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Interactions between an insect sleeper pest, *Spodoptera litura*, and future forages under climate change in New Zealand

A thesis

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of the requirements for the degree of

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By

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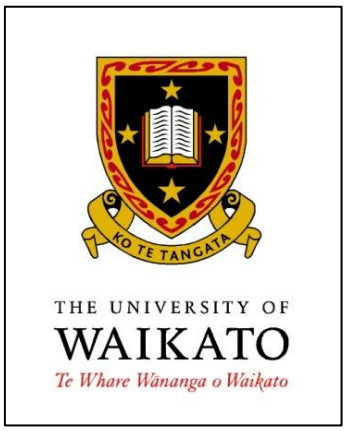

2026

‘The earth-bound early stages built enormous digestive tracts and hauled them around on caterpillar treads. Later in the life-history these assets could be liquidated and reinvested in the construction of an essentially new organism – a flying-machine devoted to sex’ - Carroll M. Williams (1959).





Abstract

A major driver of the success of New Zealand agricultural systems is the ability to grow plentiful, high-quality forage. As with agricultural systems world-wide, New Zealand pastures face increasing pressures due to climate change. Pre-breeding research to predict future stresses and identify valuable forage fitness traits is needed. This will underpin decision-making around pasture management under future climate change conditions and aid the development of resilient future forages.

Future forages will be exposed not only to increased pressure from herbivorous pest species, but also a different suite of pests due to the direct and indirect effects of climate change. Some insect species that are currently considered to be non- or minor pests because of their restricted geographical ranges, low population sizes and/or damage levels will be increasingly important as conditions become more favourable for their growth and survival. Identification and study of interactions between these so-called 'sleeper pests' and their host forages is key to supporting future farming systems.

Spodoptera litura (Lepidoptera: Noctuidae) has been identified as a potential sleeper pest in New Zealand. Pastoral systems are likely to be vulnerable given *S. litura*'s broad host range and highly fecund, mobile adults. Despite being well-studied globally, little is known about this species in New Zealand or about its interactions with plant species relevant to New Zealand pastoral systems. This study sought to investigate the geographical and temporal distribution of *S. litura* in New Zealand using existing data sources, and to conduct fundamental research into *S. litura* biology and interactions with forage plant hosts relevant to New Zealand pastoral systems.

A search for publicly available information on *S. litura* in New Zealand showed such data to be sparse, however two online platforms, iNaturalist and GBIF, provided useful information. Following appropriate checks and analyses, public observations of *S. litura* confirmed that the species occurs most commonly in the northern North Island and showed that population outbreaks occurred in 2018 and again in 2022-23 but indicated that the species remains a 'sleeper pest' at present. This study demonstrated the usefulness of online tools like the two online platforms for monitoring some sleeper pests in the absence of more targeted survey data.

Laboratory studies showed that several plant species commonly found in pasture, plantain, chicory, dock and white clover, are good-quality hosts for *S. litura*. Larvae fed excised

leaf material from one of these forage species from 6-days old had similarly high larval and pupal weights, development rates, egg-laying and survival. Larvae consumed more dock, plantain and chicory per day than clovers, but with lower feeding conversion efficiencies. Red clover, however, was a significantly poorer host, negatively impacting *S. litura* growth, survival and reproductive output. The negative effects of feeding on red clover had intergenerational consequences, through decreased performance and gut microbiota diversity of the offspring generation. Feeding on red clover for two generations significantly reduced beta diversity of mid-gut microbiota and promoted the dominance of *Enterococcus* spp. Despite the difference in host quality, young larvae offered a choice of red clover or plantain in a choice experiment did not initially choose to settle on the higher quality host. This early behaviour resulted in lower larval weights through to pupation.

Perennial ryegrass, the dominant pasture grass in New Zealand pasture, was confirmed to be a very poor host for *S. litura*, however it is not completely resistant to attack. Although feeding on perennial ryegrass severely reduced survival and growth, larvae could feed and survive on this plant, particularly at earlier instars and if additional, higher quality food sources were also available. Larvae fed on perennial ryegrass from neonate survived for longer (mean = 24 days) than those that were switched from white clover to ryegrass at 14 or 28 days (mean survival time = 8.9 and 8.0 days respectively). This was shown not to be due to enhanced starvation resistance with age, but I was unable to identify the mechanisms behind this result.

This study provides valuable information about *S. litura*'s distribution in New Zealand and identifies vulnerable and less susceptible forage species. These findings, together with future identification of traits that make some forages less suitable as hosts, will assist in developing resilient future pastures. Incorporating less preferred or poor-quality hosts in mixed pasture is likely to reduce pest population sizes and damage levels. Additionally, this study extends current understanding of the biology and behaviour of this species, in particular the effects of early feeding choices, and intergenerational impacts of host forage species on *S. litura* and its associated mid-gut microbiota. Monitoring surveys, field verification and identification of resistant cultivars are important next steps towards mitigating the risks posed by *S. litura* to future New Zealand agriculture.

Acknowledgements

I have taken a slightly unconventional pathway to doing a PhD. After completing my MSc I accepted a job as a science research technician with AgResearch Ltd (now Bioeconomy Science Institute). I have wanted to do a PhD for years, but I lacked self-confidence and doubted my ability to be a scientist. I also struggled to find enough funding to support my study, and I took some time off to have a family. But eventually, with the support of some amazing AgResearch colleagues, my family and friends, I've finally done it!

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1. Introduction, literature review and thesis aims

1.1. Introduction

Pastoral agriculture in New Zealand relies on our ability to grow plentiful and high-quality forage. Breeding forage plants that are well adapted to climate, nutrient and pest pressures is therefore vital to ensure its ongoing success. It is becoming increasingly evident, however, that future farm systems will face a different suite of conditions to those faced at present. Plant breeding programmes therefore need to anticipate future conditions such as those imposed by climate change, government policy and new technologies.

Bioeconomy Science Institute (formerly AgResearch)'s 'Forages for the Future' Strategic Science Investment Fund (SSIF) programme aimed to develop forage plant resources that are genetically better adapted for lower nutrient inputs on-farm and breed for improved resilience to emerging and future abiotic (e.g., drought), disease and pest threats. A critical aspect of this research was to identify future pest threats and to understand how they might interact with future forages.

A literature search conducted for the SSIF programme identified the lepidopteran herbivore, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), as a probable 'sleeper pest' in New Zealand (Orre-Gordon & Jensen, 2025). A sleeper pest is an organism that is already present in the environment but is likely to increase in abundance and/or in the severity of its damage due to predicted changes in climate (Bradley et al., 2018; Hulme, 2017). *Spodoptera litura* has been established in New Zealand for over 50 years. Although it causes serious harm throughout Asia and Oceania it is currently considered to be a minor pest in New Zealand because it reaches economically significant thresholds only sporadically and in geographically restricted areas. This is likely to change as climatic conditions become more favourable (Kean et al., 2015). Little research has been dedicated to this herbivore in New Zealand pasture systems. Gaining knowledge on its distribution and how it interacts with forage plant species will be an important aid for decision-making around pasture planting and management under future climate change conditions, and for developing robust future forages.

1.2. Literature review

1.2.1. Introduction

Primary industries such as agriculture are of key importance to the New Zealand economy. In the year ending June 2025 earnings from dairy, meat and wool contributed $39.5 billion, or 53% of New Zealand's total goods export earnings (MPI New Zealand, 2025). A major factor in the

success of New Zealand agriculture is our ability to grow plentiful and high-quality forage. To maintain this high quality, farmers, scientists and plant breeders need to predict the stresses that are likely to impact future forages and, where possible, to test the performance of potential novel forages against these stresses. Insect pest pressure and the direct and indirect effects of future climate variables will be key considerations. Future focussed pre-breeding research will help identify valuable fitness traits and provide confidence that new forages can tolerate future conditions.

Pressure from herbivorous pests is expected to increase in agricultural systems worldwide (Giron et al., 2018; Ma et al., 2025). Climate change and increased international trade will promote invasions of some pest species to new regions. Climate change will disrupt some natural biological controls, and it will allow some pest species that are currently constrained by climate or host availability to increase in geographical range or damage potential while others will face extinction (Rooney et al., 1996; Sharma & Dhillon, 2020). Novel insect-plant interactions will arise. Chemical control of pests will become more difficult as farmers face increasing social, economic and environmental pressures to forgo these methods (Goldson et al., 2015). Some current pesticides are being removed from the market or are becoming less useful due to evolved resistance (Duke & Dayan, 2022).

Breeding quality forages for future farms relies on in-depth knowledge of plant-insect interactions. Plant breeders and scientists must make good, fact-based predictions of future climatic, socio-economic, technology and policy conditions, how these might interact to affect plants and insects, and have strong evidence of the benefits of genetic traits to be incorporated into elite cultivars (Giron et al., 2018). Insect pest populations and their impact on pastoral farming are affected by multiple interacting factors including climate, pasture species, cultivars, symbiotic endophytic fungi, natural enemies, plant nutrient status, soil fertility and moisture levels, soil type, stock type and management, cropping and pasture renovation practices and farmer awareness of pest issues (Mansfield et al., 2021).

While system-wide changes will be required to adapt and respond to future conditions (Mansfield et al., 2021), ensuring the availability of suitable forage plants is critical. With decreasing reliance on chemical control, development of plant genotypes that have stable resistance or tolerance to herbivorous insects becomes more important. Plant resistance is one of the most environmentally friendly, sustainable, economical and effective methods of pest management (Giron et al., 2018). Therefore, while it is ‘naïve to think that we can predict exactly what the pasture pests of tomorrow will be’ (Mansfield et al., 2021), it is important that we use current knowledge to anticipate which species are likely to become more widespread and/or

damaging in the future and target research towards understanding how they might interact with future forages. This knowledge can then be used to breed future forages that will be more resistant to them.

The polyphagous and highly invasive lepidopteran species *Spodoptera litura* (Lepidoptera: Noctuidae) is a significant pest across tropical and temperate regions (Maes, 2022). It will become increasingly important in regions that are currently marginal for its survival as temperatures rise globally (Fu et al., 2025). *Spodoptera litura* is one of several species that have been identified as 'sleeper pest' species in New Zealand pastures (Kean et al., 2015; Orre-Gordon & Jensen, 2025). It is currently thought of as a minor pest in New Zealand because its geographical distribution and the frequency with which it reaches damaging population sizes is constrained by climate conditions. As conditions become more favourable under predicted climate change scenarios *S. litura* is likely to become more numerous and damaging (Kean et al., 2015). Despite the risk posed by this species we have very little knowledge about its biology and interactions with forage plants in New Zealand pastures.

In the following review I discuss in brief what future New Zealand pastures might look like, including the impact of climate change on forage plants and the herbivorous invertebrates that feed on them. Current literature and knowledge gaps about the sleeper pest of interest to this study, *S. litura*, will be presented. I will discuss why further study of this species is important, and aspects of plant-insect interactions that will provide important guidance towards designing resilient forages and management tools for future New Zealand agriculture.

1.2.2. Future New Zealand agriculture

New Zealand agriculture will be strongly affected by a changing climate. Climate changes are expected to vary regionally and will depend on the speed and extent of any mitigating actions put in place (Lawrence et al., 2022; Lilburne et al., 2025). Current predictions are that mean temperatures will continue to rise, and there will be more frequent and intense hot extremes and less frequent and intense cold extremes (Bodeker et al., 2022). Temperatures are expected to increase more in summers than in winters, and more in the south and at high elevations (Kean et al., 2015). Annual rainfall is predicted to increase in the west and south of New Zealand, particularly in the winter and spring. Conversely, there may be less winter and spring rainfall in the east and north. More summer rainfall is expected in the east of both the North and South Islands, with less in the west and central North Island. River flooding is expected to increase in most areas of the country (Bodeker et al., 2022). There is also increasing evidence of associated increases in the frequency and severity of extreme climate events such as droughts, heatwaves and floods (Lawrence et al., 2022). Mean wind patterns in New Zealand are expected to become

more north-easterly in summer, and westerlies will become more intense in winter (Bodeker et al., 2022; Lawrence et al., 2022).

A changing climate will impact the geographical distributions of some organisms (Sharma & Dhillon, 2020; Wang et al., 2015). Performance and survival of both plants and insects are constrained by temperature and rainfall patterns; thus, under future climatic conditions, populations may survive in some previously unsuitable regions while other regions become untenable. Extreme events such as droughts and floods will lead to regional extinctions (Sharma & Dhillon, 2020). Wind pattern changes will affect dispersal of insects, pollen and wind-borne seeds (Rooney et al., 1996). Changes in the distributions and efficacy of natural enemies will impact both plants and invertebrates (Mansfield et al., 2021). Distributions of herbivorous insect species will change in direct response to environmental conditions but also indirectly, through changes in host plant distribution, quality and quantity. Changing environmental variables will alter individual plant growth rates, carbon:nitrogen ratios, chemical defences, stomatal conductance, drought responses and water use efficiency, biomass partitioning (e.g. root to shoot ratios), seed production and grain quality. Effects will vary regionally, seasonally and between plant functional types (e.g. grasses vs 'forbs', C3 vs C4) and between species. The same environmental variables will also impact insects, both directly and indirectly, through plant responses (DeLucia et al., 2012; Hamann et al., 2021; Sharma & Dhillon, 2020). Predicting likely future herbivore-host plant distributions, and interactions between them, is key to future pest mitigation strategies such as breeding resilient pasture species (Mansfield et al., 2021).

1.2.2.1. Future forages

New Zealand pastures are traditionally based on ryegrasses, *Lolium perenne*, *L. multiflorum and L. perenne x L. multiflorum,* and clovers, *Trifolium repens* and *T. pratensis* (Ferguson et al., 2019). These species are tolerant to grazing and have typically performed well in New Zealand, generating large yields of good quality feed (Minnee et al., 2017). However, changing environmental conditions are impacting the performance and resilience of current forages. Farmers are increasingly looking to diversify their pastures to maintain productivity and quality during summer and autumn, to improve pasture sustainability and to future-proof against climate change related stresses. Plant breeders must consider traits such as improved heat stress, drought stress and insect pressure tolerance when developing new cultivars (Caradus et al., 2021). New Zealand pastures in future will therefore comprise both improved cultivars of currently important species and increased use of alternative forage species. Screening both new and improved forages against potential future insect pests will be vital to ensure their ability to persist.

Perennial ryegrass (*Lolium perenne*) is currently our most widely sown temperate (C3) grass. It grows well in a wide range of conditions, establishes easily, and provides high animal performance. It requires moist, fertile conditions and can perform poorly during hot, dry conditions. In areas where there are dry summer conditions and significant pest pressure, such as some sheep systems and some dairy farms in the upper North Island, pastures may only persist for 3-5 years (Stewart et al., 2022). A decline in the persistence of non-irrigated perennial ryegrass pastures in New Zealand, especially in the North Island, has become increasingly evident (Chapman, 2021). There is speculation that this is associated with the increased frequency of summer droughts (Mansfield et al., 2021), or with a combination of simultaneous stresses such as drought, insect damage and overgrazing (Chapman, 2021). Despite these challenges, perennial ryegrass is likely to remain a vital component of NZ pastoral agriculture (Caradus et al., 2021). Efforts to develop more resilient perennial ryegrass cultivars are therefore ongoing (e.g. Faville et al., 2020). Monitoring potential vulnerability to herbivorous insect pests must be a part of this.

White clover (*Trifolium repens*) is the dominant legume grown in New Zealand pastures, but it may become an increasingly less sustainable component of pastures in the future, unless more drought and insect resistant varieties can be developed. Like perennial ryegrass, white clover performs best in temperate areas with good soil fertility and adequate soil moisture (Brock et al., 2003). Because it has a shallow root system, summer-autumn drought is a key driver for the loss of white clover in pasture (Knowles et al., 2003). White clover is attacked by a large number of invertebrate pests including *Heterodera trifolii* (clover cyst nematode), *Meloidogyne* spp. (root knot nematodes), *Costelytra giveni* (grass grub), *Wiseana* spp. (porina), *Sminthurus viridus* (clover flea), *Teleogryllus commodus* (black field cricket) and *Sitona obsoletus* (clover root weevil) (Gerard et al., 2017; Woodfield & Caradus, 1996). Although the insect sleeper pest of interest in this thesis, *S. litura*, has not been noted as a pest of white clover in pasture, laboratory studies have shown that it can successfully develop through to adulthood on it (Huang et al., 2008; Lindroth et al., 2000).

Red clover (*Trifolium pratense*) may become more important in future New Zealand pastures due to its better persistence and performance in dry conditions than white clover (Brown et al., 2003). As with white clover, it performs best in wet to moderately dry soil with moderate fertility (Brock et al., 2003), but red clover's deep tap root allows it better access to soil moisture. It is spring and summer active and is often grown in monoculture, which may make it vulnerable to attack from invertebrate herbivores (Salazar & Marquis, 2022) such as *Wiseana*

spp., *S. obsoletus* and several nematode species. *Spodoptera litura* has been shown to complete its life cycle on red clover in the laboratory (Huang et al., 2008).

Alternative forage species such as lucerne/alfalfa (*Medicago sativa*) (Gerard et al., 2013; Ward, 2008) and more drought-tolerant *Trifolium* spp. are likely to become more common throughout New Zealand (Williams et al., 2007). Two broadleaf species, chicory (*Cichorium intybus*) and narrowleaf plantain (*Plantago lanceolata*), are being used increasingly. These species have higher herbage yield and nutritive value than ryegrass in summer (Minnee et al., 2017) and are more tolerant to drought and heat (Caradus et al., 2013). *Spodoptera litura* has been recorded as causing severe damage to lucerne crops and to *Plantago* spp. and chicory in pastures (Gerard et al., 2011) and to successfully utilise chicory as a host in the laboratory (Sushilkumar & Ray, 2007). Pastures that include these forage species are therefore likely to be vulnerable to *S. litura*.

1.2.2.2. Future insect pests

Invertebrate populations in New Zealand pastures will change as a direct result of a changing climate. There are several mechanisms whereby this might occur. Insects are ectothermic so, within species-specific thermal extremes, growth rates increase with increasing temperature. For some species, this will allow their populations to grow faster or achieve more generations per year. Overwintering populations of species whose numbers have typically been limited by cold winter temperatures will be larger with climate warming. This greater base population in spring will allow for larger numbers to build up over the spring-summer (Rooney et al., 1996). Outbreaks may therefore occur more often, and in regions where populations have previously fallen under pest threshold status. Changing wind patterns could affect dispersal of flying insect species and allow invasive species to expand their ranges. Conversely, species which are vulnerable to high temperatures may decrease in abundance or distribution (Bale et al., 2002; Sharma & Dhillon, 2020). Slow-growing species with long maturation periods are likely to be more vulnerable to changes in temperature than faster developing species. Slow development will prevent rapid adaptation and result in longer exposure to adverse conditions, and warming winters may disrupt diapause strategies (Bale et al., 2002). Where these traits are combined with low dispersive ability, slow growing species may become regionally extinct (Rooney et al., 1996; Sharma & Dhillon, 2020). Potential improvements in performance with temperature changes may be constrained by associated reductions in rainfall, particularly for species with soil dwelling life stages (Hulme, 2017). Climate change will also impact invertebrates indirectly, through factors such as host plant availability and quality, the abundance and species composition of natural

enemies, and changes in pasture management (Mansfield et al., 2021; Orre-Gordon & Jensen, 2025).

Some insect species are currently not considered to be 'pests', or to be minor or sporadic pests, in New Zealand due to their restricted geographical ranges, low population sizes and/or damage levels. Climate change is likely to provide more favourable conditions for some of these species, thus elevating their pest status or potential. These species are referred to as 'sleeper pests' (Frank & Just, 2020; Mansfield et al., 2021). As part of a wider project seeking to develop strategies for improving resilience of pastures in northern New Zealand (McCahon & Dale, 2025), a list was developed of 12 arthropods that are considered to be either sleeper pests or current pests that will increase in numbers and/or distribution in New Zealand pasture as a result of climate change (Orre-Gordon & Jensen, 2025). The sleeper pest *Spodoptera litura* was selected from this list for further study and is described in greater detail below.

1.2.3. *Spodoptera litura*

Synonyms: *Mamestra albisparsa* Walker, *Noctua elata* Fabricius, *Noctua histrionica* Fabricius, *Noctua litura* Fabricius, *Prodenia ciligera* Guenée, *Prodenia declinata* Walker, *Prodenia evanescens* Butler, *Prodenia glaucistriga* Walker, *Prodenia litura* Fabricius, *Prodenia subterminalis* Walker, *Prodenia tasmanica* Guenée (Holloway, 1989), *Prodenia testaceoides* Walker.

1.2.3.1. General

Spodoptera litura (Fabricius, 1775) (Lepidoptera: Noctuidae) is a globally well-studied species. However, being a sporadic pest in New Zealand, there are large gaps in our understanding of its distribution and host preferences here, particularly with respect to agricultural pasture plants. This species was chosen for further study because outbreaks are likely to occur more often in New Zealand in future as winters become milder and summer and autumn temperatures increase (Fu et al., 2025; Kean et al., 2015), and it has the potential to cause severe damage to pastures. It is an aboveground feeder that is reported to feed on several pasture plant species relevant to future farming systems. These traits, combined with *S. litura*'s short generation time, already established methods for rearing in the laboratory, and eggs/larvae that can be obtained year-around from laboratory colonies, make it a highly relevant and suitable insect for the work in this thesis.

The adult *S. litura* is a nocturnal, noctuid moth and the larvae cause severe damage to many different crops across much of the world. It is considered to be one of the most important pests of agricultural crops in the Asian tropics (Maes, 2022). This is reflected in its many common names, which include tobacco cutworm, tobacco leaf caterpillar, cluster caterpillar, taro

armyworm, tropical armyworm, oriental leafworm moth, cotton leafworm, rice cutworm, common cutworm, and eiernestrups in Dutch. Large populations of later instar larvae can almost completely strip the host plants, and may form groups that move together, feeding as they go. This has given rise to their common name, 'armyworm', a name they share with other species that share this habit, including *Spodoptera frugiperda* (fall armyworm) and *Mythimna separata* (northern or cosmopolitan armyworm).

1.2.3.2. Global distribution

Spodoptera litura is native to South-East Asia and now has a broad geographical distribution, being found widely in tropical and temperate regions throughout South and East Asia, and Oceania, including New Zealand (Figure 1-1) (Bragard et al., 2019; Fand et al., 2015). Outbreaks have been reported from Africa, the EU and America, where *S. litura* is listed as a Harmful Organism. Many of these outbreaks were eradicated but the CABI factsheet lists *S. litura* as present in France, Portugal, localised parts of the United States, Central African Republic and Ghana (Figure 1-1). Maes (2022) notes, however, that *S. litura* belongs to a species complex of very similar species with similar ecology and warns that correct identification is essential for correctly establishing its distribution.

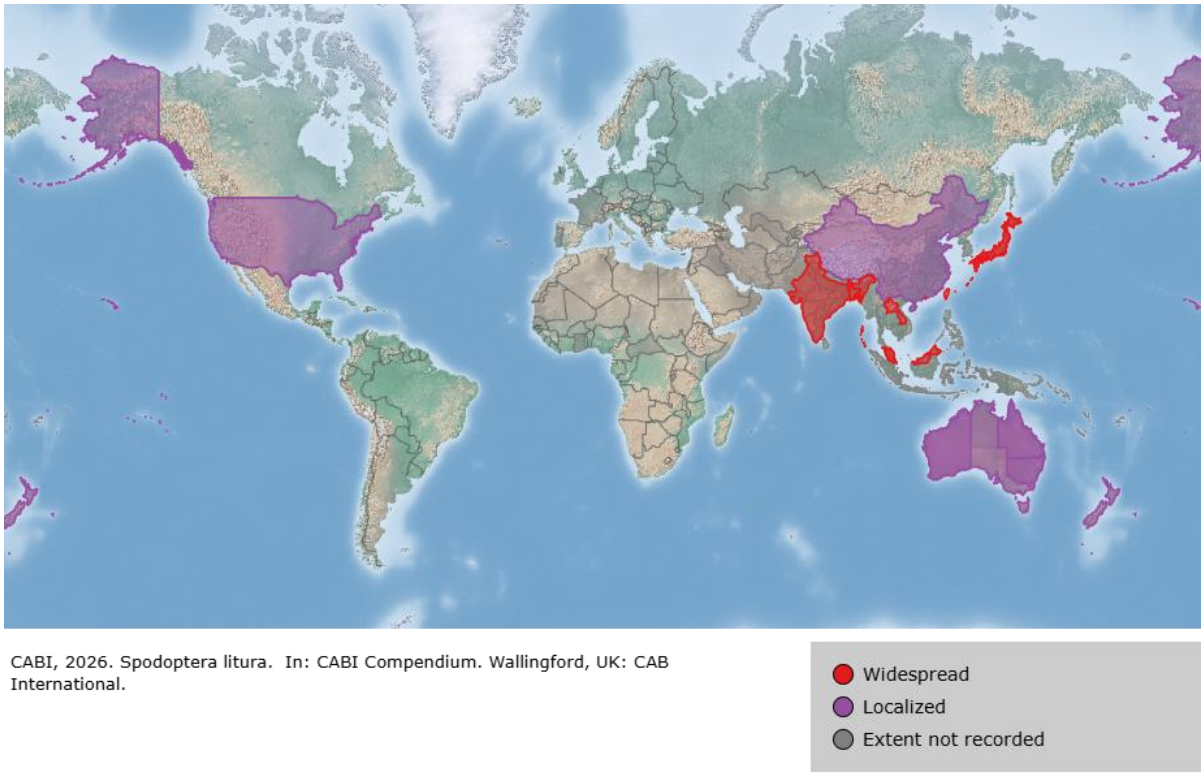

Figure 1-1 – Global distribution of Spodoptera litura *(map obtained from CABI factsheet, October 2026 https://www.cabidigitallibrary.org/doi/full/10.1079/cabicompendium.44520).*

Spodoptera litura populations tend to establish in tropical and subtropical regions with no, or few, frost days (Bragard et al., 2019). As with other species with no known diapause, low winter temperatures are an important limiting factor affecting its distribution (Bale, 1991). The species can, however, extend its range into cooler temperate regions during summer months (Bragard et al., 2019) and reports of *S. litura* in cooler temperate regions, i.e. regions where frosts occur, could be due to finding transient populations. For example, in summer *S. litura* migrates to approximately 30°N in China to breed but it cannot survive winters there and therefore has not established (Fu et al., 2015).

Spodoptera litura is a highly invasive species. The moths are extremely mobile; three-day-old, unfed males and females flew continuously for over 18 and 12 hours respectively in tethered flights in the laboratory (Murata & Tojo, 2004). From 1-day old, tethered male and female moths were able to fly for a total of 19.6 hours and 83.3 km, and 24.0 hours and 105.4 km, respectively over 72 hours (Tu et al., 2010). Wind-borne migration would allow for even longer distance migration (Fu et al., 2015). International trade gives increased potential for the extension of the geographical range of this species through transportation of eggs, larvae or pupae in soil, flowers

and vegetation (Maes, 2022). This mobility, along with the species' wide host plant range and the fecundity of the females, make *S. litura* a highly invasive species that can easily adapt to a wide range of ecological niches.

1.2.3.3. Biology

Threshold and optimal temperatures for development for this species have been well studied. A meta-analysis by Fu et al. (2025) revealed optimal temperature and humidity ranges of 30–35°C and 60–83%. Temperature increases up to a maximum of 35°C accelerated developmental rate and reproductive potential thereby shortening their lifecycle and increasing population size. The analysis did not investigate lower developmental threshold temperatures, however laboratory reared larvae fed *Glycine max* (soybean) leaves were shown to have lower developmental threshold temperatures of 10.2°C, 9.9°C and 9.8°C for eggs, larvae and pupae respectively. At 15°C, egg and larval survival ranged between 18–23%, and only 1.0% of the pupae survived to adulthood. Those adults that did emerge were 'very small, feeble and inactive', and died soon after emergence. Females were only able to produce eggs between 20°C and 30°C (Fand et al., 2015). Slightly different, but similar threshold and optimum temperatures were found for larvae fed *Ricinus communis* (castor bean) (Zakria et al., 2022) or *Corchorus olitorius* (jute) (Thakur et al., 2017). Developmental rates of the various life stages differ with temperature (Fand et al., 2015), with host plant species (Xue et al., 2010) and host nutritional quality (Subramanian et al., 2006). The number of generations completed per year varies geographically depending on climate but can reach up to 12 generations a year (Maes, 2022). None of the life stages are known to enter diapause.

There are no data to show how many generations are completed per year in New Zealand or how and where *S. litura* overwinters. Projections based on the CLIMEX model published by Jung et al. (2019) and 4 km x 5 km resolution climate data for 2020 obtained from Earth Sciences NZ (NIWA) indicate that *S. litura* may currently complete up to 4 or 5 generations per year in the most northerly regions of New Zealand (Figure 1-2). Given the species' preference for warm temperatures, *S. litura* is likely to become more numerous and damaging in New Zealand under predicted climate warming scenarios and the potential number of generations per year is likely to increase in many regions (Figure 1-2).

Female *S. litura* typically lay their eggs in clusters ranging from about 20 to over 350 eggs per batch (EPPO, 2015) on the undersides of leaves (Bragard et al., 2019). Eggs are laid in up to four layers and are usually covered with hair scales from the female's abdomen (Bragard et al., 2019; EPPO, 2015). There is some evidence that the hairs can help to protect the eggs from parasites and predators (Zakria et al., 2022). A single female typically lays from about 2000 to

2600 eggs (Maes, 2022) but fecundity varies with temperature, humidity, host species, diet quality and quantity, and a single female can lay over 5000 eggs (Zakria et al., 2022). Developmental time for egg hatch varies with temperature — from 3 days at 33°C to 9.5 days at 18°C (Thakur et al., 2017).

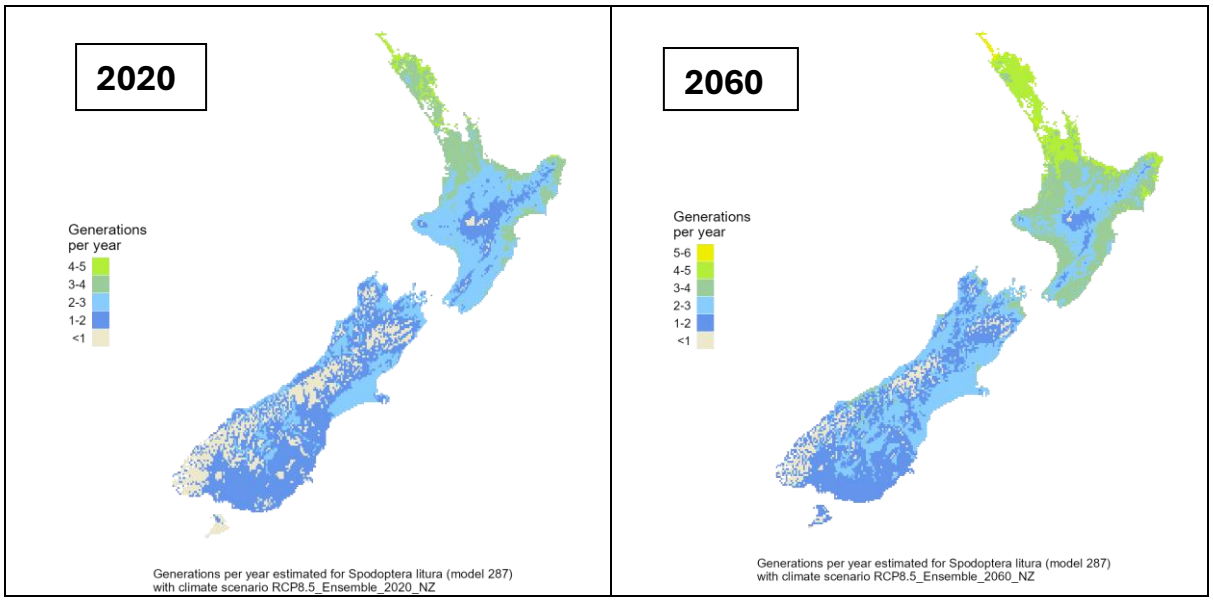

Figure 1-2 – Predicted number of Spodoptera litura *generations per year in New Zealand under climate scenario RCP8.5 (high risk/worst-case), based on CLIMEX modelling (Jung et al., 2019). Modelling conducted by Dr John Kean (Bioeconomy Science Institute, New Zealand).*

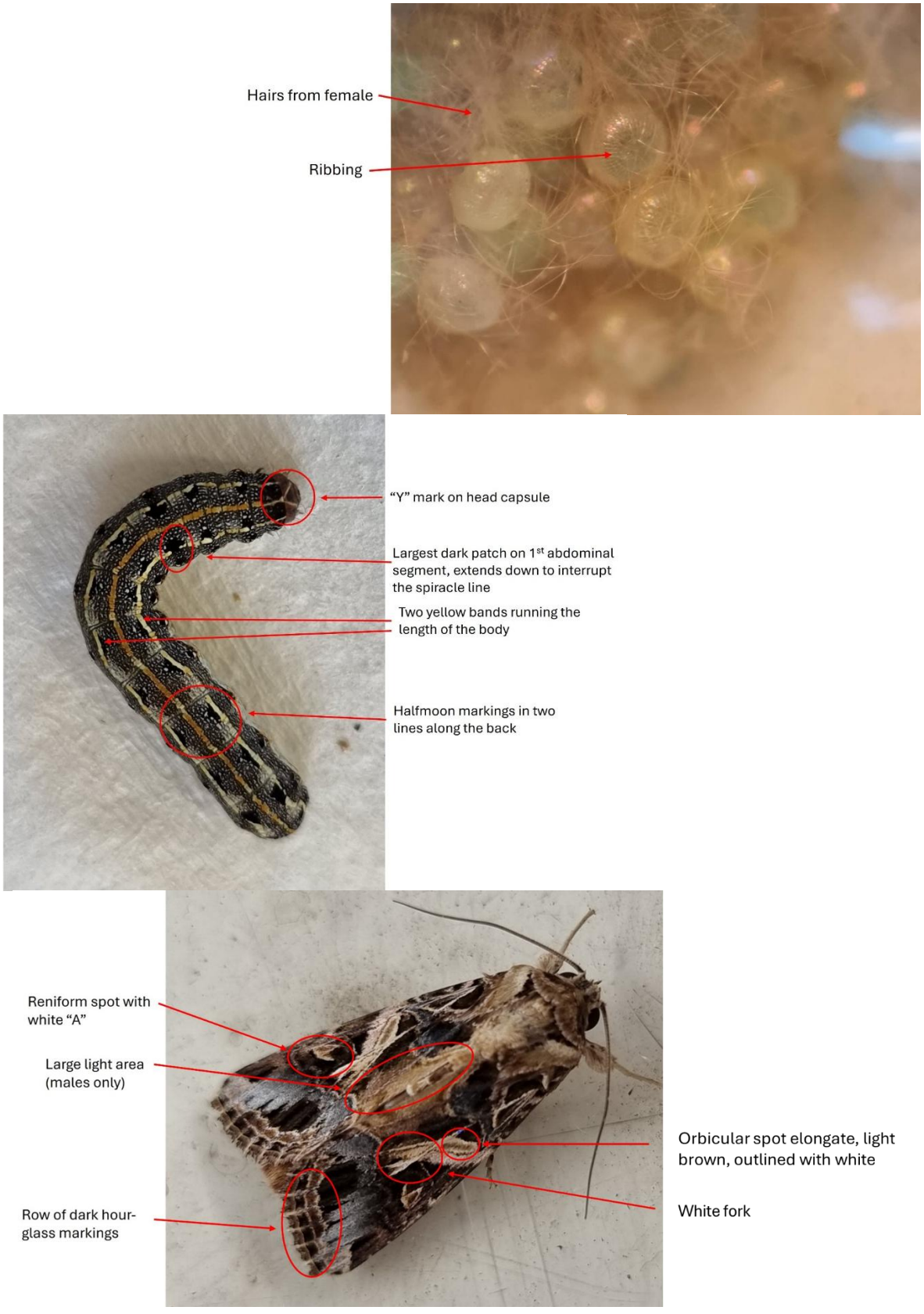

Figure 1-3 – Key identifying features of Spodoptera litura *eggs, larvae and adults (Adapted from (Jensen et al., 2025; Chapter 2).*

Neonate *S. litura* larvae are 1–2.5 mm long, 'whitish' coloured with a black head (EPPO, 2015). Mature larvae reach approximately 32 mm long and weigh around 800 mg (Maes, 2022). There are typically 6 larval instars but Tuan et al. (2015) showed that the number of instars can vary from 5 to 8 on some host plant species. Larvae can be brown or greenish in colour and often have black patches and bright orange or yellow longitudinal stripes. White ecdysial lines on the head and thorax shield form a distinctive Y-shape (Figure 1-3) (EPPO, 2015; Jensen et al., 2025). Larvae feed at night and during the day can be found in the soil around the base of the host plant (Maes, 2022). Larval duration depends strongly on temperature. Larvae fed on *Corchorus olitorius* leaves took 27.5 days to complete development at 18°C but only 13.5 days at 33°C (Thakur et al., 2017). Development rate also varies with plant host (Subramanian et al., 2006; Tuan et al., 2015; Xue et al., 2010), and food availability. Female larvae eat more than males, regardless of the host plant (Tuan et al., 2015).

Early instar-larvae create small 'windows' in leaves by scraping the softer and more digestible tissue from the surface. As they grow, larvae become able to eat the entire leaf lamina but tend to avoid the larger leaf veins and mid-ribs, thereby 'skeletonising' the leaves. Mature larvae eat the whole leaf except for the toughest parts, and can also eat buds, flowers and fruit. Individual *S. litura* larvae can consume a total of around 4 g fresh weight of groundnut foliage, with 80% of this being in the final instar (Maes, 2022). Developmental rate, survival and fecundity are also affected by host plant species (Shekhawat et al., 2018; Tuan et al., 2015; Xue et al., 2010).

