yielded to 18% fewer eggs (Sqrt Fecundity = 10.20 – 2.20Plant– 1.84Dose).

The odds that the eggs of a female would hatch decreased with the inoculation dose (Figure 3.5F, Table A2.1H). The impact of host plant was inconclusive with a p-value 0.05611 (Table A2.1H) Fertility probability = -0.04Dose + 0.086Plant). The eggs of 17 females hatched (out of 29), 13 females out of the 17 (76 %) were from *S. jacobaea* control group (Dose 0). Two females among 4 (50%) *S. triangularis* uninfected females laid eggs that hatched.

Block and plant-dose interaction were not significant for both fertility and fecundity analyses (Table A2.1E, 2F, 2G, 2H).

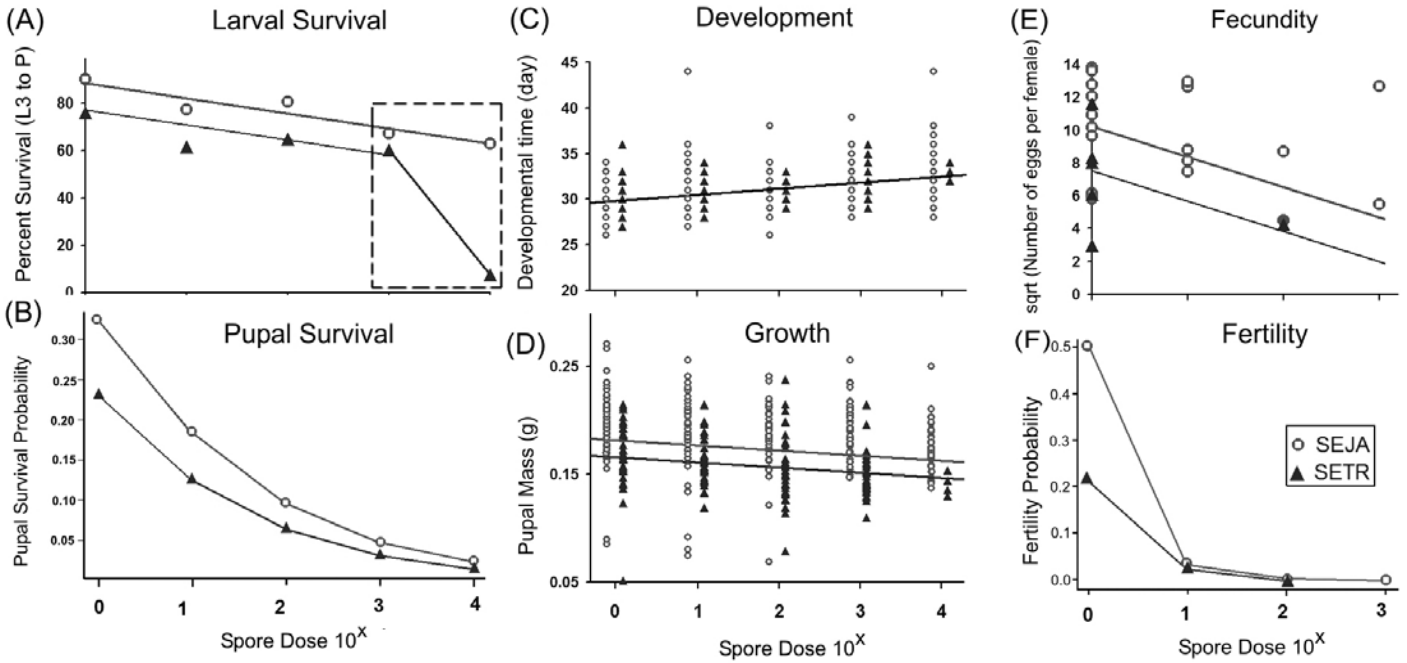


Figure 3.5: Performance results for the disease experiment. Larvae feeding on *S. jacobaea* were more resistant to disease compared to the ones feeding on *S. triangularis* for some fitness measures. A) Percent Survival of larvae from L3 to P is diagramed. Trendlines are added to show the trend of the data. Dose 0 to 103 were analyzed separately from Dose 103 to 104 to show the interaction appearing at the higher doses. B) Logistic regression fitted curves showing the estimated pupal survival probability for each test plant (Probability of Pupal Survival = -0.63Dose + 0.40Plant). C) Scatterplot for the developmental time (from L1 to P) (Developmental Time = 29.7 + 0.71Dose – 0.15Block1 – 0.15Block2). D) Scatterplots showing the pupal mass of the moths (Pupal Mass = 0.1839 - 0.0053Dose - 0.0132Plant). E) Fecundity as the proportion of female cinnabar moths laying eggs (Fecundity = 10.20 – 2.20Plant– 1.84Dose). F) Logistic regression fitted curves showing the estimated probability of a female being fertile reared on two major host plants (Fertility Probability = -0.04Dose + 0.086Plant). *S. jacobaea* (SEJA- empty circles) and *S. triangularis* (SETR – filled triangles).

Table 3.2: Number of females in fertility and fecundity experiments. As experiment continued we had lower sample sizes in the higher doses because these individuals couldn't survive or complete the life cycle.

Senecio jacobaea							
Inoculated Dose	**Number of Females**	**Number of ovipositing females**	**Proportion of oviposition females**	**Total Number of eggs laid (all eggs)**	**Total Number of hatches (all hatching eggs)**	**Eggs per female**	**Number of viable eggs per female**
Control	17	14	0.82	2630	2140	154.7	125.9
10^1 Spores	8	6	0.75	779	233	97.4	29.1
10^2 Spores	6	2	0.33	96	0	16	0
10^3 Spores	3	2	0.67	191	0	63.7	0
10^4 Spores	1	0	0.00	0	NA	0	NA
Senecio triangularis							
Inoculated Dose	**Number of Females**	**Number of ovipositing females**	**Proportion of oviposition females**	**Total Number of eggs laid (all eggs)**	**Total Number of hatches (all hatching eggs)**	**Eggs per female**	**Number of viable eggs per female**
Control	7	4	0.57	315	50	45	7.1
10^1 Spores	2	0	0	0	NA	0	NA
10^2 Spores	1	1	1	18	0	18	0
10^3 Spores	0	NA	NA	NA	NA	NA	NA
10^4 Spores	0	NA	NA	NA	NA	NA	NA

Projected population growth rates

Case 1 Horizontal transmission only

We first assumed that insects acquired the pathogen by horizontal transmission in the third instar and none of the eggs had received the pathogen by vertical transmission from their mothers. Population growth rates of the cinnabar moth declined with increasing *Nosema* spore concentration; the negative slope of this relationship indicates that the pathogen has adverse effects (Figure 3.6A). The New World Host (*S. triangularis*) is inferior to the Old World host (*S. jacobaea*); the lower intercept indicates that population growth is lower on New compared to Old host species. The lines for each host are parallel, suggesting that diet and pathogen do not interact in their effects. However, qualitative description of the relationship between population growth, spore concentration, and host plant species changes when we increase realism by adding vertical transmission.

Case 2: Horizontal and vertical transmission combined

When we combine horizontal and vertical transmission and included the actual survival rates of the eggs into the model, diet and pathogen interacted in their effects (Figure 3.6B). At low spore doses (left side of the graph), there was no detectable effect of pathogen infection in caterpillars on the Old World species *S. jacobaea* and a devastating effect of pathogen infection on the New World host species *S. triangularis*. The host-plant-species effect was nil in uninfected insects, and huge in infected insects. At high spore doses (right side of the graph), the effect of pathogen infection is so overpowering that no effect of diet (host plant species) can be expressed.

To summarize the results so far, at the individual level, high infections were devastating to cinnabar moth populations on the New World host; larvae suffered high mortality at higher doses in the laboratory when reared on *S. triangularis*. At the population level, mild pathogen infections were devastating to cinnabar moth populations on the New World host and inconsequential on Old World host Plants; by contrast severe pathogen infections were devastating on both New and Old World host plant species.

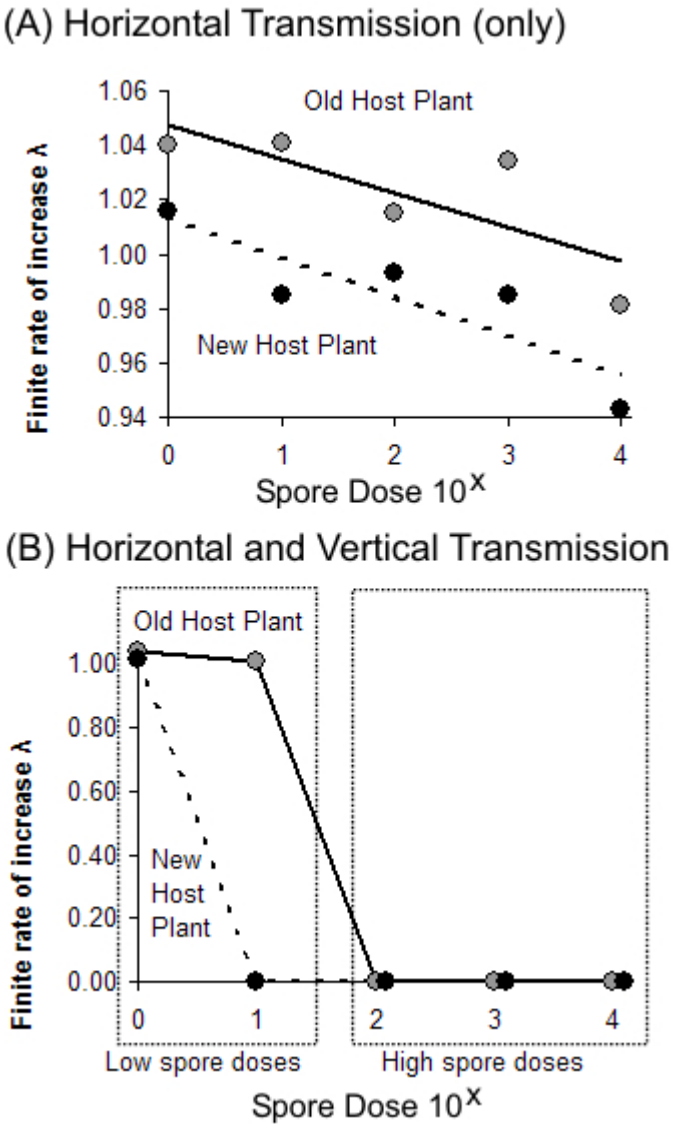


Figure 3.6: The relationship between population growth (finite rate of increase λ) of the cinnabar moth population and the treatment factors diet (foliage of New and Old Host plant species) and pathogen infection (spore concentration). (A) for the case of horizontal transmission only (B) for the case combining horizontal and vertical transmission.