Prior to pupation, larvae expel water and gut contents and then burrow into the soil where they form a protective cell (J.G. Jensen personal observation). The pupa is brown and approximately 15–22 mm long, with two small spines at the tip of the abdomen (EPPO, 2015; Maes, 2022). Pupal duration was 6 days at 33°C but extended to 15.33 days at 18°C when larvae were fed *C. olitorius* (Thakur et al., 2017). Female pupae tend to be heavier than males (Tuan et al., 2015), but this difference may be diet-dependent (Xue et al., 2010).

Adults are about 15–20 mm long; their forewings are 14–17 mm and 15–18 mm long in males and females respectively. The body is grey brown, the forewings grey to reddish brown with a bold, variegated pattern, and the hindwings are greyish-white with grey margins, often with dark veins (Maes, 2022) (Figure 1-3; Jensen et al., 2025). Adult females tend to emerge earlier than males (Sharma et al., 2025) and live for around 10 days at 25°C (Zakria et al., 2022). Some studies show that males live longer than females. For example, Xue et al. (2010) found that adult longevity varied significantly depending on the host plant species and that females lived on average for 6.6 to 7.7 days, (depending on host species), compared with 7.4 to 8.8 days for males

at 26°C. Ye et al. (2022), however, found no significant difference in development times between the sexes; males and females lived for 9.66 and 9.69 days respectively when reared on asparagus stems at 27°C, and 9.47 and 8.52 days respectively when fed asparagus leaves.

1.2.3.4. Host plants

Spodoptera litura are highly polyphagous. The number of reported host plants varies from 'at least 120 species' (Maes, 2022) to 389 host plant species (Yinghua et al., 2017) from at least 40 different plant families (Bragard et al., 2019). Many of its host plants are economically important, for example *Gossypium* sp. (cotton), Fabaceae (beans), *Nicotiana tabacum* (tobacco), *G. max*, *Arachis hypogea* (groundnut), *R. communis* and *Oryza sativa* (rice) (Bragard et al., 2019; Fand et al., 2015; Xia et al., 2020). Yield losses can be severe; Choudhary and Shrivastava (2007) reported average pre-treatment larval numbers of 15.6 to 28.2 per meter row length on *G. max* in Madhya Pradesh, India resulting in 22.2% pod damage. One larva per plant on *A. hypogea* seedlings in Orissa, India reduced pod yield by 25.8% and leaf area by 54.7%. At flowering, pod yield reduction and leaf area consumption were 19.1% and 49.1% respectively (Dhir et al., 1992).

In New Zealand, *S. litura* has been reported from *Andropogon sorghum* (broom corn), *Beta vulgaris* (silverbeet), *Brassica oleracea* (cabbage), *T. repens* (white clover), *Apium graveolens* (celery), *Dahlia* sp., *Phaseolus vulgaris* (beans), *Phytolacca octandra* (inkweed), *Ipomoea batatas* (kumara), *Gladiolus* sp., lucerne, plantain and chicory (Dale et al., 1976; Gerard et al., 2011). According to Berry and Walker (2004) it can completely defoliate clover and broadleaf weeds in pastures. However, many of these are observational associations. Lindroth et al. (2000) reared *S. litura* on two white clover cultivars in a laboratory study and Huang et al. (2008) showed that *S. litura* can successfully develop through to adulthood on both white and red clover. Sushilkumar and Ray (2018) found that chicory and broad-leaved dock (*Rumex obtusifolius*) were 'preferred' hosts based on visual feeding scores and that *S. litura* could be reared through to adulthood on these species (Sushilkumar & Ray, 2007). Unpublished research by Dr. S. Orre-Gordon (Bioeconomy Science Institute, New Zealand) has shown that the pasture grasses *L. perenne* (perennial ryegrass), *Phalaris aquatica* (phalaris) and *Dactylis glomerata* (cocksfoot) are not suitable hosts, but they may possibly be vulnerable as seedlings or when there are large populations of late instar larvae and limited access to preferred hosts.

1.2.3.5. History in New Zealand

The first recorded breeding population of *S. litura* in New Zealand was in March 1955 from tobacco crops in the Nelson District (Cottier & Gourlay, 1955). This population was apparently eradicated by the farmer using 'vigorous measures with DDT' and no further caterpillars were

found in the area. The species was subsequently noted on occasion as a migrant species, for example by Fox (1970) who reported three adult *S. litura* from the north-west coast of the South Island 'after several days of strong westerly winds'. Dale et al. (1976) described the finding of a larva in Te Kao (Northland) by B.B. Given in 1973 as the 'first indication of the larva overwintering in New Zealand', and by 1976, Fox (1976) expressed 'special interest and concern' at the 'epidemic proportions' reached by *S. litura* in Auckland and Northland from February to May, and noted it as a pest from Taranaki northwards. Dale et al. (1976) and Boardman (1977) likewise described the species as causing serious damage to pastures, crops such as lucerne and rape (*Brassica napus*) and home gardens in Northland and Auckland, and less serious damage in Taranaki and Levin. Dale et al. (1976) also reported that, in 1975, egg masses had been sent in from various localities as far south as Tauranga.

In April and early May 2011, *S. litura* caused widespread serious damage to Bay of Plenty lucerne crops and *Trifolium* spp., *Plantago* spp. and chicory in pastures, reaching populations as high as 100 m^{-2} (Gerard et al., 2011). A mild and wet winter in 2010 followed by above average spring and summer temperatures and near record summer rainfall were thought to have allowed for the completion of four generations and thus for populations to build up to outbreak levels. Poor weed control due to dry spring soil conditions during maize establishment may also have contributed. The authors noted that similar weather patterns are expected to increase in frequency with climate change.

1.2.3.6. Population control

Populations of *S. litura* in New Zealand are thought by some to be largely kept in check by natural enemies. Adverse climate events such as drought or high rainfall often seem to be followed by outbreaks, however, and this is theorised by some authors to be largely due to a disruption of the normal parasitoid life cycles. For example, the Northland Regional Council website (www.nrc.govt.nz/environment/weed-and-pest-control/pest-control-hub) says 'Moist, humid and warmer than average temperatures can lead to outbreaks with high numbers of armyworm caterpillars consuming large quantities of pasture and maize crops. Climatic conditions, such as high rainfall/flooding in January and February can disrupt the parasitoid life cycle, reducing their impact on armyworm populations.' Similarly, AgPest (2026) states that 'Occasional outbreaks occur but are rare and are likely to be initiated by climatic disruption to natural enemies and crop weed control, followed by above average summer and autumn temperatures.' However, there does not appear to be any published evidence to support these claims. Peacock et al. (2006) used discriminant analysis to investigate the influence of climate variables on insect

establishment patterns in New Zealand and found that the distribution of *S. litura* was best predicted by monthly summer temperature.

A world review of natural enemies and diseases of *S. litura* listed 71 species of insect parasitoids, 48 species of predatory insects and spiders, and a further 23 protozoan, fungal, bacterial, viral and nematode pathogens (Ranga Rao et al., 1993). Of these, five larval and four egg parasitoids, two predators, and 18 of the pathogens are known to occur in New Zealand. Therefore, New Zealand populations of *S. litura* are thought to be kept generally in check by a suite of generalist natural enemies, including *Meteorus pulchricornis* (Hymenoptera: Braconidae) but there are no published records of this. Although Berry and Walker (2004) found *S. litura* to be a preferred host of *M. pulchricornis* in the laboratory they reported only one occasion of *M. pulchricornis* being reared from a field collected *S. litura*.

Worldwide, field populations of *S. litura* have developed resistance to a wide range of insecticides including organophosphates, carbamates and pyrethroids (Tong et al., 2013). In New Zealand, the most effective insecticides for this pest are thought to be alpha cypermethrin, chlorpyrifos, diazinon, methomyl, methamidophos and carbaryl, but the latter three are currently under review by the New Zealand Environmental Protection Authority (EPA) (AgPest, 2026). *Spodoptera litura* has low susceptibility to transgenic Bt cotton (Wan et al., 2008; Yinghua et al., 2017).

1.2.3.7. Current and predicted distribution in New Zealand

In New Zealand, *S. litura* is thought to occur predominantly in the North Island and outbreaks are reported sporadically from northern North Island regions (Orre-Gordon & Jensen, 2025). However, the exact geographical distribution of this species in New Zealand is unknown. CLIMEX modelling indicates that conditions are suitable or favourable for *S. litura* throughout the North Island and northern and eastern regions of the South Island (Figure 1-4). Projections based on climate scenario RCP8.5 indicate that these areas will expand significantly by 2060.

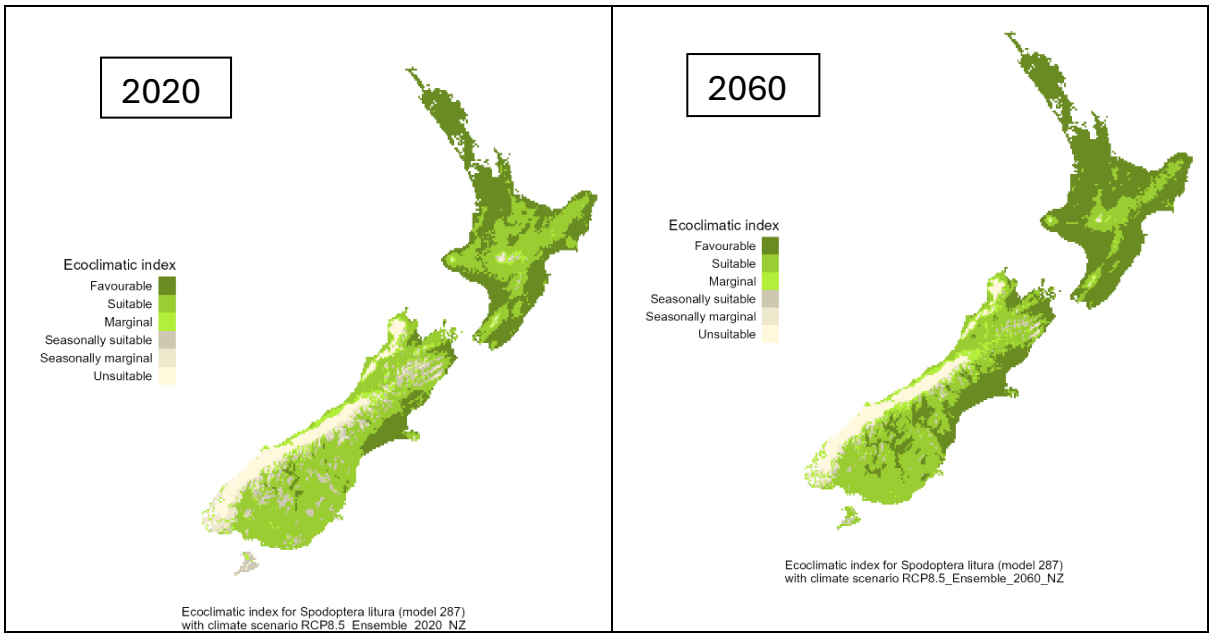

Figure 1-4 – Predicted regional ecoclimatic suitability in New Zealand estimated for Spodoptera litura *under climate scenario RCP8.5 (high risk/worst-case), based on CLIMEX modelling (Jung et al., 2019). Modelling conducted by Dr John Kean (Bioeconomy Science Institute, New Zealand).*

1.2.4. Insect-plant-gut microbiota interactions

Plants, herbivorous insects and microbes form a complex ecological network. Understanding their interactions is critical for predicting future species distributions (Wisz et al., 2013), for predicting potential pest species, and for identifying plant species and plant traits that will be resistant or tolerant to damage from these pests (Giron et al., 2018). Most herbivorous insect species are specialists, restricted to a single plant taxonomic family or species but there is a 'long, thin tail' of species, such as *S. litura*, with more generalised feeding habits (Forister et al., 2015). Although in the minority, the larger host range of generalist feeders increases their potential invasiveness and geographical spread (Gougherty & Davies, 2022; McKinney & Lockwood, 1999). The ability to utilise multiple hosts allows for a more temporally and geographically guaranteed food supply and makes them harder to control through crop rotation.

The term 'plant host' is used rather broadly among researchers. In theory a host plant is one that supports the development of larvae and contributes to the number of reproducing adults in the population (Cunningham & Zalucki, 2014). However, some records simply reflect observed associations between insect and plant in the field (Ward, 1988). Others use a more selective definition which considers the degree to which the plant contributes to the insect's fitness, how regularly the plant species is used as a host and the relative abundance of eggs and larvae. Plant host lists compiled from multiple sources often do not define the criteria used for each entry or

distinguish between high- and low-quality hosts. Furthermore, they are likely to be biased towards plants of economic significance (Rafter & Walter, 2020).

Studies that have quantified relative host use in the field show that most polyphagous insect species tend to associate closely with only a small subset of their recorded potential hosts (Rafter & Walter, 2020). These 'primary host species' are defined as those on which the insect species is found most often and in relatively high numbers in the field. Hosts that are used less frequently and at relatively lower intensities can be described as 'secondary hosts' and a final category, 'incidental hosts' is used for host plants that are used very infrequently and at extremely low intensity.

Understanding relative host plant quality is a crucial component in determining the local ecology of a polyphagous insect such as *S. litura*. Identifying primary, secondary, incidental and non-hosts helps us to predict seasonal and annual spatio-temporal dynamics of a herbivore (Rafter & Walter, 2020). Identification of favoured or non-favoured host species and assessing how these influence polyphagous pest insects such as *S. litura* will help us design pastures that will be sustainable and resilient to future conditions (Giron et al., 2018). Host plant suitability is a function of a wide range of plant-centred traits including location and availability, nutritional quality, and chemical and physical defences. Traits of the insect itself are also key determinants, both at the species and at the individual insect level, for example age, behaviour, compensation strategies and gut microbiota. These factors are discussed below.

1.2.4.1. Host plant availability

Insect survival in a given location is constrained by the presence of a suitable food source (Wisz et al., 2013). Alongside top-down forces like predation by natural enemies, bottom-up forces such as the abundance and quality of that food source will be key drivers of the insect's population growth (Scriber & Slansky, 1981). For herbivorous insects, therefore, knowledge of their host plant species range is key to predicting their potential geographical spread, and where and when populations might reach pest proportions. Where there are changes in the use or geographical range of plant species, we can expect to see a corresponding change in the presence of herbivorous insects for which these plants are hosts (Sharma & Dhillon, 2020). A good example of this was seen when northern North Island farmers planted more plantain in response to increased summer temperatures and drought and two previously innocuous native plantain moths, *Epyaxa rosearia* and *Scopula rubraria*, emerged as significant pests (Kean et al., 2015; Mansfield et al., 2021). Favourable climate changes that increase plant growth or the length of a crop growing season will increase abundance and severity of insect pests of that crop (Sharma & Dhillon, 2020). A longer crop season may allow for more insect generations in a

cropping season or year and thus greater potential for populations to build up in number to damaging levels. We need to understand what the preferred forage host plants are for *S. litura* to help predict where and when they might reach damaging populations. Current information on this is largely anecdotal and unpublished and is therefore a large gap in our understanding of this species.

1.2.4.2. Host plant quality

Host plant quality is a key determinant of a herbivore's performance and is shaped by both physical and chemical attributes that can impact a herbivore in positive or negative ways (Awmack & Leather, 2002). For example, *S. litura* feeding and growth on different cotton cultivars was positively correlated to carbohydrate, protein and water content but negatively correlated with lipid and phenolic content (Subramanian et al., 2006). Specific requirements for a high-quality host vary depending on the herbivore in question (Awmack & Leather, 2002).

Nutritional content is a major determinant of host plant quality and has been shown to be a major driver of insect physiology and behaviour (Scriber & Slansky, 1981). Nitrogen in particular has long been considered critically important to herbivores due to its role in all metabolic processes, cellular structure and genetic coding, and insufficient nitrogen can limit growth, reproduction and survival (Mattson, 1980). Nitrogen content of plant biomass is generally lower than that of insects, therefore herbivores use a variety of mechanisms such as high feeding rates, prolonged feeding, diet mixing or use of symbionts to acquire sufficient quantities (Hansen et al., 2020).

Recent research has shifted its emphasis from elemental analysis to the nutritional components of plants, particularly proteins and carbohydrates. Soluble protein and digestible carbohydrates may be better measures of nutritional quality than elemental nitrogen and carbon because they quantify the actual nutritional content available to the insect. In contrast, a proportion of total plant nitrogen and carbon is likely to include components such as cellulose, lignin and allelochemicals that are non-nutritive for some insect species (Wilson et al., 2019). While the amount of each macronutrient is important, so too is the nutrient balance; thus, insect performance can vary with changes in protein and carbohydrate ratios (Aceituno-Medina et al., 2024; Behmer, 2009; Le Gall & Behmer, 2014; Roeder & Behmer, 2014). Specific amino acids may have to be acquired dietarily and other nutrients such as lipids, sterols, vitamins and minerals may be critical or important components of a herbivore's diet, with these requirements varying between species and life stages (Hanife, 2006).

Non-nutritive plant traits such as food water content and indigestible fibre content also strongly affect herbivorous insects (Mattson, 1980; Scriber & Slansky, 1981). Leaf structural

characteristics such as trichomes and leaf toughness can cause significant difficulties for herbivores and often correlate more strongly with spatial and temporal patterns of herbivory than chemical composition (Clissold et al., 2009). Leaf toughness is generally associated with low water content. Slow passage of food through the gut due to food toughness may alter nutrient assimilation ratios and has consequences for development and metabolic requirements. Experiments on the Australian plague locust (*Chortoicetes terminifera*) showed that increased food toughness extended development times and decreased the efficiency of food conversion (Clissold et al., 2009).

Plant defensive chemistry is also an important component of interactions between plants and herbivorous insects. Plant secondary metabolites such as phenols, tannins and flavonoids have been shown to influence preference and performance in many herbivores but effects vary depending on the feeding guild (Lavola et al., 1998), herbivore species, and environmental conditions (Stamp & Yang, 1996). For example, the increasingly used pasture plant species, plantain, (Caradus et al., 2021; Minnee et al., 2017) contains the iridoid glycoside, aucubin. The compound has been shown to reduce feeding and growth of another *Spodoptera* species, *Spodoptera exigua* (beet armyworm) (Biere et al., 2004; Bryant et al., 2020). The bioflavonoid rutin is toxic to the grasshopper, *Oedaleus asiaticus* (Wang et al., 2020), but had positive, negative or neutral effects on the growth rate of *S. exigua* depending on the temperature regime and on the presence or absence of another allelochemical, chlorogenic acid (Stamp & Yang, 1996).

1.2.4.3. Insect responses

Inadequate nutrition, exposure to plant chemical and physical defences or low food availability during the larval stage of holometabolous insects has potential impacts at both the individual and the population level. Reduced larval development, lower quality lipid reserves and deficiencies in macro- and micronutrients have been associated with smaller adult size, reduced reproductive capacity, poorer immune system function and lower resistance to environmental stress (Awmack & Leather, 2002; Nestel et al., 2016). Herbivorous species can use a variety of pre-ingestive and post-ingestive mechanisms to try to counter poor host quality (Behmer, 2009; Boggs, 2009; Couture et al., 2016). These strategies must be balanced against the need to avoid nutritional imbalance, toxic chemistry, natural enemies and to complete development within suitable environmental windows.

One strategy to compensate for low nutritional value is to increase the quantity of food ingested either through a faster ingestion rate or longer feeding period (Lee et al., 2012; Slansky Jr. & Feeny, 1977). Alternatively, herbivores may switch food sources to regulate nutritional components (Le Gall & Behmer, 2014; Roeder & Behmer, 2014). Such dietary mixing has been

shown to enhance the performance of some insects such as the generalist herbivore *Schistocerca americana* (grasshopper) (Bernays et al., 1994) and *S. litura* (Mitra et al., 2021). Some insect herbivores may change their diet by eating different plant species or plant tissues (Behmer, 2009) and some polyphagous insect 'herbivores' have been shown to supplement a poor quality diet through cannibalism, predation or scavenging, including feeding on exuviae or egg shells (LoPresti, 2018).

Post-ingestive mechanisms including changes in midgut biomass allocation, digestive processing efficiency and growth rate have been shown to help regulate nutritional intake (Couture et al., 2016; Lee, 2007). Measurement of food, faeces and body weights allow us to calculate and compare an insect's food processing efficiency through formulae developed by Waldbauer (1968). Widely used food utilization indices include approximate digestibility (AD = how much of the food that was eaten was digested), the efficiency of conversion of ingested food (ECI = how much of the food that was eaten was turned into body weight) and the efficiency of conversion of digested food (ECD = how much of the food that was digested was turned into body weight) (Figure 1-5). Post-ingestional allocation of nutrients may differ in response to nutritional challenges. Aceituno-Medina et al. (2024) found that protein:carbohydrate:lipid ratios in *Anastrepha ludens* larval diet affected the assignment of macronutrients that persisted to the adult stage and differed for males and females. Differential resource allocation may facilitate improved migratory capability through changes in body size and proportion (Angelo & Slansky, 1984). Effects of host plant quality on reproductive fitness may be mediated through resorption of eggs, adjusting egg number, size or nutritional content, and changes to sex ratios (Awmack & Leather, 2002). Among insect species that enter diapause, poor nutrition can induce or avert dormancy depending on the timing and degree of nutritional deficit (Short & Hahn, 2023).

Resource acquisition and allocation responses to poor host quality vary not only between but also within species (Boggs, 2009). Larval age can affect a herbivore's responses to plant chemical and physical defences through variation in head and body size, mandible shape, symbiotic microbial bacteria, detoxification enzymes, behavioural and physiological changes (Quintero & Bowers, 2018). Most studies on nutritional ecology examine only a single developmental stage and do not distinguish between sexes. The work of Roeder and Behmer (2014) showed the importance of monitoring across an insect's lifespan and also suggested an intergenerational effect that is rarely studied. Intergenerational effects can be described as the influence of parental phenotype or environment on offspring traits such as growth, survival, reproduction or physiology (Mousseau & Dingle, 1991; Paul et al., 2022). These effects may play

a key role in insect ecology and evolution and in their ability to respond to changing environments (Badyaev & Uller, 2009).

Nutritional regulation strategies often come with costs such as increased exposure to predators, increased ingestion of plant defence chemicals or consumption of excessive protein (Behmer, 2009; Lee, 2007). Diet mixing or feeding on alternative hosts can have a metabolic cost that can negatively affect the insect's fitness (Cunningham et al., 2001; Karowe & Martin, 1989). Even when successful in the short term, insects may pay a substantial long-term cost for nutrient regulation and the impacts of poor host quality can be intergenerational (Mousseau & Dingle, 1991). These negative effects may be outweighed if preferred food sources are scarce or environmental conditions are challenging. In these situations diet switching will help the insect to survive adverse conditions and expand their host range (Mitra et al., 2021). Trade-offs between life history traits such as longevity, migration and reproductive strategies are important outcomes to understand with respect to polyphagous pest insects such as *S. litura*.

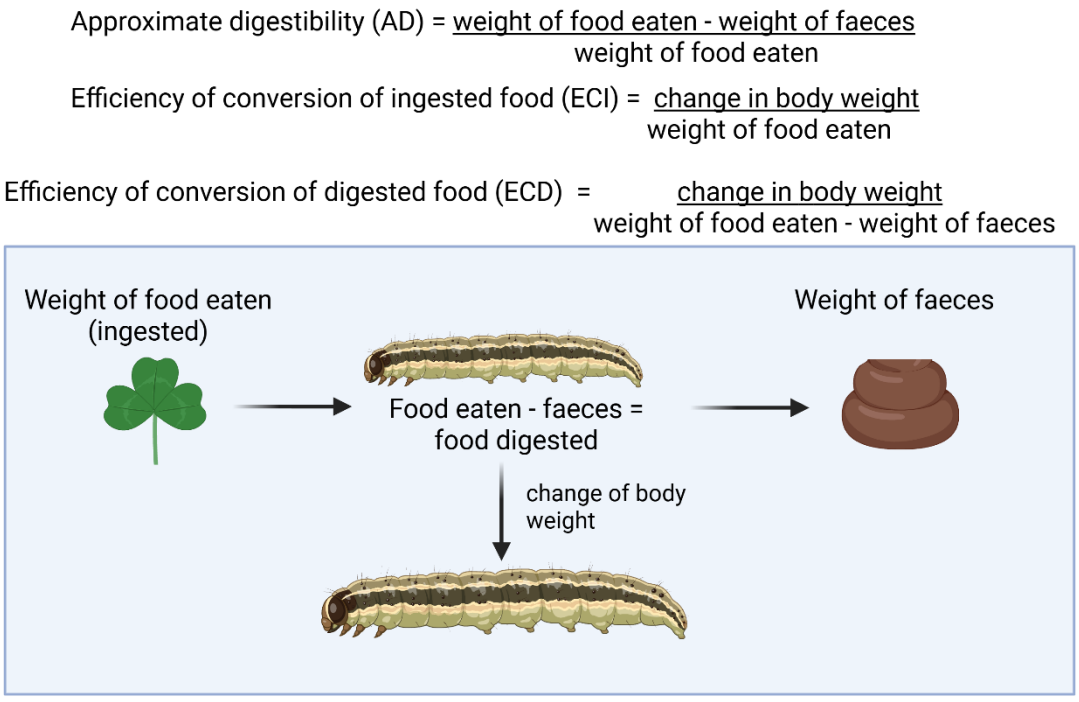

Figure 1-5 – Summary diagram explaining feed conversion indices developed by Waldbauer (1968). Picture created in BioRender.com

1.2.4.4. Insect gut microbiota

Many insects benefit from associations with microorganisms through their ability to synthesise essential nutrients or digest and detoxify ingested food (Douglas, 2009; Giron et al., 2017). For example, the bacterial symbiont *Buchnera aphidicola* provides its host aphid with essential amino acids and vitamins that are rare in phloem sap (Engel & Moran, 2013). Treating *S. litura* with antibiotics reduced feeding and growth in a study by Xia et al. (2020), suggesting the importance of gut bacteria for this species. Metagenomic sequencing showed that the gut bacteria encoded the genetic capacity for many enzymes involved in digestion, detoxification, and nutrient supply. Another study by Thakur et al. (2016), however, showed that *S. litura* larvae developed more quickly when fed a diet containing the antibiotic streptomycin sulphate, probably due to the removal of pathogenic bacteria. Variation in insect-microbiome interactions may in part explain variation in the abundance and distribution of insects (Douglas, 2009). The ability of microorganisms to affect insect host plant range, feeding efficiency and metabolism make them an important consideration when trying to understand plant-insect interactions and how these might be affected by environmental variables such as climate change (Enders & Begcy, 2021).

Microbial gut symbionts can be acquired environmentally or through maternal transmission (Giron et al., 2017). Environmental transmission occurs horizontally through contact with infected individuals or from the diet (Paniagua Voirol et al., 2018). Alternatively, microbes can be vertically transmitted from one generation to the next (Paniagua Voirol et al., 2018). This may occur intracellularly within the egg. The symbiont *Ishakawaella capsulata* lives in special capsules in the guts of *Megacopta punctatissima* (stinkbug) which are released when the female *M. punctatissima* defecates on the outside of her egg cases. On hatching, the larval stinkbugs thus consume the capsule and acquire the symbiont (Engel & Moran, 2013).

How lepidopterans acquire and retain gut microorganisms is poorly understood (Paniagua Voirol et al., 2018), and gut bacterial communities vary widely both between and within species (Mason et al., 2020). The causes of this variation are not clear but Mason et al. (2020) speculated that it may be in part due to sensitivity of resident gut bacteria to variation in diet. These authors found strong interactions between gut microbes and plant diet and a positive relationship between larval growth and gut microbial biomass for *S. frugiperda*. They also found that gut microbes persisted through larval moults, suggesting that they are not simply transient communities. Maternal transmission of microbes could allow improved utilisation of non-preferred hosts over successive generations (Engel & Moran, 2013). On the other hand, maternal transmission could limit diversification by restricting individuals to the same hosts as their

parents while environmental transmission could allow insects to adapt more quickly to changing food and abiotic conditions (Paniagua Voirol et al., 2018). Further work is needed to understand the gut microbiota of herbivorous insects, the effects of host plant species and environmental conditions, and how these all interact to affect their ecology. Although there are published records of the gut microbiota of *S. litura* larvae feeding on taro or artificial diet (Xia et al., 2020) and soybean (Bapatla et al., 2018), intergenerational effects and the mode of transmission of gut microbes are not known for this species.

1.2.5. Summary

Pasture farming in New Zealand is ever evolving, with farmers facing a plethora of environmental, social and biological pressures. Climate change brings new challenges and will have significant impacts on pasture forage plants and the insects that feed on them. To maintain quality feed and sustainable pastures under future climate scenarios, farmers, plant breeders and scientists will need to anticipate these changes and identify fitness traits that enable plants to tolerate them. A first step towards this is to identify herbivorous insects that are likely to become important pests in future, and to understand how they will interact with potential future forages.

Spodoptera litura has been identified as a 'sleeper pest' which is likely to become more damaging and/or abundant in New Zealand pastures under climate change. Although well studied internationally, very little is known about *S. litura* in New Zealand pasture systems. Currently available information on its spatial and temporal abundance is sparse and largely anecdotal. Published research largely focuses on its internationally important host crops such as tobacco and cotton. We have little current knowledge about favoured pasture plant hosts, and how different plant species relevant to New Zealand pastoral systems will affect its survival, development, reproductive capacity and behaviour. Intergenerational effects due to phenotypic or gut-microbiota responses to host differences may also impact insect population dynamics and distributions, so studying these adds a valuable level of understanding.

1.3.Thesis aims

1.3.1. Hypotheses and objectives

The overarching goals of this thesis were to:

- Investigate the availability and usefulness of existing sources of *S. litura* distribution data in New Zealand.
- Conduct fundamental research into *S. litura* biology and interactions with forage plant hosts relevant to New Zealand pastoral systems.

I hypothesised that:

(1) — In the absence of sufficient resources to conduct population surveys at multiple sites and across multiple years, online tools such as iNaturalist/GBIF could provide useful information for monitoring sleeper pest species, with *S. litura* as a case study.

(2) — *Spodoptera litura* will be able to cause considerable damage to New Zealand pastures and pasture crops through feeding on broadleaf pasture plants, such as plantain and clover, but some plant host species will be higher quality than others.

(3) — Feeding on low-quality host plants will affect *S. litura* survival, behaviour, growth, reproduction and gut microbiota, and will have intergenerational effects on the gut microbiota and performance of the subsequent generation.

1.3.2. Thesis structure

The structure of this thesis is summarised in Figure 1-6. The four experimental chapters are based on the objectives outlined in Section 1.3.1 and describe investigations into potential monitoring tools and plant host interactions with the sleeper pest, *S. litura*. All experimental chapters have either been published (Chapters 2 and 3) or will be submitted to a journal for publication (Chapters 4 and 5) in international or national journals.

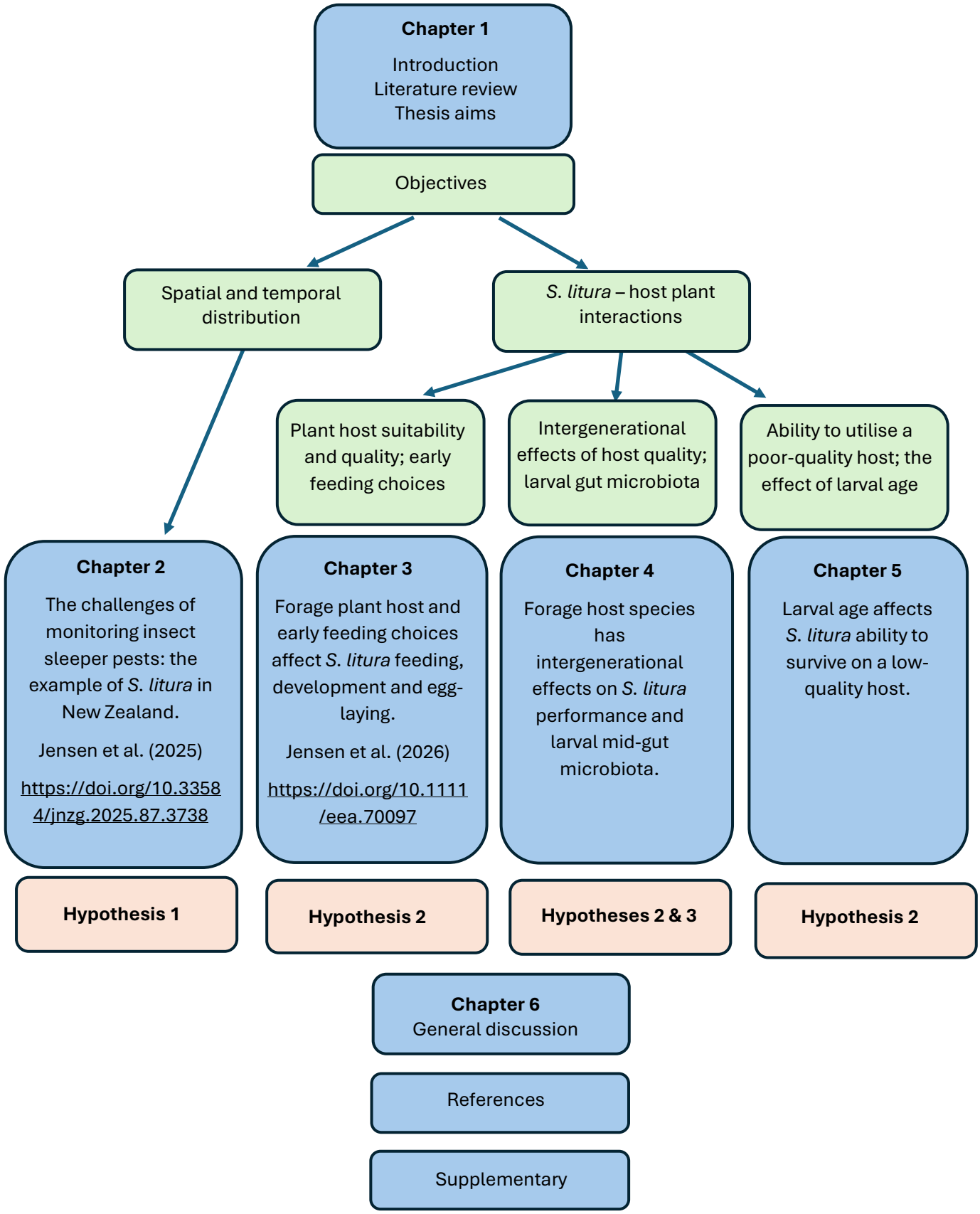

Figure 1-6 – Diagrammatic summary of the thesis structure and how each chapter aligns with the broader research themes.

2. The challenges of monitoring insect sleeper pests: the example of *Spodoptera litura* (tropical armyworm) in New Zealand

This chapter has been published in the Journal of New Zealand Grasslands (2025) (https://doi.org/10.33584/jnzg.2025.87.3738) and reformatted to fit the thesis structure. The chapter addresses the hypothesis that, in the absence of sufficient resources to conduct population surveys at multiple sites and across multiple years, online tools such as iNaturalist and the Global Biodiversity Information Facility (GBIF) could provide useful information for monitoring sleeper pest species. The example of *S. litura* in New Zealand was used to test this hypothesis.

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The challenges of monitoring insect sleeper pests: the example of *Spodoptera litura* (tropical armyworm) in New Zealand

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2.1. Highlights

Farmers will face a different suite of insect pests under climate change. Monitoring their population dynamics over time is important for risk assessment and mitigation, but it can be expensive and time-consuming, and published information can be sparse. To highlight this issue, I investigated suitable sources of such information on *Spodoptera litura* (tropical armyworm), a potential sleeper pest in New Zealand. After appropriate quality checks and analyses, data from two online platforms, iNaturalist and the Global Biodiversity Information Facility (GBIF), indicated higher numbers and an extended geographical range for *S. litura* in 2018 and 2022-23. This example suggests that these platforms could be a useful tool for future pest monitoring.

Keywords: Citizen science, Global Biodiversity Information Facility, iNaturalist, pasture pests

2.2. Introduction

Future agricultural systems will face a novel suite of insect pest species due to factors such as climate change and changing host plant availability (Mansfield et al., 2021). The range and abundance of some current pest species is likely to change, and other species that are not currently considered pests may emerge as such as conditions become more favourable. The latter group, known as 'sleeper pests', can be defined as species that are already present and causing only periodic or geographically restricted damage but that are predicted to increase their range, and/or outbreak size and frequency, under climate change (Orre-Gordon & Jensen, 2025).

Monitoring potential sleeper pest populations, determining their dynamics and delimiting their range can be important in terms of recognizing an emerging threat and responding to it. However, with a large number of introduced insects present in New Zealand (Brockerhoff et al., 2010), and limited research resources, most potential sleepers are poorly understood, with few resources directed at intentional monitoring (Mansfield et al., 2021). This is true to the extent that many taxa are likely present but remain undocumented. For those we know something about, information may be restricted to specimens in natural history collections, reports, or, increasingly, citizen science data platforms. These sources of data or information may represent "presence only" data sets and may appear to be haphazard by their nature, and their use and interpretation are challenging (Phillips et al., 2006; Roigé & Phillips, 2021). I hypothesise, however, that citizen science platforms such as iNaturalist and the Global Biodiversity Information Facility (GBIF) are likely to be the best available source of data for monitoring an emerging threat like a sleeper pest and that, with appropriate checks and analyses, they have the potential to provide valuable information for understanding and tracking their spread.