Field observations

Prevalence (proportion of infected individuals) declined with elevation and at elevations where two hosts overlapped; prevalence was higher at *S. jacobaea* sites compared to *S. triangularis* sites (Figure 3.7, Table A2.1I).

Little Crater Lake and Timothy Lake were the only two populations where disease was present among sites with New World Hosts. Only one infected individual was found from each of these locations out of the total individuals sampled at each site ($N_{\text{Timothy Lake}}$ = 28 and $N_{\text{Little Crater Lake}}$ = 17). Therefore we didn't have sufficient sample size to apply statistical tests to compare and contrast the severity of disease in new vs. old host plant sites. However, these two locations had very low levels of disease (10^3 spores per ml of larva); whereas on *S. jacobaea* sites spore dose per ml of larval tissue ranged in between 10^5 and 10^8. We found a strong positive correlation with the prevalence and intensity of disease (linear regression; F = 22.8; d.f = 6, 1; p = 0.003). Severity = 720175 + 15765763 Prevalence (Figure 3.6).

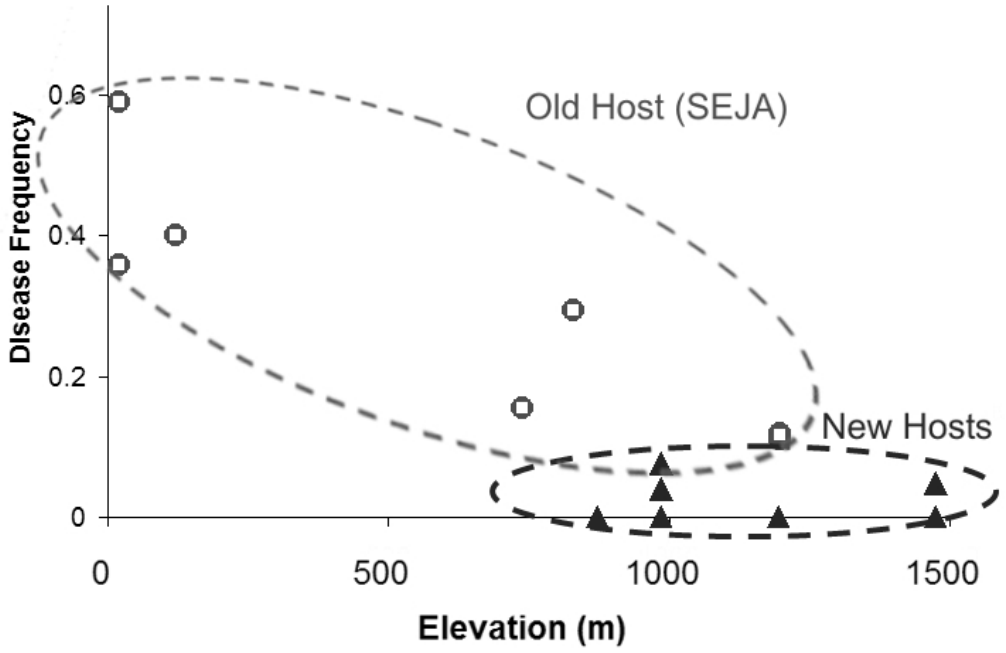


Figure 3.7: Scatterplot demonstrating the prevalence of disease on Old World and New World Host sites. Prevalence (proportion of infected individuals) declined with elevation and at elevations where two hosts overlapped; prevalence was higher *at S. jacobaea* sites compared to *S. triangularis* sites (Table A2.1I). S. jacobaea (SEJA): empty circles. New host sites: filled triangles.

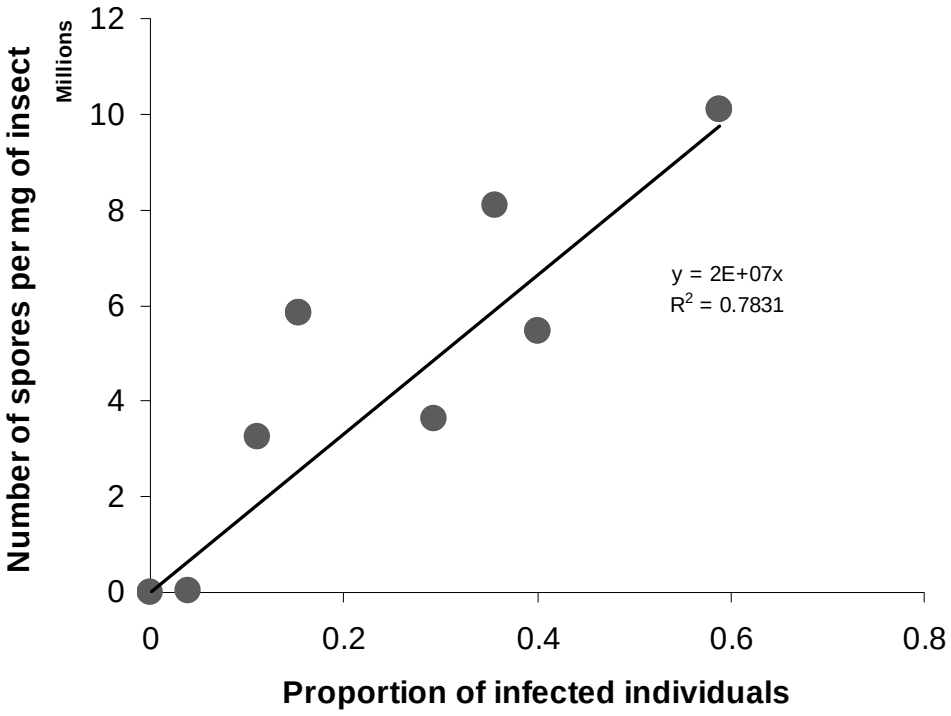


Figure 3.8: Correlation of prevalence and severity. Severity (number of spores per mg of insect) and prevalence (proportion of infected individuals) and were positively correlated (linear regression; F = 22.8; d.f. = 6, 1; p = 0.003). Severity = 720175 + 15765763 Prevalence (Proportion of infected individuals).

DISCUSSION

The relationships between the pathogen, *Nosema tyriae* and the cinnabar moth *T. jacobaeae* differ on two of the moth's host plant species, one a New World host *S. triangularis* and the other the Old World host *S. jacobaea* We concluded that (1) *Nosema* had negative influence on all cinnabar moth performance parameters on both host plant species; (2) the Old World host *S. jacobaea* was a better host compared to the New World host *S. triangularis*; (3) at the individual level results are conflicting for different vital rates and net effect of conflicting changes in vital rates is made clear only by the analysis of population growth; (4) at the population level, mild pathogen infections were devastating to cinnabar moth populations on New World host and inconsequential on Old World host Plants and severe pathogen infections were devastating on both New and Old World host plant species.

Effect of host quality

In the absence of disease, *S. jacobaea* was a superior host compared to *S. triangularis*: more larvae and pupae survived, pupae were bigger and fertility was higher on the moths that were reared on *S. jacobaea*. Nutritional quality of the plants might be the major reason for this difference. Nitrogen is a critical limiting nutrient in terrestrial ecosystems, and superior growth, development, and reproduction often occur in insects on plants with higher nitrogen content since nitrogen is the building blocks for the amino acids and thus proteins (Scriber and Slansky 1981b). Prior studies investigating the relationship between the nutritional quality of host plants and its correlation to performance in the cinnabar moth found that nitrogen is a limiting element in cinnabar moth diet (Dempster 1970a, 1971, 1975, 1979, Myers and Post 1981, Pajutee 1981, van der Meijden et al. 1984). Yet our previous study (Chapter 2) concluded that *S. triangularis* and *S. jacobaea* had no detectable difference in nitrogen levels, averaging 2.8% per mg of dry sample which is above the minimum required for herbivorous insects' growth (~1%) (Strong et al. 1984). On the other hand, in our previous study we also found that another *Senecio* species, *S. integerrimus*, which was an unsuitable plant, had adequate levels of

nitrogen for insect growth (Chapter 2). We conclude that *S. triangularis* is nutritionally less suitable than *S. jacobaea*, but nitrogen content does not explain the observed differences in performance on the two host plants, and we must look for other explanations.