Researchers internationally have increasingly turned to citizen science (Cull, 2021). Data from platforms, such as iNaturalist and GBIF, have been used to assess phenology (Drury et al., 2019), monitor biodiversity (Alfeus et al., 2025), track invasions (Werenkraut et al., 2020) and aid in the development of species distribution models (Hochmair et al., 2020). Many authors caution, however, that care must be taken with analysis and interpretation of the data due to the unstructured, opportunistic nature of the sampling and reporting process (Di Cecco et al., 2021; Isaac et al., 2014).

iNaturalist.org is an online platform where anyone, anywhere, can share observations of nature. The platform originated in 2008 as a Master's project at UC Berkely but has since grown and became an independent nonprofit organization in 2023 (iNaturalist.org/pages/about). The New Zealand branch of iNaturalist, iNaturalistNZ - Mātaki Taiao, had its origins in the NZ Bio-Recording Network Trust (NZBRN) in 2005. This became 'NatureWatchNZ' in 2012 and finally in

2018 moved to the inaturalist.nz site. Observers can upload photos or sound files to the iNaturalist site, along with the date and location of the observation, and any other relevant information. An artificial intelligence (AI) interface suggests species identification based on location and image analysis. Other users may then either suggest, agree or disagree with species identification. A 'verifiable' observation (i.e. one that has a date, is georeferenced, has photos or sounds and is neither a captive nor a cultivated organism) becomes 'research grade' when two-thirds of identifiers agree on the identification. Observations that have not reached research grade are classified as 'needs identification (ID)'.

GBIF also provides open access data about all life forms. The network is funded by 'the world's governments', drawing data from diverse sources, including iNaturalist (gbif.org/what-is-gbif). Data are filtered to exclude observations such as those with missing date or location information or, for iNaturalist observations, specimens that are not 'research grade', or are not associated with one of three Creative Commons licenses (CC0, CC BY, CC BY-NC) (Hochmair et al., 2020). Links to a variety of resources are available within GBIF to help with gathering and interpreting data.

Here, I demonstrate the challenges in finding published population data for a potential New Zealand sleeper pest, *Spodoptera litura,* and explore the opportunities and risks of using alternative data types such as those found on iNaturalist and GBIF.

Tropical armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae), has been identified as a likely sleeper pest of pasture in New Zealand (Orre-Gordon & Jensen, 2025). This noctuid moth species is considered to be one of the most important pests of agricultural crops in the Asian tropics, where the larvae feed on a broad range of economically important plant species and its exposure to pesticides has led to insecticide resistant populations (CABI, 2014). The species is highly invasive. The adults are extremely mobile and females typically lay 2,000-2,600 eggs (Zakria et al., 2022). In New Zealand, *S. litura* is currently considered to be a problem only sporadically and mainly in the northern North Island (Kean et al., 2015). However, outbreaks are likely to occur more often and its distribution to move further south as winters become milder and summer and autumn temperatures increase with climate change (Kean et al., 2015). As a pest that is periodic and geographically restricted in New Zealand, studies and publications on the population dynamics of *S. litura* have been sporadic since its initial establishment.

2.3. Study approach

As a test case and example of the challenges involved in sleeper pest monitoring in pastoral systems, I searched multiple sources for information on *S. litura* in New Zealand. Online

searches were conducted using the citation databases Google Scholar and Scopus® for published information, the search engine Google for mention of *S. litura* in online news articles and industry outputs, and within the social media app, Instagram.

Ministry of Agriculture and Fisheries (MAF) Research Division Reports from 1965 to 1986, MAF Agricultural Research in Progress reports from 1981 to 1986 and a selection of MAF Technical Reports held in the AgResearch Ruakura library were inspected.

I conducted an unfiltered search for '*Spodoptera litura'* on the citizen science platform iNaturalistNZ and inspected these over several months in 2024–25. Search results included all life stages (eggs, larvae, adults) and observations with tentative ('needs ID') or corroborated ('research grade') species identification. Monthly totals of the number of observations were obtained from iNaturalistNZ 'History' charts for *S. litura*, Noctuidae (the family that *S. litura* belongs to) and Lepidoptera (all moths and butterflies). Noctuid observation data from 2024 were exported directly from iNaturalist on 18 March 2025 for any life stage and any quality grade ('research grade' and 'needs ID') in New Zealand between 1 January 2024 and 31 December 2024. These data included the proposed species identification, and the date and location of each observation.

I downloaded records of *S. litura* in New Zealand from the data aggregator, GBIF, via rgbif in R (https://github.com/ropensci/rgbif) on 12 March 2025 (GBIF Occurrence Download https://doi.org/10.15468/dl.q4kn9r on 2025-03-12, download link https://api.gbif.org/v1/occurence/download/request/0003617-250310093411724.zip).

In addition to visual examination of the number of observations over time I conducted a simple statistical analysis to account for temporal differences in observer effort. I calculated the proportion of noctuid and Lepidoptera observations each month that were *S. litura* and analysed these separately using Binomial General Linear Models using a logit link (R package glmmTMB; Brooks et al., 2017). The models contained 'year' representing a long-term trend and 'month' representing a seasonal component. The first 4 years of data were excluded from the final analysis because there were very few *S. litura* observations from 2014 to 2017 and their inclusion resulted in complete separation errors. Post-hoc pairwise comparisons with Bonferroni-adjusted *p*-values were conducted using R package emmeans (Lenth et al., 2022).

iNaturalist observations are usually accompanied by latitudinal and longitudinal data describing where the specimen was observed. I used these data to examine potential geographical bias in reporting by visually comparing observations of Noctuidae, and the subset of these that were *S. litura,* from mainland New Zealand in 2024. I grouped the observations by location using regions defined by Crosby et al. (1998). These 'Crosby regions' are of

approximately equal area and are used for specimens in the New Zealand Arthropod Collection and its associated databases and publications. Maps showing the locations of individual *S. litura* observations reported in iNaturalist each year from 1 January 2015 and 31 December 2024 were examined for evidence of changes in distribution over time.

All images associated with the 185 observations from iNaturalistNZ in 2024 with a suggested species identification of *S. litura* were inspected and classified by image quality and certainty of identification by the primary author (J.G. Jensen). Images and descriptions from EPPO (2015) and Brambila (2013) were used to guide species identification. Key morphological characteristics are shown in Figure 2-1. *Spodoptera litura* eggs can be tentatively identified based on features such as the typical noctuid ribbing and the covering of hairs from the female's abdomen, but molecular methods are required for reliable identification (EPPO, 2015). Likewise, very young larvae cannot be accurately identified without molecular methods. Host plant was often not reported or present in the image, so was not included in this study.

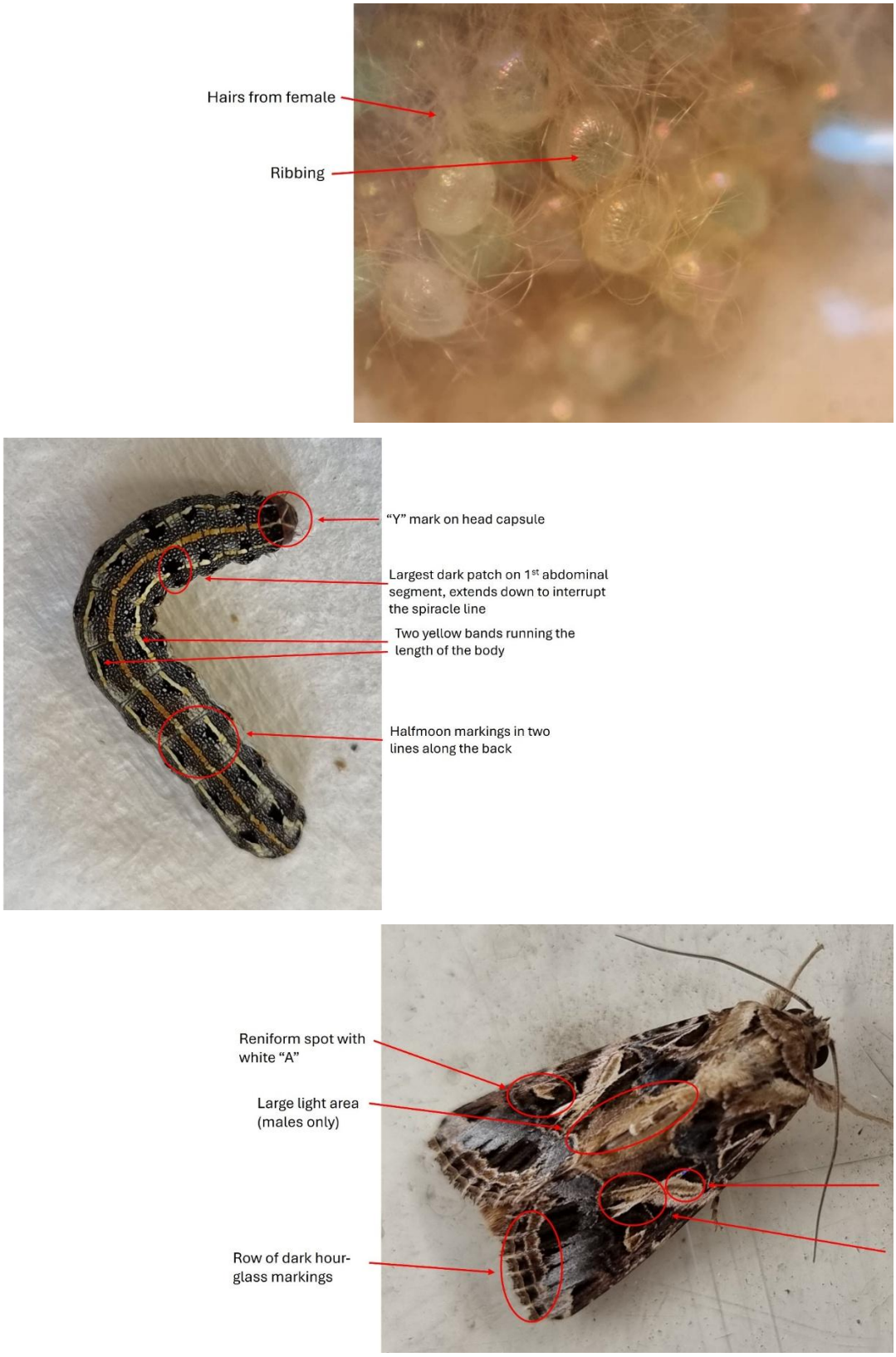

Figure 2-1 – Egg mass, larva and adult Spodoptera litura *with key characteristics useful for species identification.*

2.4. Study findings

2.4.1. Published *S. litura* population information

Most of the information published to date on *S. litura* in New Zealand is from before 1980 and comprises brief observations describing the insect's arrival and establishment. The first breeding population of *S. litura* was noted in New Zealand in March 1955 in the Nelson District (Cottier & Gourlay, 1955). The population was eradicated by the farmer using 'vigorous measures with DDT' and for almost the next 20 years the species was noted only occasionally as a migrant species, with captured adults being assumed to have blown over from Australia (Fox, 1973b). An annual series of papers by Dr K. J. Fox in the New Zealand Entomologist described the subsequent establishment of *S. litura* in New Zealand in the mid-1970s. Three specimens (listed under its former genus name, *Prodenia litura*) were recorded in March 1970 from Farewell Spit and Waimangaroa (Fox, 1970) and a further two specimens were listed for each of the following two years (Fox, 1971, 1973a). In 1973, Fox (1973b) noted that 'the tropical army worm and serious pest, *Spodoptera litura,* was rather alarmingly common during [mid-March through to early May]', but it was still considered a migrant rather than an established pest. By 1975 *S. litura* was noted to be breeding in the Auckland area (Fox, 1975) and in 1976 to be a major pest from Taranaki northwards. Dale et al. (1976) and Boardman (1977) likewise described the species as causing serious damage to pastures, crops such as *Medicago sativa* (lucerne) and *Brassica napus* (rape) and home gardens in Northland and Auckland, and less serious damage in Taranaki and Levin.

Subsequent published reports of *S. litura* in New Zealand are sparse. Gerard et al. (2011) briefly described an outbreak in the Bay of Plenty in the autumn of 2011. Widespread, serious damage was observed in *M. sativa* crops and *Trifolium* spp., *Plantago* spp. and *Cichorium intybus* (chicory) in pastures, with larval numbers reaching as high as 100/m^2. A mild and wet winter in 2010 followed by above average spring and summer temperatures and near record summer rainfall were thought to have allowed for the completion of four generations and thus for populations to build up to outbreak levels. The authors noted that they expected these weather patterns to occur more often with climate change.

2.4.2. Unpublished population information

Historical MAF reports contain a wealth of information about the research MAF conducted, together with the challenges faced by the primary industries and their proposed solutions. Reports from 1965 to 1986 were inspected for mention of *S. litura* but none were found. 'Armyworm' infestation areas, damage and control costs were reported in a comprehensive survey of New Zealand pests conducted by MAF in 1979 (Schofield, 1979) but this was probably

either *Persectania aversa* (southern armyworm) or *Mythimna* (*Pseudaletia) separata* (northern or common armyworm). The absence of *S. litura* from these reports indicates that it was not causing enough damage to warrant further study at that time.

Anecdotal information about insect pests can occasionally be gleaned from news and social media, personal observations, and conversations with industry partners (e.g. DairyNZ, PGGWrightsons staff), farmers and the general public. My online search yielded only two news articles (Stuff, 2022, 2023) and one Instagram post from May 2022, all describing large numbers of *S. litura* at that time. These reports corroborated observations of *S. litura* in plantain crops at Ōhaupō and *Ipomoea batatas* (kumara) crops in Ruawai reported to the primary author (J.G. Jensen) in summer/autumn 2023. The author also observed large numbers of larvae in Ruakura, Hamilton pastures that year.

2.4.3. iNaturalist/GBIF data

GBIF data can include observations from multiple sources but in the case of *S. litura* only 59 of the 721 records from New Zealand until 12 March 2025 were from sources other than iNaturalist. Other sources included the Auckland Museum Entomology Collection, Lepidoptera Observations from New Zealand by Donald Hobern and the New Zealand Arthropod Collection. Only 24 *S. litura* observations were recorded in GBIF and only one in iNaturalist before 2014; therefore, only data from 2014 onwards is shown.

2.4.3.1. *Spodoptera litura* seasonal and annual abundance

Seasonal peaks in observation numbers occurred each year in April (Figure 2-2A). The annual number of observations appeared to increase in 2018 and again in 2022.

The iNaturalist platform has become more widely known and more popular with time, leading to an increase in the number of users and/or more observations per user. This can be seen from a visual comparison of monthly noctuid and Lepidoptera observations reported on iNaturalistNZ (Figure 2-2B, C). Both increased over the 2014-2024 period, suggesting an increase in observer 'effort'. The apparent increase in the number of *S. litura* observations (Figure 2-2A) must therefore be interpreted carefully to account for these differences in overall observer effort. A simple way to do this is to calculate the number of *S. litura* observations in proportion to the total number of observations, as iNaturalist does. I refined this calculation slightly by comparing the proportion of *S. litura* observations to observations for the family (Noctuidae) and order (Lepidoptera). Analysis of these proportions showed that there were statistically significant effects of both month and year on the proportion of all Lepidoptera observations that were *S. litura* (X^2_{11} = 606.7, p<0.001 and X^2_6 = 162.9, p<0.001 for month and year, respectively) and the

proportion of all noctuid observations that were *S. litura* (X^2_{11} = 559.7, p<0.001 and X^2_6 = 167.7, p<0.001 for month and year, respectively). The effect of month on the number of observations is to be expected for a seasonally active insect like *S. litura* as seasonal peaks in population numbers can occur at different times for different lepidopteran species. When considered in relation to the numbers of overall Lepidoptera (Figure 2-3A) and noctuid (Figure 2-3B) observations per year, higher proportions of *S. litura* were reported in 2018 and in 2022-23, indicating that these were likely to have been genuine outbreak years in which *S. litura* numbers were higher than in non-outbreak years. Further monitoring is required to determine if there is also an underlying trend upwards that has been masked by the outbreak.

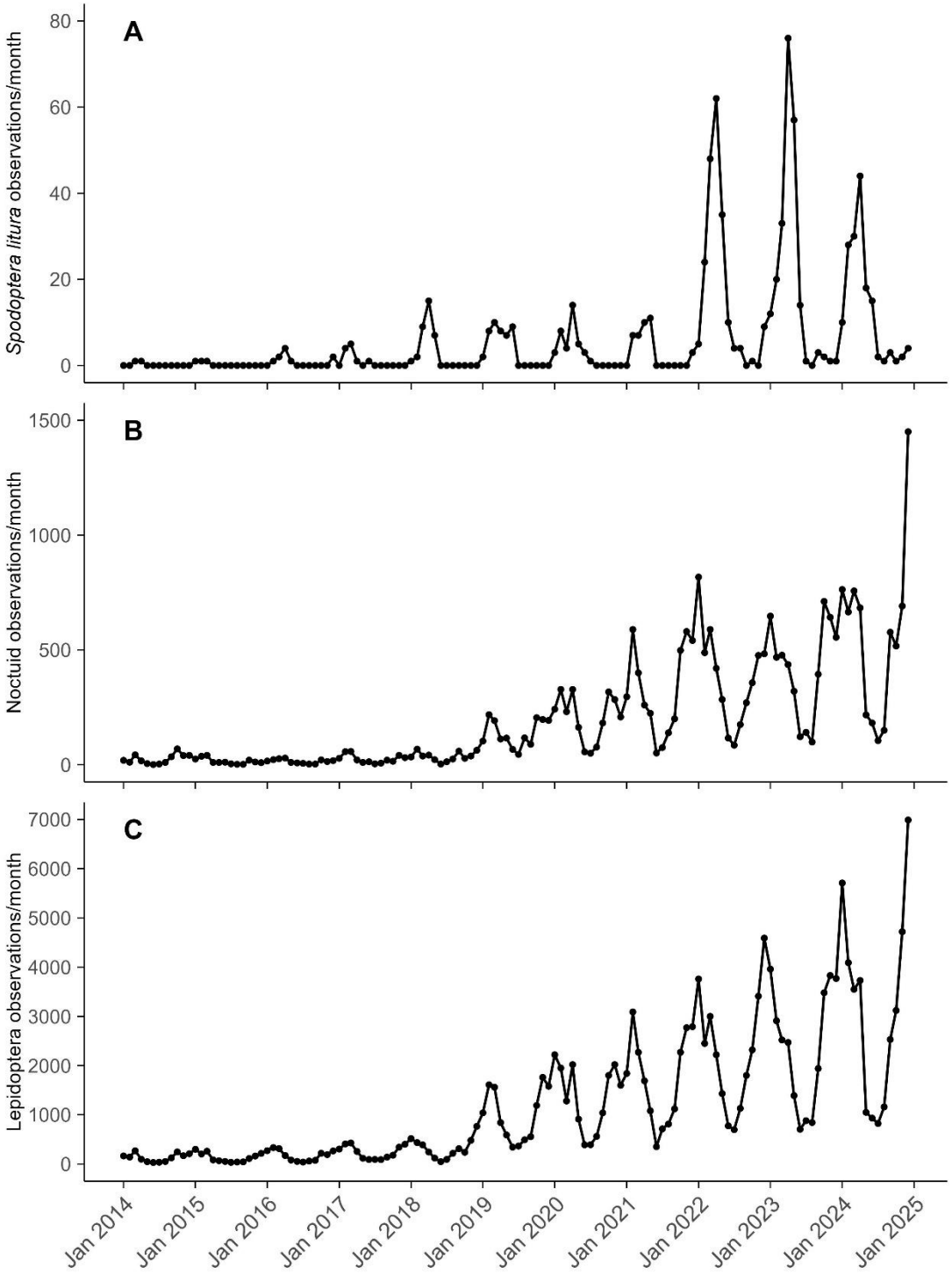

Figure 2-2 – The number of research grade observations reported for New Zealand in iNaturalistNZ each month from January 2014 to December 2024 for (A) Spodoptera litura*, (B) Noctuidae and (C) Lepidoptera. Observations are research grade when two-thirds of identifiers agree on the species identification.*

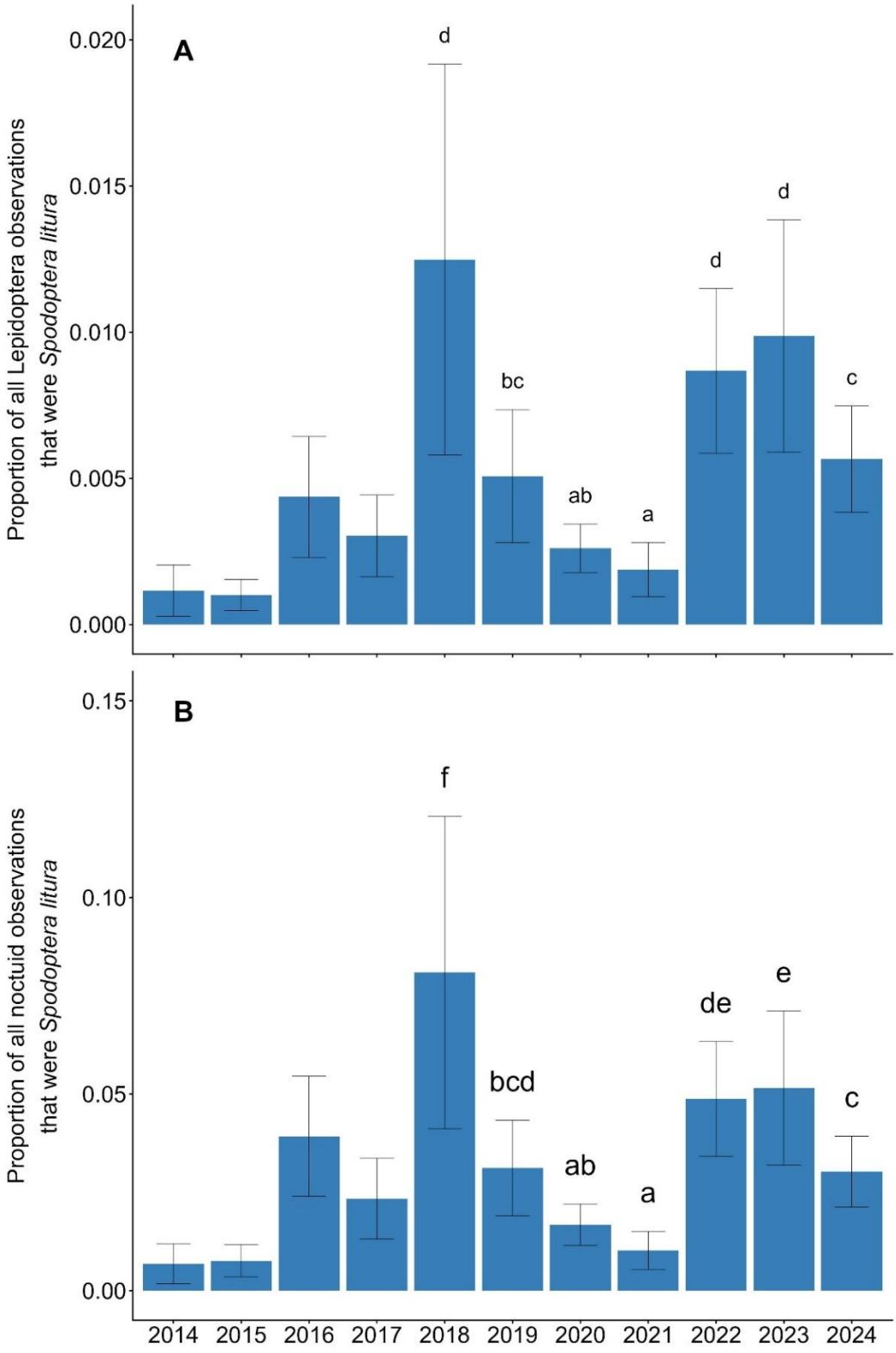

Figure 2-3 – Annual means of research grade Spodoptera litura *observations reported each month in iNaturalistNZ for mainland New Zealand from January 2014 to December 2024 as a proportion of (A) Lepidoptera and (B) Noctuidae observations. Observations are research grade when two-thirds of identifiers agree on the species identification. Error bars are ± one standard error of the mean. Observations from 2014 to 2017 were omitted from the statistical analysis model due to low observation numbers. Means with different letters are significantly different ($p < 0.05$).*

2.4.3.2. *Spodoptera litura* geographical range

Most of the *S. litura* observations reported in iNaturalistNZ/GBIF are from the northern North Island – 670 of the 894 iNaturalist observations to the end of 2024 were from Auckland, Northland or the Far North – although there were occasional reports from the South Island, including two adults seen as far south as Doubtful Sound in early 2022. My simple comparison of *S. litura* and Noctuidae observation locations (Figure 2-4) showed that, in contrast to *S. litura*, noctuid reports in 2024 were spread reasonably evenly across the country. The potential for bias in user contributions in the iNaturalistNZ data was evident, however, in the large number of observations from Dunedin, which were due mainly to one user who contributed 988 of the 1,787 noctuid observations in that region.

Visual comparison between years of the location of each *S. litura* observation (Figure 2-5) indicated that higher numbers of observations south of Auckland may have coincided with the 'outbreak years' of 2018 and 2022-23.

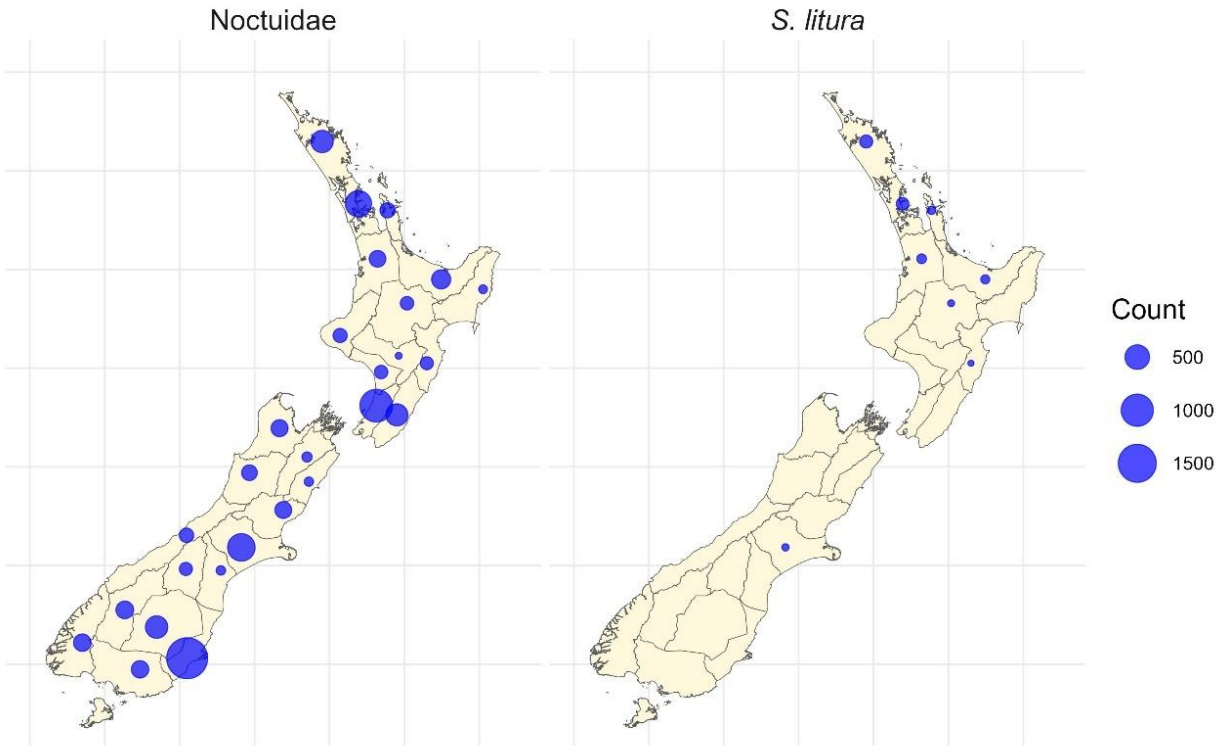

Figure 2-4 – Total number of recorded observations of Noctuidae and Spodoptera litura *per 'Crosby region' in iNaturalistNZ in 2024. The size of each point is proportional to the number of observations in that region.*

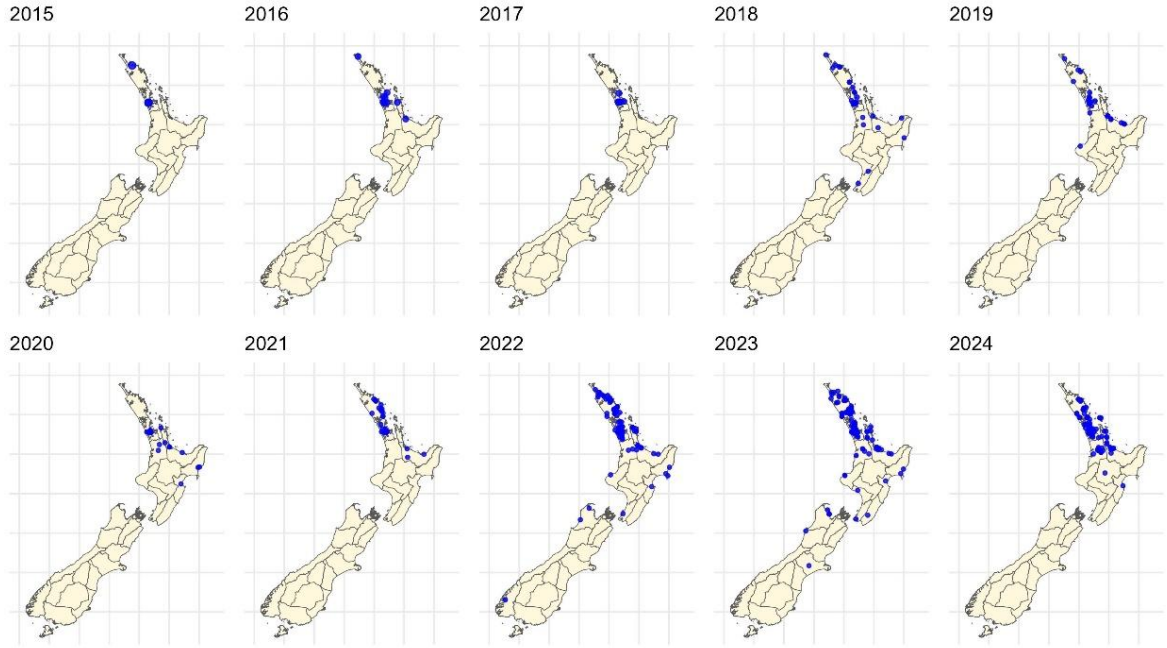

Figure 2-5 – Locations of individual Spodoptera litura *observations in iNaturalistNZ per year.*

2.4.3.3. Image diagnostic quality

Three of the 185 *S. litura* observations reported for New Zealand in 2024 were of eggs, 74 of larvae (including seven of eggs with newly hatching larvae) and 107 were adults. The remaining specimen could not be identified and may not have been an insect at all. Most (113) of the observations had only one species identification suggestion from a user who was not the original observer – 91 of these were 'research grade'. Through inspection of the images associated with each observation, and looking for identifying features such as those shown in Figure 2-1, I determined that most of the observations (153) were 'definitely' or 'probably' *S. litura*. Only 34% of the observations were accompanied by 'good' images that clearly showed distinguishing features. I did not consider species identification to be possible for fourteen (8.8%) of the 'research grade' observations. One poor quality 'needs ID' observation suggested by the observer as *S. litura* was most likely *S. frugiperda* so this is not included in Table 2-1. In iNaturalist, users may add comments when they contribute an identification. Two comments of note that accompanied suggested identifications observations were 'probably, but hard to be sure without

an image of the pattern on its back', and 'I have little idea about identifying larvae' (accompanying a research grade larval observation for an image that this author considered too poor to identify accurately).

Table 2-1 – Quality rating of images, and certainty of specimen identification as assessed by J.G. Jensen for all 184 Spodoptera litura *observations from iNaturalistNZ in 2024.*

Image quality	**iNaturalist observation grade**	**Certainty of identification**			
		Definitely	Probably	Possibly	Can't id
Poor	Research	25	13	5	12
	Needs ID	4	5	0	6
Ok	Research	47	1	3	2
	Needs ID	3	3	1	2
Good	Research	49	1	0	0
	Needs ID	2	0	0	0
Total		130	23	9	22
Percentage		70.7%	12.5%	4.9%	12.0%

2.5. Discussion

My search for published and unpublished work on *S. litura* populations in New Zealand generated sparse information with little detail. The most comprehensive source of data was through the online platforms, iNaturalist and GBIF. Citizen science projects such as iNaturalist offer a continuous, low-cost, large-scale method for collecting data compared with traditional active surveillance techniques (Cull, 2021). These types of data are freely available worldwide, and with new ways of exploring large data sets its value is clear. My example of *S. litura* in New Zealand demonstrates the potential usefulness of these data for population monitoring of species for which other sources of data are scarce. It also highlights the need for care to be taken with its analysis and interpretation.

Sampling and reporting in citizen science data is often uneven over time and space (Boakes et al., 2016). More observations may be recorded from densely populated areas, from sites that are easy or enjoyable to access, or from organised survey events such as BioBlitz (https://www.landcareresearch.co.nz/tools-and-resources/education/bioblitz/) or the City Nature Challenge (https://inaturalist.nz/projects/city-nature-challenge-2025-bend). There can also be a wide variation in effort between observers. This was demonstrated by my examination

of noctuid data from Dunedin in 2024, where over 55% of the observations were reported by a single iNaturalist user. Overall contributions to iNaturalist have grown exponentially over the past decade (Di Cecco et al., 2021) and this temporal trend must be considered when analysing population dynamics. A significant strength of iNaturalist, however, is that observations of any life form can be reported. Statistical models can thus incorporate observation data for other species or higher taxonomic groups to account for uneven sampling. Furthermore, these data can be used to augment data from other sources when multiple sources are integrated to provide a more cohesive picture, such as in Bayesian methods (Gelman et al., 2013). I have used very simple analyses and visual assessments to demonstrate potential observer bias, but for comprehensive studies more in-depth analyses that model the data collection process and appropriately control for biases should be used (e.g. Isaac et al., 2014).

Some species or life stages are easier to find, photograph or identify than others, and some are of more interest to individual observers. This interest may be linked to the 'charisma' or novelty of specific species, or may be increased in response to high profile surveys or media coverage of invasive species (Boakes et al., 2016). Both adult and larval *S. litura* are relatively large and striking, are found in easily accessible locations and the larvae cause significant damage to crops and home gardens. These factors make the species more likely to be noticed and reported on and therefore a good candidate for study through citizen science platforms.

Publicity and industry (e.g. Ministry for Primary Industries, Foundation for Arable Research) responses following the discovery of the related noctuid, *Spodoptera frugiperda* (fall armyworm), in February 2022 may have led to an increase in reported observations of *S. litura*, through an increased public awareness of these pests. Increased reporting can be positive or negative depending on the intended use of the data. Drury et al. (2019) used citizen science data to investigate phenotype variation in three species of damselfly. They were able to improve their outreach through active recruitment strategies, resulting in a larger data set for studying wing pigmentation. In contrast, when looking for differences in population dynamics over time, such as for this sleeper pest study, a change in outreach effort during the study period can lead to a bias in sampling effort that can skew the results and must be accounted for in any analysis.

As with the analysis of species abundance over time, we need to be cautious in our interpretation of a species' geographical distribution from citizen science data. It is important to recognise that a lack of observations does not necessarily mean an absence of the species at that time/location (Gerard et al., 2024). Other site sampling methods like trapping, or mark and recapture are more definitive but do not preclude a failure to detect an organism when it is there either. A well-designed study, however, ensures that sampling effort is distributed evenly, and

sampling effort can be accurately judged. Geographically widespread reporting of Lepidoptera and Noctuidae in iNaturalistNZ gives us some confidence that users who are likely to be interested and motivated to report *S. litura* are active across all regions of New Zealand. There were 8,519 observers reporting Lepidoptera across New Zealand and 488 of these detected *S. litura*. We can therefore tentatively infer that the species is usually either absent, vagrant or in very low numbers in the South Island rather than simply not being found or reported.

A significant advantage of the iNaturalist platform is the association of each record with an image that allows researchers the opportunity to check the assigned species identification. The species identification of a 'research grade' observation must have the agreement of at least two-thirds of the identifiers. Many identifications are the work of a few very active users. These identifiers have extensive expertise and diagnostic experience, and their contribution is invaluable. However, any user may suggest, agree or disagree with a species identification – no qualifications are required and one of the identifications required for 'research grade' observations may be (and often is) provided by the original poster. In 2017, iNaturalist added computer vision image recognition to aid in species identification. This can be a useful tool but may result in under-qualified identifiers agreeing with incorrect identification suggestions. Thus, although a specimen might be tagged 'research grade', in practice identifications may be unreliable. Photos may be blurry or not show the key identification features required for that species or life stage, and many species cannot be accurately identified based on external morphology alone (a problem for any study or monitoring effort). For small or cryptic species, identification based on hand-held cameras is particularly difficult.

Identification can be based on the appearance of the specimen, where and when it was found and, for herbivorous species, what plant it was associated with. Identifiers will also consider what other similar looking species are known to occur in New Zealand. Before 2022, to a reasonably trained eye, species identification from photographs of *S. litura* in New Zealand seemed relatively easy as there were no known similar looking species in the country. The arrival of the related and similar-looking species, *S. frugiperda,* has made accurate identification more difficult. It is also possible that *S. frugiperda* was present earlier than recognised and was being misidentified as *S. litura* or that other, similar-looking species such as *S. littoralis*, could be present without our knowledge.

The number of *S. litura* observations reported in the citizen science databases iNaturalistNZ and GBIF were higher from 2022 than in previous years. Analysis of these observations in proportion to observations of Lepidoptera and Noctuidae, however, indicated that while the apparent increase in numbers in 2022 and 2023 was probably 'real', it was in part

skewed by a temporal increase in observer effort. The analysis also highlighted an earlier potential outbreak year in 2018. I was not able to account for all the potential variation and bias in the data, so these results can only be considered indicative. However, news and social media records from 2022-23, as well as personal observations/communications appear to corroborate my results for that period. Continued monitoring through tools such as iNaturalist will help us determine if this was a 'real' but temporary increase due to favourable climatic conditions in those years, or part of a trend upwards for a potentially wakening sleeper pest.