The term host quality does not only include nitrogen content, but also the secondary metabolites, or other trace elements that affect the performance of herbivorous insects (Awmack and Leather 2002). These traits might act as attractants or stimulants affecting feeding rates; they might act toxins or digestibility reducers which can exert sublethal effects by impairing growth and reducing fecundity (Price et al. 1980, Awmack and Leather 2002). Plants in the genus *Senecio* contain pyrrolizidine alkaloids and previous studies show that the diversity and concentration of pyrrolizidine alkaloids in genus *Senecio* differ from species to species (Christov et al. 1997, Bourauel et al. 1998, Christov et al. 2002, Macel et al. 2002, Christov and Evstatieva 2003, Conforti et al. 2006). Yet many studies demonstrated that variation in cinnabar moth larval performance is not correlated to the subtle variation in pyrrolizidine alkaloid composition (Vrieling and Boer 1999, Macel et al. 2002, Macel and Vrieling 2003). For example, Macel et al. (2002) studied eight different *Senecio* species, found differences in larval performance between species, but no correlation between larval performance and pyrrolizidine alkaloid composition. In this study we only concentrated on the nutritional quality of the host plants since other studies already showed the lack of correlation between the alkaloids and performance of insects. Meijden et al. (1984) showed that nitrogen and alkaloid concentrations are positively correlated in *S. jacobaea*. They argue that high nitrogen levels usually means high alkaloid levels, and high levels of alkaloids can be poisonous even for the cinnabar moth which can sequesters these alkaloids. Diversity and concentrations of alkaloids might explain the relatively poor performance of the cinnabar moth on *S. triangularis*. It has been found that *S. triangularis* has four major types of alkaloids, 7-angelyretronecine, 7-senecioylretronecine, 7-angelyl-9-sarracinylretronecine and 7-senecioyl-9-sarracinylrotrenecine (Rueger and Benn 1983). Rueger and Benn (1983) argue that the last three of these alkaloids are new natural products, not previously reported from *S. jacobaea* or other plant species. Future studies including the impact of composition, concentration and diversity of pyrrolizidine alkaloids on the cinnabar moth might still

reveal the underlying mechanisms of the observed lower performance on *S. triangularis*. At this point, the chemical and physical mechanisms explaining the lower performance of the cinnabar moth on *S. triangularis* compared to *S. jacobaea* are unknown. Although *S. triangularis* is a less suitable host compared to *S. jacobaea*, population level studies suggest that larvae reared on *S. triangularis* projected a finite rate of increase (λ) greater than 1 and there are cinnabar moth populations in nature that feed solely on *S. triangularis* (Diehl and McEvoy 1990).

Effect of disease

Nosema tyriae had negative effects on the performance of the cinnabar moth. Survival decreased, development slowed, pupae got smaller, fecundity and fertility, and projected population growth rates decreased with increasing spore doses in experimental cinnabar moth populations. At low doses of the pathogen the effect of the pathogen on cinnabar moth depended on host. The impact of high doses of pathogen on the cinnabar moth populations was overwhelmingly high on both old world and new world host plants and projected a population decline. Ours appears to be the first experimental study evaluating the impact of *Nosema tyriae* on cinnabar moth demography and population dynamics. Yet Bucher and Harris (1961) observed similar findings when they accidentally observed the pathogen in their lab-reared cinnabar moth populations; they state that disease progress is slow and lightly infected larvae might produce normal pupae, but the disease seemed to increase mortality during pupal stage, and it causes death when infection is widespread enough to interfere seriously with the infected tissues. Studies with other *Nosema* species infections also recorded reduced adult mating success, reduced longevity and fecundity, prolonged larval development and failure in pupation was well as pupal deformities (Solter and Maddox 1998b, Inglis et al. 2003).

We studied this insect-pathogen interaction isolated in the lab environment. Three simplifying assumptions implicit in these experiments may not hold in natural populations. First, we reared larvae individually in our lab yet many stages of the cinnabar moth are gregarious in nature. We assumed a pulse of horizontal transmission. Aggregation of

insects increases contacts between uninfected insects and pathogen particles from carcasses, feces, and exuviae of infected insects and may therefore increase transmission of the pathogen. Secondly, we conducted our experiments in a closed environment that contrasts with the open environment in nature, where insects move freely in search of food and avoid competition. Movement increases contacts with spores and thereby increases transmission rates. Thirdly, we assumed that our insects were exposed to pulse of spores in a single stage, yet in nature they are continuously exposed to spores throughout the life cycle, leading to higher infection levels. Finally we conducted these experiments under optimal conditions yet conditions in field are suboptimal.

To relax these simplifying assumptions, we recommend conducting transmission experiments in cinnabar moth populations on two host plant species in the field like those used by Dwyer in studying baculovirus epidemics in gypsy moths (Dwyer et al. 2005). Laboratory experiments like ours measure the probability of infection given known amount of pathogen consumption. Field transmission experiments measure the overall probability of infection. Dwyer et al. (2005) argues that if overall probability of infection is $p(\mathrm{I})$, the probability of infection given pathogen consumption is $p(\mathrm{I/C})$, and the probability of pathogen consumption is $p(\mathrm{C})$, then

$$p(\mathrm{I}) = p(\mathrm{I/C})p(\mathrm{C}).$$

Field and laboratory transmission experiments that estimate the probability of pathogen consumption on different hosts, together with our dose-response experiments, would allow us to estimate the overall probability of infection in cinnabar moth populations on Old and New World host plants, and thereby demonstrate the behavior of disease on the cinnabar moth populations in nature.

Interaction of host plant and disease

The New World Host, *S. triangularis* had substantial direct and indirect effects on the survival of the cinnabar moth, direct effects through host plant and indirect effects by

increasing their susceptibility to the pathogen, *Nosema tyriae*. At the individual level results are conflicting for different vital rates. For example the survival dropped significantly on the moths reared on the *S. triangularis* at higher doses. Yet for other vital rates this interaction impact was not significant. The net effect of conflicting changes in vital rates is reflected in the population growth rates. At the population level mild infections were benign in cinnabar moth populations on *S. jacobaea*, the Old World Host, while comparatively virulent in cinnabar moth populations feeding on *S. triangularis*. The strength of the pathogen- insect interaction depended on the plant species; it was weaker on the Old World host (*S. jacobaea*) than on the New World host (*S. triangularis*) both at individual and population levels. We expected and demonstrated that the negative effect of malnutrition was magnified with the *Nosema* infection and vice versa. Other studies on other *Nosema* species found similar conclusions; adverse effects on herbivores from weather extremes, malnutrition, and presence of insecticides elevated the impacts of the *Nosema* infection (Solter and Maddox 1998b, Inglis et al. 2003).

Host plant quality can affect the interactions between herbivorous insects and microbial control agents via the diet of the herbivore (Awmack and Leather 2002). A plant might either be nutritionally poor and enhance susceptibility of an insect to disease (Price et al. 1980, Cory and Hoover 2006), or might contain some secondary metabolites that reduce the digestibility of food (Price et al. 1980) or enhance the effectiveness of entomopathogens in killing the host (Cory and Hoover 2006). For example alkaloids or other secondary metabolites or phytochemicals can bind to occlusion bodies in the larval midgut and reduce the infectivity of the pathogen (Cory and Hoover 2006). Studying combined effects of alkaloids and other possible chemical factors might be important to understand the biochemical interactions of entomopathogen and disease. While the mechanisms have not been confirmed, the observed increased susceptibility of cinnabar moth populations to disease on *S. triangularis* compared to *S. jacobaea* might indicate that *Nosema tyriae* might constrain host shifts and help prevent cinnabar moth from imposing adverse effects on a nontarget plant species *S. triangularis*.

Entomopathogen-Insect-Plant interactions in the field

Our field survey measured prevalence and severity of microsporidiosis in cinnabar moth populations on *S. jacobaeae* and *S. triangularis* along an elevation gradient. We expected prevalence to be higher on *S. triangularis* since new hosts increased cinnabar moth susceptibility to disease. Yet, the opposite was true. At elevations where two hosts overlapped; prevalence was higher at *S. jacobaea* sites compared to New World Host sites. Prevalence and severity were positively correlated. In New World Host sites, disease was a very rare occasion and when present, it was less severe compared to the Old World host sites.

Two conditions have to be met for the persistence of a pathogen (1) a minimum, threshold host population size must be exceeded (Anderson and May 1979, Onstad et al. 1990) (2) the disease should not be too virulent or it will depress the host population below the threshold (Onstad et al. 1990). If the threshold cinnabar moth population is very low on the new host, transmission rates might be slow; and the disease becomes less prevalent and less severe. This might be a possible scenario since higher elevation New World Host moth populations might be below the threshold due to environmental factors. Second scenario dictates that if the pathogen is more virulent in cinnabar moth populations on new host, then transmission may be reduced and the prevalence of the pathogen may decline. Unfortunately, it is difficult to establish a cause effect relationship from observational data. More field and laboratory studies are required to resolve the question of which scenario operates in the field.

We found that prevalence declined with elevation, but we acknowledge that many potentially influential variables covary with elevation. Confounded variables include the distribution and abundance of host plant species, the distribution and abundance of cinnabar moths, and temperature. We expect cinnabar moth populations to become smaller with increasing elevation due to unfavorable abiotic conditions and lower abundance of high quality food resources. Temperatures become lower and growing seasons become shorter with increasing elevation. Ragwort populations become smaller

and *S. triangularis* populations become larger with increasing elevation. Global warming is presently pushing distributions to higher latitudes and elevations and advancing phenologies of some insects (Parmesan 2006). Similar changes may be occurring in this system of interactions, putting more native plant species at risk to cinnabar moth attack. Despite evidence of prevalence and severity of parasite infection in the field, relatively little is known about the effects of this parasite on the population dynamics of its host in the field.