Winter temperatures were the 'warmest on record' for the three years from 2020 to 2022 (NIWA, 2000–2025), and the 2022-23 summer was exceptionally wet for the North Island. Summer rainfall in 2021-22, however, was below normal for Northland, Auckland and parts of Waikato (NIWA, 2000–2025). Gerard et al. (2011) speculated that a mild, wet winter and warm spring and summer led to the *S. litura* outbreak in the Bay of Plenty in 2011, while Kean et al. (2015) and Peacock et al. (2006) considered warmer summer and autumn temperatures to be key, as they allow for additional generations to develop. More accurate presence/absence data and more sophisticated modelling is needed for proper examination of any correlation with changes in climate for this species.

Increasing public awareness through outreach initiatives, recruitment of observers, education on species identification and increased contributions from subject experts and taxonomists can all help improve engagement with, and accuracy of, citizen science platforms such as iNaturalist (Drury et al., 2019; Hochmair et al., 2020). This should ideally be accompanied by statistically sound validation of the data through carefully designed and conducted studies such as physical survey/s of the species of interest. Unfortunately, for many researchers, this level of effort and cost is out of reach but with careful analysis that accounts for all variation, e.g. the number and location of users and uneven recording intensity, citizen science data can make a valuable contribution to studies such as mine.

2.6. Conclusions

In the absence of sufficient resources to conduct population surveys at multiple sites and across multiple years, my study has shown that useful indicators of population size and geographical spread can be inferred from iNaturalist/GBIF data, with the caveat that researchers must conduct quality assessments and care should be taken with the analysis and interpretation. Populations of *S. litura* in New Zealand appear to have been larger during the summer-autumn periods of 2018, 2022 and 2023; however, further monitoring is required to determine if there is an overall trend upwards, and if its distribution is shifting southwards. This information would provide a useful warning tool for farmers under changing climate conditions.

2.7. Acknowledgements

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2.8. Authorship contributions statement:

Joanne Jensen: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing-original draft; review and editing (lead). **Michael Clearwater**: Supervision (equal); writing – original draft; review and editing (supporting). **Andrew Barnes**: Supervision (equal); writing – original draft; review and editing (supporting). **Sofia Orre-Gordon**: Funding acquisition (lead); supervision (equal); writing – original draft; review and editing (supporting).

3. Forage plant host and early feeding choices affect *Spodoptera litura* feeding, development and egg-laying

This chapter has been published in the journal Entomologia Experimentalis et Applicata (2026) (https://doi.org/10.1111/eea.70097) and reformatted to fit the thesis structure. The chapter addresses the hypothesis that *S. litura* will have the potential to cause considerable damage to New Zealand pastures and pasture crops through feeding on broadleaf pasture plants, such as plantain and clover, but that some plant host species will be higher quality than others. Feeding on non-preferred host plants will affect *S. litura* behaviour, growth and reproduction.

Entomologia Experimentalis et Applicata
Experimental and Applied Entomology

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Forage Plant Host and Early Feeding Choices Affect *Spodoptera litura* Feeding, Development and Egg-Laying

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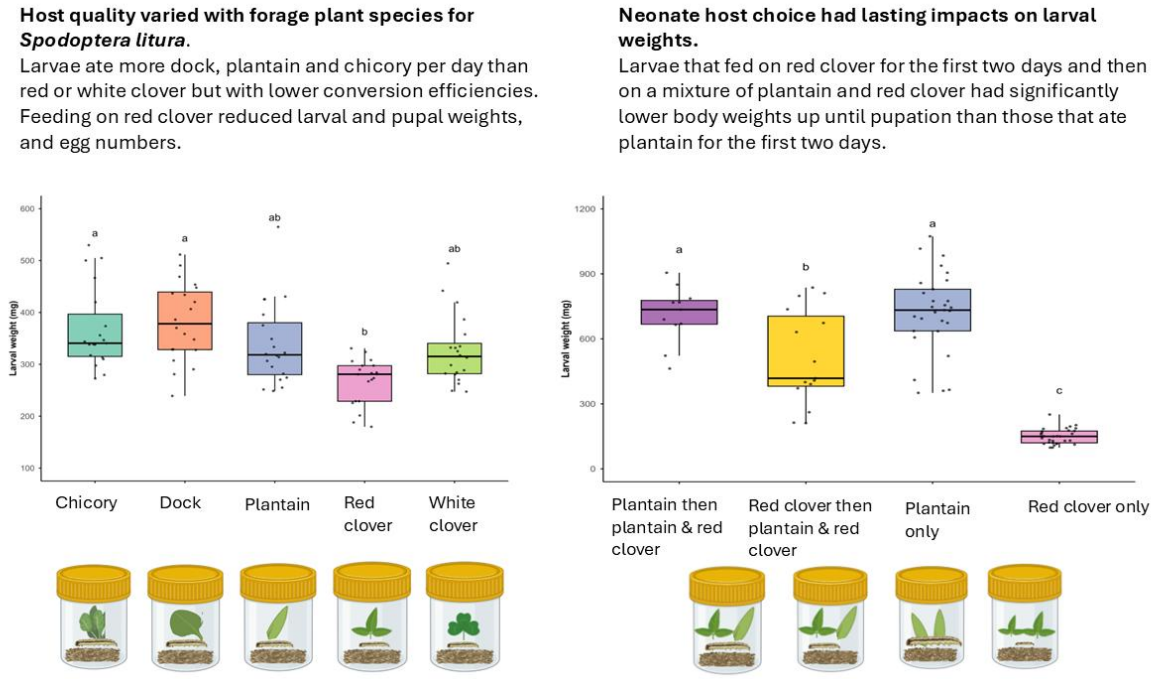
Keywords: agriculture | climate change adaptation | host plant quality | pasture | sleeper pest | tropical armyworm

3.1. Graphical abstract



3.2. Abstract

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a significant pest across tropical and temperate regions. Although present in New Zealand since the 1970s its range and impact are expected to grow with climate change. However, little is known about its distribution or host preferences in New Zealand, particularly regarding pasture plant species.

I conducted laboratory experiments to assess *S. litura* feeding, development, and reproduction on forage species that are increasingly favoured for pastures due to their stress tolerance, nitrogen-fixation, or leaching mitigation abilities. The forage species tested were plantain (*Plantago lanceolata* L.), chicory (*Cichorium intybus* L.), red clover (*Trifolium pratense* L.), and white clover (*T. repens* L.). The common weed species, dock (*Rumex obtusifolius* L.), was used as a positive control. Larvae were fed with cut leaves from one of the plant species to compare host quality. A second trial compared plantain, red clover, and both together. Leaf consumption, growth, and egg-laying were monitored. Larvae consumed more dock, plantain, and chicory per day than clovers, but with lower conversion efficiencies. Larvae reared on red clover from newly hatched took an average of four days longer to pupate, weighed 33% less at pupation and laid 92% fewer eggs than those fed plantain. When given a choice, neonates initially

settled equally on plantain and red clover. However, early feeding on red clover resulted in persistently lower larval weights, even after switching to better hosts. These effects did not carry through to pupation. These findings highlight the importance of host plant quality for insect herbivore performance. Identification of high- and low-quality host plants is a key step in mitigating the risks posed by this invasive and damaging pest. These results have important implications for pasture species selection and breeding under future climates.

Keywords: agriculture, climate change adaptation, host plant quality, pasture, sleeper pest, tropical armyworm

3.3. Introduction

Agricultural systems are highly susceptible to climate variability. Addressing the risks associated with climate change is becoming increasingly urgent (Howden et al., 2007). Rising global temperatures and the growing frequency and severity of extreme climate events, such as droughts, floods and heatwaves, are expected to directly and indirectly impact fitness variables such as the survival or distribution of some plant species, the insects that depend on them, and their natural enemies (Mansfield et al., 2021; Sharma & Dhillon, 2020). Predicting and understanding these changes are crucial steps towards ensuring the resilience and long-term sustainability of agriculture worldwide.

Pastures in New Zealand are traditionally based on ryegrasses, *Lolium perenne*, *L. multiflorum* and *L. perenne* × *L. multiflorum,* and clovers, *Trifolium repens* and *T. pratense* (Ferguson et al., 2019), but changing environmental conditions are impacting their performance and resilience (Chapman, 2021). Farmers are increasingly looking to diversify or adapt their pastures to maintain productivity and quality during summer and autumn, improve pasture sustainability, and future-proof against climate change-related stresses (Caradus et al., 2021). In particular, drought- and heat-tolerant plant species are becoming more common (Stewart et al., 2022).

In addition to changes in plant composition, climate change will impact herbivorous insect populations, both in direct response to changes in regional climate conditions and indirectly, through different host plant availabilities. Some species that are currently not considered to be ‘pests’ because of their restricted geographical ranges, low population sizes and/or damage levels will be increasingly important as conditions become more favourable for their growth and survival. These species are referred to as ‘sleeper pests’ (Frank & Just, 2020;

Mansfield et al., 2021). One species identified as a potential sleeper pest in New Zealand is *Spodoptera litura* (Lepidoptera: Noctuidae) (Kean et al., 2015; Orre-Gordon & Jensen, 2025).

Spodoptera litura is one of the most important pests of agricultural crops in the Asian tropics (CABI, 2014). The species is broadly distributed in tropical and temperate regions throughout South and East Asia and Oceania (Bragard et al., 2019; Fand et al., 2015). Temperatures in many regions of New Zealand are marginal for survival, thus while the species was first noted in New Zealand in 1955 (Cottier & Gourlay, 1955), it did not establish overwintering populations until the mid-1970s (Dale et al., 1976; Fox, 1970) and it continues to be a sporadic pest. Outbreak-level populations appear to occur after mild winters and warm spring/summer seasons with high rainfall (Gerard et al., 2011). These conditions are likely to occur more frequently in New Zealand in the future, as winters become milder and summer and autumn temperatures increase (Kean et al., 2015). By inference, with climate warming *S. litura* is likely to extend its range globally into previously marginal regions.

Larval *S. litura* are highly polyphagous, feeding on hundreds of plant species in at least 40 plant families (Bragard et al., 2019). Many of these host species are economically important, for example *Gossypium* spp. (cotton), *Nicotiana tabacum* (tobacco), *Glycine max* (soybean), *Arachis hypogea* (groundnut), *Oryza sativa* (rice), *Brassica* spp., *Zea mays* (maize), and *Camellia sinensis* (tea) (Bragard et al., 2019; Fand et al., 2015; Xia et al., 2020). Although well-studied globally, there are large gaps in our understanding of the distribution and host preferences of *S. litura* with respect to agricultural pasture plants. Many of the host pasture species reported for New Zealand are observational associations (Berry & Walker, 2004; Chapman, 1984; Dale et al., 1976; Gerard et al., 2011). Lindroth et al. (2000) reared *S. litura* on two white clover (*T. repens*) cultivars in a study looking at the effects of UVB on clover and Huang et al. (2008) showed that *S. litura* can successfully develop through to adulthood on both white and red (*T. pratense*) clover. Sushilkumar and Ray (2018) found that chicory (*Cichorium intybus*) and broad-leaved dock (*Rumex obtusifolius*) were 'preferred' hosts based on visual feeding scores and that *S. litura* could be reared through to adulthood on these species (Sushilkumar & Ray, 2007). *Spodoptera litura* is therefore potentially capable of causing considerable damage to New Zealand pastures and pasture crops through feeding on commonly occurring broadleaf plant species.

Although many forage species are likely to be vulnerable to this pest, I hypothesised that some plant species or cultivars would be better hosts than others. This will affect feeding choices, and growth and development parameters of *S. litura*. Identification of vulnerable or resistant host forages will provide valuable information for farmers and plant breeders and help future-proof pastures against this sleeper pest. Chicory, narrowleaf plantain (*Plantago*

lanceolata), red and white clover, and dock were chosen as test plant species in this study because of their prevalence in New Zealand pastures. White clover is the most commonly sown perennial forage legume and is typically planted alongside perennial ryegrass (*Lolium perenne* L.). It plays an important role in maintaining high feed quality in late spring and summer and in nitrogen fixation (Stewart et al., 2022). Chicory, plantain and red clover are becoming increasingly important in response to climate change because of their drought and heat tolerances (Kean et al., 2015; Stewart et al., 2022) and because chicory and plantain have high herbage yields and nutritive values (Minnee et al., 2017). Chicory and plantain may also mitigate nitrogen leaching (Stewart et al., 2022). Broad-leaved dock is a weed species commonly found in pastures throughout New Zealand (Popay et al., 2010). *Spodoptera litura* is known to do well on this host (Sushilkumar & Ray, 2007), therefore it was included in my testing as a positive control.

Here I present the results of two laboratory experiments. In the first experiment (Host Quality) I assessed the host suitability and quality of the five forage plant species. In the second experiment (Host Choice), I reared *S. litura* from neonate to adulthood either on a high (plantain) or low (red clover) quality host plant species, as identified in the Host Quality Experiment, or on both plantain and red clover. This allowed me to assess feeding choices and the effects of diet mixing on development and egg-laying.

3.4. Methods

3.4.1. *Spodoptera litura* rearing

Adult *S. litura* were captured by light trap in late summer at Ruakura Research Centre, Hamilton, New Zealand. Adults were placed in large, clear Perspex containers and supplied with tap water via an absorbent cotton dental roll or damp, 2-ply facial tissue (Tork Premium) and with crumpled paper towels (TORK Xpress hand towel multifold) for refugia and egg-laying surfaces. On hatching, larvae were transferred to 5 L plastic containers with 2 cm depth of damp vermiculite (Exfoliators (Aust) Pty Ltd, Premium Grade 4, 3-6 mm) on the base. Container lids had fabric mesh inserts to allow air circulation. The containers were placed in open-ended plastic bags to maintain humidity. Larvae were fed ad libitum: a mixed diet of artificial diet modified from Singh and Moore (1985) supplied by Plant and Food Research Ltd. combined with fresh dock, plantain, chicory and white clover leaves.

Larvae used for the Host Quality Experiment were third-generation laboratory-reared. The colony was initially kept under natural light and temperature conditions in a laboratory, but second-generation adults were transferred to an incubator at 25°C with 16:8 h light:dark to

ensure consistent egg laying. The larvae used for the Host Choice Experiment were offspring of wild-caught moths and were placed in a 25°C incubator with a 16:8 h light:dark cycle on hatching.

3.4.2. Plant material

Narrowleaf plantain (cultivar Ecotain), chicory (cultivar Grasslands Puna), red clover (cultivar Relish) and white clover (cultivar Grasslands Kopu II) seeds were obtained from the Margot Forde Germplasm Centre, Palmerston North, New Zealand. Broad-leaved dock seed was collected from pasture at Ruakura Research Centre. Seeds were sown directly into individual 0.7 L plastic pots (11 cm W x 10 cm H) in potting mix (Dalton's® grass and clover mix). The experiments were run between February 2022 and March 2023 at Ruakura Research Centre New Zealand (-37° 46' 24.28171", 175° 18' 24.78687"). During this period, plants were kept under semi-natural conditions in a screen house to better reflect the conditions of plants in the field. An onsite weather station (Station: Hamilton, Ruakura 2 EWS, agent #26117) recorded a mean temperature of 16.0°C (min. -2.3°C, max. 30.4°C) and bright sunshine hours between 130 h (June) and 244 h (March) per month. Average monthly humidity in Hamilton is 84.9% (78.3-91.1%) (NIWA, 2000–2025). Daylight hours varied from 9 h 33' in winter to 14 h 47' in summer (Timeanddate.com, 1995–2025). The plants were top watered every 2–3 days as required.

Plants for each of the five plant species treatments used for the Host Quality Experiment were kept in randomised positions within replicate blocks (randomised block design) on a screenhouse bench (Figure 3-1A). For the Host Choice Experiment, red clover and plantain plants were grouped randomly (complete randomised design) (Figure 3-1B).

Leaf segments were cut from the plants as required for feeding to *S. litura* larvae.

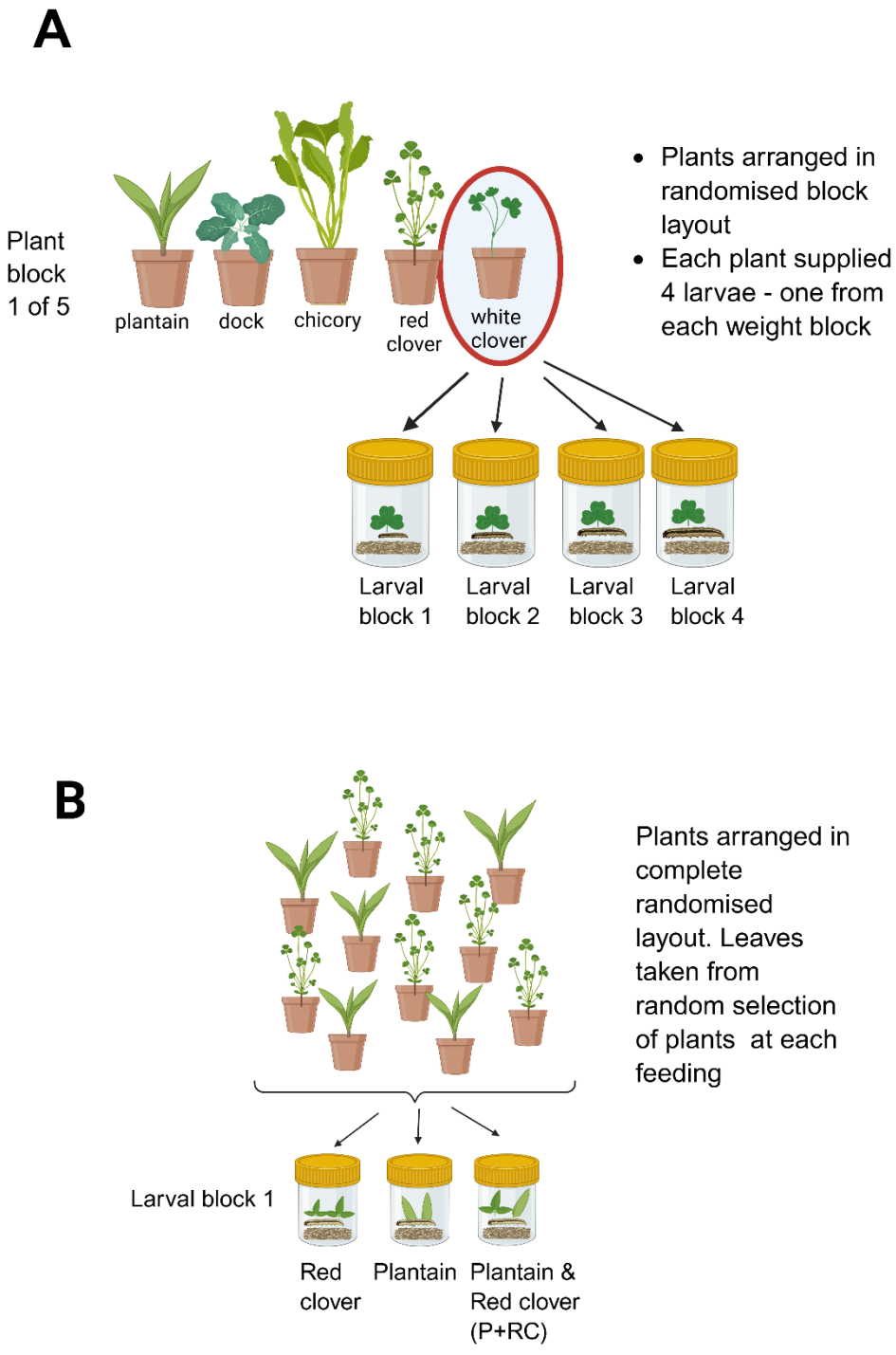


Figure 3-1 – Experimental setup for (A) the Host Quality Experiment in which Spodoptera litura *larvae were fed foliage from one of five forage plant species (total number replicate larvae per treatment = 20) and (B) the Host Choice Experiment where 30 replicate larvae per treatment were fed either red clover leaves, plantain leaves or both. Images were created in https://BioRender.com.*

3.4.3. Host Quality Experiment

I conducted a no-choice, laboratory experiment to determine if the five forage plant species were suitable hosts for *S. litura* and if they differed in host quality. Quality was assessed by comparing feeding, growth, survival and egg-laying of *S. litura* fed cut herbage from each of the plant host species.

3.4.3.1. Larval feeding and development

Newly hatched larvae were placed in rearing containers with damp vermiculite and fed an artificial diet ad libitum. All larvae had hatched on the same day. After 6 days, larvae were transferred to individual 30 mL clear plastic containers and starved overnight at 20°C. Damp paper towels were wrapped around the outside of the containers to maintain a high humidity environment, but free water was not provided to larvae during this period. The following day, 100 larvae were weighed and transferred to individual 120 mL clear plastic (polystyrene) specimen vials with 20 mL (approx. 1 cm depth) slightly damp vermiculite. Larvae were selected by size to restrict the weight range to between 5.5 and 12 mg. They were then ranked and split into four blocks by weight such that larvae in block 1 were the lightest and larvae in block 4 were the heaviest. Each of the larvae in a weight block was randomly assigned to be supplied with herbage from one of 25 plants (five replicate plants x five plant host treatments). Thus, the number of larvae per host plant treatment was 20 (four larval weight blocks x five replicate plants per treatment; Figure 3-1A). As larvae were in separate vials I was able to identify and monitor them individually throughout the experiment. The larvae were placed in polystyrene trays in a randomised block design and transferred to an incubator set at 25°C with 16:8 h light:dark.

Variables measured in the experiment are summarised in Table 3-1. Larvae were provided with fresh herbage from their respective plant treatment daily for 5 days. Any uneaten material was removed at the same time. Where possible, the larvae were fed leaf segments from the same plant for the entire experimental period. Spare plants from the original 20 sown per treatment were used as substitutes when a plant had insufficient leaf material, but in all cases the four larvae assigned to an individual plant were fed by the same plant at a given time. The quantity of leaves provided was estimated to be more than each larva would require for a 24-hour period, so that they would not run out of food, and to be similar between larvae. At each feeding time, an equivalent quantity of weighed leaf segments from each plant was placed in a specimen vial with vermiculite but without a larva to monitor moisture loss. These 'control' leaf segments were dried overnight in an oven at 55°C and dry weights used to calculate herbage moisture content.

After 5 days, (at approximately 4th instar of six instars for this species), the larvae were weighed, starved overnight to clear gut contents and reweighed the following day (Day 6). The

vermiculite was then removed from the vials and fresh leaf segments were provided. An equivalent sample of leaf material from each plant was collected and frozen at -20°C for later total nitrogen and total carbon analyses. Larvae, remnant leaf segments and frass were weighed after 24 hours (Day 7). Larva 4 from each plant/treatment group and frass from all larvae were freeze-dried and re-weighed. The freeze-dried larvae were stored for subsequent nitrogen and carbon analyses.

The remaining (live) larvae were returned to their empty vials to starve overnight again. Larvae were reweighed on Day 8, frass was collected and frozen, and larvae were returned to vials with fresh damp vermiculite and fresh leaf material. The larvae were then fed ad libitum until pupation with leaf segments from their plant species treatment. Directly after pupation, individuals were weighed, their sex was determined by visual examination under a stereomicroscope (10x magnification), and they were returned to their individual vials until they eclosed. The time (days) to pupation and adulthood was recorded.

Table 3-1 – Summary of variables measured for the Host Quality and the Host Choice Experiment with Spodoptera litura.

Experimental measurements	Host Quality Experiment	Host Choice Experiment
Number larvae per treatment	20	30
Larval age from which host plant treatments supplied	7 days	neonate
Larval weights	Days 5–8 (12–15-day old larvae)	Days 5,6, 8,9,12 & 13 (5–13-day old larvae)
Herbage fresh and remnant weights	Days 6 & 7	Days 5,6, 8, 9, 12, 13
Frass	Day 7, 8	Days 9, 13
Pupal weights	✓	✓
Days to pupa & adult	✓	✓
Survival	✓	✓
Egg-laying	✓	✓
Herbage moisture content	✓ (control)	✓ (remnant)
Plant & larval Total N & C	✓	X

3.4.3.2. Mating and egg-laying

On eclosion, adults were transferred to 750 mL plastic mating containers with damp tissue and crumpled paper towels for egg-laying and refugia and were paired as soon as an adult of the opposite sex from the same treatment was available. Six adult pairs were set up for each of the

dock, red clover and white clover treatments and five pairs for each of the plantain and chicory treatments. The containers were checked daily, and any eggs laid were counted.

3.4.3.3. Nitrogen and carbon analyses

Freeze-dried larvae and herbage samples were analysed for total carbon and nitrogen at Plant and Food Research Ltd., Lincoln, New Zealand. Plant samples were dried, ground, and then oven-dried overnight at 60°C. Larvae were left intact. Total carbon and nitrogen were determined on 0.1–0.2 g sub-samples by an automated dry combustion method (Dumas) using a Leco TruMac CN analyser (Leco Corporation, MI, USA). Samples were combusted in a pure oxygen atmosphere at 1,250°C. Excess water was removed using a thermoelectric cooler, and the gases were collected in a ballast chamber. A 10 mL subsample was cut and passed through a non-dispersive infrared detector to measure carbon, then scrubbed of carbon dioxide, water and oxygen and passed through a thermal conductivity detector to measure nitrogen.

3.4.4. Host Choice Experiment

Having identified plantain as a higher quality host than red clover for *S. litura*, I conducted a second laboratory experiment that compared larval behaviour and performance of *S. litura* feeding on the two host species separately with that of larvae offered both host species together. This allowed me to investigate larval choices and the effect of diet mixing on feeding and development.

3.4.4.1. Larval feeding and development

Newly hatched *S. litura* larvae were placed in individual 120 mL specimen vials with 20 mL damp vermiculite in the base. Thirty replicate larvae per treatment were provided with leaf segments from either plantain, red clover or both plantain and red clover (P+RC). Larvae in the P+RC treatment were placed on the vermiculite between the two host plant species segments, allowing them to choose their host. Containers were placed in a randomised block design in polystyrene trays in an incubator set at 25°C with a 16:8 h light:dark cycle. For the first 5 days, larval position was recorded daily, and leaf segments were checked for feeding damage (yes/no binary data). Leaf segments were replaced every 2–3 days as required for the first 10 days and daily thereafter.

On Days 5, 8 and 12 (at approximately 2nd, 3rd and 5th instars respectively) larvae were weighed and supplied fresh, weighed leaf material from their assigned treatment. After 24 hours, larvae and remnant herbage were weighed. The remnant herbage was frozen then later freeze-dried and re-weighed to determine dry weights. For the Day 5 assessment larvae were left in vermiculite,

but for the Day 8 and Day 12 assessments vermiculite was removed from the vials to allow for recovery, weighing and freeze-drying of frass.

Between assessment days and until pupation, food from the relevant treatment was supplied daily ad libitum and remnant food removed. On pupation, individuals were weighed, sexed and returned to their vials until adult eclosion. Time to pupation, adult eclosion and adult death were recorded.

At eclosion adults were paired for mating and egg-laying as described in section 3.4.3.2. Thirteen pairs were set up for the plantain treatment, nine for red clover and 15 for the P+RC treatment.

3.4.5. Feed conversion calculations

Conversion efficiencies of ingested (ECI) and digested food (ECD), and approximate digestibility (AD) were calculated using Waldbauer's formulae (below; Waldbauer, 1968). In these calculations, Δw_l is the change in larval weight over a 24-hour period, w_h is the weight of host food eaten and w_f is the weight of frass produced over the same period. Note that larvae were starved for 24 hours prior to the feeding efficiency assessments in the Host Quality Experiment but not in the Host Choice Experiment. Wet weights were used for all calculations presented in this paper but estimated dry weight values for the Host Quality Experiment were also calculated for the five larvae per treatment that were dried after the 24-hour feeding conversion assessment. Dry weight efficiency calculations used measured final larva and frass dry weights, estimated initial larval dry weights based on the measured dry weight: wet weight ratios, and estimated herbage initial and final dry weights based on control herbage wet and dry weight values.

$$\mathrm{ECI} = \frac{\Delta w_\mathrm{l}}{w_\mathrm{h}} 100 \qquad (1)$$

$$\mathrm{ECD} = \frac{\Delta w_\mathrm{l}}{(w_\mathrm{h} - w_\mathrm{f})} 100 \qquad (2)$$

$$\mathrm{AD} = \frac{(w_\mathrm{h} - w_\mathrm{f})}{w_\mathrm{h}} 100 \qquad (3)$$

3.4.6. Statistical analyses

All statistical analyses and graphical outputs were performed using R version 4.4.2 (R Core Team, 2024).

For both the Host Quality and Host Choice Experiments, larval, pupal, food and frass weight, and herbage dry matter content data were analysed using linear mixed-effects models (LMMs) estimated by restricted maximum likelihood (REML) with plant species included as a fixed effect and plant number specified as a random effect. ANOVA models were used to test for differences in plant and larval nitrogen and carbon among host plant species treatments. Feeding score data were analysed using LMMs with plant species included as a fixed effect and replicate specified as a random effect. For analysis of feeding efficiency calculations and proportion of red clover eaten, I used an arcsine square root transformation before analysis using a linear mixed model. Plant species was a fixed effect and plant number was incorporated as a random effect.

A generalised linear model (GLM) with zero-truncated negative binomial distribution and log link function was used to analyse count data representing the number of days at each life stage. For egg-count data, a GLM with a negative binomial distribution and a log link function was used to account for overdispersion. Larval survival (0 = dead, 1 = alive) was analysed using GLMs with bias-reduced maximum likelihood estimation following the method of Firth (1993), as complete separation was present in the data

For the analyses of the Host Quality Experiment, initial larva weight was included as a covariate to account for any differences between larvae. Data measured on a continuous scale and analysed using linear mixed models were log transformed where necessary to satisfy the assumptions of normality and homoscedasticity. Where available, sex was included as a fixed effect in the initial model but was removed if found not to be significant.

The lack of movement of the young larvae between plant species in the first few days of the Host Choice Experiment allowed me to divide larvae from the P+RC treatment into two subsets — larvae that ate plantain for the first 2 days ((P+RC) plantain) and those that ate red clover for the first 2 days ((P+RC) red clover). Analysis of data from the two subsets allowed me to determine how early feeding choices affect later feeding and growth. Thus, analyses were performed using LMMs with the main treatment level (red clover, plantain, P+RC) and at the subset level by nesting 'initial feeding' within main treatment included as fixed effects and replicate modelled as a random effect. Only two larvae in the P+RC treatment fed on both plantain and red clover within the first 2 days. This was insufficient data to model as a third nested 'initial feeding' subset robustly, so data from these larvae were omitted from the analysis (Kuznetsova et al., 2017).

For variables measured on more than one date (e.g. larval weight), a repeated measures analysis was initially explored. However, the estimated treatment effects from this model were

consistent with those obtained from mixed-effects models fitted separately at each time point, which included initial weight as a covariate to account for baseline differences. Therefore, the simpler time point-specific mixed-effects models were selected to analyse these data.

For LMMs, the significance of fixed effects was assessed using Type II F statistics with Satterthwaite's approximation for degrees of freedom implemented via the lmerTest package in R (Kuznetsova et al., 2017). For GLMs and GLMMs, fixed effects were evaluated using Type II Wald Chi-square tests as implemented in the car package (Fox & Weisberg, 2011). The significant terms were followed up with multiple comparison tests based on the estimated marginal mean differences applying a Bonferroni correction to adjust *p*-values for multiple comparisons using the emmeans package (Lenth et al., 2022). Where transformation of data was necessary prior to analysis by LMM or GLMM, results were backtransformed to the original scale and used for presentation throughout this paper.

In all LMM analyses, graphical model validation tools were used to assess the underlying assumptions of variance homogeneity and normality (plots of residuals against fitted values, standardised residuals versus fitted values, and quantile–quantile plots). For the GLMM analyses, model validation was conducted by examining plots of Pearson residuals against fitted values and versus each explanatory variable included in the model, following the approach described by Zuur et al. (2009). Additionally, simulation-based diagnostic checks were performed using the *DHARMa* package (Hartig, 2016).

3.5. Results

3.5.1. Larval feeding – quantity and conversion efficiency

The quantity of leaf material eaten by *S. litura* larvae depended on the plant species provided. In the Host Quality Experiment, the wet weight of herbage eaten on Day 6–7 differed significantly between treatments ($F_{4,19.1} = 22.9$; $p < 0.001$), with larvae eating more plantain, chicory or dock than white or red clover (Figure 3-2A). Differences in the Host Choice Experiment were also statistically significant and, as with the Host Quality Experiment, larvae ate more plantain than red clover (Day 13 assessment — $F_{2,58.6} = 167.9$; $p < 0.001$; Figure 3-2B).

Most of the larvae offered a choice between plantain and red clover in the Host Choice Experiment fed on only one of the two plant species for the first 3 days, with roughly equal numbers choosing to feed on each. As they matured, larvae began to move between and feed on both plant species. By Day 8 all larvae had fed on both red clover and plantain at some time during the 8 days, with 76% feeding on both species within the Day 6 to 8 feeding assessment period. By Day 13, red clover made up only a small proportion of the larval diets (mean proportion of diet

that was red clover = 5.9% ± 1.4% wet weight mg/day for those that had fed on red clover for the first 2 days and 2.1% ± 1.0% for those that had fed on plantain, $F_{1, 24} = 4.7$, $p = 0.04$).

Among P+RC treatment larvae, those that only ate plantain for the first 2 days ate more in total per day for the rest of the experiment than those that had eaten only red clover for the first 2 days, but this difference was not statistically significant at Day 13 ($p > 0.05$) (Figure 3-2B).

The efficiency of conversion of the ingested food (ECI) and approximate digestibility (AD) calculated from food eaten (wet weights) by larvae in the Host Quality Experiment between Days 6 and 7 were higher for red and white clover than for plantain (ECI: $F_{4, 19.8} = 7.2$, $p < 0.001$; AD: $F_{4, 19.2} = 7.1$, $p = 0.001$; Figure 3-3A, B). AD was likewise higher for larvae feeding on red clover than for larvae feeding on plantain or P+RC in the Host Choice Experiment ($F_{2, 150} = 65.8$, $p < 0.001$; Figure 3-3E) but for ECI this was only true for the first and third assessment dates ($F_{2, 236} = 14.8$, $p < 0.001$; Figure 3-3D). The efficiency of conversion of digested food (ECD) did not differ significantly between treatments in the Host Quality Experiment ($F_{4, 20.2} = 2.1$, $p = 0.113$; Figure 3-3C) but was lowest for larvae feeding on red clover in the Host Choice Experiment ($F_{2, 151} = 32.4$, $p < 0.01$; Figure 3-3F). Initial feeding choice (i.e. whether larvae offered a choice ate plantain or red clover for the first 2 days) did not significantly affect any of the feeding efficiency measures (data not shown).

Estimated feeding efficiency calculations based on dry weights were used to investigate the possibility that my wet weight feeding efficiency calculations were biased by the different dry matter values of the host plant species in the Host Quality Experiment. Analysis of the ratio between dry weight and wet weight values for each efficiency measure showed no significant differences between treatments ($p > 0.05$), indicating a lack of bias.