Summary

We used a Life Table Response Experiment combining a factorial experiment and a matrix model to estimate the independent and interacting effects of malnutrition and disease on an herbivore in a tritrophic entomopathogen – herbivore – plant system consisting of the microsporidian pathogen *Nosema tyriae*; a phytophagous insect the cinnabar moth *Tyria jacobaeae* (introduced from Europe to North America for biological control of tansy ragwort, *Senecio jacobaea*); and two host plant species, one native to Europe *Senecio jacobaea* (target plant) and one native to North America *Senecio triangularis* (non-target host plant). The factorial experiment estimated the effects of five doses of the pathogen combined compounded with two host species on the demographic performance of the cinnabar moth. All cinnabar moth vital rates (rates of growth, development, survival, and reproduction) decreased with the increasing dose of pathogen *Nosema* spores. Vital rates generally were lower on the New World host *S. triangularis* compared to Old World host *S. jacobaea*. At individual level, the pathogen x host interaction was significant on survival at higher doses, but we didn't observe any effect of the interaction term on other performance parameters (development, growth, fertility, fecundity). At the population level, the effect of one factor depended on the level of the other factor at lower doses of the pathogen (*Nosema tyriae*). The projected population growth rates of cinnabar moths were more sensitive to low infection dose in cinnabar moth populations on the New World host, *S. triangularis* compared to the Old World host, *S. jacobaea.* Field prevalence (proportion of infected individuals) of the *Nosema* declined with elevation and at elevations where two hosts overlapped, prevalence was higher at *S.*

jacobaea sites compared to *S. triangularis* sites. The presence of disease might constrain hosts shifts by the cinnabar and provide protection for the non-target plant species against adverse effects of this biological control organism. Detailed field and lab studies are required to reveal the dynamics between moth abundance, plant abundance, disease frequency and host plant damage.

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CHAPTER 4: GENERAL CONCLUSIONS

We evaluated the interactions within a tritrophic system composed of the cinnabar moth (herbivorous insect), its Old and New World Hosts, and its pathogen (*Nosema tyriae*). We first concentrated on the two-trophic (herbivore-host plant) interactions and their strength and later we incorporated the impact of the third trophic level and measured the individual and interacting impacts of the pathogen and host plants on the performance of the cinnabar moth.

A recap

In the first part of the thesis, we concentrated on the two-trophic level insect herbivore interactions of the cinnabar moth with its Old World and New World host plants. We demonstrated the host choice by adults and larvae, host suitability, the correlation between preference and performance parameters, and the impact of nutrients levels as a component of host quality. We could include 6 (*P. bolanderi, P. subnuda, P. flettii, P. pseudaurea, S. integerrimus and S. triangularis*) out of 9 North American species that were exposed to the cinnabar moth in Oregon. Both the larvae and adults were highly selective and they had similar choices except minor conflicts (larvae were less discriminating that adults), which actually might pose a survival advantage for starving larvae wandering in search of food. We measured host suitability in terms of finite intrinsic rate of the moth populations reared on the test plants, since the true reflection of the host quality would be visible on the cinnabar moth population dynamics. Although all the test plants supported development at least to the pupal stage, we concluded that not every native relative of the *S. jacobaea* was acceptable and suitable. Preference and performance of the cinnabar moth (both larvae and adults) were positively correlated. If moths selected the unsuitable plants, they would have a lower survival and colonization capacity especially in novel environments. This was not the case in our system. The cinnabar moths selected the

suitable plants, and thus preference performance correlation posed no obstacle in colonization of new habitats with new host plant species. Nitrogen, a limiting element in cinnabar moth diet, might be one of the factors determining host selection, as adult cinnabar moths selected the high nitrogen containing plants. In summary the cinnabar moth appeared to have the ability to form successful new host-plant associations in novel environments.

In the second part of the thesis, we incorporated the impact of the third trophic level, the pathogen (*Nosema tyriae*). We concentrated on only two host plant species, one New World Host (*S. triangularis*) and one Old World Host (*S. jacobaea*) as they were the most acceptable and suitable test plants. We demonstrated the independent and interacting effects of malnutrition and disease on the cinnabar moth. *S. triangularis* was a suitable host even though it was inferior compared to *S. jacobaea*; *Nosema* decreased the fitness of the insects both at individual and population levels, and *S. triangularis* had substantial effects on the survival of the cinnabar moth by increasing their susceptibility to the pathogen. Field studies showed that at elevations where two hosts overlapped, prevalence was higher at *S. jacobaea* sites compared to New World Host sites. Teasing apart the confounded environmental factors will require more detailed field and lab experiments. In summary, in our system the new host increased the susceptibility of the herbivore to adverse effects of a pathogen (*Nosema tyriae*).

In conclusion, we observed strong correlation between preference and performance of the cinnabar moth and nutrient quality on the New World and Old World test plants. Yet even the most successful new host-herbivore association was more vulnerable to the impact of the natural enemy.

Future studies

This study illustrates the importance of tritrophic interactions, by the first showing how herbivore behavior, demography, and population growth vary between two host plant species in a two-trophic-level interaction and then showing how herbivore demography and

population growth vary across host plant species in a three-trophic-level interaction created by adding an entomopathogen as a natural enemy. We supported our experiments with the field observations and previously conducted field studies. Yet, our findings yield new questions. The need for the detailed field and lab studies emerged to reveal (1) the population dynamics of the cinnabar moth feeding on the unsuitable hosts in the field, (2) the impact of the cinnabar moth on populations of the native hosts, (3) the influence of elevation and temperature on the pathogen-cinnabar moth interaction, (4) variation in disease transmission rates on different host plant species and (4) the feasibility of using *Nosema tyriae* for mitigating the nontarget impacts of the cinnabar moth.

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Vrieling, K., and N. J. d. Boer. 1999. Host-plant choice and larval growth in the cinnabar moth: do pyrrolizidine alkaloids play a role. Entomologia Experimentalis et Applicata. **91**:251-257.

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APPENDICES

APPENDIX 1

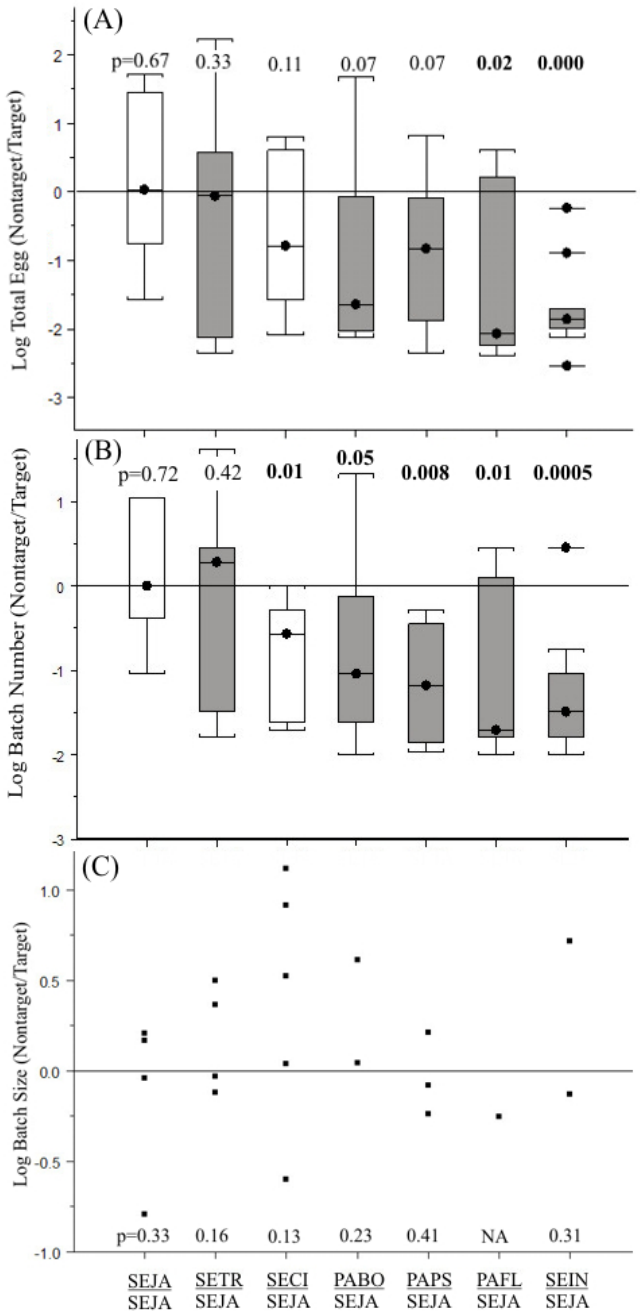

Figure A1.1: Adult oviposition paired choice results showed that females became more selective when given a choice. a) Log (total egg number on nontarget)/ (total number on target) b) Logratio of total egg number of nontarget over target c) Scatterplot showing the Log batch size ratio of nontarget over target (one sided, paired t test, null: parameters on nontarget is greater or equal to target). Figure a and b Boxplot showing the distribution of data. Median, and first-third quantiles were shown, lines with dots show outliers.

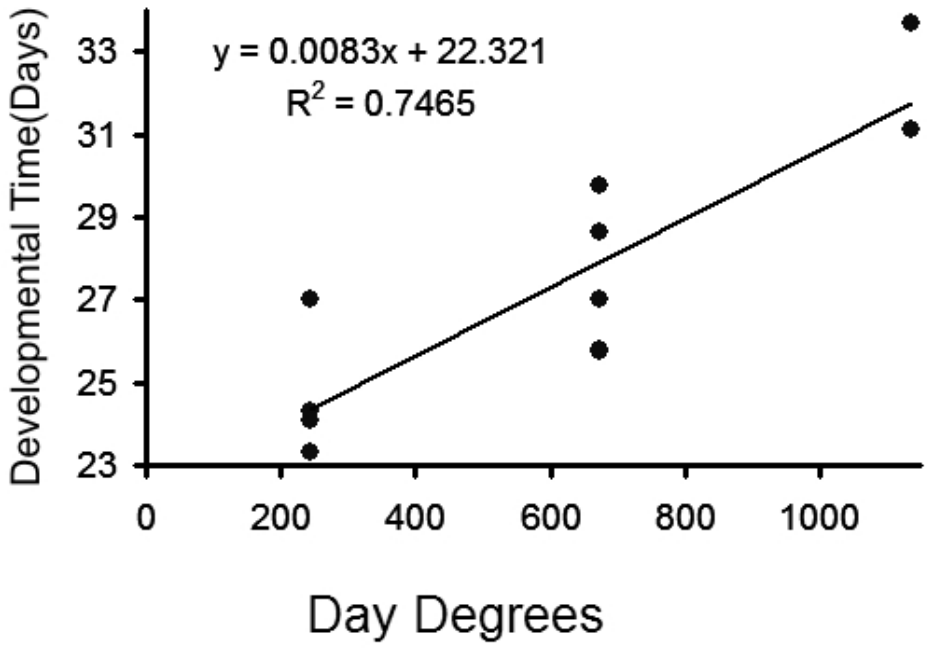

Figure A1.2: Developmental time increases with the available day degrees (ANOVA with Tukey's Multiple Adjustment, $p = 5.61 \times 10^{-5}$, $F = 14.6039$, d.f. = 2, 26). Willamette Valley (Baskett Slough), Coast (Neskowin) and Cascades (Santiam Pass) were the major source populations for the larvae during 2004 Larval Performance experiments.

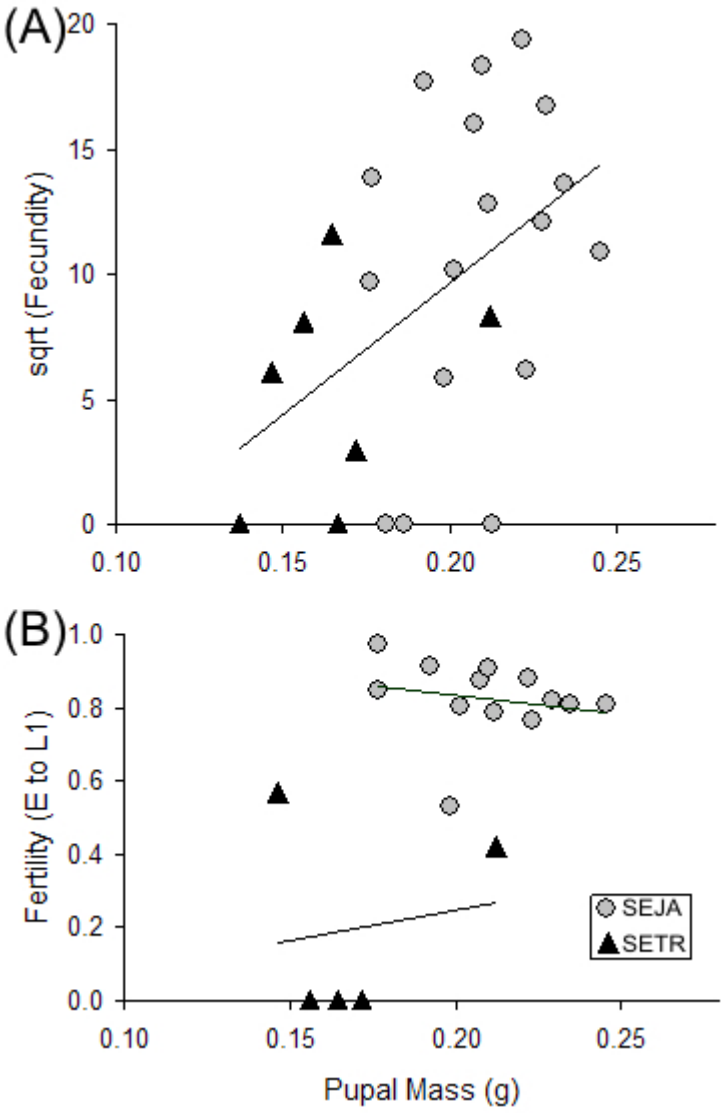

Figure A1.3: Scatterplots illustrating the relationship between Pupal mass and Fertility and Fecundityg . (A) As pupae got bigger fecundity increased fecundity (log (total number of eggs per female)) vs. Pupal Mass (F = 6.381015, d.f. = 2, 21, p-value = 0.0196384, residual standard error = 5.788, Multiple R^2 = 0.2404). (B) There wasn't a significant linear relationship between fertility and pupal mass (F = 0.0714, d.f. = 2, 15. p = 0.944) Gray Circles: *S. jacobaea*, Black triangles: *S. triangularis*.

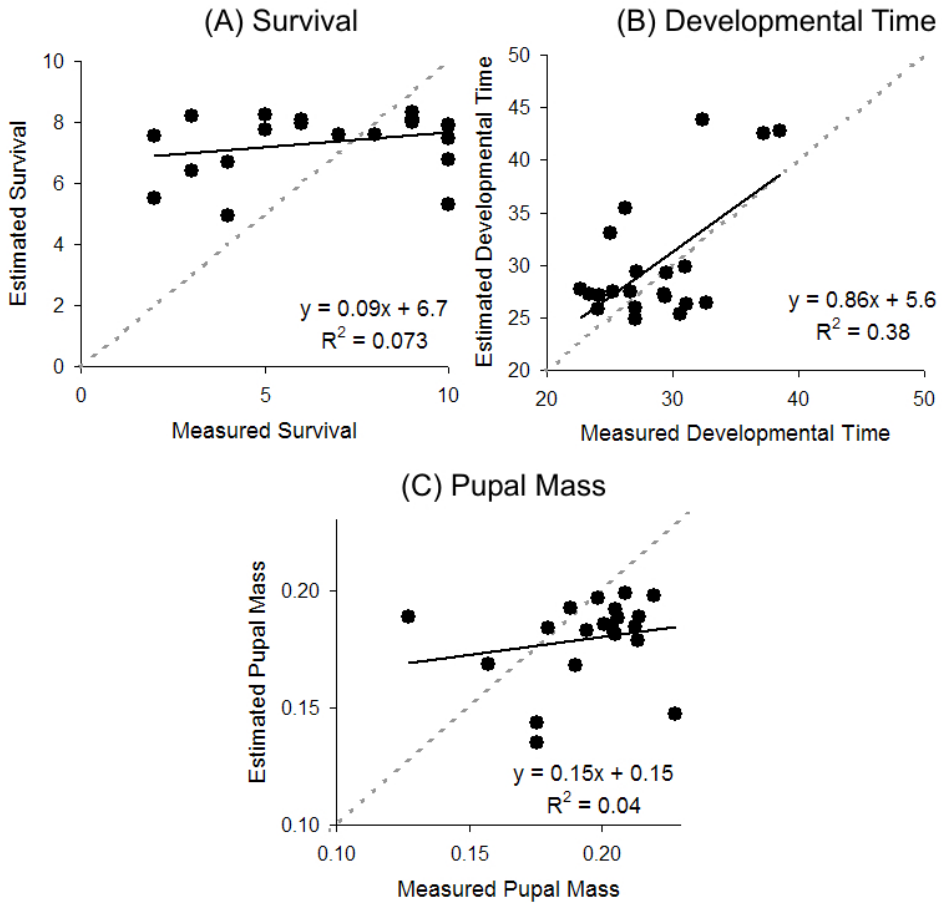

Figure A1.4: The relationship between Estimated and Measured parameter values for the larval performance 2004 paired choice experiments : We plotted insect density as a function of time for each host plant species and integrated the area under the curve to obtain "density duration" – a measure that combines intensity and duration of the response. We then calculated weighted mean performance on the pair of species as the sum of performances on each host species weighted by the fraction of total density duration accounted for by each host species. We calculated the fraction of larvae on each plant (and off the plants) and obtained a fractional density duration value by simply calculating the area under the fraction of larvae vs. time curve (as in Larval Preference experiments but covering whole larval development period). We used this value was used to compare the estimated and measured performance parameters (survival, developmental time and pupal mass) to the mean values obtained in no choice experiments using the formula:
Estimated Parameter = (Fractional Density Duration on Ragwort x Mean parameter on Ragwort) + (Fractional Density Duration on Nontarget x Mean parameter on Nontarget).
We tested the correlation of estimated vs. measured values for each parameter. (A) There was no correlation between the estimated vs. measured fraction of surviving larvae ($F = 1.494$; d.f. = 1, 19, $p = 0.2365$). (B) There was a correlation between the estimated vs. measured developmental time in days ($F = 11.53$; d.f. = 1, 19, $p = 0.003$). (C) There was no correlation between estimated vs. measured pupal mass ($F = 0.77$; d.f. = 1, 19, $p = 0.39$).

Table A1.1: The list of native *Senecio* and *Packera* taxa in Oregon. Taxa listed in bold are exposed to the cinnabar moth judged from mapping the overlap of moth and plant distributions (Fuller 2002). *preference and performance tests were completed in this manuscript, ** only performance experiments were completed in this manuscript.

Species	Authority	Life History
Packera bolanderi*	**(Gray) W.A. Weber & A. Love**	**Perennial**
Packera cana	**(Hook.) W.A. Weber & A. Love**	**Perennial**
Packera subnuda*	**(Buek) W.A. Weber & A. Love**	**Perennial**
Packera eurycephala	(Torr. & Gray ex Gray) W.A. Weber & A. Love	Perennial
Packera flettii*	**(Weig.) W.A. Weber & A. Love**	**Perennial**
Packera hesperia	(Grene) W.A. Weber & A. Love	Perennial
Packera indecora	(Greene) A. & D. Love	Perennial
Packera macounii	**(Greene) W.A. Weber & A. Love**	**Perennial**
Packera pseudaurea*	**(Rydb.) W.A. Weber & A. Love**	**Perennial**
Packera streptanthifolia	(Greene) W.A. Weber & A. Love	Perennial
Senecio aronicoides	DC.	Biennial/perennial
Senecio crassulus	Gray	Perennial
Senecio ertterae	T.M. Barkl	Annual
Senecio fremontii	Torr. & Gray	Perennial
Senecio hydrophiloides	Rydb.	Biennial/perennial
Senecio hydrophilus	**Nutt.**	**Biennial/perennial**
Senecio integerrimus*	**Nutt.**	**Biennial/perennial**
Senecio serra	Hook.	Perennial
Senecio sphaerocephalus	Greene	Perennial
Senecio triangularis*	**Hook.**	**Perennial**