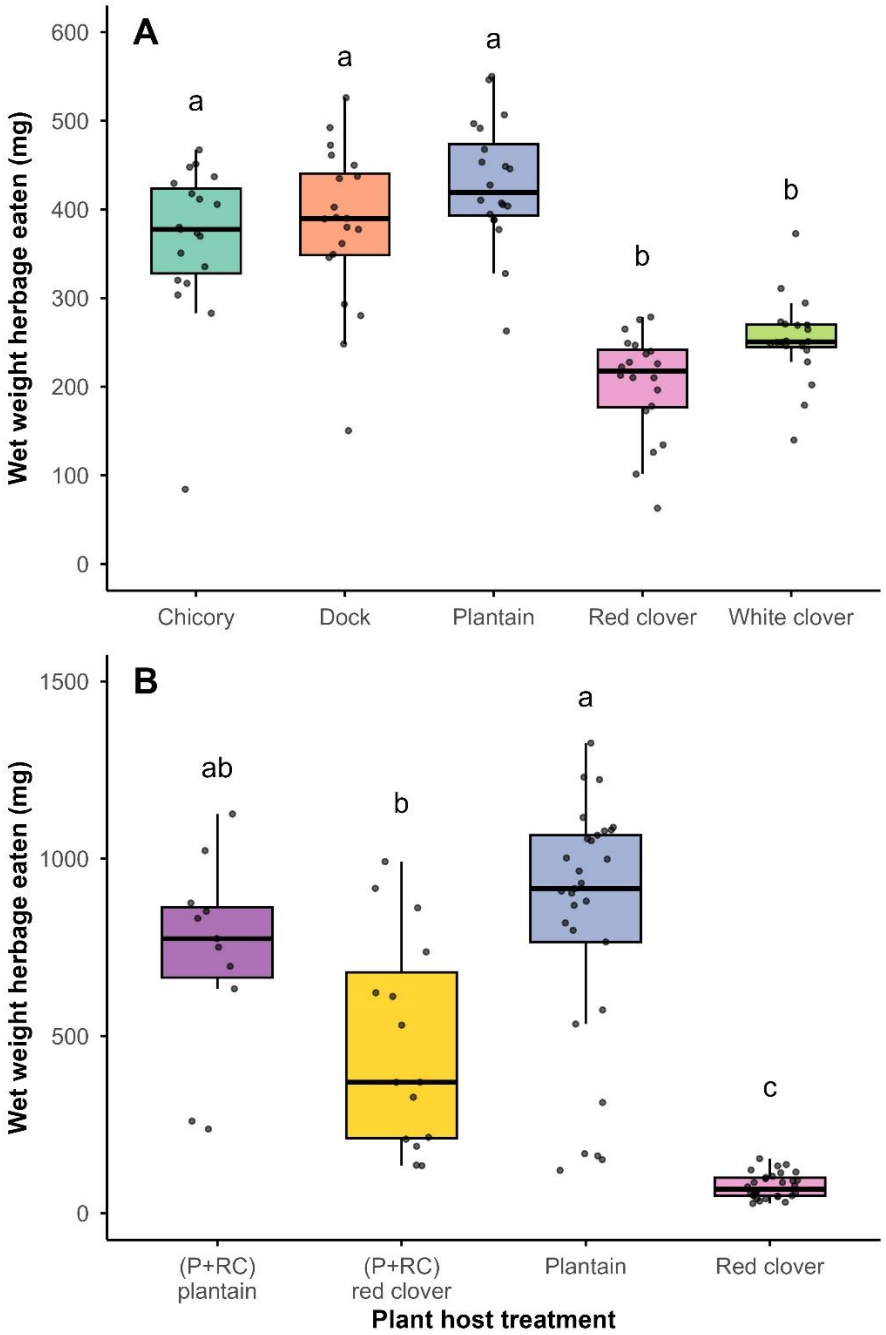


Figure 3-2 – Wet weight of food eaten in 24 hours by Spodoptera litura *larvae fed (A) chicory, dock, plantain, red clover or white clover (Day 7 assessment, 14-day old larvae in a Host Quality Experiment) or (B) fed plantain, red clover or both plantain and red clover (P+RC) (Day 13 assessment of 13-day old larvae in a Host Choice Experiment). P+RC treatment larvae were further divided into the subset that ate plantain for the first 2 days ((P+RC) Plantain) and the subset that ate red clover for the first 2 days ((P+RC) red clover). Different letters indicate statistically significant differences between treatments at a level of $p < 0.05$. In all boxplots, horizontal lines correspond to the median, 1st and 3rd quartiles and vertical lines show smallest and largest values no further than 1.5*Interquartile range (IQR). Each data point in the boxplots represents a replicate.*

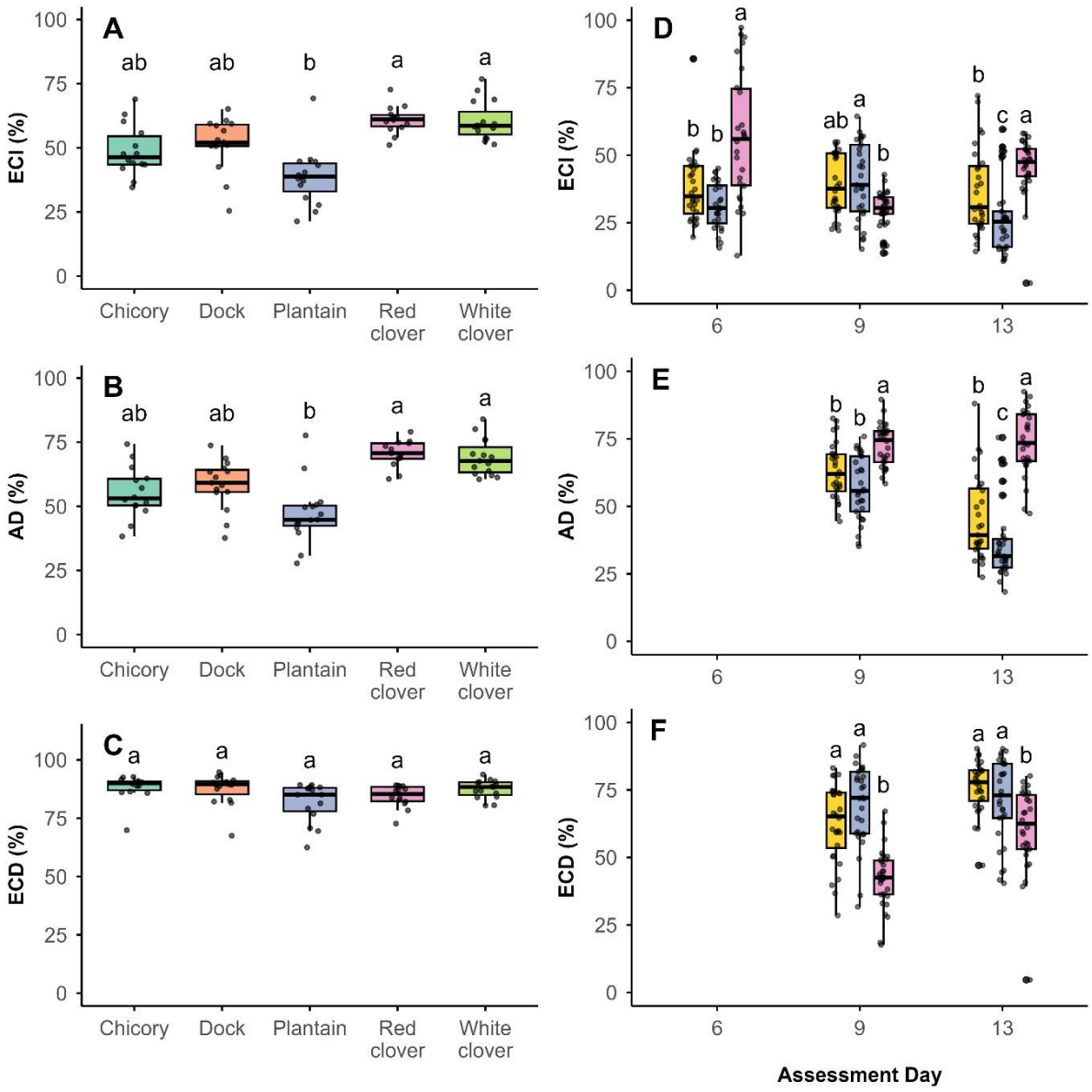


Figure 3-3 – (A and D) Efficiency of conversion of ingested food (ECI), (B and E) approximate digestibility (AD) and (C and F) efficiency of conversion of digested food (ECD) calculated for a 24-hour feeding period for Spodoptera litura *larvae (A–C) after 7 days of feeding on one of five plant treatment species (Host Quality Experiment) or (D–F) after 6, 9 and 13 days of feeding on plantain or red clover, or both plantain and red clover (P+RC) (Host Choice Experiment). Boxes within each assessment day in figures D–F are, from left to right, P+RC (yellow), plantain (blue) and red clover (pink). Calculations for both experiments used live larval weight change, wet weights of fresh and remnant food, and wet weight of frass. ECD and AD were not measured on Day 6 because frass was not collected. Different letters within a given assessment day indicate statistically significant differences between treatments at a level of $p < 0.05$.*

3.5.2. Larval growth, development and survival

In both experiments, larval weights generally reflected consumption data. After 7 days of feeding, the mean weights of larvae in the Host Quality Experiment differed significantly between treatments ($F_{4,20.1} = 5.3$, $p < 0.001$), with larvae fed red clover being significantly smaller than those fed chicory or dock (Figure 3-4*A*). Similarly, larvae fed only red clover in the Host Choice Experiment weighed significantly less than larvae fed plantain or P+RC ($F_{2,60.8} = 250.2$, $p < 0.001$; Figure 3-4B). Larvae offered P+RC weighed less than those on plantain due to the subset of larvae that chose to feed on red clover for the first 2 days, and which remained lighter for the rest of the trial than those that chose to feed on plantain for the first 2 days (Figure 3-4B).

In the Host Quality Experiment, individuals fed red clover were significantly smaller at pupation than those from other treatments (Table 3-2).

Survival was high in all treatments in the Host Quality Experiment and similarly high in the plantain and P+RC treatments in the Host Choice Experiment (Table 3-2). Five out of 30 larvae fed red clover in the Host Choice experiment survived and appeared to develop normally for 25–30 days but died without pupating. Thus, while survival to pupation did not differ between treatments in the Host Quality Experiment there was a statistically significant difference in the Host Choice Experiment ($X^2_{2,N=87} = 6.4$, $p = 0.041$).

Neither time to pupation nor time from pupation to eclosion differed between treatments in the Host Quality Experiment. Adult survival time was not recorded in this experiment. In the Host Choice Experiment larvae fed on red clover took longer to pupate and weighed less at pupation than larvae fed on plantain or offered a choice (regardless of their initial feeding choice) (Table 3-2). Time to eclose (i.e. time as pupa) was not significantly different between treatments. Although adult survival time was shorter for individuals fed red clover in the Host Choice Experiment, overall survival time from hatching was still significantly longer for this treatment ($p < 0.05$).

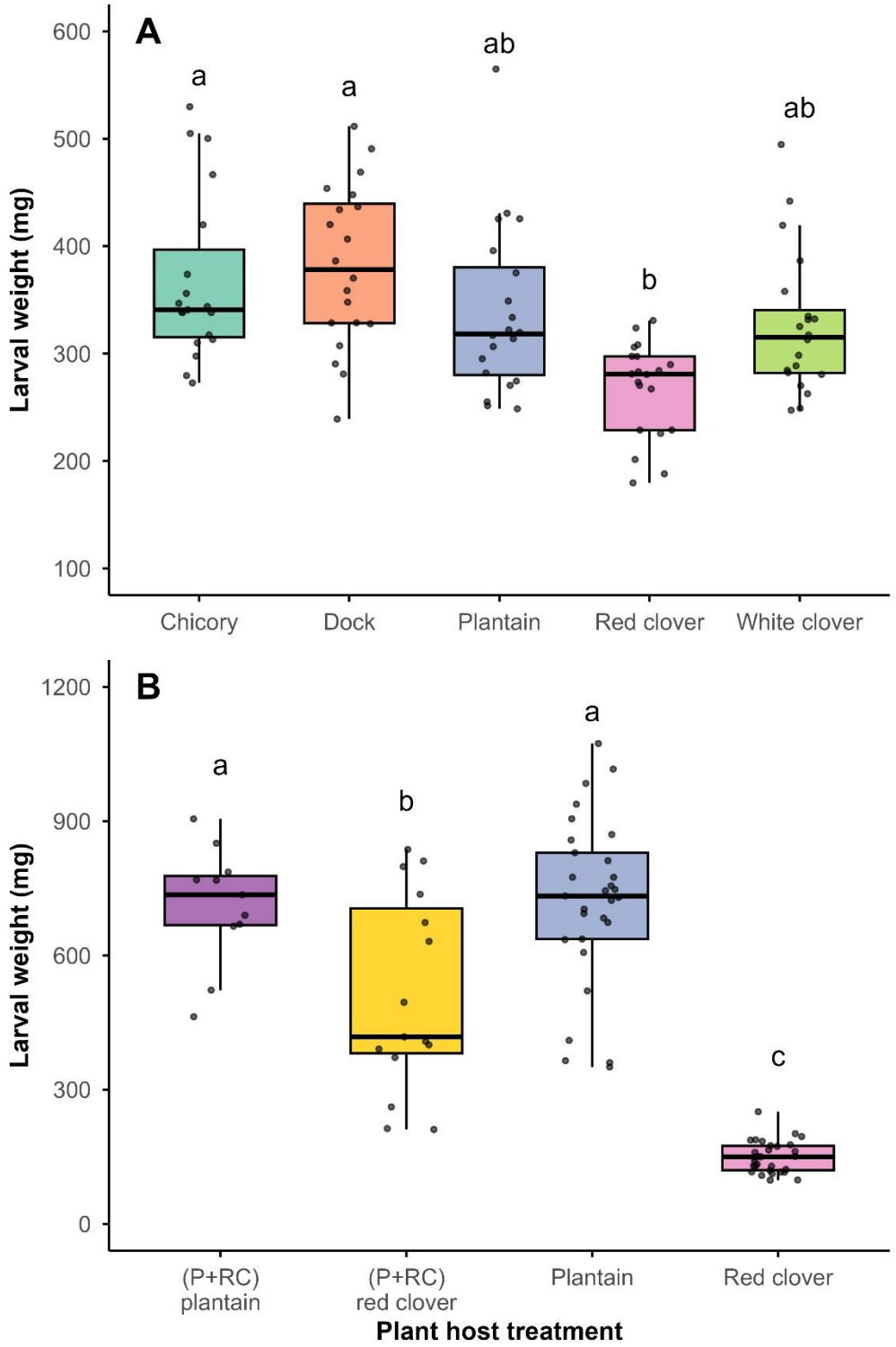


Figure 3-4 – Spodoptera litura *larval weights after 7 days of feeding on (A) chicory, dock, plantain, red clover or white clover (Host Quality Experiment) or 13 days of feeding on (B) plantain, red clover or plantain and red clover (P+RC) from newly hatched (Host Choice Experiment). P+RC treatment larvae were further divided into the subset that ate plantain for the first 2 days ((P+RC) plantain) and the subset that ate red clover for the first 2 days ((P+RC) red clover). Different letters indicate statistically significant differences between treatments at a level of $p < 0.05$.*

Table 3-2 – Estimated means (and their standard error) for pupal weight (mg) and number of days from larval hatching to pupation, pupation to adulthood and, for the Host Choice Experiment, adult eclosion to death for Spodoptera litura *fed leaves from one of five plant species from 6 days old (Host Quality Experiment) or when fed either plantain, red clover, or plantain and red clover (P+RC) from hatching (Host Choice Experiment). In the Host Choice Experiment, larvae offered a choice were further divided into those that ate only plantain for the first two days ((P+RC) plantain) and those that only ate red clover for the first two days ((P+RC) red clover).*

Plant host treatment	N* (pupa/ adult)	Pupal weight (mg)	Egg to pupa (days)	Pupa to adult (days)	Adult survival (days)
Host Quality Experiment					
Chicory	14/13	324.8 (13.6) a	22.3 (1.3) a	11.7 (1.0) a	—
Dock	15/15	310.9 (12.6) a	23.0 (1.2) a	12.0 (0.9) a	—
Plantain	15/15	335.8 (13.6) a	22.5 (1.2) a	11.9 (0.9) a	—
Red clover	14/13	248.8 (10.4) b	23.1 (1.3) a	12.0 (1.0) a	—
White clover	15/15	325.4 (13.1) a	21.6 (1.2) a	11.7 (0.9) a	—
Test statistic		$F_{4,67}$=8.52, p<0.001	$X^2_{4,\ N=73}$=0.93, p=0.921	$X^2_{4,\ N=70}$=0.08, p=0.999	—
Host Choice Experiment					
Plantain	30/29	351.2 (10.0) a	18.8 (0.8) b	11.3 (0.6) a	6.3 (0.5) a
Red clover	25/25	233.0 (7.2) b	22.8 (1.0) a	12.3 (0.7) a	4.6 (0.5) b
P+RC	28/28	336.2 (10.3) a	19.2 (0.9) b	11.2 (0.7) a	6.5 (0.5) a
Test statistic		$F_{2,76}$=53.1; p<0.001	$X^2_{2,\ N=80}$=11.76; p=0.003	$X^2_{2,\ N=78}$=1.58; p=0.453	$X^2_{2,\ N=76}$=8.55; p=0.014

Note: For each experiment, different letters within columns indicate statistically significant differences between treatments at a level of $p < 0.05$.

** N is the number survived to pupa/adult out of 15 per treatment for the Host Quality Experiment and of 30 for the Host Choice Experiment.*

3.5.3. Egg-laying

Moth pairs that had been fed red clover in the Host Quality Experiment laid significantly fewer eggs than those fed plantain ($X^2_{4,N=28}$ = 9.8, p = 0.045; Figure 3-5A). Likewise, moth pairs fed red clover as larvae in the Host Choice Experiment laid significantly fewer eggs than those on plantain and fewer than those offered a choice ($X^2_{2,N=36}$ = 14.6, p < 0.001; Figure 3-5B).

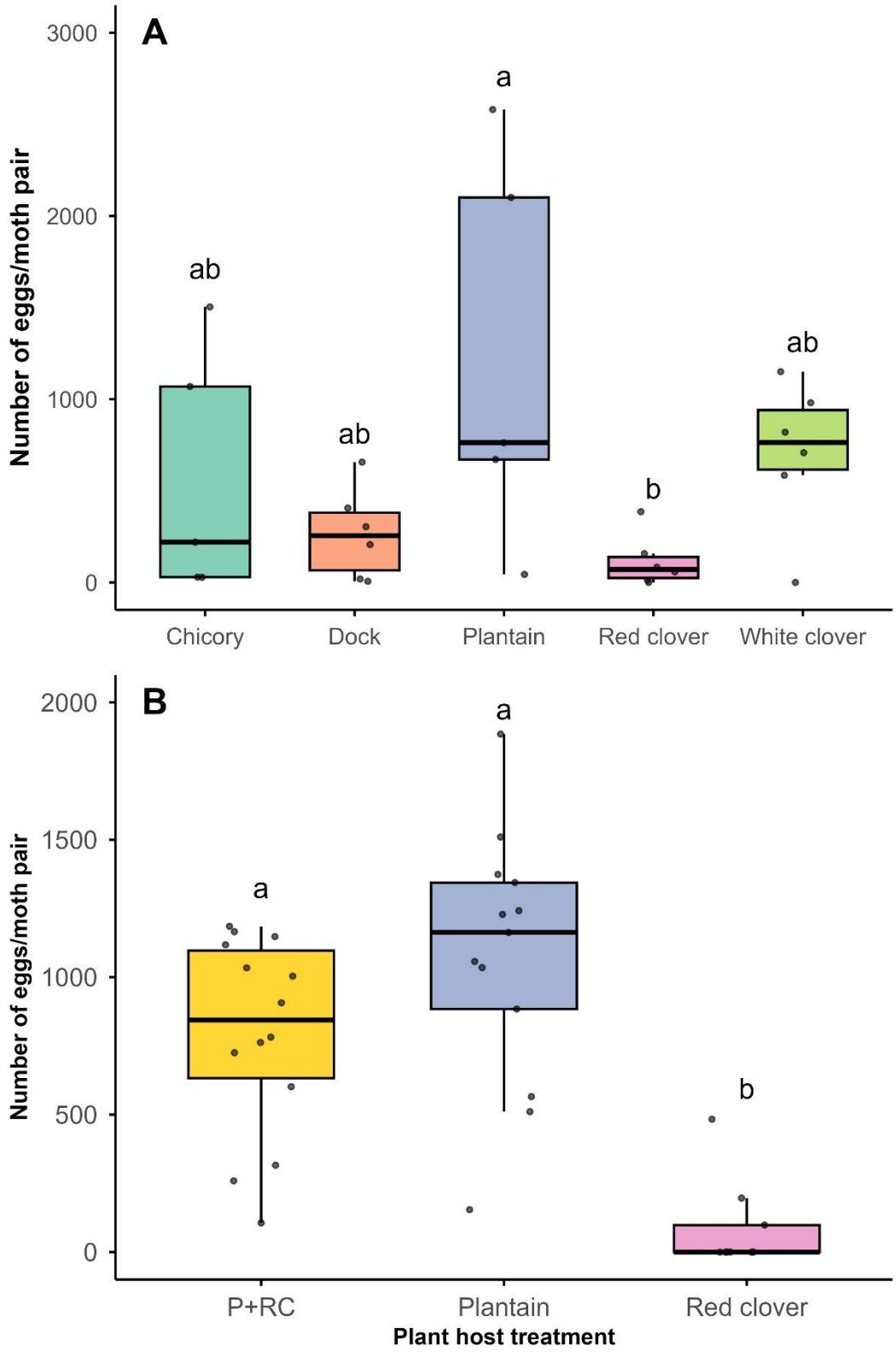


Figure 3-5 – The number of eggs laid per Spodoptera litura *moth pair after feeding on (A) chicory, dock, plantain, red clover or white clover (Host Quality Experiment) or on (B) plantain, red clover or plantain and red clover (P+RC) (Host Choice Experiment) as larvae. Different letters indicate statistically significant differences between treatments at a level of $p < 0.05$. Each datapoint represents eggs from one replicate moth pair.*

3.5.4. Differences between sexes

The sex of each individual could only be determined after pupation; therefore, this information was not available for the larvae in the Host Quality Experiment that were frozen on Day 7 or for two larvae that died before pupation. Of the 73 individuals that pupated in this experiment, 33 (45.2%) were female. There was no statistically significant effect of sex on larval or pupal weights, the amount of food eaten, or any of the feeding efficiency measures. Sex did not significantly affect the length of time to pupation, but male pupae took longer to eclose — 12.8 ± 0.6 days compared with 10.9 ± 0.5 days for females ($X^2_{1, N=70} = 5.4$, $p = 0.020$).

The sex ratio in the Host Choice Experiment was similar to that of in the Host Quality Experiment, with 39 of the 83 pupae being female (47.0%). There was no significant effect of sex on larval or pupal weight, amount of food consumed, or any of the food conversion efficiency measures. Sex also did not significantly affect the length of time for larvae to pupate in this experiment, but female moths emerged earlier than males due to a shorter time as pupae (females = 0.7 ± 0.5 days, males = 12.6 ± 0.6 days, $X^2_{1,N=78} = 5.5$, $p = 0.019$). Adult survival time did not differ significantly between the males and females ($p = 0.207$). There were no statistically significant sex*treatment interactions for any of these developmental times.

3.5.5. Moisture, carbon and nitrogen content

Relative daily weight change (i.e. moisture loss or gain) of herbage in the Host Quality Experiment control vials (without larvae) ranged from -6.0% to +4.2% when vermiculite was present in the vials, and -8.4% to -2.8% between Days 6 and 7 when vermiculite was absent. There were statistically significant differences between plant species ($p < 0.001$), assessment day ($p < 0.001$) and an interaction between the two ($p = 0.007$). Red clover, white clover and dock leaves tended to lose more moisture than the other plant species, but these differences were not statistically significant for all treatment comparisons or for all days.

Across the 4 days it was measured, red clover had a significantly higher % dry matter content than dock, with the other plant species being intermediate between the two (dock = 13.4% ± 0.7%, chicory = 15.9% ± 0.7%, plantain = 14.7% ± 0.7%, white clover = 14.9% ± 0.7% and red clover = 17.1% ± 0.7%, $F_{4, 20.2} = 3.78$; $p = 0.02$). The higher dry matter content of red clover affected the moisture content of the frass, which was measured on Day 7 of the experiment. Frass from larvae fed red clover had a significantly higher % dry matter (30.8% ± 2.0%) than that from larvae fed chicory, dock and plantain (21.3% ± 2.0, 20.3% ± 2.0 and 19.2% ± 2.0%, respectively), with white clover being in between (25.0 ± 2.0%) ($F_{4,20.2} = 5.7$; $p < 0.001$).

Analysis of a subsample of herbage taken from each plant on Day 6 and of one of the larvae fed from each of these plants (frozen on Day 7) showed that the plantain had lower %

nitrogen and lower % carbon than all other plant species apart from chicory (Table 3-3). Larvae fed plantain had lower % nitrogen than those fed red clover but % carbon did not differ between the two.

The relative daily weight change of herbage in the Host Choice Experiment could not be measured as there were no control (without larvae) vials but as the experimental setup used was the same as for the Host Quality Experiment it was assumed to be similarly low. Percentage dry matter content of the two plant species used in the Host Choice Experiment was measured using wet and dry weights of remnant herbage at each of the three assessment dates. The dry matter content varied significantly between the three assessment dates ($F_{2, 315.5} = 44.2$; $p < 0.001$) and between treatments ($F_{1, 315.5} = 670.5$, $p < 0.001$) but was consistently higher for red clover than for plantain. The mean % dry matter content of plantain remnants averaged 13.9% ± 0.3% (across the three dates and only for the no-choice treatment) compared with 25.7% ± 0.3% for red clover remnants. The dry matter content of plantain remnants did not differ significantly when they were in a container with red clover (i.e. in the P+RC treatment vials) but red clover remnants in a container with plantain had a lower percentage dry matter (24.4% ± 0.3%) than red clover on its own (i.e. in the red clover treatment vials) (25.7% ± 0.3%; $p < 0.05$).

As with the Host Quality Experiment, the dry matter content of frass from larvae feeding on red clover was significantly higher than for those feeding on plantain or P+RC on both of the dates it was measured (means for Days 8 and 12 combined: Red clover = 35.0% ± 0.7, P+RC = 15.3% ± 0.7, Plantain = 12.9% ± 0.6; $F_{2, 59.9} = 313.5$; $p < 0.001$).

Table 3-3 – Estimated means for % (dry weight) nitrogen and carbon in herbage (leaf) subsamples from each of five chicory, dock, plantain, red clover and white clover plants on Day 6 and in five Spodoptera litura *larvae fed from those plants (sampled on Day 7).*

Plant species	**Plant N %**	**Plant C %**	**Larval N %**	**Larval C %**
Chicory	3.12 cd	42.58 ab	9.58 ab	45.70 a
Dock	3.85 bc	44.28 a	9.62 ab	44.50 ab
Plantain	2.25 d	40.76 b	9.16 b	43.16 b
Red clover	4.54 ab	44.08 a	10.80 a	42.60 b
White clover	4.98 a	43.78 a	10.72 ab	43.60 ab
SE	0.222	0.615	0.356	0.524
Test statistic	$F_{4,20}$=24.5; p<0.001	$F_{4,20}$=5.67; p<0.001	$F_{4,20}$=4.31; p=0.01	$F_{4,20}$=5.39; p<0.001

Note: Different letters within each column indicate statistically significant differences at a level of $p < 0.05$.

3.6. Discussion

Spodoptera litura larvae ate more chicory, dock and plantain herbage per day than either of the clover species tested here. However, larvae feeding on white clover had similar body weights, developmental rates and egg-laying, indicating that *S. litura* larvae can convert white clover herbage to body mass more efficiently. This is supported by the lower efficiency of conversion of ingested food (ECI) and approximate digestibility (AD) for larvae feeding on the non-legume forage species, although this was statistically significant only when compared with plantain. Dry matter content was similar for these four forage species, so it seems likely that the differences in conversion efficiency were attributable to the significantly higher nitrogen content in white clover. Food conversion efficiency tends to increase with increasing food nitrogen content, but other variables such as food water content and indigestible fibre content also have strong effects (Mattson, 1980; Scriber & Slansky, 1981). Targeted, controlled experiments are therefore required to clearly identify the underlying mechanisms.

Interestingly, while larvae consumed similar quantities of red and white clover per day, and less of both than chicory, dock and plantain, larvae fed white clover developed into heavier pupae than those fed red clover. Conversion efficiencies, nitrogen and carbon levels were similar between the two treatments, so did not explain this difference. Red clover tended to have higher dry matter content which, although not always statistically significant, is likely to have contributed to the poorer larval growth (Scriber, 1977). Lower levels of feeding on red clover compared with white clover have been noted for other insect pests, for example clover root weevil (Cooper et al., 2003), with the isoflavones such as formononetin, genistein and biochanin being implicated as responsible (Gerard et al., 2005; Quiroz et al., 2017; Wang et al., 1998). Concentrations of formononetin in white clover leaves tend to be extremely low or undetectable (Tava et al., 2022; Wu et al., 2003), therefore although levels in the red clover cultivar Relish that was used in my study are low compared with other red clover cultivars (Gerard et al., 2017) secondary metabolites such as formononetin are likely contributors to the poorer growth and survival of *S. litura* on red clover (Tava et al., 2022; Wu et al., 2003). Leaf physical characteristics may also affect herbivory (Clissold et al., 2009; dos Santos et al., 2020). Leaf toughness can lead to decreased meal sizes and feeding rates, slower gut passage rates and reduced nutrient assimilation efficiency. It can also alter the ratios of protein to carbohydrate that are assimilated (Clissold et al., 2009). Wilman and Altimimi (1984) found that red clover (cv. Norseman) leaves were less digestible than those of white clover (cv. Grasslands Huia) because of lower cell wall digestibility, and that red clover digestibility declined more quickly with crop maturity. The negative effects of red clover on *S. litura* may be due to a combination of some or all of these

factors. Rearing experiments on different red clover cultivars with varying physical and chemical properties or using artificial diets would be a valuable next step towards understanding the exact mechanisms.

Effects of food quality on utilisation efficiency, larval development and egg-laying have intergenerational and population-level implications (Awmack & Leather, 2002). Feeding on red clover reduced egg-laying to one-tenth that of individuals fed on plantain, which will clearly negatively impact the population growth rate of *S. litura*. Conversely, larvae feeding on plantain grew larger, ate more, and were considerably more fecund as adults. Plantain crops may therefore suffer greater herbage losses than red clover, both within and between *S. litura* generations.

I hypothesised that *S litura* larvae would choose to feed on the highest quality host plant species available. While this may be the case for older larvae, the young larvae in my Host Choice Experiment, in which larvae were offered a choice between plantain and red clover, appeared to feed on whichever acceptable plant host they encountered rather than seeking a preferred host. As they matured, larvae became more mobile and began to switch between hosts and, by late-instar, were consuming more plantain than red clover. For some insect species, host selection may be a consequence of female oviposition preference rather than larval feeding preference, while for others neonate larvae may leave their original host plant to seek a more favourable feeding site (Ortiz-Carreón et al., 2025). My study did not measure female oviposition preferences, but other researchers have shown that *S. litura* may prefer to oviposit on some hosts over others (Singh et al., 2015; Xue et al., 2010). Neonate *S. litura* may therefore rely on the maternal oviposition choice in scenarios such as in my trial, where the host was adequate and the cost of moving to a higher quality host may have been greater than the cost to their developmental rate on a less preferred host. Alternatively, the red clover in my Host Choice Experiment may have had defence chemicals that *S. litura* larvae were unable to detect.

The lack of movement to a higher-quality host by young larvae had repercussions for their growth that persisted through most of the larval phase. Larvae that were offered both red clover and plantain but fed only on red clover for the first two days remained smaller throughout most of the trial than those fed on plantain. These larvae appeared to eat less per day (although not significantly so by the end of the trial); however, there were no statistically significant differences in feeding conversion efficiencies. Nevertheless, there were no differences in pupal weight or in the length of time taken to pupate between the two groups. Some lepidopteran species, including *S. litura*, have been shown to use diet choice, or strategies such as accelerated growth rate and extended larval periods to overcome nutritional imbalance (Lee, 2007; Lee et al., 2012). Taylor

(1989) showed that nitrogen utilization efficiencies of salvinia moth larvae, *Samea multiplicalis* Guenée, were impaired by early exposure to poor diet and that these effects could persist through to the final instar. In addition to feeding behaviours such as ingestion rate the authors suggested gut enlargement or changes in gut enzyme secretion as two possible mechanisms by which larvae could compensate for early food quality deficiency. They also showed that in some cases adaptive responses are insufficient to completely compensate for early diet deficiencies. Abbas et al. (2025) found that *S. litura* consumption efficiencies were highest in the 6th instar. While my data did not explicitly identify the physiological mechanism of compensation, it is likely that compensatory feeding and/or higher feeding efficiency occurred after the last larval weight measurement, in the last few days before pupation.

Host plant quality is a function of physical and chemical traits that can impact the herbivore in question, either positively or negatively, and is a key determinant of the herbivore's performance (Awmack & Leather, 2002). 'High' and 'low' quality traits will vary between herbivore species so are often assessed using food utilisation indices such as the efficiency of conversion of ingested or digested food and approximate digestibility. These are based on measurements including changes in larval weight, food intake and amount of faeces produced and may be calculated based on wet or dry weights (Waldbauer, 1968). I chose to present feeding efficiency calculations based on wet weights to avoid the inherent inaccuracy involved in having to estimate initial dry weights of larvae and herbage. However, using wet weights could potentially bias feeding efficiency comparisons in studies such as mine that have variation in diet dry matter content between treatments (Scriber, 1977; Scriber & Slansky, 1981). Intuitively, utilization of plant material with a higher water content could seem less efficient when calculated using wet weights because a lower proportion of the food weight contributes to energetic or structural components of larval growth. On the other hand, some of the measured larval wet weight gain would be through hydration of tissues and haemolymph volume, thus potentially balancing the higher food wet weight. I compared the wet and dry weight feeding efficiency ratios between treatments to check for evidence of bias and found no significant differences. Furthermore, plantain and white clover had similar dry matter contents, but ECI and AD were significantly lower for larvae feeding on plantain than on white clover. I therefore infer that the relatively small differences in % dry matter between treatments in this study were not mathematically great enough to cause a significant bias to the wet weight efficiency calculations.

Most of an insect's consumption and growth usually happens in the penultimate and final instars (e.g. Abbas et al., 2025), so growth and utilisation values calculated for those instars are generally considered to be representative of the entire larval stage (Scriber & Slansky, 1981).

Moreover, measurements of growth and feeding are easier for larger, more mature larvae. Therefore, food utilisation data in the literature are most often for these later instars. The effects of different food qualities and other factors may be greater for early instars, however, so quantifying performance values for younger larvae can be beneficial (Scriber & Slansky, 1981). I measured feeding efficiency of individual larvae at three time points in the Host Choice Experiment and found that while ECI was greater for larvae feeding on red clover than on plantain at the Day 6 and Day 13 assessments, the opposite was true at Day 9. Growth, consumption and metabolic rates can vary between and within instars (Scriber & Slansky, 1981). Some researchers choose to measure feeding efficiency for an entire instar to mitigate against within-instar differences (e.g. Karowe & Martin, 1989; Lindroth et al., 2000), while others measure over a 24-hour period as was done in my study. Therefore, while they provide an interesting 'point in time' view, caution must be taken in comparing food utilisation measures between studies, particularly where it is not clear if wet or dry weights have been used, for example, Lindroth et al. (2000), and they should be considered alongside growth and development measures such as those presented in my study.

For herbivores such as *S. litura*, differences in host quality are influenced not only by plant species but also by factors such as cultivar and plant resource availability (e.g. nitrogen or moisture) and may vary seasonally or in response to environmental stress (Awmack & Leather, 2002). Cultivar differences may partly explain why my results differ from those of some published studies. Sushilkumar and Ray (2007) showed that larval and pupal periods were longer for larvae fed chicory than those fed dock, although as with my results they found no significant differences in the length of any other life stages, in larval or pupal weights or in larval survival. In Sushilkumar and Ray (2007), however, plant material was collected from the field and therefore the genotype is unknown. They stated that *S. litura* had a short developmental period on dock and chicory, but they did not specify the rearing methods or temperature. Consequently, I cannot directly compare their results with my own. My results also differ from those of Huang et al. (2008) who found no significant differences in development or nutrition utilisation between *S. litura* fed red or white clover. However, as with Sushilkumar and Ray (2007), the authors did not specify which clover cultivars they used for their trial. Cultivars of forage plants are known to vary in their production of metabolites, including defence chemicals such as formononetin in red clover and iridoid glycosides in plantain, and thus in their susceptibility to insect pests (Gerard et al., 2005; Harvey et al., 2005).

My finding that female *S. litura* emerged earlier than males is typical for this species. It is thought to be a strategy to reduce inbreeding risk, given the large size of the egg clusters, whereby

early-emerging females can disperse before their brothers emerge (Herrero et al., 2018; Li et al., 2014). The delayed emergence for males (1–2 days) is relatively small compared with the adult lifespan (Bragard et al., 2019; Sharma et al., 2025) so it is unlikely to negatively impact the reproductive rate of *S. litura* populations.

Overall, my study confirmed that *S. litura* can complete its development on five common pasture plant species: plantain, dock, chicory, red and white clover. I also showed differences in host quality between these plant species, and that host choice affects *S. litura* growth and development. This has the potential to affect them at population and intergenerational levels. Understanding how *S. litura* will feed and develop on forage plant species is important for farm management decisions and accurate population growth modelling throughout the introduced range of this potential agricultural pest species. Worldwide, agricultural pasture composition will change under predicted climate change scenarios (Howden et al., 2007). Climate change adaptation strategies must include an understanding of the interactions between future forage plant species and their associated herbivores (Sharma & Dhillon, 2020). Identification of vulnerable or tolerant host forages will provide valuable information for farmers and plant breeders and help to future-proof pastures against herbivorous pests. *Spodoptera litura* is a globally important pest species and, in currently marginally suitable regions worldwide, it is likely to pose an increasingly significant risk to pasture persistence as our climate warms (Kean et al., 2015; Orre-Gordon & Jensen, 2025). Future work aimed at identifying the factors influencing resistance to *S. litura,* for example leaf toughness and secondary metabolites like formononetin, will help plant breeders develop more resilient cultivars and inform farmers in vulnerable regions about suitable crop planting. Typically, sowing rates of 1–3 kg/ha for chicory and plantain, 4–6 kg/ha of red clover and 3–5 kg/ha of white clovers are recommended in standard grass-based mixed pastures in New Zealand (Agricom, 2025) but rates vary depending on factors such as pasture purpose, soil fertility and moisture, and management system, and all these plant species can be sown as pure swards. Tolerance factors identified under constant conditions in laboratory experiments such as mine need to be verified with field experiments. These would allow us to understand population-level effects for both the plant species and *S. litura*, the influence of mixed-species planting and help to calculate planting ratios that would maintain an optimal balance between improved climate resilience and the potential for increased pest pressure. These are important steps towards the development of a suitable climate mitigation strategy for agricultural systems.

3.7. Author contributions

Joanne Jensen: Conceptualization (equal); data curation (lead); formal analysis (supporting); investigation (lead); methodology (lead); project admin (equal); writing-original draft; review and editing (lead). **Chanatda Somchit**: Formal analysis (lead); methodology (supporting); writing – original draft; review and editing (supporting). **Michael Clearwater**: Supervision (equal); writing – original draft; review and editing (supporting). **Andrew Barnes**: Supervision (equal); writing – original draft; review and editing (supporting). **Sofia Orre-Gordon**: Conceptualization (equal); funding acquisition (lead); project admin (equal); supervision (equal); writing – original draft; review and editing (supporting).

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3.10. Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3.11. Conflicts of interest statement

The authors declare no conflicts of interest.

4. Forage host species has intergenerational effects on *Spodoptera litura* performance and larval mid-gut microbiota

This chapter is intended for submission to a scientific journal for publication. It addresses the hypothesis that feeding on non-preferred host plants will affect *S. litura* behaviour, growth, reproduction and gut microbiota and have intergenerational effects.

4.1. Abstract

Herbivorous pest species such as *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) will become increasingly important in currently marginal regions due to climate change. Identifying forage plant species that will be less susceptible to feeding damage and understanding the effects on *S. litura* of feeding on them is an important step towards ensuring pasture resilience in the face of increasing *S. litura* pressure.