Table A1.2: The origin of the test plant materials . For adult oviposition experiments, we used only greenhouse grown plants. We collected *S. jacobaea* and *S. triangularis* from field and re-potted these plants in the greenhouse for larval performance experiments. We were unable test three other species (*S. hydrophilus*, *P. cana*, *P. macounii*) due to difficulties in locating and/or culturing these plants

Plants	Origin of Plants	Coordinates	Field OR Greenhouse
S. jacobaea	Neskowin	45°6'23"N, 123°58'46"W	Field
S. triangularis	Mary's Peak	44°34'14" N, 123°16'33"W	Field
P. bolanderi	Veda Lake	45° 20' 35"N, 121° 56' 28"W	Greenhouse
P. flettii	Onion Peak	45° 48' 58"N, 123° 53' 05"W	Greenhouse
P. subnuda	Square Lake		Greenhouse
P. pseudaurea	Mt. Hood	45° 06' 51"N, 121° 48' 20"W	Greenhouse
S. cineraria	Shonnard's Nursery, Corvallis, OR		Greenhouse
P. cana	NA	NA	NA
P. macounii	NA	NA	NA
S. hydrophilus	NA	NA	NA

Table A1.3: The origins of the cinnabar moths used in adult preference experiments. We started a colony from cinnabar moth pupae collected from Neskowin, Oregon. We then supplemented our starter colony with additional collections from other locations including sites from Willamette Valley to Cascades to obtain a sufficient number of individuals for all experiments. We collected moths over the season and divided the collection date into four categories (from May (early season for the cinnabar moth) to August (late season)). We included the date of collection and origin of the moths as explanatory variables in the statistical analysis.

	Low Elevation Willamette Valley Early Season	Low Elevation Coast Mid Season	High and Low Elevation Coast and Cascades Mid Season	High Elevation Cascades Late Season
Date Code	1	2	3	4
First Collection Date	12-May-04	25-May-04	4-Jun-04	29-Jun-04
Last Collection Date	30-May-04	4-Jun-04	29-Jun-04	6-Jul-04
Sites	Fern Ridge (44°05’14”N, 123°17’58”W) Laboratory (from Neskowin) (45° 06' 25"N, 123° 58' 59"W) Baskett Slough (44°57’08” N 123° 16’09”W) Veneta (44°3′0″N, 123°21′9″W) Corvallis (44° 33' 59"N, 123° 17' 58"W)	Neskowin	Neskowin Mary’s Peak	Mount Hood (45° 06' 51"N, 121° 48' 20"W) Mary’s Peak (44°34’14” N, 123°16’33”W)

Table A1.4: The results for the adult preference tests wit different statistical analyses. . In adult preference paired choice tests, we compared the number of eggs laid, number of egg batches and number of eggs per batch on ragwort and on non-target test plants within each cage using a log ratio test (one sided; null: log Ratio > 0) and with a Wilcoxon-Rank-Sum-TestBoth parametric and non-parametric tests yielded to the same results nonparametric test results. *Batch size variables included the 21 cages where females laid eggs onto both of the plants and these values were compared within each cage.

	Number of Eggs		Number of Batches		Number of eggs per batch	
	Log Ratio	Wilcoxon Rank Sum Test	Log Ratio	Wilcoxon Rank Sum Test	Log Ratio	Wilcoxon Rank Sum Test
SEJA	0.67	0.7229	0.72	0.7517	0.33	1.0000
SETR	0.33	0.2771	0.42	0.6333	0.16	0.6250
SECI	0.11	0.1359	0.01	0.0136	0.13	0.6250
PABO	0.07	0.0542	0.05	0.0638	0.23	0.5000
PAPS	0.07	0.0711	0.008	0.0180	0.41	0.5862
PAFL	0.02	0.0380	0.01	0.0377	NA	NA
SEIN	0.00001	0.0046	0.0005	0.0064	0.31	1.0000

Table A1.5: The cages with the pathogen infection in 2004 larval preference and performance experiments.. The numbers of cages bearing infected and spore-free insects are listed below. In larval preference statistical analyses all disease categories were included, whereas in performance analyses infected cages were removed (because of the strong outlier impact observed in preliminary data analysis), leaving two categorical explanatory variables in the tests (spore-free and N/A). "Other single choices" represents the cases when plants were all consumed and new test plants were needed to be added.

	Single Choice			**Paired Choice**			
	Spore Free	**N/A**	**Infected**	**Spore Free**	**N/A**	**Infected**	**Total**
SEJA	6	0	1	5	0	3	14
SETR	4	0	3	5	0	2	14
SEIN	4	2	1	3	0	3	13
SECI	6	6	1	2	0	7	22
PAFL	2	0	2	2	0	0	6
PABO	1	0	0	1	0	0	2
PAPS	4	0	3	4	0	4	15
Total	27	8	11	22	0	19	86

Other Single Choices

	Uninfected	**NA**	**Infected**
PABO + SEJA	1	0	0
SEIN +SETR	1	0	0

Table A1.6: Larval performance experiments main performance parameters (survival, developmental time, and pupal mass) and their confidence intervals. . *S. jacobaea* appeared to be the most suitable host, while *S. integerrimus* was the least. "Other single choices" represents the cases when plants were all consumed and new test plants need to be added. (N/A = Not Available due to sample size, N/T = Not Tested)

		Larval Survival		Developmental Time (days)		Pupal Mass (g)	
		2004	2005	2004	2005	2004	2005
Single Choice	SEIN	0.00	0.03	N/A	33 (NA)	NA	0.09547
	SECI	0.05	N/T	46.7 (43.7, 50.0)	N/T	0.069 (0.009, 0.389)	N/T
	PAPS	0.23	N/T	58.5 (40.9, 82.5)	N/T	0.095 (0.055, 0.144)	N/T
	PABO	0.40	0.37	35.0 (N/A)	29.3 (± 0.37)	0.177 (NA)	0.154675 (± 0.01)
	PAFL	0.48	0.63	30.0 (21.5,42.0)	28.6 (± 0.63)	0.190 (0.149, 0.235)	0.161079 (± 0.01)
	SETR	0.78	0.77	27.6 (24.0, 31.9)	27.8 (± 0.77)	0.167 (0.140, 0.196)	0.163729 (± 0.01)
	PASU	N/T	0.57	N/T	28.6 (± 0.57)	N/T	0.154281 (± 0.01)
Control	SEJA	0.82	0.93	27.1 (25.1, 29.3)	27.0 (± 0.93)	0.197 (0.178, 0.215)	0.196909 (± 0.01)
Paired Choice	PABO	0.80	N/T	22.6 (NA)	N/T	0.205 (NA)	N/T
	SETR	0.76	N/T	27.6 (23.7, 32.0)	N/T	0.187 (0.142, 0.236)	N/T
	SECI	0.60	N/T	28.3 (16.5, 48.5)	N/T	0.187 (0.105, 0.291)	N/T
	PAPS	0.60	N/T	30.1 (21.9, 41.3)	N/T	0.184 (0.142, 0.237)	N/T
	SEIN	0.50	N/T	31.0 (26.8, 35.8)	N/T	0.196 (0.176, 0.218)	N/T
	PAFL	0.30	N/T	28.8 (11.0, 74.8)	N/T	0.212 (0.125, 0.321)	N/T
Other Single Choices	PABO+SEJA	0.90	N/T	28.67 (N/A)	N/T	0.171 (N/A)	N/T
	SEIN + SETR	0.40	N/T	32.75 (N/A)	N/T	0.154 (N/A)	N/T

Table A1.7: ANOVA Tables for all of the statistical analysis in Chapter 2.

(A) Adult Oviposition Single Choice Tests – Number of Eggs Laid: One Way ANOVA with Tukey's Multiple Adjustment indicated that, *S. integerrimus* was not acceptable by the cinnabar moth females. Origin of females and the average leaf width of plants were influential on the number of eggs laid (Figure 3A).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	649.3271	108.2212	9.568599	0.000010
Origin of Females	7	370.3214	52.9031	4.677533	0.001423
Leaf Width	1	52.4041	52.4041	4.633419	0.040120
Date Code	2	8.7372	4.3686	0.38626	0.683159
Age of plant (1 yr or 2 yrs old)	1	1.8212	1.8212	0.161029	0.691257
Developmental Stage (Flw or vegetative)	1	27.8453	27.8453	2.462003	0.127863
Stem Height	1	14.3574	14.3574	1.269439	0.269436
Residuals	28	316.681	11.31		

(B) Adult Oviposition Single Choice Tests – Number of Batches: One Way ANOVA with Tukey's Multiple Adjustment indicated that, *S. integerrimus* received the fewest number of batches (Figure 3B).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	19.82192	3.303654	5.290305	0.001002
Origin of Females	7	5.3481	0.764014	1.211679	0.329109
Leaf Width	1	2.25851	2.258508	3.581854	0.068795
Date Code	2	1.17103	0.585516	0.928592	0.406938
Age of plant (1 yr or 2 yrs old)	1	0.48402	0.484022	0.767629	0.388406
Developmental Stage (Flw or vegetative)	1	2.1072	2.107204	3.341894	0.078211
Stem Height	1	0.12144	0.121443	0.192601	0.664128
Residuals	28	17.65517	0.630542		

(C) Adult Oviposition Single Choice Tests – Number of Eggs per Batch: One Way ANOVA with Tukey's Multiple Adjustment indicated that batch size did not differ from plant to plant (Figure 3C).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	8.71911	1.453185	0.398825	0.871753
Origin of Females	7	24.40524	3.486463	0.956855	0.485324
Leaf Width	1	7.99322	7.993218	2.193728	0.152755
Date Code	2	6.56509	3.282546	0.90089	0.420674
Age of plant (1 year or 2 years old)	1	2.97078	2.970781	0.815327	0.376331
Developmental Stage (Flowering or Vegetative)	1	2.09397	2.093972	0.574688	0.456447
Stem Height	1	1.7897	1.789696	0.49118	0.490748
Residuals	22	80.16071	3.643669		

Table A1.7 (Continued)

(D) Larval Preference: One Way ANOVA with Tukey's Multiple Adjustment indicated that test plants influenced larvae's preference but not infection or date code (origin or season of the mother moths) (Figure 4C).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plants	6	10.78064	6.654959	6.13288	0.0001663
Infection	1	0.00840	0.008396	0.031098	0.8612467
Date Code	2	1.25248	0.626238	2.319486	0.1162925
Residuals	29	7.82971	0.269990		

(E) Larval Performance 2004 Greenhouse Experiments – Developmental Time: One Way ANOVA with Tukey's Multiple Adjustment indicated developmental time was longest on *S. cineraria*, shortest on *S. jacobaea* (Figure 5B).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	13	0.376229	0.028941	20.25923	0.000001
Origin of Larvae	2	0.039438	0.019719	13.80391	0.000131
Location of the Cage	4	0.00368	0.00092	0.64394	0.636939
Residuals	22	0.031427	0.001429		

(F) Larval Performance 2004 Greenhouse Experiments – Survival: One Way ANOVA with Tukey's Multiple Adjustment indicated survival lowest on *S. integerrimus*, highest on *S. jacobaea* (Figure 5A).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	14	77.97048	5.56932	24.5614	0.000001
Origin of Larvae	2	1.20553	0.602764	2.65827	0.083425
Location of the Cage	4	0.53185	0.132962	0.58638	0.674492
Infection	1	0.10824	0.108243	0.47736	0.493931
Residuals	37	8.38978	0.226751		

(G) Larval Performance 2004 Greenhouse Experiments – Pupal Mass: One Way ANOVA with Tukey's Multiple Adjustment indicated pupae were the smallest on *S. cineraria*, biggest on *S. jacobaea*) (Figure 5C).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	13	0.101964	0.007843	9.562213	0.000003
Origin of Larvae	2	0.005102	0.002551	3.110067	0.064640
Location of the Cage	4	0.00261	0.000652	0.795386	0.540883
Residuals	22	0.018046	0.00082		

(H) Larval Performance 2005 Incubator Experiments – Developmental Time: One Way ANOVA with Tukey's Multiple Adjustment indicated developmental time differed from one test plant to the other (longest on *S. integerrimus*, shortest on *S. jacobaea*) (Figure 5B).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	5	82.58397	16.51679	16.99871	< 0.0000
Block	2	3.17773	1.58887	1.63523	0.200583
Residuals	91	88.42011	0.97165		

Table A1.7 (Continued)

(I) Larval Performance 2005 Incubator Experiments – Survival: According to the logistic regression results, the odds that a larvae would complete its development differed on each test plant. Highest survival was on S. jacobaea, lowest on S. integerrimus (Figure 5A).

Null Deviance: 247.73 on 179 degrees of freedom
Residual Deviance: 175.3658 on 172 degrees of freedom

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			179	247.73	
Test Plant	5	71.75628	174	175.9737	< 0.0001
Block	2	0.60791	172	175.3658	0.737894

(J) Larval Performance 2005 Incubator Experiments – Pupal Mass: One Way ANOVA with Tukey's Multiple Adjustment indicated pupal mass differed from one test plant to the other (smallest on *S. integerrimus*, biggest on *S. jacobaea*) (Figure 5C).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	5	0.034979	0.006996	13.77252	< 0.0001
Block	2	0.000431	0.000215	0.4241	0.655648
Residuals	91	0.046224	0.000508		

(K) Adult Performance Laboratory Experiments – Adult Emergence: According to the logistic regression analysis, the odds that an adult would emerge from a pupa were lower for the pupae reared on *S. triangularis* (Figure 6A).

Null Deviance: 155.9337 on 112 degrees of freedom
Residual Deviance: 149.4411 on 109 degrees of freedom

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			112	155.9337	
Test Plant	1	5.40030	111	150.53340	0.02013
Block	2	1.092249	109	149.44110	0.57919

(L) Adult Performance Laboratory Experiments – Fecundity: According to the logistic regression analysis, the odds that a female moth would lay eggs were not influenced by the host plant (Figure 6B).

Null Deviance: 26.99209 on 23 degrees of freedom
Residual Deviance: 21.18446 on 20 degrees of freedom

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			23	26.99209	
Test Plant	1	1.587399	22	25.40469	0.207698
Block	2	4.220224	20	21.18446	0.121224

Table A1.7 (Continued)

(M) Adult Performance Laboratory Experiments – Fertility: According to the logistic regression analysis, the odds that an egg-laying female's egg would hatch were not influenced by the host plant.

Null Deviance: 16.2202 on 17 degrees of freedom
Residual Deviance: 8.823715 on 14 degrees of freedom

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			17	16.2202	
Test Plant	1	3.470104	16	12.7501	0.062487
Block	2	3.926384	14	8.82372	0.14041

(N) Nutritional Quality of Plants – Total Nitrogen: One Way ANOVA with Tukey's Multiple Adjustment indicated total nitrogen differed from one test plant to the other (lowest on *S. cineraria*, highest on *S. jacobaea*) (Figure 7A).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	24.24024	4.040039	62.05341	<0.00001
Origin of Plants	1	1.25475	1.254755	19.27253	0.00004
Residuals	75	4.88294	0.065106		

(O) Nutritional Quality of Plants – Total Carbon: One Way ANOVA with Tukey's Multiple Adjustment indicated total nitrogen differed from one test plant to the other (lowest on *S. cineraria*, highest on *S. triangularis,* and *S. integerrimus*) (Figure 7A).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	94.99488	15.83248	14.76574	<0.00001
Origin of Plants	1	2.1747	2.1747	2.02818	0.158553
Residuals	75	80.41834	1.07224		

(P) Nutritional Quality of Plants – Water Content: One Way ANOVA with Tukey's Multiple Adjustment indicated water content differed from one test plant to the other (lowest on *S. cineraria*, highest on *S. jacobaea*) (Figure 7A).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Test Plant	6	1389.126	231.5211	30.65915	>0.00001
Origin of Plants	1	34.554	34.5541	4.57582	0.03613
Residuals	75	498.396	7.5515		

Table A1.8: Main Matrices for the 2004 Larval performance Experiments.

S. jacobaea, Cage 1, λ = 1.0287

	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.24	0.67	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.33	0.67	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.33	0.82	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.18	0.91	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.09	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93

S. jacobaea, Cage 2, λ = 1.0240

	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.78	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.20	0.69	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.28	0.78	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.22	0.81	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.16	0.90	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.09	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93

S. jacobaea, Cage 3, λ = 1.0281

	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.24	0.82	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.18	0.71	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.29	0.82	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.18	0.89	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.11	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93

S. jacobaea, Cage 4, λ = 1.0319

	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.78	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.22	0.67	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.33	0.67	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.33	0.73	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.24	0.73	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.24	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93

Table A1.8 (Continued)

S. jacobaea, Cage 5, λ = 1.0336								
	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.29	0.50	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.50	0.75	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.25	0.67	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.33	0.90	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.10	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93
S. jacobaea, Cage 6, λ = 1.0235								
	E	L1	L2	L3	L4	L5	P	A
E	0.77	0.00	0.00	0.00	0.00	0.00	0.00	2.89
L1	0.19	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.24	0.62	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.25	0.67	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.33	0.91	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.09	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93
S. triangularis, Cage 1, λ = 1.0187								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.67	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.27	0.57	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.38	0.71	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.29	0.71	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.29	0.92	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.08	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94
S. triangularis, Cage 2, λ = 1.0231								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.60	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.40	0.67	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.33	0.71	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.29	0.78	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.22	0.87	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.12	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

Table A1.8 (Continued)

S. triangularis, Cage 3, λ = 1.0165								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.67	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.27	0.70	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.23	0.71	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.29	0.75	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.25	0.93	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.07	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94
S. triangularis, Cage 4, λ = 1.0221								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.67	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.33	0.60	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.40	0.67	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.33	0.75	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.25	0.92	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.07	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94
S. cineraria, Cage 1, λ = 1.0030								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.60	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.20	0.79	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.16	0.64	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.16	0.90	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.10	0.95	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.05	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94
S. cineraria, Cage 2, λ = 1.0090								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.68	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.11	0.67	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.33	0.78	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.22	0.82	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.18	0.96	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.04	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

Table A1.8 (Continued)

S. cineraria, Cage 3								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	NA	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	NA	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 4								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.50	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.13	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 5								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	NA	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	NA	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 6								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.08	0.75	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.25	0.83	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.00	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94

Table A1.8 (Continued)

S. cineraria, Cage 7								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	NA	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	NA	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 8								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.00	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 9								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	NA	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	NA	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94
S. cineraria, Cage 10								
	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	NA	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	NA	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94

Table A1.8 (Continued)

S. cineraria, Cage 11

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.72	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.21	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94

S. cineraria, Cage 12

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.56	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.19	NA	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	NA	NA	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	NA	NA	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	NA	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	NA	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	NA	0.94

P. flettii, Cage 1, $\lambda = 1.0174$

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.75	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.25	0.47	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.47	0.60	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.40	0.67	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.33	0.93	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.05	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

P. flettii, Cage 1, $\lambda = 1.0206$

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.71	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.29	0.75	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.25	0.67	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.33	0.82	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.18	0.88	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.10	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

Table A1.8 (Continued)

P. bolanderi, Cage 1, λ = 1.011954641

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.69	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.23	0.80	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.18	0.68	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.23	0.82	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.18	0.93	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.07	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

P. pseudaurea, Cage 1, λ = 1.0083

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.66	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.12	0.88	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.13	0.75	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.25	0.91	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.09	0.96	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.04	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

P. pseudaurea, Cage 2, λ = 1.0112

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	0.72	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.21	0.68	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.27	0.75	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.25	0.85	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.11	0.95	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.05	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

P. pseudaurea, Cage 3, λ = 1.0181

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	6.47
L1	0.16	0.69	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.23	0.83	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.17	0.92	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.08	0.88	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.12	0.89	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.06	0.99	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

Table A1.8 (Continued)

P. pseudaurea, Cage 4, λ = 0.0000

	E	L1	L2	L3	L4	L5	P	A
E	0.63	0.00	0.00	0.00	0.00	0.00	0.00	2.95
L1	0.16	1.27	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.32	1.11	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.32	1.00	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.08	0.98	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.07	NA	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	NA	0.00	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94

Table A1.9: Elasticity Matrices for the 2004 larval performance experiments.