Having previously identified red clover (*Trifolium pratense*) as a less favourable host, I investigated the intergenerational consequences of feeding on red clover compared with a more favourable host, plantain (*Plantago lanceolata*). I reared *S. litura* on red clover or plantain for two generations and compared the growth, feeding and mid-gut bacteria of second-generation larvae with that of individuals whose parents were raised on the same or the alternative plant host.

Host plant species significantly impacted the performance of the subsequent *S. litura* generation. Individuals whose parents had fed on red clover were smaller and had reduced feeding efficiencies compared with those whose parents fed on plantain. This was true both for offspring feeding on plantain and for those on red clover. Parent host also affected offspring mid-gut microbiota. Offspring generation larvae feeding on red clover, and whose parent had fed on plantain, had significantly lower alpha (Shannon) diversity than other treatments. Feeding on red clover for two generations significantly reduced beta diversity, and larval mid-gut microbiota were strongly dominated by *Enterococcus*.

These results indicate the potential for red clover to reduce *S. litura* population diversity, resilience and adaptability. Field-verification and investigation of the mechanisms behind this effect are important next steps.

Keywords: Tropical armyworm, sleeper pest, host plant quality, red clover, plantain, parental diet, intergenerational acclimation, host-mediated intergenerational responses, 16S sequencing

4.2. Introduction

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a highly polyphagous and invasive species that will become increasingly important in currently marginal regions due to climate change (Fu et al., 2025; Jensen et al., 2025; Orre-Gordon & Jensen, 2025). In addition to the direct effects of climate variables such as temperature and rainfall populations of this species will be indirectly impacted through factors such as host plant availability. *Spodoptera litura* can utilise many different forage species as hosts but some are higher quality than others. In a previous study I showed that *S. litura* that were fed only red clover (*Trifolium pratense*) had smaller body weights and laid fewer eggs than those on plantain (*Plantago lanceolata*) (Chapter 3; Jensen et al., 2026). Although New Zealand pastures are traditionally dominated by *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover), farmers are increasingly incorporating alternative forage species such as red clover and plantain to improve pasture resilience (Caradus et al., 2021; Stewart et al., 2022). Understanding which alternative forage species will be more resistant to attack, and the effects on *S. litura* of feeding on them, is an important step to ensuring pasture resilience in the face of increasing *S. litura* pressure.

Parental diet has been shown to influence progeny fitness for some insect species (Mousseau & Dingle, 1991). While poor parental diet can negatively impact offspring performance (e.g. Keena et al., 1998), in some cases an acclimatory response occurs, whereby offspring tolerance to a poor food is improved compared to individuals with no familial exposure. For example, Rotem et al. (2003) found that *Pieris rapae* larvae grew best when reared on diet containing the same amount of protein as their mothers. For some insects, this acclimatory effect has been linked to the inheritance of microbiota (Giron et al., 2017).

Gut microbiota are known to have an important influence on nutrition and digestion (Chen et al., 2022; Dillon & Dillon, 2004) and can help insects to rapidly respond to plant secondary metabolites (Javal et al., 2023). These microbes are therefore likely to play a key role in diet change and niche expansion for herbivorous insects (Chu et al., 2013; Lange et al., 2023; Lu et al., 2016; Salem et al., 2020). Individuals can acquire their gut microbiota from various sources. Microbes can be spread horizontally via the environment, diet or contact with other individuals and/or vertically from one generation to the next (Bright & Bulgheresi, 2010; Giron et al., 2017; Paniagua Voirol et al., 2018; Shapira, 2016). Qi et al. (2024) showed that *Spodoptera frugiperda* can accumulate *Pantoea dispersa*, a prevalent endophytic bacterium in maize. The accumulated *P. dispersa* aided in the metabolism of anti-herbivore benzoxazinoid compounds, thereby improving the insect's growth. The authors also showed that *P. dispersa* could be passed onto the next generation through vertical transfer through *S. frugiperda* eggs (Qi et al., 2024).

Published studies on *S. litura* feeding on soybean (Bapatla et al., 2018), castor (Thakur et al., 2015), taro (Xia et al., 2020) and tomato (Dhayalan et al., 2025) highlight the functional diversity and contribution to nutrition and detoxification of their associated gut microbiota. Differences in microbial community composition between the studies demonstrate the impact of factors such as diet and environment on gut microbiota and also a potential pathway by which polyphagous insects such as *S. litura* could develop improved tolerance to poor quality hosts.

Having previously shown red clover to be a poor host to *S. litura*, I hypothesized that successive generations might become better able to tolerate the poorer quality host through inheritance of gut microbiota. To investigate this, I reared *S. litura* on red clover or an alternate control species, plantain, for two generations and compared the growth, feeding and mid-gut bacteria of second-generation larvae with that of individuals whose parents were raised on the same or the alternative plant host (Figure 4-1).

4.3. Methods

4.3.1. *Spodoptera litura* rearing

A laboratory colony of *S. litura* was generated from adults caught in light traps in Hamilton, New Zealand in late summer (March) 2023. For mating and egg-laying, adults were placed in fabric mesh cages (50 cm wide x 50 cm deep x 60 cm high) to mate and oviposit. They were provided with dampened 2-ply facial tissue (TORK Premium) or dental wick as a source of water and crumpled paper towels (TORK Xpress hand towel multifold) for refugia and laying surfaces.

Newly hatched larvae were transferred to 5 L plastic rearing containers with 15 cm x 8.5 cm holes cut in the lid and covered with fabric mesh inserts, for air flow. Clear plastic bags (35 µm thickness) were loosely placed over the mesh to maintain higher humidity in the containers. A layer of damp vermiculite (Exfoliators (Aust) Pty Ltd, Premium Grade 4, 3–6 mm) on the base of each container provided moisture and refugia. Freshly cut herbage from broad-leafed dock (*Rumex obtusifolius*), white clover (*Trifolium repens*), red clover (*T. pratense*), plantain (*P. lanceolata*) and chicory (*Chichorium intybus*), collected from nearby pasture, was placed in the containers every 1 to 2 days, for the larvae to feed on. This was done to prevent feed habituation and to maintain the ingestion of naturally occurring microbes present in the pasture ecosystem through successive generations. Uneaten food was removed regularly to maintain hygiene.

Two generations of colony *S. litura* were reared under ambient temperature (mean = 17.5°C, min. = 2.5°C, max. = 28.2°C) and light (light hours = 11 h 43’ – 10 h 35’) conditions on a laboratory bench. Thereafter colony rearing and experiments were conducted in a 25°C incubator with a 16:8 h light:dark cycle. Newly hatched larvae from the third colony generation were used

for the Offspring Performance Experiment described below and sixth generation larvae for the Offspring Mid-Gut Microbiota Experiment.

4.3.2. Offspring Performance Experiment

As a first step towards understanding potential intergenerational effects to *S. litura* of feeding on a poor host, red clover, compared with a good host, plantain, I conducted a laboratory experiment in which *S litura* were reared through two generations on each of the two pasture plant species. Offspring generation larvae were fed either the same host plant as their parents or on the alternative host (Figure 4-1). In this experiment I assessed the impact of parental diet on offspring performance by measuring the growth, feeding and survival of the offspring.

To rear the parent generation, 50 newly hatched, third generation colony-reared *S. litura* larvae were placed into each of six 5 L rearing containers. Larvae in three of the containers were supplied with freshly cut red clover (cultivar Relish) leaves and larvae in the other three containers with plantain (cultivar Ecotain). Fresh herbage obtained from individually potted plants was supplied as required until all the *S. litura* larvae had pupated. Plants were grown in 0.7 L plastic pots (11 cm W x 10 cm H) in potting mix (Dalton's grass and clover mix) and kept in a screenhouse at Ruakura Research Centre, New Zealand under natural light (light hours 10 h 35' – 9 h 35') and temperature (mean = 12.0°C, min. = -0.2°C, max. = +22.6°C) conditions. At eclosion moths were left in the rearing containers and supplied with water and crumpled paper towels acting as an egg-laying surface. I did not supply adults with a food source to avoid masking treatment effects through adult compensatory feeding. Eggs were monitored daily for hatching.

Newly hatched offspring generation larvae from red clover and plantain parent-generation containers were transferred to one of four clean rearing containers. Fifty offspring of larvae fed plantain were placed in one container and supplied with fresh plantain (P-Plantain treatment), and a further 50 were placed in a separate container and supplied with fresh red clover (P-Red clover treatment). Similarly, offspring of larvae fed red clover were placed in one of two containers and were fed either plantain (RC-Plantain treatment) or red clover (RC-Red clover) (Figure 4-1). Fresh herbage was supplied as required every 1–2 days.

After one week, 30 larvae were removed from each of the four rearing containers and placed in individual 30 mL plastic containers, without food, to void their gut contents overnight. Larvae were weighed and 20 from each of the four treatments were selected based on weight so that the average larval weight for each treatment was as similar as possible. Larvae across the four treatments weighed between 10 mg – 38 mg and were approximately third instar of six for this species.

The weighed larvae were then transferred to individual, labelled, 120 mL clear plastic specimen vials with 25 mL damp vermiculite in the base. They were fed excised leaf material from one of 10 assigned plantain or red clover plants, thus receiving food from the same plant throughout the following one-week period. Vials were placed in trays in randomised positions blocked by plant number. Fresh leaf material was supplied each day, and uneaten herbage was removed after 24 hours.

Larval feeding conversion efficiencies of 14-day old (approximately 5th instar) offspring generation larvae were measured over a 24-hour period as described in section 4.3.4. Larvae were subsequently fed ad libitum from the appropriate plant host species until they pupated. Pupae were weighed and their sex determined by examination under a stereo microscope. They were then returned to their individual vials and survival and the time to adult eclosion were recorded.

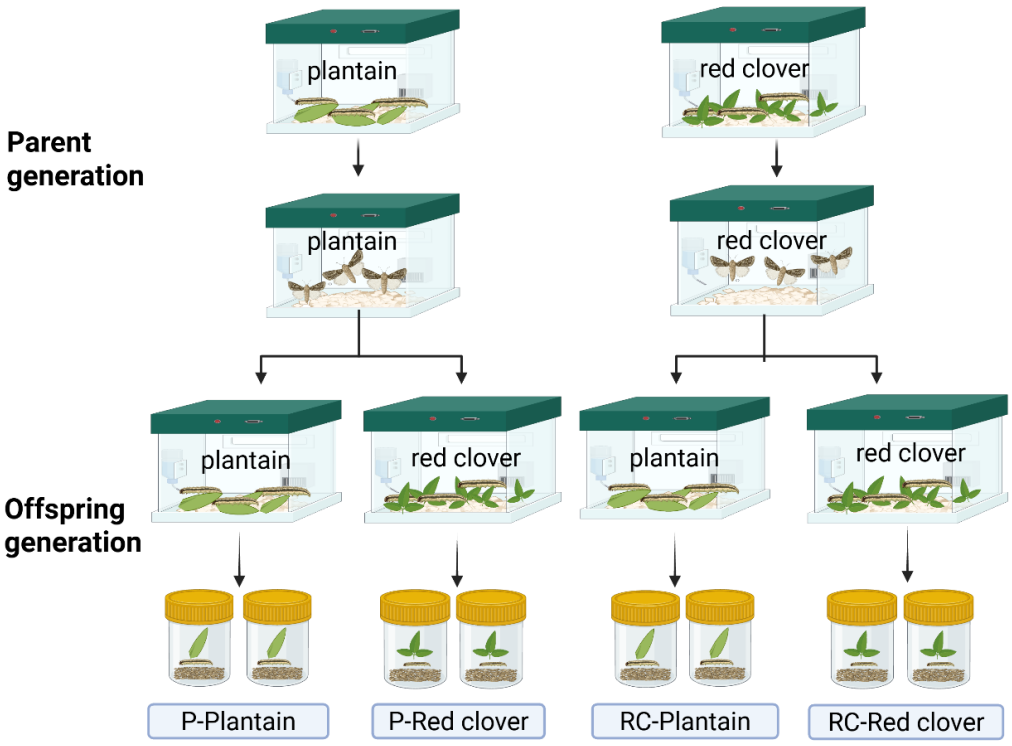


Figure 4-1 – Experimental setup for two experiments in which Spodoptera litura *larvae were reared for two generations on red clover or plantain foliage, and offspring were fed on the same or alternate plant species as their parents. P=parent generation fed plantain, RC=parent generation fed red clover. (Image created in https://BioRender.com).*

4.3.3. Offspring Mid-Gut Microbiota Experiment

A second, similar experiment was conducted to investigate if parental diet effects on offspring performance could be related to changes in gut microbiota, and to identify a potential pathway for intergenerational effects on *S. litura*. As per the previous experiment, second generation larvae were fed either the same host plant as their parents or on the alternative host (Figure 4-1). At late-instar the mid-gut was removed for comparison of gut microbiota between treatments using 16S sequencing.

To rear the parent generation, twenty newly hatched, 6th-generation laboratory reared *S. litura* larvae were placed in each of six 5 L rearing containers. They were held in a 25°C incubator with 16:8 h light:dark cycle and were fed with either red clover or plantain leaves through to pupation. As adults emerged, they were transferred to clean 5 L mating/laying containers with other adults from the same plant host treatment.

Newly hatched larval offspring from these adults were transferred to 1 L plastic containers with damp vermiculite, with 10 larvae per container. As in the Offspring Performance Experiment, offspring from each of the parent diet treatments were assigned to one of the two offspring diet treatments (Figure 4-1). Larvae hatching from the same egg batch were assigned evenly to each of the two offspring treatment groups, and larvae in the same offspring rearing container were all from one egg batch. New containers of larvae were set up over a 4-day period, with hatching date noted. Offspring larvae were provided with leaves excised from potted red clover or plantain plants as required.

After 13 days, offspring generation larvae were transferred from the rearing containers to individual 120 mL specimen vials for a feeding conversion efficiency evaluation (described in section 4.3.4). This was set up over 4 days to account for the different larval hatch dates. A mite infestation during the experiment reduced egg hatching and larval survival in the offspring generation. As a result, there was uneven replication between treatments, with 11 replicate larvae in the P-Red clover treatment compared with 22–27 for the other three treatments. Larvae were selected to minimise differences in larval weights between treatments. Following the feeding efficiency assessment a subsample of the larvae was selected for dissection and gut-microbiota sequencing, as described in sections 4.3.5 and 4.3.6.

4.3.4. Feed conversion calculations

For both experiments the efficiency with which 14-day old larvae processed food into biomass was calculated using formulae described by Waldbauer (1968) and methods described by Jensen et al. (2026; Chapter 3). Larvae were starved for 24 hours prior to the feeding efficiency measurement period, then weighed and provided with a known weight of food. After 24 hours of

feeding larvae, remnant food and any frass produced over that period were weighed. The proportion of ingested food that was digested (Approximate Digestibility = AD) was calculated as the weight of food eaten minus the frass weight, divided by the weight of food eaten. The proportion of food eaten that was turned into body weight (Conversion Efficiency of Ingested food = ECI) was calculated by dividing the increase in larval weight by the weight of the food eaten (ingested). The Efficiency of Conversion of Digested food (ECD) is the larval weight gain divided by the amount of food eaten less the amount of frass produced. All calculations used wet weights (Jensen et al., 2026).

An additional sample of leaf material from each plant was added to a vial with no larva inside to monitor herbage moisture loss over the 24-hour feeding period. These samples were then oven-dried and re-weighed to calculate dry matter content, and the values obtained were used estimate the dry weight of food eaten by the larvae.

4.3.5. Larval gut dissection

Five larvae per treatment were selected for sequencing of gut bacterial microbiota. Where possible, larvae from different egg batches and rearing containers were chosen and those that were noted to be prepupal were avoided. Larvae were starved for 24 hours prior to dissection to clear their gut.

Larvae were placed in individual plastic zip-lock bags and surface sterilised by soaking for 45 s, with occasional agitating, in 70% ethanol. They were then rinsed three times in sterile water. Larvae were severed near the second pair of thoracic legs and approximately 2–3 mm from the terminal abdominal segment with a sterile scalpel and the mid-gut carefully removed with sterile forceps. These were placed in separate sterile Eppendorf tubes with 60% glycerol solution and frozen in a -80°C freezer.

4.3.6. Gut microbiota

Metagenomic DNA was extracted from individual larval mid-gut samples using the Qiagen DNeasy® PowerSoil Pro Kit according to the manufacturer's instructions. Cell lysis and homogenization was performed by vortexing in PowerBead® Pro Tubes with Solution CD1 for 10 minutes. The extracted DNA was eluted in 100 µl of elution buffer. DNA quality and quantity were measured using a Nanodrop® spectrophotometer (ThermoFisher Scientific, NZ) and Qubit® fluorometer (ThermoFisher Scientific, NZ) respectively. Samples were stored at -20°C until further use.

DNA samples were diluted to 30 ng/µl with sterile dH_2O and submitted to Custom Science Ltd (New Zealand) for sequencing targeting the 16S rDNA V3-V4 region for microbiota analysis.

The universal primer set 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5' GGACTACHVGGGTWTCTAAT-3') was used for amplification and amplicon sequencing performed by Illumina MiSeq. The 338F/806R primers were selected for amplification due to their high coverage of bacterial taxa and proven performance in Illumina MiSeq workflows (Chen et al., 2023; Du et al., 2023). The original image data were analysed using BCl2fastq (v2.17.1.14) for base calling and preliminary quality analysis.

4.4. Statistical analyses

All statistical computations were performed using R version 4.5.1 (R Core Team, 2025).

4.4.1. Larval performance

Larval, pupal and food weight data were analysed using linear mixed models (LMMs) estimated by restricted maximum likelihood (REML). Feeding efficiency calculations were analysed using a generalised linear mixed model (GLMMs) (R package glmmTMB) assuming a beta distribution based on the parameterization of Ferrari and Cribari-Neto (2004) and logit link function. Days to pupation and adulthood were also analysed by GLMM, using a zero-truncated negative binomial type-2 response and a log link. Survival (binomial response) was modelled using bias-reduced logistic regression using brglm2 (R package brglmFit). For all assessments, parent and offspring diet were included as fixed effects. For the Offspring Performance Experiment, where larvae were fed from the same plant throughout the feeding assessment period, plant number was included as a random effect, and the weight of the larva when it was transferred to an individual vial was included as a covariate. Sex was included in the initial model but dropped if not significant.

For the Offspring Mid-Gut Microbiota Experiment herbage was sourced randomly from multiple plants therefore plant number could not be included as a random effect, and larvae were not reared through to pupa so sex could not be determined. However, in this experiment the egg batch from which larvae had hatched was noted and this was included as a random effect. For both experiments data from larvae noted as prepupal or that moulted during the feeding conversion evaluation period were omitted from those analyses. Data were log transformed where necessary to stabilise the variance, but back-transformed means and standard errors are presented throughout this paper.

For model validation, graphical model validation tools were used to test the underlying assumptions of variance homogeneity and normality. For LMMs the significance of the fixed terms was assessed using Type II F-tests based on Satterthwaite's approximation for degrees of freedom (R package lmerTest; Kuznetsova et al., 2017). For GLMMs I used Type II Wald χ^2 (R

package car; Fox & Weisberg, 2011) and likelihood ratio tests based on single-term deletion (drop1, test = “Chisq”) were used for survival analyses. When results were statistically significant I conducted a multiple-comparison procedure based on the significance of estimated marginal mean differences (R package emmeans; Lenth et al., 2022). *P*-values were adjusted for multiple comparisons using a Bonferroni correction or, for the survival analysis, a Holm adjustment.

4.4.2. Microbial community analysis

Initially the raw reads were trimmed, paired, error-corrected, and associated with representative Amplicon Sequence Variants (ASVs) and chimaera-filtered using the DADA2 pipeline (Callahan et al., 2016) following the pipeline tutorial as of 2022 (https://benjjneb.github.io/dada2/tutorial.html). All ASVs with less than 10 copies were discarded, to avoid transient DNA and possible sequencing errors. The taxonomic lineage of the 16S ASVs were obtained using the Qiime version 1.9.0 "assign taxonomy" program with standard parameters (Caporaso et al., 2010) and the Silva database version 138 (Quast et al., 2013) as a reference database.

To check these results, the ASVs were searched against the Bacteria and Archaea 16S ribosomal RNA database of the NCBI RefSeq Targeted Loci Project. As a further check, reads were joined using PEAR (version: v0.9.6, parameters: default) and ASVs generated in the same manner as before, this time using the joined reads treated as single end reads. All methods resulted in near identical annotation.

Alpha diversity metrics (species richness and diversity) were estimated using Observed ASVs, Shannon and Simpson diversity indices. Initial treatment effects were assessed by Kruskal-Wallis and Wilcoxon non-parametric rank sum tests, which provide conservative, distribution-free comparisons of group differences. Effect size and direction were calculated using Cliff’s delta. Having established group differences using non-parametric tests, generalised linear models (GLMs) were then fitted for each diversity metric to evaluate the effects of parent and offspring diet. Parametric models allow the testing of specific hypotheses by incorporating multiple predictors, interactions and covariates. Shannon diversity was analysed using a linear model (Type II ANOVA) with log-transformed sequencing depth as a covariate, Simpson diversity using beta regression and Observed richness using a Poisson regression with log offset for sequencing depth. Analyses were calculated using the R packages phyloseq, dplyr, car, betareg and effsize.

Following alpha diversity analysis, the ASV table was collapsed to genus level for analysis of microbial community composition. ASVs were normalised to relative abundances to account for differences in sequencing depth between samples. Bray–Curtis distances were calculated

using the vegdist function (vegan R package) and checked for within-treatment variation in dispersion (PERMDISP) using betadisper. I then used PERMANOVA (Permutational Multivariate Analysis of Variance) to test for differences in genus-level microbial community composition between treatments (beta diversity). Analyses were implemented in adonis2 (vegan package**)**. Significance was assessed using 999 permutations.

Principle Coordinates Analysis (PCoA) was performed using the pcoa function (ape package). The first two axes, explaining the greatest proportion of variation, were visualized using ggplot2 with 95% confidence ellipses.

To evaluate whether treatment influenced the relative abundance of the two most common genera in my dataset (*Enterococcus* and *Erysipelatoclostridium*), I fitted beta-binomial regression models using maximum likelihood (bbdml function in the corncob R package) (Martin et al., 2020). For each genus, effects of parent diet, offspring diet and their interaction were modelled in the abundance (mean) sub-model, with the dispersion sub-model specified according to the best-supported structure for each genus. Model significance was assessed using Wald chi-square tests (waldchisq). Where appropriate, I also performed parametric bootstrap versions of these tests to confirm the robustness of the result. For *Enterococcus*, I conducted a joint Wald test of the parent x offspring diet interaction across both the mean and dispersion components, whereas for *Erysipelatoclostridium* dispersion was modelled additively and interaction effects were tested in the mean component only.

4.5. Results

4.5.1. Larval Performance Experiment

4.5.1.1. Offspring generation - larval and pupal weights

Larvae feeding on plantain were on average significantly heavier at 15 days old than those feeding on red clover ($F_{1,19} = 11.0$; $p = 0.004$; Figure 4-2A). Larvae were also significantly affected by what their parents had eaten; larvae whose parents fed on plantain were heavier than those whose parents had fed on red clover ($F_{1,52} = 8.3$; $p = 0.006$). However, post hoc pairwise comparisons within each offspring diet treatment showed that this was statistically significant ($p < 0.05$) only for offspring feeding on red clover.

At pupation, average weights were significantly larger for individuals whose parents had fed on plantain ($F_{1,52} = 23.8$; $p < 0.001$), but there was no statistically significant effect of offspring diet ($F_{1,17} = 1.6$; $p = 0.222$) (Figure 4-2B).

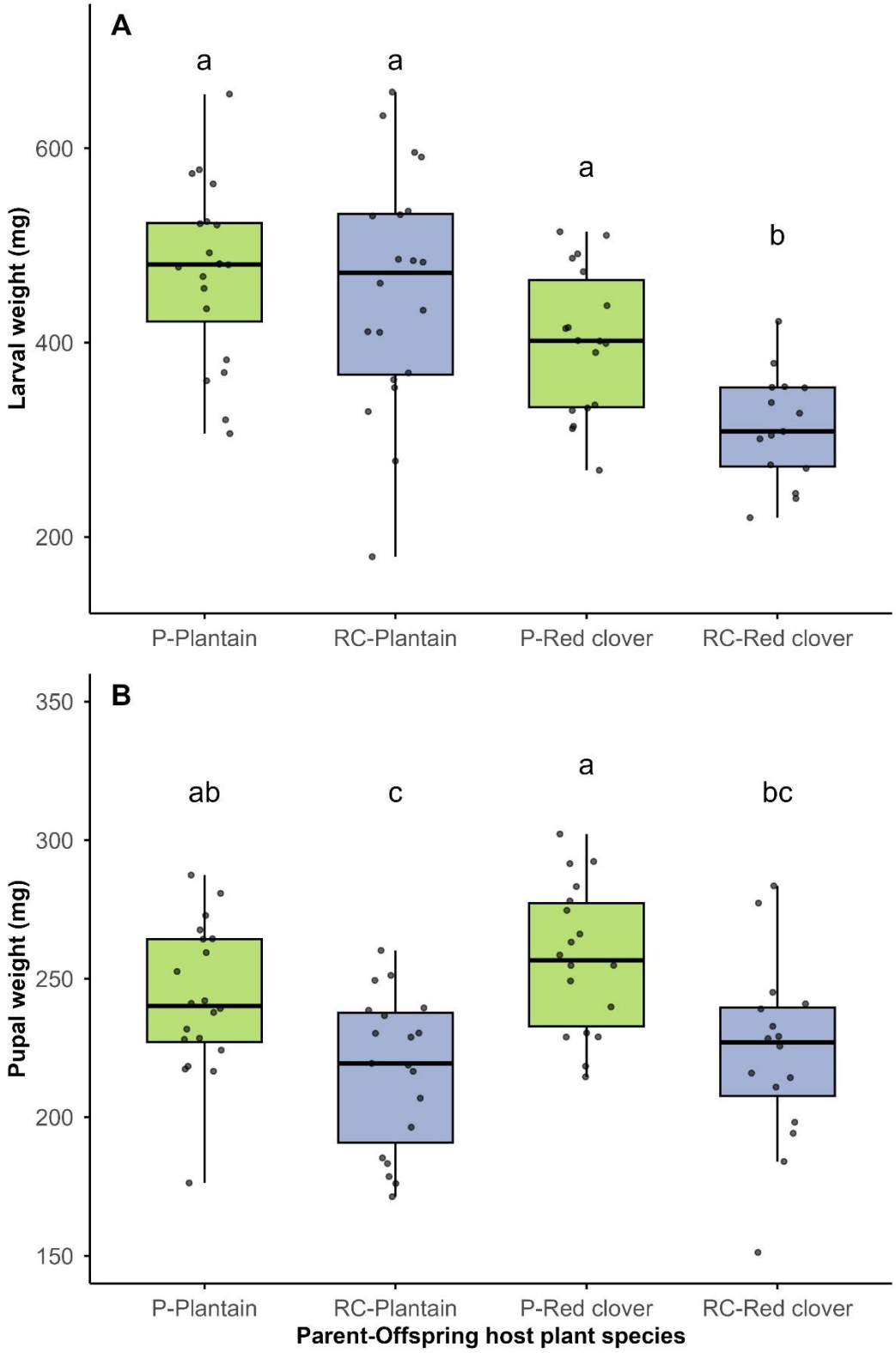


Figure 4-2 – (A) Weight (mg) of 15-day old Spodoptera litura *larvae and (B) pupae that had been fed either plantain or red clover (offspring diet) and whose parents had fed on either plantain (P) or red clover (RC) (parent diet).Green boxes represent offspring of parents fed plantain, and blue boxes for parents fed red clover. Letters in common show no statistically significant difference (*p > 0.05*). In all boxplots, horizontal lines correspond to the median, 1st and 3rd quartiles and vertical lines show smallest and largest values no further than 1.5*Interquartile range (IQR).*

4.5.1.2. Offspring generation larval feeding

Larval weight change, the amount of herbage eaten (fresh weight) and the amount of frass produced over a 24-hour period were measured when larvae were two weeks old. These data were used to calculate the efficiency with which the larvae converted the food to body mass (Figure 4-3). Treatment effects on the weight of herbage eaten over this period were similar to larval weights (Supplementary data; Table 8-1). Larvae feeding on red clover ate significantly less than those eating plantain ($F_{1,19} = 45.3$; $p < 0.001$). The effect of parent diet on consumption by the offspring generation larvae was not statistically significant when measured using herbage wet weights ($F_{1,52} = 2.5$; $p = 0.121$) but when analysed using estimated dry weight consumption data the differences were statistically significant ($F_{1,52} = 5.8$; $p = 0.020$; Supplementary; Table 8-1). Offspring of parents fed red clover ate significantly less (dry weight) than those of parents fed plantain ($p < 0.05$).

Larval feeding efficiencies were affected by both what larvae were eating (offspring diet) and what their parents had eaten (Figure 4-3). Larvae feeding on red clover converted a greater proportion of the food eaten (ingested) to body weight (i.e. had a higher ECI; $X^2_{1, N=73} = 244.4$, $p < 0.001$), digested a greater proportion of that food (i.e. higher AD; $X^2_{1, N=73} = 419.4$, $p < 0.001$) and converted a greater proportion of the digested food to body weight (i.e. ECD; $X^2_{1, N=73} = 4.8$, $p = 0.029$) compared with larvae feeding on plantain. For all three measures the offspring of parents fed on plantain had higher feeding efficiencies than those that fed on red clover (ECI: $X^2_{1, N=73} = 11.9$, $p < 0.001$; ECD: $X^2_{1, N=73} = 8.7$, $p = 0.003$; AD: $X^2_{1, N=73} = 7.1$, $p = 0.008$; Figure 4-3).

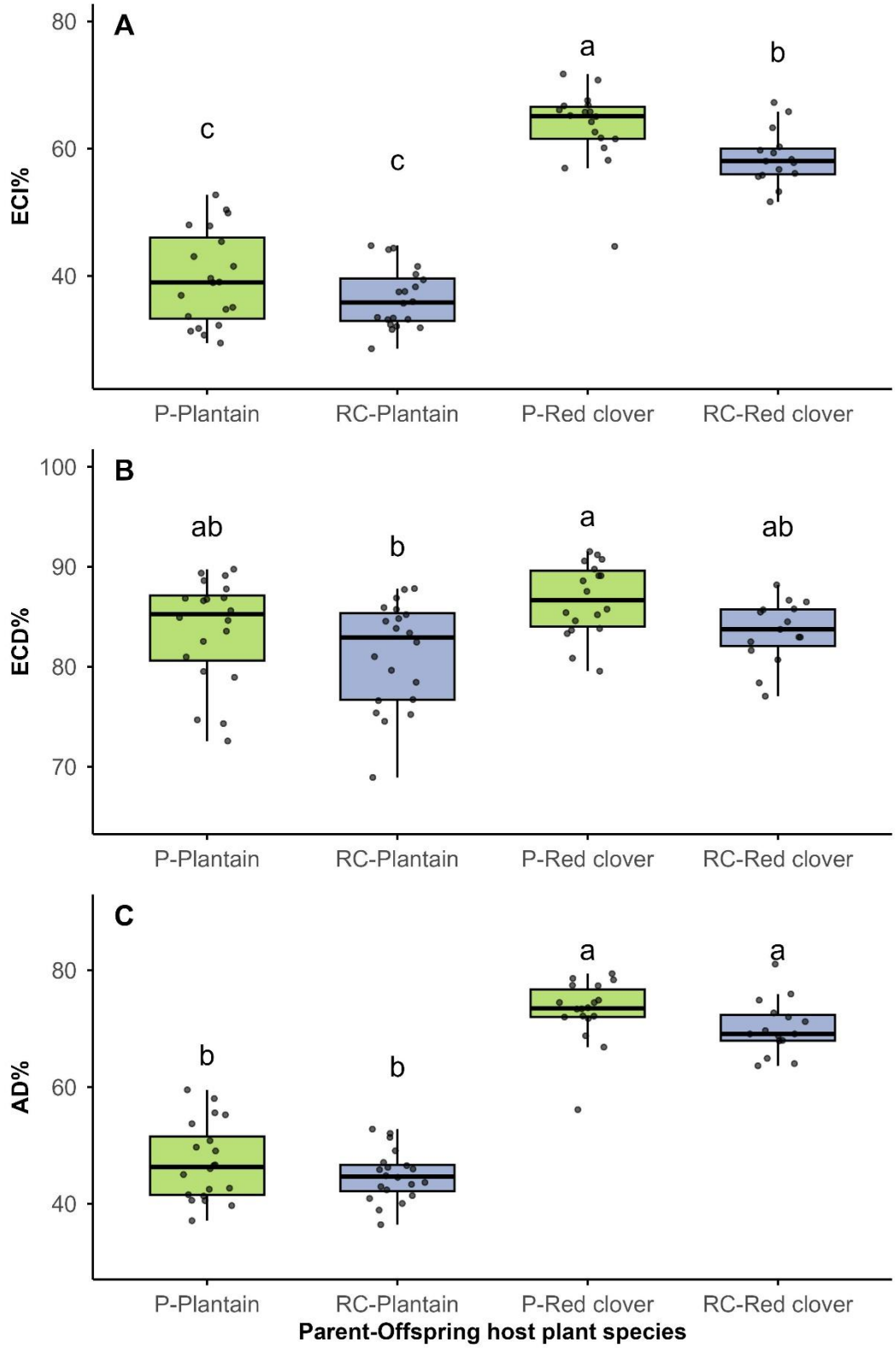


Figure 4-3 – Feeding efficiency measures for 2-week-old Spodoptera litura *larvae fed on plantain (P) or red clover (RC) (offspring diet), and whose parents had fed on the same or alternate host plant species. Green boxes represent offspring of parents fed plantain (P), and blue boxes for parents fed red clover (RC). ECI = Efficiency of Conversion of Ingested food, ECD = Efficiency of Conversion of Digested Food, AD = Approximate Digestibility. Letters in common show no statistically significant difference ($p > 0.05$).*

4.5.1.3. Offspring generation – pupation, eclosion and survival

The mean time (days) to pupation and to adulthood was not significantly affected by parent or offspring diet treatment, nor by an interaction between the two ($p > 0.05$; Supplementary Table 8-2).

Only 75% (15/20) individuals in the RC-Red clover treatment survived to adulthood, compared with 90, 95 and 100% from the P-Red clover, P-Plantain and RC-Plantain treatments respectively. Statistical analysis indicated borderline evidence for a parent × offspring diet interaction ($X^2_{1, N=80} = 3.4$, $p = 0.065$) but post-hoc comparisons did not show significant pairwise differences.

4.5.2. Offspring Mid-Gut Microbiota Experiment

4.5.2.1. Larval survival, weights and feeding

Larval mortality occurred in three of the four treatments during the 3-day period over which feeding efficiency was measured. Thirty six percent (4/11) of individuals died in the P-Red clover treatment, 19% (5/27) in RC-Plantain, 18% (4/22) from RC-Red clover and 0% (0/22) from P-Plantain. Statistical analysis showed a significant parent × offspring diet interaction ($X^2_{1, N=82} = 5.3$, $p = 0.021$) but post-hoc comparisons did not show significant pairwise differences.

Offspring larvae feeding on red clover were significantly smaller than those feeding on plantain ($p < 0.05$; Figure 4-4). In this experiment, however, there were no statistically significant effects ($p > 0.05$) of parent diet on any of the variables measured by the end of the 3-day feeding conversion analysis period. Feeding quantity and efficiency results are provided in Supplementary; Table 8-3.

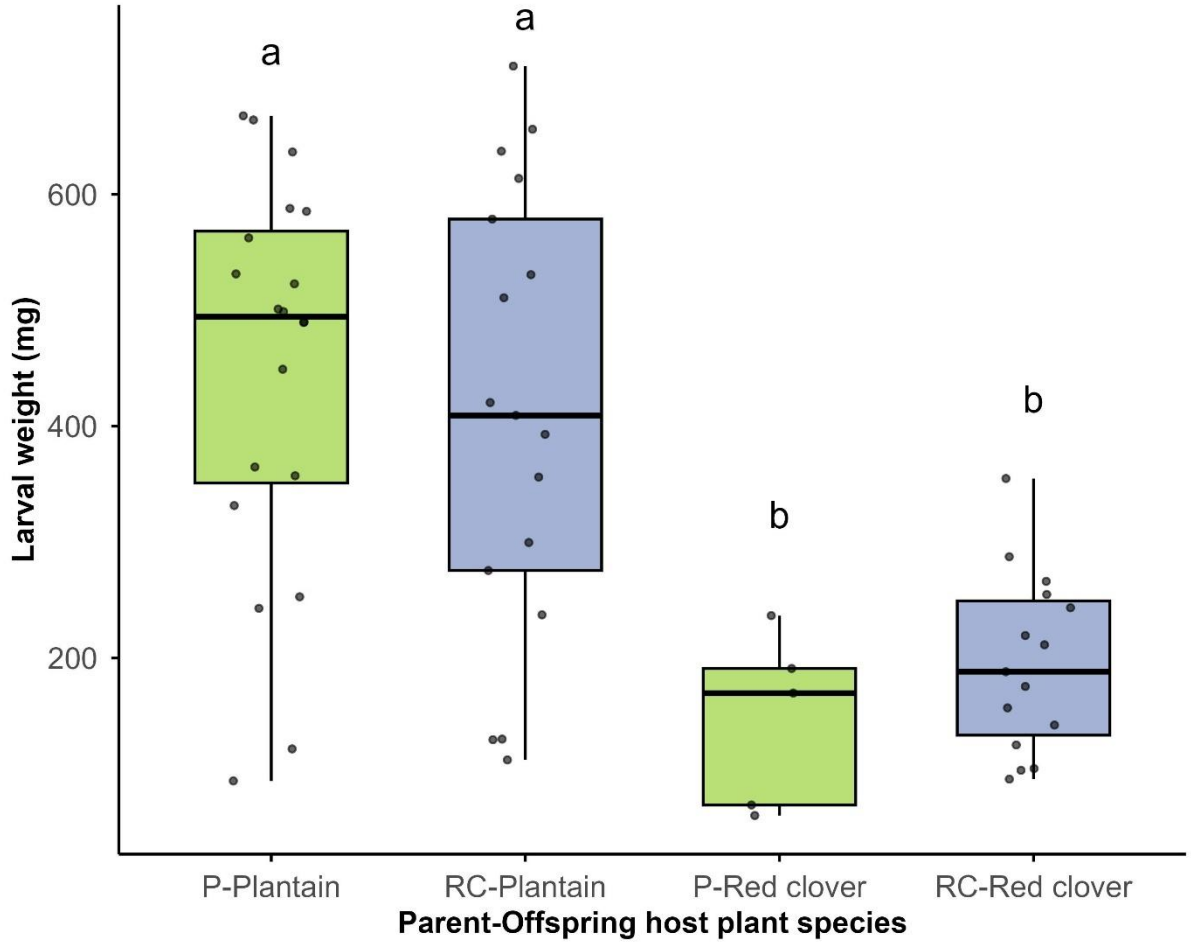


Figure 4-4 – Weight (mg) of 16-day old Spodoptera litura *larvae fed either plantain or red clover and whose parents fed on plantain (green boxes) or red clover (blue boxes) in an Offspring Mid-Gut Microbiota Experiment. Letters in common show no statistically significant difference (p > 0.05).*

4.5.2.2. Gut microbiota

Sequencing quality was high, with Q30 scores ranging from 86% to 89% across samples, indicating >99.9% base call accuracy.

Parent diet had a statistically significant ($p < 0.05$) effect on the richness and evenness of larval gut communities (alpha diversity), whereas offspring diet had no significant effect ($p > 0.05$). A Wilcoxon rank-sum test (W = 26, $p = 0.045$) and Hodges–Lehmann estimate (HL = -0.259; 95% CI = -0.504, -0.017) indicated that larvae whose parents ate red clover had higher Shannon diversity. Median Shannon values suggested this difference corresponded to approximately 1.3-fold greater diversity, and Cliff's delta (-0.527) indicated a large effect size. Linear modelling of Shannon alpha diversity (and complementary beta regression for Simpson

and Poisson regression for observed richness) confirmed a significant effect of parent diet (Shannon: $F_1 = 6.2$, $p = 0.024$; Simpson: $X^2_{1, N=21} = 4.4$, $p = 0.036$; Poisson: $X^2_{1, N=21} = 4.9$, $p = 0.027$), with no effect of offspring diet or parent × offspring interaction ($p > 0.05$). Post hoc pairwise comparisons showed that larvae from the P–Red clover treatment had lower Shannon diversity than those from RC–Red clover, whereas no significant differences were detected among larvae feeding on plantain regardless of parent diet (Figure 4-5).

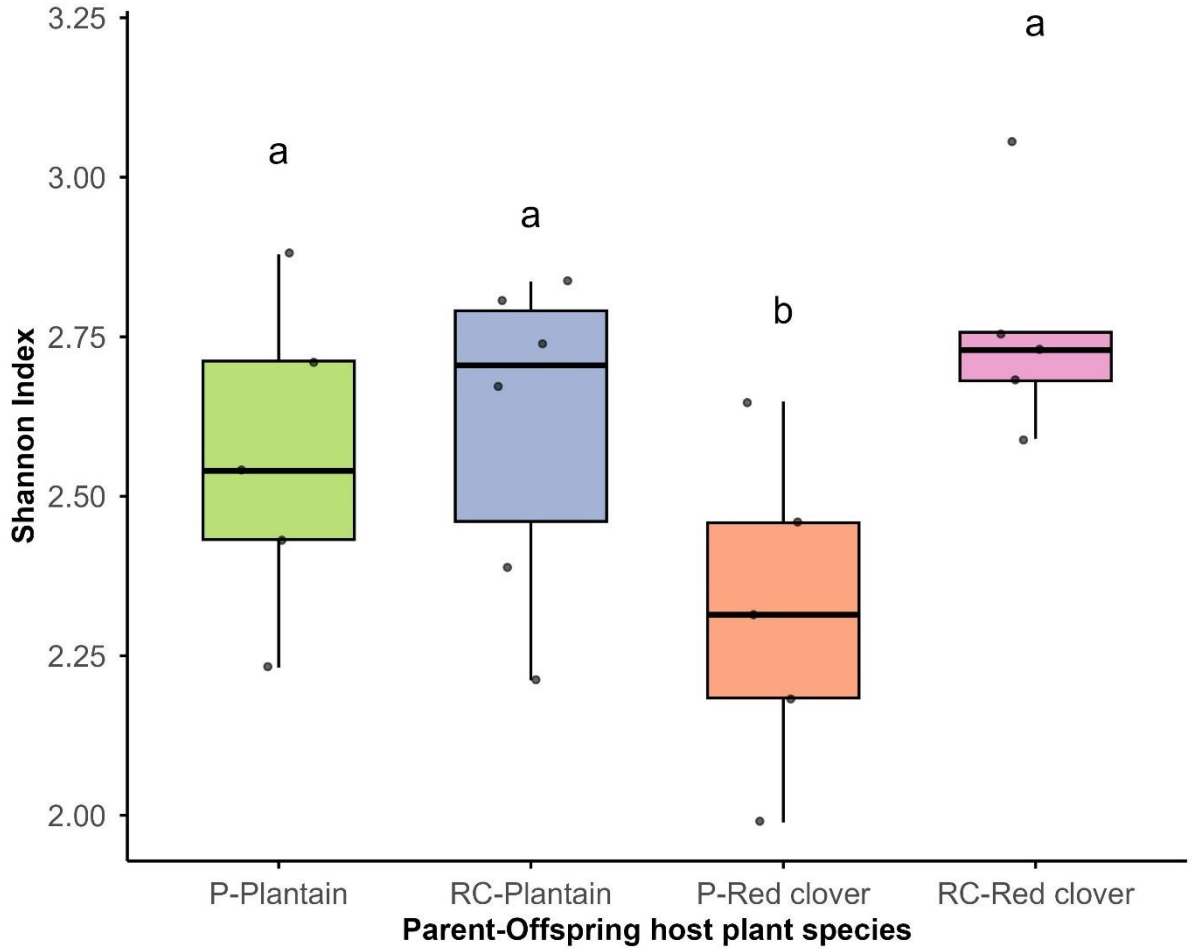


Figure 4-5 – Shannon alpha diversity of gut microbiota of 2-week-old Spodoptera litura *larvae fed either plantain or red clover, and whose parents fed on plantain or red clover. Letters in common within an offspring diet treatment show no statistically significant difference (p > 0.05).*

ASVs were collapsed to genus level and converted to relative abundance prior to beta-dispersion analysis. Relative abundance of the top ten genera is shown for each larva in Figure 4-6. Permutation tests on Bray-Curtis distances showed non-significant differences in dispersion ($p > 0.05$). This confirmed that group variances were comparable and therefore the assumption of

homogeneity was met for PERMANOVA. PERMANOVA (adonis) showed no statistically significant main effects of parent diet (p = 0.250) or offspring diet (p = 0.180), but there was a significant interaction between the two factors (F_1 = 4.8, R^2 = 0.186, p = 0.022). Non-adjusted pairwise post-hoc comparisons showed that larvae in the RC-Red clover treatment differed from all other treatments (unadjusted p = 0.039 – 0.048; pairwise R^2 = 0.37 – 0.40), but these comparisons were not significant after BH adjustment (q = 0.096). Visualisation through PCoA ordination (Figure 4-7) did not show separated treatment clusters, however the different orientation of the confidence ellipse for the RC–Red clover treatment is consistent with the significant interaction detected by PERMANOVA. This suggests that while overall community composition overlapped among treatments, there was a directional compositional shift for larvae in the RC-Red clover treatment compared with the other treatments.

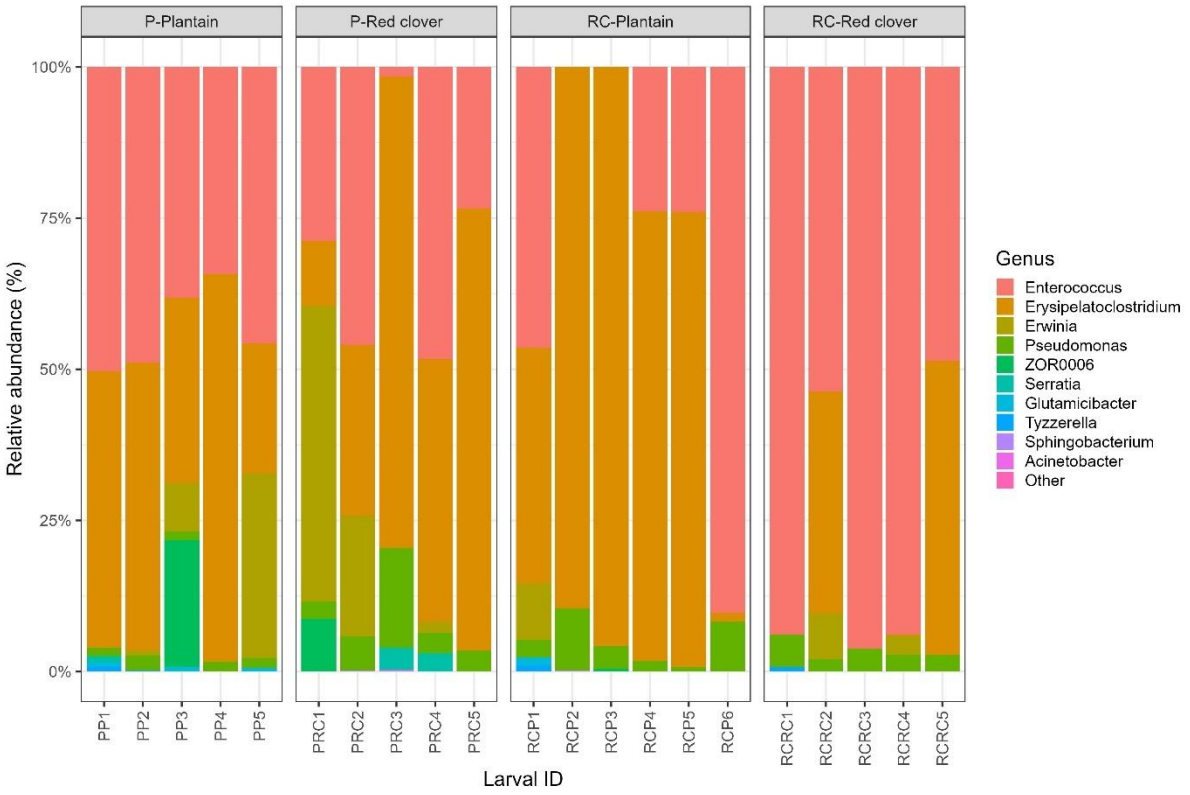


Figure 4-6 – Percentage relative abundance of top ten mid-gut microbial genera for each Spodoptera litura *larva. Treatment labels indicate parent-offspring generation host plant treatments. P = parent generation fed on plantain, RC = parent generation fed on red clover.*

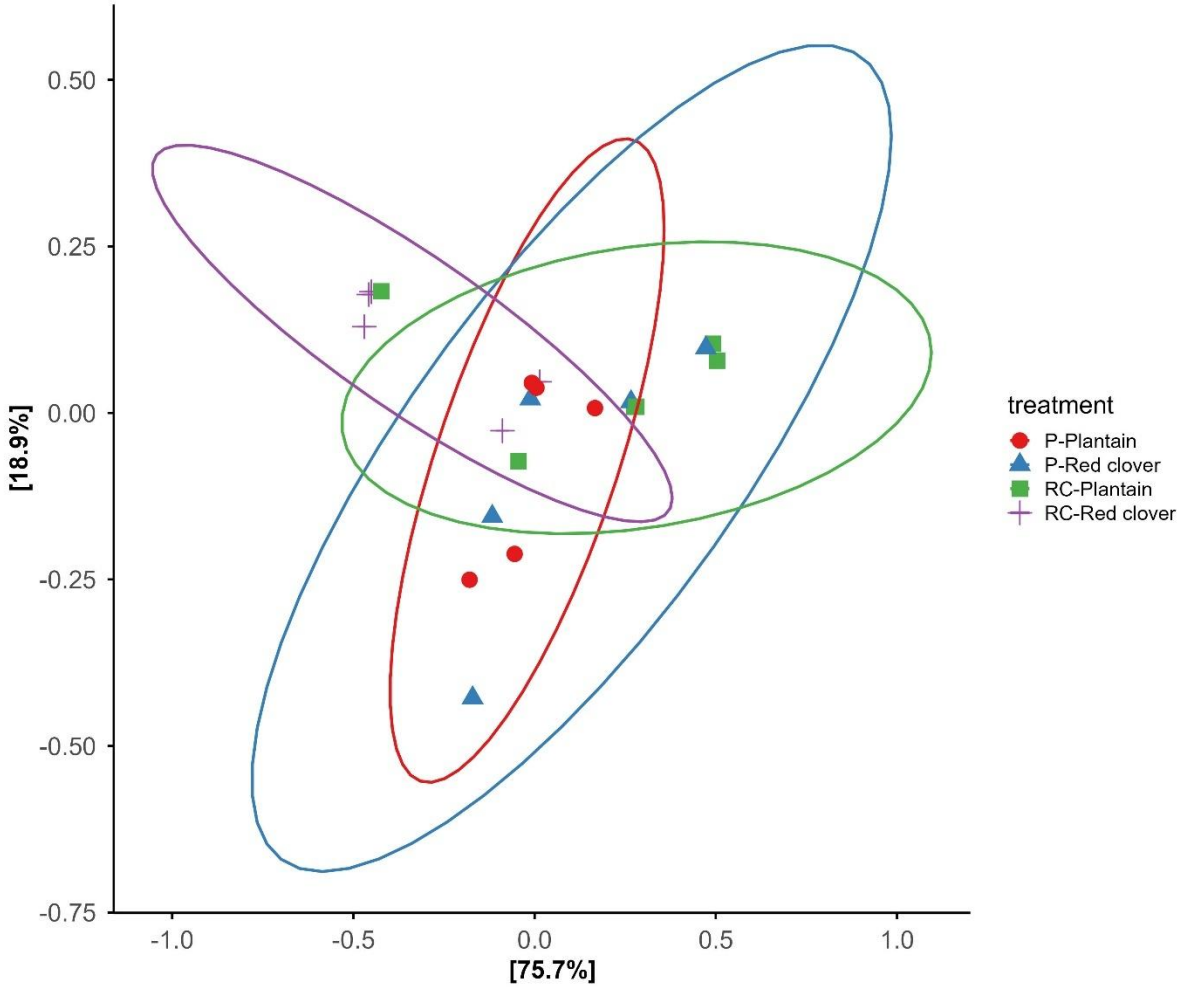


Figure 4-7 – PCoA of Bray-Curtis dissimilarities computed from genus-level relative abundances of Spodoptera litura *larval mid-gut microbiota. Ellipses show 95% t-based confidence regions of group centroids; they visualise dispersion and do not imply significance.*

The most abundant bacterial taxa were *Enterococcus* and *Erysipelatoclostridium* spp., together comprising over 65% of the total reads in all but one larva (larva PRC1; Figure 4-6). None of the *Erysipelatoclostridium* and only 25 of 119 *Enterococcus* ASVs could be identified to species level. The *Enterococcus* species identified were either *Enterococcus casseliflavus* or *Enterococcus flavescens;* these are now considered to be synonyms (Naser et al., 2006). Other bacterial taxa were sparse in comparison and included *Erwinia iniecta* (25 ASVs) and *Pseudomonas poae* (18 ASVs). The trimmed raw ASV table with matched taxa is available in Figshare (Jensen, 2026).

There was a statistically significant parent x offspring diet interaction affecting the relative abundance of *Enterococcus* spp. (mean + dispersion Wald test: $X^2_{2,\ N=21} = 30.6$, $p < 0.001$). Predicted mean abundance was highest when both parent and offspring were fed red clover

(77%; 95% CI = 57 – 90%), whereas all other diet combinations had substantially lower abundances (P-Plantain = 43%, P-Red clover = 28%, RC-Plantain = 21%). Conversely, larvae from the RC-Red clover treatment had marginally lower abundance of *Erysipelatoclostridium* spp. (mean = 11%, 95% CI =2 – 45%) compared with other treatments (P-Plantain = 42%, P-Red clover=46%, RC-Plantain= 55%). *Erysipelatoclostridium* spp. relative abundance was also significantly affected by a parent x offspring diet interaction (mean + dispersion Wald test: $X^2_{1, N=21} = 4.3$, $p = 0.039$).

4.6. Discussion

This study showed that host plant species has a significant intergenerational impact on *S. litura* performance. Larvae whose parents had fed on the poorer host species, red clover, were smaller and had reduced feeding efficiencies compared with those whose parents fed on plantain. This was true both for offspring feeding on plantain and for those on red clover, a result that was contrary to my hypothesis that larvae would be better able to utilise the host that their parents fed on.

As in my previous research (Jensen et al., 2026), larvae feeding on red clover digested a greater proportion of their ingested food (AD) and converted their ingested food more efficiently to body mass (ECI) than those feeding on plantain. However, the offspring of larvae that had fed on red clover had reduced feeding efficiencies, including ECD (the efficiency with which digested food was converted to body mass), regardless of the offspring larval diet. In addition to changes in feeding efficiency, parent diet significantly impacted the dry weight of food eaten. Together, these effects significantly reduced the body weights of offspring of larvae that were fed red clover.

Digestive enzymes and gut microbiota are key factors in nutrient acquisition and counteracting plant defence chemicals and they can vary in response to dietary challenges (Zhang et al., 2024). Gut microbial communities have been shown to differ with diet for some herbivorous lepidopteran species including *Trichoplusia ni* (Leite-Mondin et al., 2021), *Helicoverpa armigera* (Gayatri Priya et al., 2012), *Spodoptera frugiperda* (Jones et al., 2019; Yuning et al., 2022) and *S. litura* (Xia et al., 2020). However, other studies have found marked variation between individuals with little to no correlation with plant host (e.g. Minard et al., 2019). I did not find any statistically significant offspring diet treatment differences in midgut microbiota between larvae feeding on red clover and those feeding on plantain. However, I did show a statistically significant effect of parent diet on alpha (Shannon) diversity and a significant parent x offspring diet interaction effect on beta diversity.

Analysis of alpha diversity helps us to understand the complexity of the microbial ecosystem within individual samples. The Shannon alpha diversity index accounts for both the richness and evenness of the community. The low Shannon alpha diversity amongst larvae in the P-Red clover treatment therefore indicates that these larvae had fewer bacterial species and/or their communities were dominated by a few species, but this dominance was uneven between individuals. Beta diversity explains the diversity between samples. The low beta diversity for the RC-Red clover treatment show that these larvae had similar mid-gut bacterial communities to one another, while larvae in the other treatments were more variable. Further analysis at the genus level showed that RC-Red clover mid-gut microbiota were strongly dominated by *Enterococcus*. Together, these results suggest that feeding on red clover can reduce gut microbial diversity within each larva but in an unpredictable way, as shown in the P-Red clover treatment. This can then allow for dominance by inherited microbial taxa such as *Enterococcus* in the successive generation, as seen in the RC-Red clover treatment. In contrast, plantain did not select for a single dominant microbial genus, allowing *S. litura* to maintain higher levels of gut microbial diversity both within and between individuals.

Enterococcus taxa are commonly associated with Lepidoptera both in the field and laboratory (e.g. Chen et al., 2016; Deguenon et al., 2021; Gayatri Priya et al., 2012; Thakur et al., 2015). Brinkmann et al. (2008) found an *Enterococcus* phylotype to be the metabolically dominant bacterium inside the gut of *Manduca sexta* feeding on tobacco. They also showed it to be associated with and metabolically active on eggs, thus demonstrating a clear pathway for vertical transmission from mothers to larvae for that species. The functional effects of *Enterococcus* in lepidopteran guts have not been fully established, but studies indicate that its impact on insect growth and metabolism varies between strains, insect host species and life stages, and dietary conditions. Chen et al. (2022) showed that the addition of some *Enterococcus* isolates to fall armyworm diet gut improved performance but only on a suboptimal pinto bean artificial diet and only in younger instars. Mason et al. (2022) found that some *Enterococcus* and *Enterobacter* isolates helped with utilization of poor diet for *S. frugiperda* but not with other more optimized diets. However, under identical conditions the *Enterococcus* isolate was antagonistic to *S. exigua*. *Enterococcus* strains such as *E. casseliflavus* have been shown to enhance insecticide resistance (e.g. Hu et al., 2022; Xia et al., 2018). Some researchers have noted an association between feeding on a diet high in toxins and gut microbiota dominated by *Enterococcus* spp., including *E. casseliflavus,* and suggest a potential role in detoxification (Deguenon et al., 2021; Vilanova et al., 2016). I found no evidence to support this theory for *S.*

litura, given the poorer performance of larvae in the RC-Red clover treatment despite their significantly higher proportion of *Enterococcus* taxa.

My study linked a reduction in *S. litura* fitness with lower gut microbial diversity and community dominance when larvae feed on red clover for multiple generations. Associations between reduced fitness and lower microbial diversity or community dominance have been shown for other systems (e.g. Krams et al., 2017; Ørsted et al., 2022). Interactions between gut microbiota and insect fitness are bidirectional. Microbes can affect nutrition, immunity, reproduction, pathogen resistance and stress tolerance, and reciprocally, changes in an insect's physiology and immune status can influence their gut microbiota (Lange et al., 2023; Shapira, 2016; Zhang et al., 2024). This suggests multiple, interacting pathways by which feeding on a poor host could reduce the resilience and adaptability of *S. litura* and the population-level impacts that would occur if this continued over successive generations (Figure 4-8). Feeding on low-quality hosts is often associated with lower body weights and reduced reproductive potential (Awmack & Leather, 2002). Smaller females may produce fewer or lower quality eggs (Aceituno-Medina et al., 2024; Boggs & Freeman, 2005) thereby reducing the number, viability and/or performance of their offspring (Mousseau & Dingle, 1991). Although I did not measure parent generation pupal weight in this experiment, and there was no significant effect of offspring diet on pupal weights, my previous research has shown that feeding on red clover can significantly reduce *S. litura* pupal weights (and therefore adult weights; Nestel et al., 2016) and the number of eggs laid per female (Chapter 3; Jensen et al., 2026). Alongside this reduced fitness and consequent possible loss of genetic diversity, an over-dominated, low-diversity gut microbial community is likely to further limit the insect's resilience and adaptability (Henry et al., 2021; Lange et al., 2023).

While my study results were counter to my hypothesis that successive generations of *S. litura* might become better able to tolerate red clover through inheritance of gut microbiota, these results must be verified through field-trials. Gut microbial communities are also affected by environment and have been shown to differ between laboratory and field-caught insects (Staudacher et al., 2016). Furthermore, my laboratory study was only conducted over two generations. *Spodoptera litura* larvae and adults are highly mobile and are likely to encounter multiple plant hosts within and between generations. My laboratory study illustrates a worst-case scenario in which the larvae and adults were not provided with alternative food. However, these results indicate that the presence of red clover in pastures may affect *S. litura* at a population level and further investigation into the mechanisms is warranted. Understanding the

physical and/or chemical drivers behind the negative effects I observed would aid in the breeding or selection of more resistant forage species and cultivars.

In addition to demonstrating the negative effects on *S. litura* of feeding on red clover, this study confirmed that the control species, plantain, is a good host. *Spodoptera litura* maintained performance and gut microbial diversity even when confined to feeding on plantain for two generations. In regions and seasons in which *S. litura* are likely to be abundant farmers may therefore need to plant less vulnerable alternatives or rely on pesticides for crop protection. This scenario will become increasingly common as climate conditions in currently marginal temperate regions become more favourable.

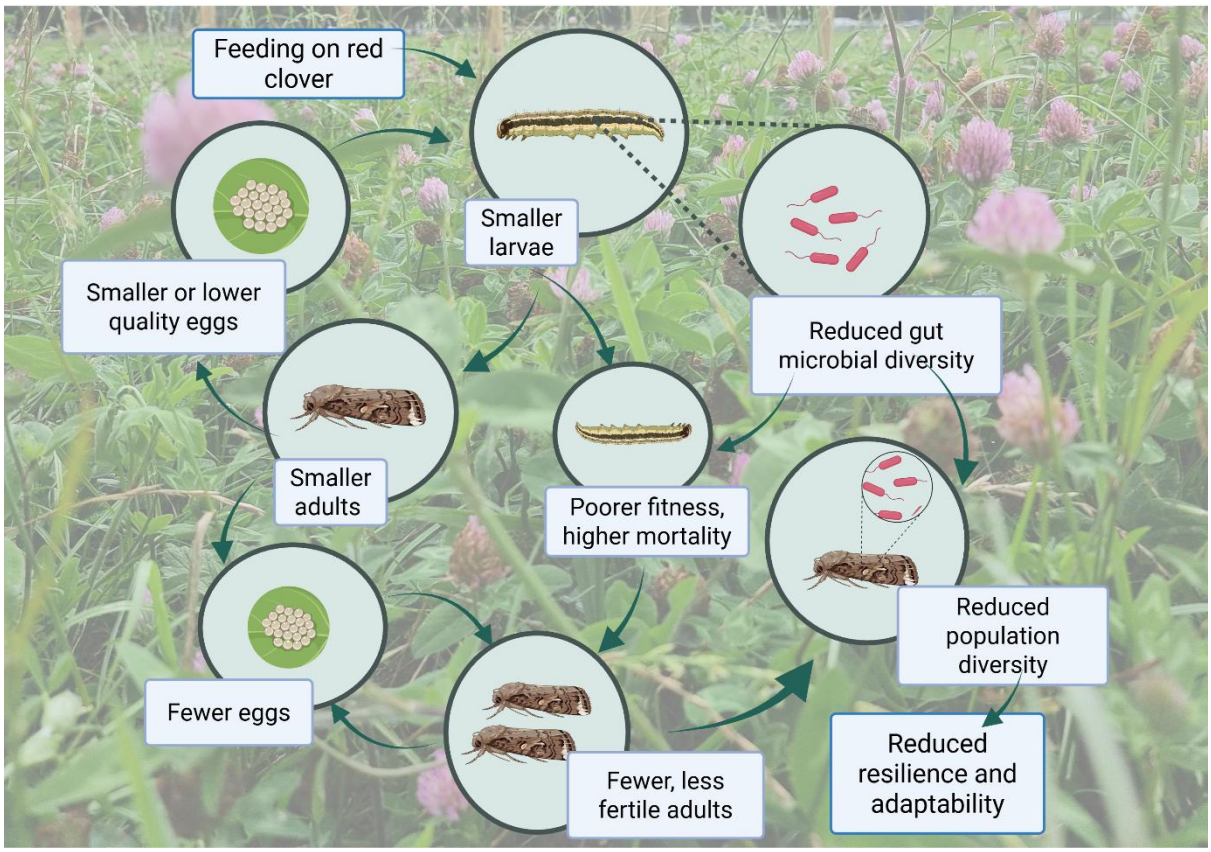


Figure 4-8 – Pathways for reduced population performance of Spodoptera litura *feeding on a poor host such as red clover for multiple generations.*

4.7. Acknowledgements

Thank you to Anne Barrington for *S. litura* eggs, Lily Ouyang for help with feeding larvae, Faith Mtandavari advice on DNA extractions and Paul MacLean for amplicon sequence analysis. Sequencing costs were funded by the University of Waikato. This project was funded by the New Zealand Strategic Science Investment Fund (SSIF) through the Bioeconomy Science Institute (AgResearch Group) (PRJ0116252 and PRJ0756153).

5. Larval age affects the ability of *Spodoptera litura* to survive on a low-quality host

This chapter is intended for submission for publication in a scientific journal. It further addresses the hypothesis that some pasture plants will be higher quality hosts for *S. litura* than others, and that feeding on non-preferred host plants will affect *S. litura* behaviour, growth and reproduction.

5.1. Abstract

The highly polyphagous lepidopteran pest, *Spodoptera litura,* is predicted to become more widespread and damaging in temperate regions at the margins of its current distribution. Pastoral agriculture in these regions, including in New Zealand, may be increasingly vulnerable to this species through direct and indirect impacts of a changing climate. I investigated whether *S. litura* larvae can utilise the important pasture forage species, *Lolium perenne* (perennial ryegrass) for all or part of its development, and the effect of larval age on this. White clover (*Trifolium repens*) was included as a positive control as it is a known suitable host and is commonly grown together with perennial ryegrass in New Zealand pastures. In laboratory studies, I monitored *S. litura* larval growth and survival on perennial ryegrass compared with white clover, and on both plants together. I also tested the ability of *S. litura* larvae to switch between ryegrass and clover, the effect of larval age on that ability and whether age-related differences in starvation resistance could explain my experimental results.

My studies confirmed that perennial ryegrass is a poor host for *S. litura*. Survival to adulthood was contingent on access to additional food sources. Larval age affected its ability to utilise ryegrass. Larvae fed ryegrass from neonate survived for longer than those that were switched from clover to ryegrass when older. Larvae were able to complete development when switched from ryegrass to white clover after feeding on ryegrass for 14 or 21 days (72% and 8% survival respectively). Starvation resistance increased with age, thus the decline in the ability to use ryegrass with increasing larval age was not solely due to deterrence and starvation.

My results indicate that perennial ryegrass may be vulnerable to damage from *S. litura* during outbreaks, despite being a poor host, but also that ryegrass may help mitigate the predicted climate change-related increases in population sizes of this pest in New Zealand. Field-based studies are required to confirm these findings.

Keywords: Tropical armyworm; sleeper pest, pastoral agriculture, climate change adaptation, host plant quality, perennial ryegrass, white clover, diet switching.

5.2. Introduction

Spodoptera litura (Lepidoptera: Noctuidae) is a highly invasive, economically important pest throughout the tropical Asia-Pacific region (CABI, 2014). The larvae are extremely polyphagous, feeding on a large number of plant species, including many that are important to pastoral agriculture (Bragard et al., 2019; Jensen et al., 2026). In New Zealand, *S. litura* is a sporadic pest, mainly in the northern North Island, but with climate change it is likely to cause more harm over a wider geographical area in the future (Jensen et al., 2025; Orre-Gordon & Jensen, 2025). To fully understand the potential future impact of *S. litura* on temperate pastoral systems we need to assess its ability to utilise perennial ryegrass (*Lolium perenne* L.), the dominant pasture grass sown in these regions (Jaramillo et al., 2021; Wilkins & Humphreys, 2003).

While Poaceae species such as rice, maize and sorghum are listed as hosts for *S. litura* (Ahmad et al., 2013; Bragard et al., 2019) perennial ryegrass is not included in its published host range lists. One study from Guizhou (Zhang et al., 2017) noted *S. litura* as a dominant pest of '*Lolium perenne* artificial grassland' in summer-autumn. My own unpublished research indicates that *S. litura* strongly prefers to feed on broadleaf species over grasses and that survival on perennial ryegrass is very poor. Anecdotally however, during outbreaks large caterpillars have been seen to cause damage to perennial ryegrass and young seedlings are reported to be particularly vulnerable.

Multispecies swards are becoming increasingly common in temperate pastoral systems as farmers seek to improve pasture persistence (McCahon et al., 2021) and to mitigate environmental concerns such as dependence on artificial fertilisers (Jaramillo et al., 2021). White clover (*Trifolium repens*) is the dominant legume grown in New Zealand pasture (Stewart et al., 2022), but alternative, more persistent legumes, and forb species such as chicory (*Chicorum intybus* L.) and narrowleaf plantain (*Plantago lanceolata* L.) are increasingly being incorporated into pasture (Brown et al., 2003; Minnee et al., 2017; Nguyen et al., 2022). Increasing plant species diversity in agroecosystems can reduce pest pressure through mechanisms such as resource dilution, but may have the opposite effect if pests are able to use these plants as alternative hosts/reservoirs (Ratnadas et al., 2012). As many broadleaf forages are known to be good hosts for *S. litura* (Jensen et al., 2026) their presence may allow for the build-up of larger populations or more generations per season of *S. litura* in pastures. This increased source of pest pressure could then spill over to perennial ryegrass, particularly when there are high numbers and other, preferred food sources become exhausted. Alternatively, the presence of a lower quality host such as perennial ryegrass could act as pest pressure sink, reducing *S. litura* populations and thereby pressure on the associated broadleaf species.

As a first step towards understanding *S. litura* pest pressure in ryegrass-based pastures I conducted laboratory experiments to evaluate its ability to feed and survive on perennial ryegrass. White clover was used as a positive control, as it has been shown to be a good host for *S. litura* (Jensen et al., 2026; Lindroth et al., 2000) and because of its importance in New Zealand pasture. I also investigated the ability of *S. litura* larvae to switch between white clover and perennial ryegrass, and whether larval age affects this ability. I hypothesised that larger larvae would be capable of feeding and potentially completing their development on ryegrass whereas smaller larvae would not. A starvation experiment was also conducted to assess a possible mechanism behind the observed poor performance of *S. litura* on ryegrass.

5.3. Methods

Four laboratory experiments were conducted (Figure 5-1).

A Whole Plant Experiment (Figure 5-1A) compared the growth and survival of *S. litura* caged on potted perennial ryegrass plants, white clover plants or both together. Larvae were placed on the plants as neonates and their performance was monitored through to adulthood. I hypothesised that larvae would not survive when restricted to feeding only on ryegrass, and that when given a choice they would feed preferentially on white clover.

Two Diet-switching Experiments (Figure 5-1C and D) assessed the effect of larval age on the ability of *S. litura* to switch plant host diet in the absence of a choice. I provided larvae with cut leaf segments from either ryegrass or white clover and at one of three ages switched them to the alternate plant species. The ryegrass to clover experiment investigated the length of time that larvae could feed and survive on the low-quality host and if it could complete development once switched to a higher quality host. The clover to ryegrass switching experiment tested my hypothesis that more mature larvae would be able to complete their development on ryegrass if previously fed clover, while younger larvae would not.

A Starvation curve experiment was also conducted (Figure 5-1B). Cut white clover leaves were provided from neonate and then food withheld from one of three larval ages. This tested the hypothesis that young larvae would be better able to resist starvation than more mature larvae.

Knowledge gained from these experiments will help us predict if ryegrass plants will be vulnerable to feeding by *S. litura* in pastures and under what circumstances.

5.3.1. Plant and insect sources

Spodoptera litura were obtained from a colony maintained at Plant and Food Research, Mt. Albert, Auckland, New Zealand. Newly hatched larvae were used for the Whole Plant Experiment

and two Diet-switching Experiments. Larvae for a Starvation Experiment were hatched from eggs laid by adults from the Whole Plant Experiment.

Perennial ryegrass and white clover seeds were germinated in Petri dishes at 20°C then transplanted to polystyrene trays with potting media (Daltons™ ryegrass and clover mix). After seven weeks, one tiller per ryegrass plant was cut with scissors near the base and used to confirm the presence or absence of *Epichloë* endophyte using an immunoblotting technique (Simpson et al., 2012). *Epichloë* endophytes have well-known anti-insect properties (Caradus & Johnson, 2020), thus I only used plants that did not contain *Epichloë* in order to focus purely on the plant species effect. Plants were grown in a screenhouse at Ruakura Research Centre, Hamilton, New Zealand under natural light and temperature conditions between October 2022 and April 2024. An onsite weather station recorded a mean temperature of 15.8°C (min. -2°C and max. +29.7°C) over this period and between 117 h and 253 h bright sunshine per month. For all experiments, I used the white clover cultivar Kopu II. Perennial ryegrass cultivar Alto was used for the Whole Plant Experiment and cultivar Samson for the Diet-switching Experiments.

5.3.2. Whole Plant Experiment

The performance and survival of *S. litura* on the test species, perennial ryegrass, compared with the positive control, white clover, was assessed using potted plants in a laboratory experiment. The plants were transplanted at 8 weeks old into 0.7 L plastic pots (11 cm W x 10 cm H) filled with potting mix so that each pot contained two plants of the same species or one of each species (Figure 5-1A). Sixteen replicate pots for each of the three experimental treatments (ryegrass-only, clover-only and ryegrass+clover) were placed on a screenhouse bench using a randomised block design. For pots with ryegrass+clover, left-right positions of the two plants were also randomised.

At 3-months old, in summer (January) 2024, the plants were reduced to no more than 20 ryegrass tillers (minimum = 5) or 20 expanded clover trifoliate leaves (minimum = 6). Damaged leaves and all stolons were removed from the clover, and ryegrass plants were trimmed to 8 cm. The plants were shifted to a controlled temperature room (25°C with a 16:8 h light:dark cycle). Each pot was in a separate drip tray and was bottom watered as required.

A segment of filter paper (Whatman® Qualitative Grade 1), cut to approximately 1 cm^2, was placed on the soil surface between the two plants in each pot, to help with placement and visibility of the *S. litura* larvae at setup. One neonate *S. litura* larva was placed on each filter paper segment. The plants were then covered with a fabric mesh bag held up with a wire hoop and fastened around the top of the pot with an elastic band.

Larvae were weighed after 7 and 14 days. Larvae were carefully removed with a soft paint brush, weighed on an analytical balance to the nearest 0.1 mg, and returned to the filter paper segment in the centre of the pot. The presence or absence of feeding scars on each plant was recorded. Each pot was monitored daily for signs that the larva was pre-pupal — before pupating, larvae cease feeding and bury themselves. Two days after this behaviour was noted, the pupa was carefully excavated, weighed and sexed, then returned to its pupal chamber.

At eclosion adults were transferred to 750 mL, plastic mating containers with damp tissue and crumpled paper towels for egg-laying and refugia. They were paired as soon as possible after adult emergence with an adult of the opposite sex from the same treatment. Four adult pairs were set up from each of the clover-only and ryegrass+clover treatments. Only one adult eclosed from the ryegrass-only pots therefore egg-laying could not be assessed for this treatment. Eggs were counted and time to death of each moth was recorded.