S. jacobaea, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.02
L1	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.02	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.02	0.12	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.02	0.43	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.14

S. jacobaea, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.10	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.45	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.14

S. jacobaea, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.06	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.10	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.42	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.14

S. jacobaea, Cage 4

	E	L1	L2	L3	L4	L5	P	A
E	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.02
L1	0.02	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.02	0.45	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.16

Table A1.9 (Continued)

S. jacobaea, Cage 5

	E	L1	L2	L3	L4	L5	P	A
E	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.02
L1	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.02	0.11	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.02	0.42	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.15

S. jacobaea, Cage 6

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.12	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.14

S. triangularis, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.16

S. triangularis, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02
L1	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.02	0.08	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.02	0.47	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.17

Table A1.9 (Continued)

S. triangularis, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.15

S. triangularis, Cage 4

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.46	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.17

S. cineraria, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.59	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.11

S. cineraria, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.17	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.51	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.13

Table A1.9 (Continued)

P. flettii, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.49	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.16

P. flettii, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.09	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.47	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.16

P. bolanderi, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.12	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.51	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.14

P. pseudaurea, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.05	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.08	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.16	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.12

Table A1.9 (Continued)

P. pseudaurea, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.16	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.49	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.13

P. pseudaurea, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.01	0.05	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.01	0.10	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.01	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.01	0.08	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.01	0.42	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.14

Table A1.10: Sensitivity Matrices for the 2004 larval performance experiments.

S. jacobaea, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.05	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.09	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.17	0.44	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.96	0.16

S. jacobaea, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.07	0.06	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.05	0.06	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.06	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.09	0.11	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.16	0.46	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.57	0.15

S. jacobaea, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.06	0.07	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.08	0.05	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.08	0.11	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.14	0.44	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.85	0.15

S. jacobaea, Cage 4

	E	L1	L2	L3	L4	L5	P	A
E	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.09	0.07	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.08	0.05	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.05	0.05	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.07	0.06	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.07	0.47	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	4.57	0.18

Table A1.10 (Continued)

S. jacobaea, Cage 5

	E	L1	L2	L3	L4	L5	P	A
E	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.09	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.06	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.03	0.06	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.07	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.05	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.18	0.44	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	4.45	0.17

S. jacobaea, Cage 6

	E	L1	L2	L3	L4	L5	P	A
E	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.04	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.16	0.49	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.74	0.16

S. triangularis, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.09	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.05	0.14	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.18	0.49	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.46	0.17

S. triangularis, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01
L1	0.10	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.05	0.05	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.07	0.10	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.13	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.93	0.19

Table A1.10 (Continued)

S. triangularis, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.04	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.05	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.05	0.14	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.18	0.50	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.20	0.17

S. triangularis, Cage 4

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.09	0.04	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.06	0.14	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.20	0.47	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.74	0.18

S. cineraria, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.04	0.02	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.07	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.14	0.60	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	1.80	0.11

S. cineraria, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.08	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.03	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.05	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.05	0.18	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.22	0.52	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	2.35	0.13

Table A1.10 (Continued)

P. flettii, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.02	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.03	0.04	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.04	0.14	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.25	0.51	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.39	0.17

P. flettii, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.09	0.05	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.07	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.08	0.10	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.15	0.48	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	3.60	0.18

P. bolanderi, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.06	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.05	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.06	0.13	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.15	0.52	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	2.76	0.15

P. pseudaurea, Cage 1

	E	L1	L2	L3	L4	L5	P	A
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.07	0.06	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.07	0.03	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.03	0.08	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.09	0.16	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.20	0.49	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	2.13	0.12

Table A1.10 (Continued)

P. pseudaurea, Cage 2

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.03	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.04	0.06	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.09	0.17	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.22	0.50	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	2.53	0.14

P. pseudaurea, Cage 3

	E	L1	L2	L3	L4	L5	P	A
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L1	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.00
L2	0.00	0.05	0.06	0.00	0.00	0.00	0.00	0.00
L3	0.00	0.00	0.07	0.11	0.00	0.00	0.00	0.00
L4	0.00	0.00	0.00	0.14	0.09	0.00	0.00	0.00
L5	0.00	0.00	0.00	0.00	0.10	0.09	0.00	0.00
P	0.00	0.00	0.00	0.00	0.00	0.19	0.43	0.00
A	0.00	0.00	0.00	0.00	0.00	0.00	2.98	0.15

APPENDIX 2

Table A2.1: ANOVA Tables for all of the statistical analyses in Chapter 3.

A) Larval Survival: Logistic regression results showed that as the dose of inoculation increased, larval survival decreased. At high doses plant-dose interaction became significant.

High Doses (10^3, 10^4 spores/μl) Analysis of deviance table

	Df	Deviance Resid.	Df	Resid.Dev	Pr(Chi)
NULL			233	324.3758	
Dose	1	15.58259	232	308.7932	0.00007898
Plant	1	26.46324	231	282.3300	0.00000027
Block	2	7.12065	229	275.2093	0.02842963
Plant:Dose	1	20.26841	228	254.9409	0.00000673

Low Doses (0, 10^1, 10^2, 10^3 spores/μl) Analysis of deviance table

	Df	Deviance Resid.	Df	Resid.Dev	Pr(Chi)
NULL			478	556.0975	
Plant	1	11.63492	476	533.1281	0.0007608
Dose	1	11.63492	477	544.4626	0.0006473
Block	2	5.70774	474	527.4203	0.0576209
Plant:Dose	1	1.47349	473	525.9469	0.2247960

B) Developmental Time: Linear regression results showed that developmental time decreased linearly as the infection dose increased.

Res. std. error: 2.147 on 389 d.f.; R^2: 0.1934; F = : 18.65 d.f = 5, 389; p = 1.11e-016

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Dose	1	377.49800	377.49810	81.90506	0.00000
Plant	1	9.96500	9.96530	2.16215	0.14226
Block	2	38.65500	19.32770	4.19350	0.01578
Plant:Dose	1	3.75100	3.75080	0.81380	0.36756
Residuals	389	1792.89000	4.60900		

C) Pupal Mass: Linear regression results showed that pupal mass decreased linearly as inoculation dose increased. Larvae reared on *S. triangularis* had smaller pupae.

Res. std. error : 0.02895 on 386 d.f. ;R^2: 0.2462; F = : 25.22; d.f. = 5, 386; p = 0

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Dose	1	0.01074	0.01074	12.81950	0.00039
Plant	1	0.09103	0.09103	108.62990	0.00000
Block	2	0.00288	0.00144	1.71890	0.18063
Plant:Dose	1	0.00101	0.00101	1.20510	0.27299
Residuals	386	0.32347	0.00084		

Table A2.1 (Continued)

D) Pupal Survival: Logistic regression results showed that pupal survival decreased linearly as inoculation dose increased. Larvae reared on *S. triangularis* had lower pupal survival.

Null Deviance: 442.5892 on 394 d.f.; Residual Deviance: 373.5583 on 389 d.f.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			394	442.5892	
Dose	1	54.20217	393	388.38710	0.00000
Plant	1	11.45661	392	376.93050	0.00071
Block	2	3.35803	390	373.57240	0.18656
Plant:Dose	1	0.01411	389	373.55830	0.90544

E) Fecundity: Logistic regression results showed the moths reared on *S. triangularis* had lower probability of laying eggs.

Null Deviance: 58.57363 on 44 d.f.; Residual Deviance: 51.9241 on 39 d.f.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			44	58.57363	
Dose	1	2.325513	42	53.3565	0.12727
Plant	1	4.002683	41	50.27247	0.04543
Block	2	0.78774	40	52.56876	0.67444
Plant:Dose	1	0.64466	39	51.9241	0.42203

G) Fecundity: Linear regression results showed that as the dose of infection increased, number of eggs per female decreased. The moths reared on *S. triangularis* laid fewer eggs.

Res. std. error : 3.92 on 24 d.f.; R^2: 0.3672; F = 2.785; d.f = 5, 24, p = 0.04029

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Dose	1	83.5297	83.5297	5.436841	0.02844
Plant	1	110.1648	110.1648	7.17049	0.01316
Block	2	19.9269	9.9634	0.648508	0.53175
Plant:Dose	1	0.3108	0.3108	0.020227	0.88809
Residuals	24	368.7274	15.3636		

H) Fertility: Logistic regression results showed that probability of eggs hatching decreased with increasing spore dose.

Null Deviance: 39.33614 on 28 d.f.; Residual Deviance: 19.83484 on 23 d.f.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			28	39.33614	
Dose	1	15.2233	27	24.11284	0.00010
Plant	1	3.64867	26	20.46417	0.05611
Block	2	0.6275	24	19.83666	0.73070
Plant:Dose	0	0.00183	23	19.83484	0.96588

Table A2.1 (Continued)

I) Disease Prevalence: Logistic regression results showed that probability of observing disease in field were higher in *S. jacobaea* sites. As elevation increased the probability of observing disease decreased.					
Null Deviance: 327.6273 on 336 d.f. ; Residual Deviance: 211.3455 on 324 d.f.					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			336	327.6273	
Host	1	83.59588	335	244.0314	0.000000
Elevation	1	21.87654	334	222.1548	0.000003
Year of collection	1	0.00377	333	222.1511	0.951027
Location	9	10.80552	324	211.3455	0.289276