5.3.3. Ryegrass to Clover Diet-switching Experiment

Given the poor performance and survival of *S. litura* on perennial ryegrass, I conducted a laboratory diet-switching experiment to determine whether larvae can recover from feeding on this poor-quality host if switched to a higher quality host such as white clover. Neonate *S. litura* larvae were placed in individual 120 mL plastic specimen vials with 1 cm damp vermiculite in the base. Larvae were assigned to one of four feeding treatments: ryegrass-only, or clover from neonate, 14-days-old or 21-days-old (Figure 5-1C). Vials were placed in a randomised block design in a 20°C controlled temperature room with a 16:8 h light:dark cycle.

The larvae were fed daily with freshly harvested leaf segments and, except for the first four days when the larvae were very small, remnant leaf segments were removed at each feeding. Leaves were harvested from approximately 1-year old perennial ryegrass and white clover plants. The plants were generated as described in section 5.3.1, then transplanted into individual pots and set up in a complete randomised design. Larval survival was checked daily. Larval weight was recorded at 14, 21 and 28 days, and at pupation. Time to pupation and adulthood, and sex were also recorded.

5.3.4. Clover to Ryegrass Diet-switching Experiment

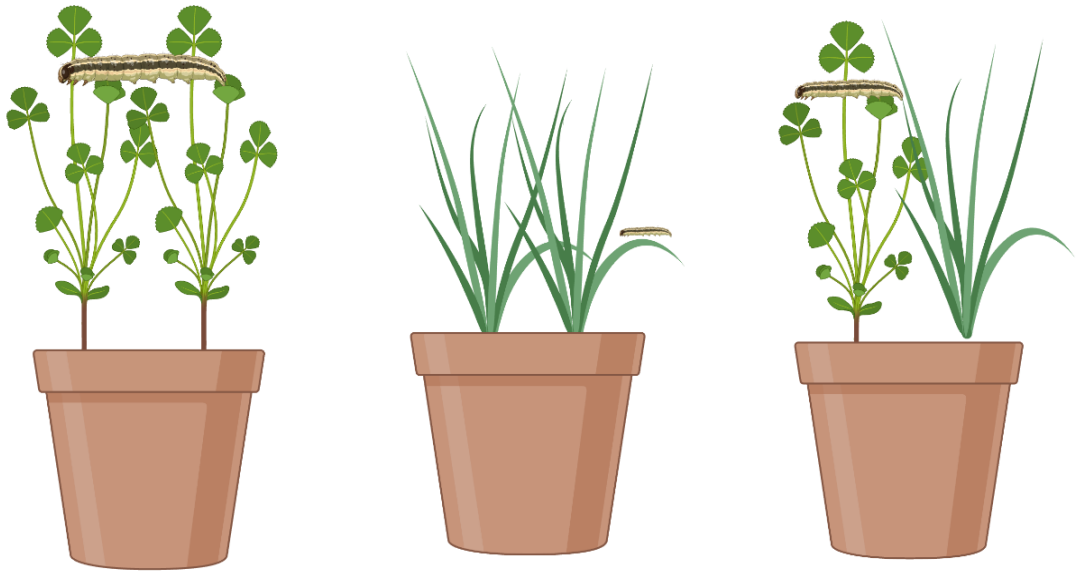
A second diet-switching experiment evaluated whether larval age would affect the ability of *S. litura* larvae to use perennial ryegrass as a host. The experiment was conducted using the same methods as described in Section 5.3.3. except that larvae were fed clover first, then transferred to ryegrass (Figure 5-1D).

5.3.5. Starvation Curve Experiment

Following the Clover to Ryegrass Diet-switching Experiment, in which I showed an effect of larval age on the length of time *S. litura* can survive on perennial ryegrass, I investigated age-related starvation resistance as a possible mechanism. Neonate larvae were placed in individual 120 mL specimen vials with a piece of synthetic mesh fabric (two layers of approximately 1 cm x 5 cm) with 250 µL milliQ water to maintain humidity and provide a source of water. Larvae were fed detached white clover leaves continuously, or starved from neonate, 7 days, 14 days or 21 days (Figure 5-1B).

The vials were placed in polystyrene trays in a randomised block design and placed in a 20°C controlled temperature room with a 16:8 hours light: dark cycle. Survival was checked daily and clover replaced with fresh leaves for larvae in 'fed' treatments. Water was added to the fabric in 150 µL aliquots as needed.

A - Whole Plant experiment

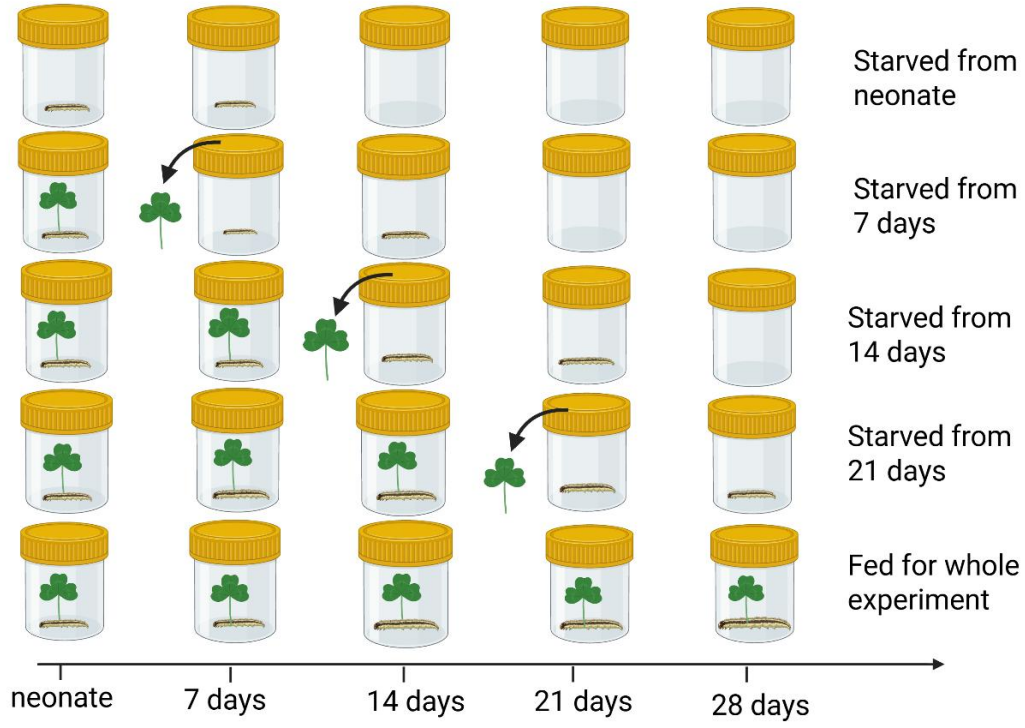


Clover-only

Ryegrass-only

Clover+ryegrass

B - Starvation Curve Experiment



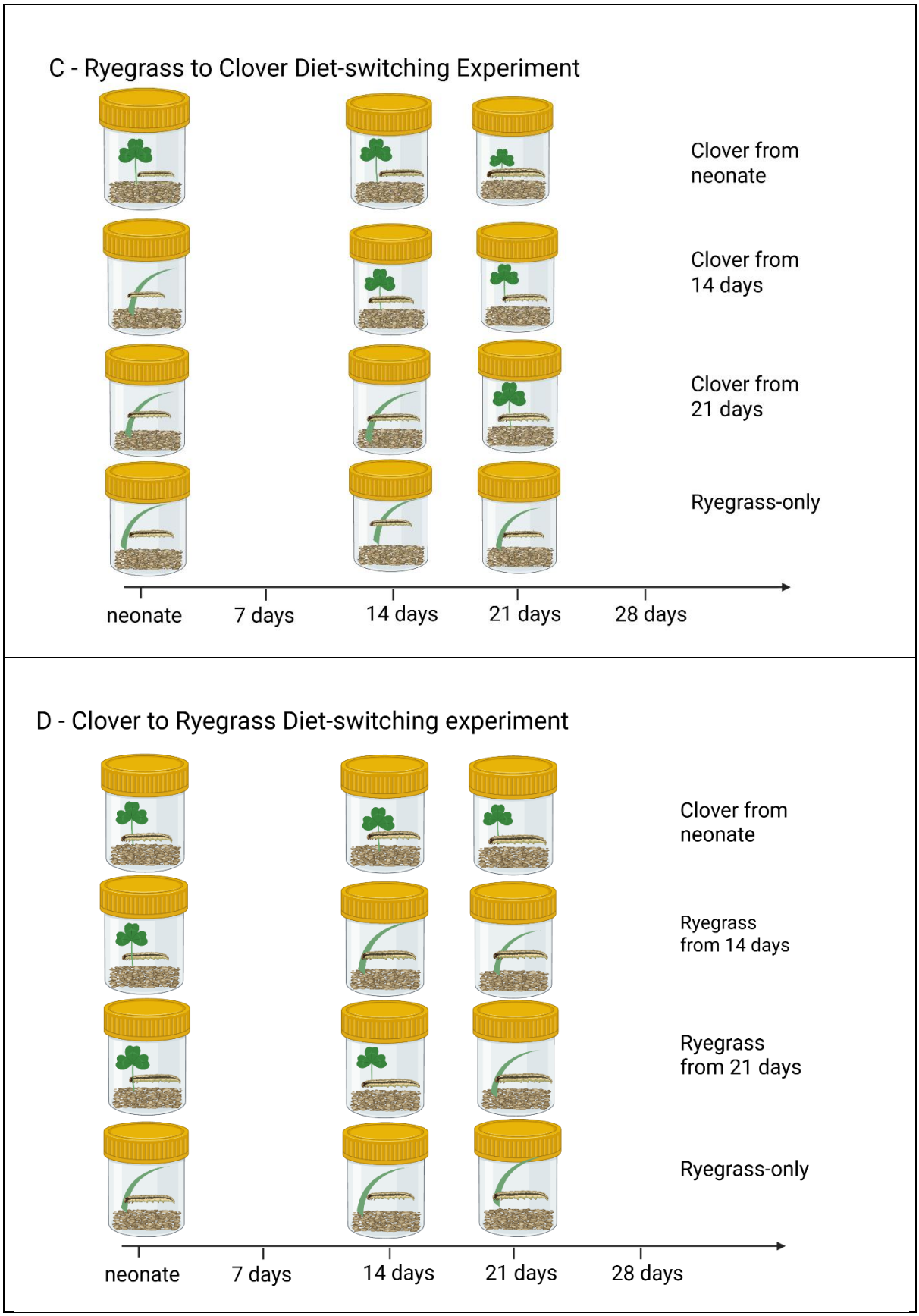


Figure 5-1 – Graphical summaries of experimental layouts for (A) Whole Plant Experiment, (B) Starvation Curve Experiment, (C) Ryegrass to Clover Diet-switching Experiment and (D) Clover to Ryegrass Diet-switching Experiment with Spodoptera litura.

5.3.6. Statistical analyses

All statistical analyses were performed using R version 4.4.2 (R Core Team, 2024).

Larval and pupal weights were analysed using linear mixed-effects models (LMMs) estimated by restricted maximum likelihood (REML). Replicate block was included in all models as a random effect. Where available, sex was included into the initial model as a fixed effect but dropped if not significant.

Count data were analysed using generalised linear models (GLM) or generalised mixed models (GLMMs). For the number of eggs laid per adult pair I used the glm.nb function from the MASS R package (Venables & Ripley, 2002). The effect of treatment on development time (i.e. the number of days at a life stage) was fitted using the glmmTMB package in R (Brooks et al., 2017). Both models assumed a truncated negative binomial distribution to accommodate overdispersed data and the absence of zero values in the response and used a log link function. Data describing survival to adulthood was also analysed by GLM, using a binomial distribution to reflect the binary nature of the outcome (survived vs. not survived), and a logit link function.

Individual treatments were omitted from analyses when low sample sizes occurred. For instance, in the Whole Plant Experiment, only two of sixteen *S. litura* individuals caged on the ryegrass-only treatment pupated. This treatment was therefore omitted from the 'days to pupation' analysis due to insufficient statistical power and convergence difficulties associated with such limited data.

For LMMs the significance of the fixed terms was assessed using Type II *F* statistics with Satterthwaite's approximation for degrees of freedom (R package lmerTest; Kuznetsova et al., 2017). For GLMs and GLMMs, fixed effects were evaluated using Type II Wald Chi-square tests (R package car; Fox & Weisberg, 2011). This was followed by a multiple-comparison procedure based on the significance of estimated marginal mean differences using a Bonferroni correction to adjust *p*-values for multiple comparisons (R package emmeans; Lenth et al., 2022).

Graphical model validation tools were used to assess the underlying assumptions. For LMM analyses, variance homogeneity and normality were checked using plots of residuals against fitted values, standardised residuals versus fitted values, and quantile–quantile plots. For the GLMM analyses, I examined plots of Pearson residuals against fitted values and versus each explanatory variable included in the model (Zuur et al., 2009) and performed simulation-based diagnostic checks using the DHARMa package (Hartig, 2016).

Data were log- or square root-transformed where necessary to stabilise the variance, but back-transformed means and standard errors are presented throughout this paper.

5.4. Results

5.4.1. Whole Plant Experiment

Spodoptera litura larval weights were strongly affected by plant host (Figure 5-2A, B). After 7 days larvae caged onto pots with white clover (either with or without ryegrass) were significantly heavier than those on pots with ryegrass only ($F_{2, 26.2} = 53.3$; $p < 0.001$; Figure 5-2A). After 14 days, weights of larvae caged on pots with both ryegrass+clover were intermediate between, and significantly different from, both those on clover-only, and those on ryegrass-only ($F_{2, 26.6} = 208.8$; $p < 0.001$; Figure 5-2B). These differences carried through to pupal weights ($F_{2, 26} = 29.3$; $p < 0.001$; Figure 5-2C).

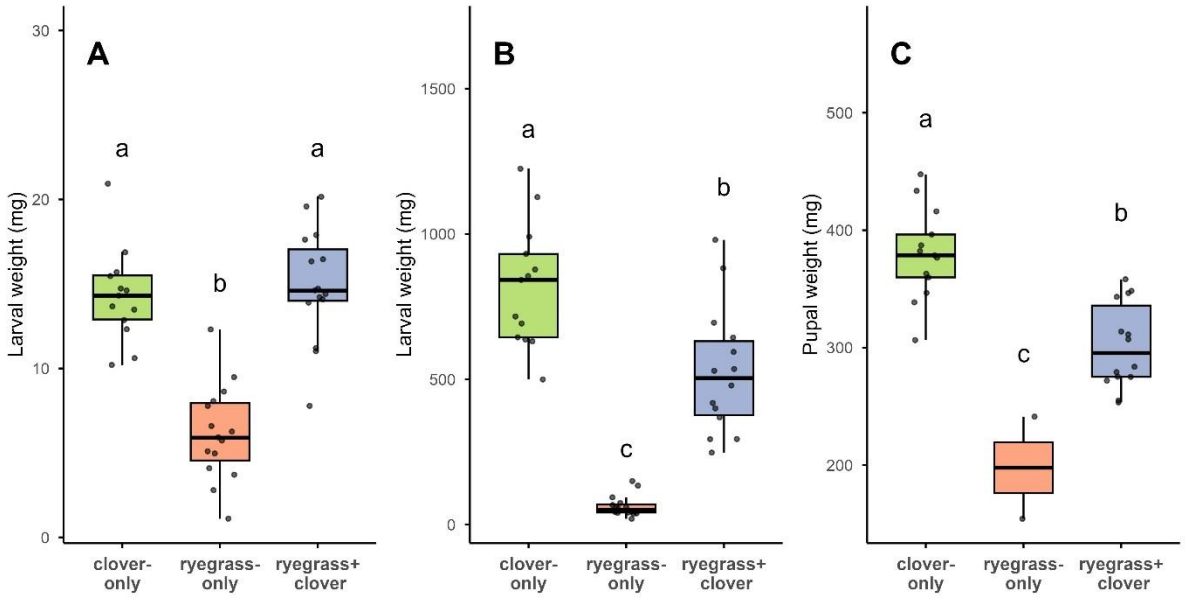


Figure 5-2 – Weights (mg) of Spodoptera litura *(A) larvae after 7 days, (B) larvae after 14 days and (C) pupae when caged on pots with clover, ryegrass or ryegrass+clover. Different letters indicate significant differences between treatments at a level of p < 0.05. In all boxplots, horizontal lines correspond to the median, 1st and 3rd quartiles and vertical lines show smallest and largest values no further than 1.5*Interquartile range.*

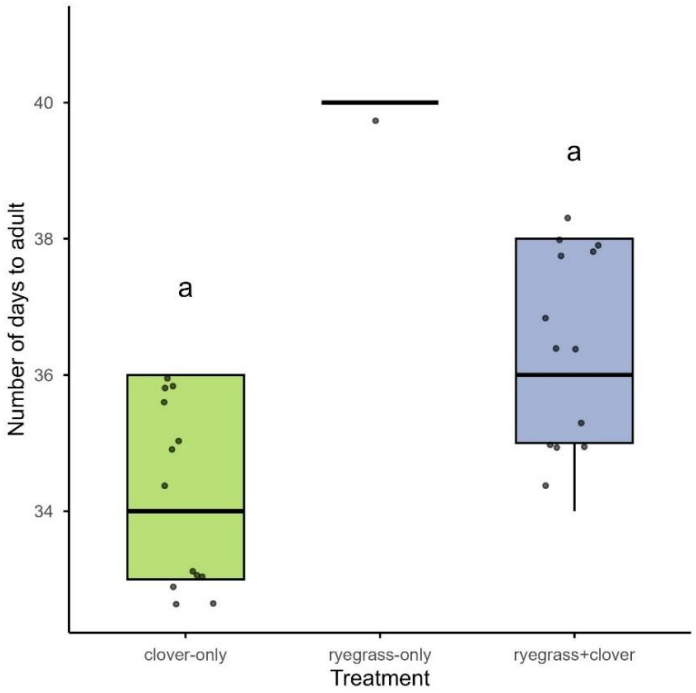


Figure 5-3 – Time (days) from neonate to adult eclosion for Spodoptera litura *caged on pots with clover, ryegrass or ryegrass+clover. Different letters indicate significant differences between treatments at a level of p < 0.05. The ryegrass-only treatment was omitted from analysis because of the low sample size.*

Plant host also had a statistically significant effect on the percentage of *S. litura* that survived to adulthood ($X^2_{2, N=47} = 26.6$; $p < 0.001$). Only two of the 16 *S. litura* caged on ryegrass-only pots pupated. One of these successfully emerged as an adult but the second died during eclosion. In comparison, 87% (13/15) of *S. litura* caged on clover-only and 93% (14/15) of those on ryegrass+clover developed through to adults, with no significant difference between these two treatments ($p > 0.05$). The mean time to adult eclosion was slightly lower for *S. litura* on pots containing only clover (34.3 ± 1.6 days) compared with 36.4 ± 1.7 days ryegrass+clover, but this difference was not statistically significant ($X^2_{1, N=26} = 0.79$, $p = 0.373$) (Figure 5-3). The only *S. litura* that survived to adulthood on the ryegrass-only treatment eclosed after 40 days (insufficient data for inclusion in the statistical analysis).

The number of eggs laid did not differ significantly between adult pairs from clover-only pots and those from ryegrass+clover pots (mean number eggs/moth pair =1381 (clover-only), 1422 (ryegrass+clover); $X^2_{1, N=8} = 0.03$; $p = 0.853$).

The very small feeding scars made by first and second instar larvae are difficult to see on ryegrass and can easily be missed. Scars were noted from the first (Day 7) assessment on ryegrass plants in all the ryegrass-only pots but not in the ryegrass plants in the ryegrass+clover pots at the Day 7 or Day 14 assessments. However, small scars that may have been larval feeding

were noted on many of these plants during a more thorough examination at the end of the experiment.

Plants in this experiment were colonized by foliar-feeding *Rhopalosiphum padi* (aphids) while in the screenhouse, and these multiplied in the warm conditions in the controlled temperature room during the experiment. Although larvae were not observed feeding on the aphids or shed skins, this cannot be ruled out.

5.4.2. Ryegrass to Clover Diet-switching Experiment

Survival of *S. litura* fed exclusively on perennial ryegrass from neonate remained high for 14 days. Of the 75 larvae fed only ryegrass for 14 days from neonate (including those in the ryegrass-only treatment, and those switched from ryegrass to clover at 14 or 21 days) 87% were still alive at the Day 14 assessment. However, survival of larvae that remained on ryegrass declined sharply thereafter, reaching 0% by Day 24 (Figure 5-4). For individuals in the ryegrass-only treatment (n = 25), excluding one individual that died on Day 1, larvae lived for between 15 and 24 days (average = 20.4 days).

Mortality among larvae being fed perennial ryegrass reduced the number that were available to be switched to clover at each treatment age: 25 larvae were fed clover from neonate, 20 from Day 14 and 5 from Day 21. Some mortality occurred after larvae were switched from ryegrass to clover. Two larvae died within 24 hours of switching to clover at 14 days, while mortality among larvae switched at 21 days occurred up to 9 days later, and only two individuals reached pupation (Figure 5-5). In comparison, all 25 larvae fed only on clover throughout the experiment survived to adulthood. There was no pupal mortality in any of the feeding treatments.

At 21 days, larvae that had fed on clover since neonate were significantly heavier than larvae switched from ryegrass to clover at 14 days (457 ± 21.6 mg and 259 ± 25.9 mg respectively; $F_{1, 41} = 35$; $p < 0.001$). At pupation, however, the opposite was true, with pupae from the clover-only treatment being significantly lighter (Table 5-1). No individuals in the ryegrass-only treatment survived to pupation and only two individuals that had been fed clover from 21 days. There were therefore insufficient data points for meaningful statistical analysis for those treatments. Sex and the interaction between sex and treatment were not statistically significant so were dropped from the models.

Larvae that were switched from ryegrass to feed on clover at 14 days pupated and eclosed slightly later than those fed clover from neonate, but these differences were not statistically significant ($p > 0.05$; Table 5-1).

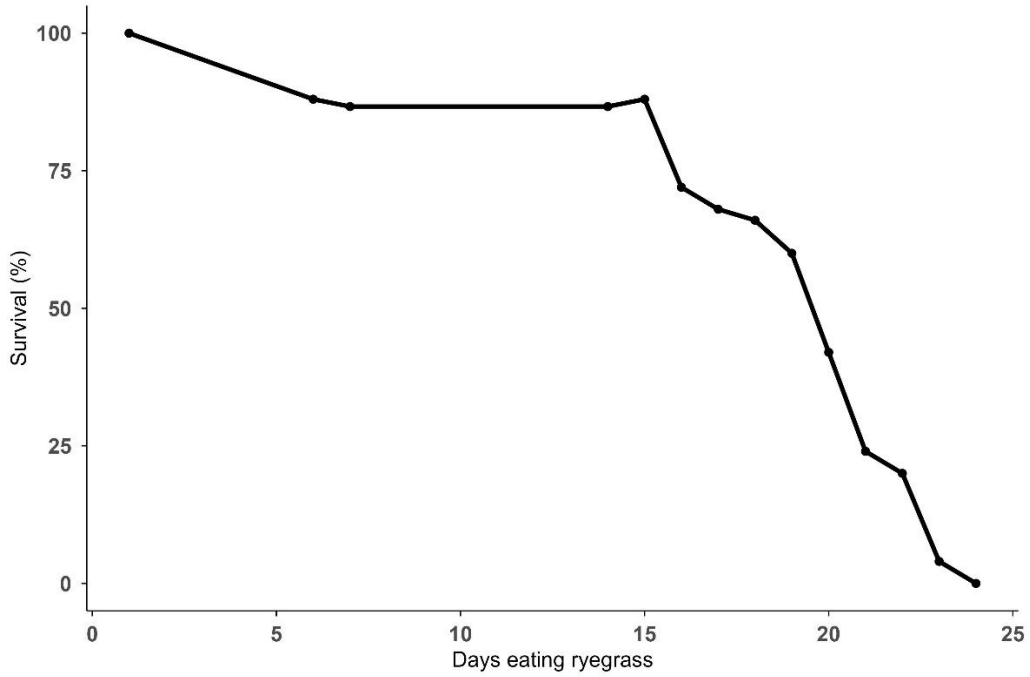


Figure 5-4 – Percent larval survival over time for Spodoptera litura *fed ryegrass from neonate (includes larvae from both the 'ryegrass-only' and the 'ryegrass to clover' switch treatments up to the date they were switched to clover; i.e. N = 75 for days 1 – 14, N = 50 for days 15 - 21, and N = 25 for >21 days).*

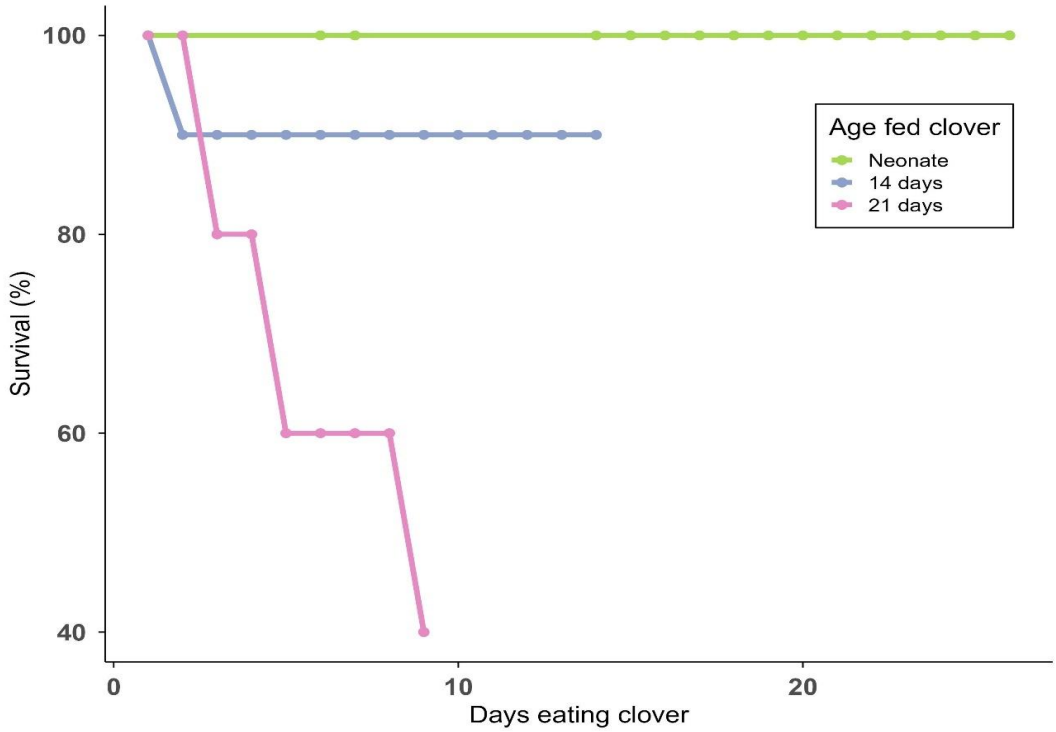


Figure 5-5 – Percent survival of Spodoptera litura *larvae fed clover from neonate or switched from ryegrass to white clover at 14 or 21 days old. Day 1 is the first day that larvae were fed clover. The sample size (n) in each treatment at Day 1 was 25, 20 and 5 for larvae fed clover from neonate, 14 days and 21 days old respectively.*

Table 5-1 – Mean (and standard error) pupal weight and time (days) from hatch to pupation and adulthood for Spodoptera litura *larvae fed clover from neonate, larvae switched from ryegrass to clover at 14 or 21 days old, and larvae only fed ryegrass.*

Age fed clover	Number of larvae (n)	Pupal weight (mg)	Time to pupation (days)	Time to adulthood (days)
Clover from neonate	25	363.8 (7.1) b	28.6 (1.1) a	46.5 (1.4) a
Clover from 14 days	18	401.8 (8.5) a	31.4 (1.3) a	49.5 (1.7) a
Clover from 21 days	2	370.5*	39.0*	56.0*
Ryegrass-only	0	—	—	—
Test statistic		$F_{1,18}$=12.8; p=0.002	$X^2_{1, N=43}$=2.65; p=0.103	$X^2_{1, N=43}$=1.94, p=0.163

**Omitted from statistical analysis because the sample size (n) was too low*

5.4.3. Clover to Ryegrass Diet-switching Experiment

Consistent with the Ryegrass to Clover Diet-switching Experiment, survival rates were high for larvae fed only clover, with 24 of the 25 (96%) larvae surviving to adulthood. In contrast, all the *S. litura* larvae that were fed perennial ryegrass died before pupation, even if they had initially fed on clover, and regardless of the age at which they were switched from clover to ryegrass (Figure 5-6A).

On average, larvae fed ryegrass from neonate survived for longer than those that switched from clover to ryegrass as more mature larvae (Figure 5-6B). Larvae that were initially fed clover died on average 8.9 ± 0.8 and 8.0 ± 0.7 days after being switched to ryegrass at 14 days and 21 days respectively ($X^2_{2, N=72}$ = 144.1; p < 0.001; Figure 5-6B). In contrast, 88% of larvae that were fed ryegrass from neonate were still alive after 14 days and their mean survival time was 24.2 ± 1.5 days (Figure 5-6A).

Larvae fed ryegrass from neonate continued to grow, although much more slowly than those fed clover, for about 21 days, after which growth and survival decreased markedly (Table 5-2). The mean weight of 21-day-old larvae switched from clover to ryegrass at 14 days had increased by 41% since switching, but they were not significantly heavier than those fed ryegrass since neonate (p > 0.05). Larvae fed ryegrass from 21 days lost weight over the following week (by 28 days old) and 74% died within the following 24 hours.

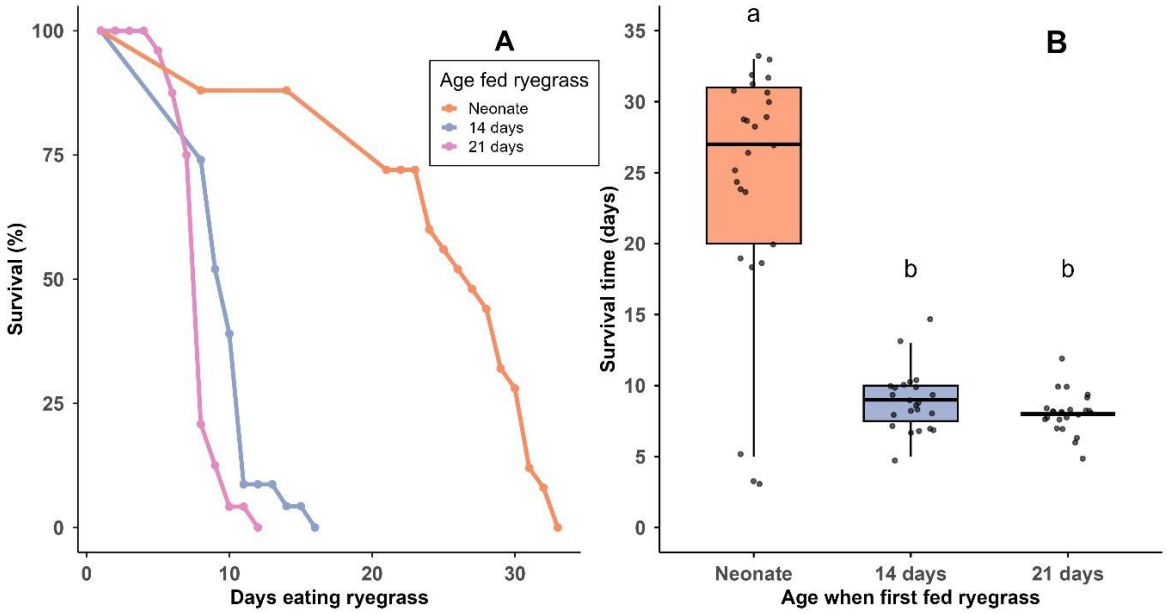


Figure 5-6 – (A) Percent survival of Spodoptera litura *larvae fed ryegrass from neonate or switched from white clover to ryegrass at 14 or 21 days. Day 1 is the first day that larvae were fed ryegrass. Sample sizes (n) per treatment at day 1 were 25, 23 and 24 for larvae fed ryegrass from neonate, 14 days and 21 days of age respectively. (B) The number of days that larvae survived from when they were first fed ryegrass. Different letters indicate significant differences between treatments at a level of p < 0.05.*

Table 5-2 – Mean weight (and standard error; n=sample size) for Spodoptera litura *larvae fed clover or ryegrass from neonate, or clover from neonate then switched to ryegrass from 14 or 21 days. Boxes in green are for larvae that had been feeding on clover for the preceding week and blue for larvae that had fed on ryegrass for the preceding week. Letters in common within a column indicate means are not significantly different (p > 0.05).*

Age fed ryegrass	Larval weight (mg) (14 days old)	Larval weight (mg) (21 days old)	Larval weight (mg) (28 days old)
Clover-only	33.2 (2.2) a (n=24)	356.1 (21.4) a (n=24)	1145.8 (47.7) a (n=7)
Ryegrass from 21 days	31.5 (2.1) a (n=24)	330.2 (19.9) a (n=24)	321.5 (15.7) b (n=19)
Ryegrass from 14 days	34.8 (2.3) a (n=23)	49.2 (4.1) b (n=17)	26.4* (n=1)
Ryegrass from neonate	19.1 (1.5) b (n=22)	48.2 (3.9) b (n=18)	49.5 (8.1) c (n=11)
Test statistic	$F_{3, 67.1}$=18.4; P<0.001	$F_{3, 61.2}$=283.9; P<0.001	$F_{2, 23.4}$=541.3; P<0.001

**Treatment omitted from the analysis due to low sample size (n).*

5.4.4. Starvation Curve Experiment

Four of the 25 larvae fed clover-only died early (as first instars) in this experiment but the remaining 21 successfully developed through to adulthood. Of the 25 larvae assigned to each of the starvation treatments, 2, 5 and 8 died before food (white clover) was withheld at 7, 14 and 21 days respectively. These mortality rates were higher than for the clover-only treatments in the Ryegrass to Clover and Clover to Ryegrass Diet-switching Experiments (0% and 4% mortality respectively).

Larvae survived for up to 7 days following removal of food, with survival time (days) increasing with larval age at the start of the starvation period (Figure 5-7; $X^2_{3, N=83} = 7.7$; $p = 0.052$).

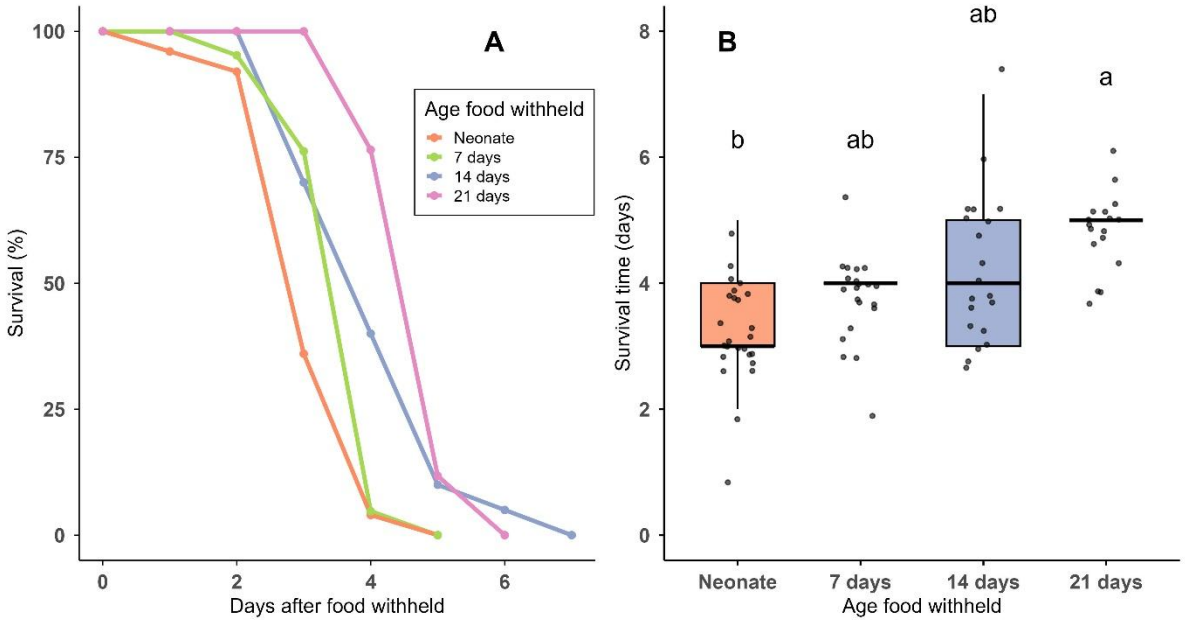


Figure 5-7 – (A) Percent survival of Spodoptera litura *larvae starved from neonate, or after 7, 14 or 21 days feeding on white clover from neonate. (B) The length of time that larvae survived following removal of food. Different letters indicate significant differences between treatments at a level of $p < 0.05$.*

5.5. Discussion

This study confirmed that perennial ryegrass is a poor host for *S. litura* larvae. While a comprehensive evaluation of host plant suitability would include adult oviposition and larval preferences, a key criterion is the ability of a host to support larval survival to adulthood (Cunningham & Zalucki, 2014). None of the larvae that were fed only perennial ryegrass and none that were switched from clover to ryegrass in my Diet-switching Experiments reached pupation.

However, one individual in the Whole Plant Experiment completed development through to adulthood. This was probably aided by the presence of *Rhopalosiphum padi* aphids on the plants. Some insect 'herbivores' are known to supplement their diet through cannibalism, predation, or scavenging, or feeding on cast skins or egg shells (Bernays, 1998). This may provide an additional source of protein or other nutrients, or dilute plant toxins, thereby allowing the insect to utilise an otherwise unsuitable host. Additional nutrients may also help an insect speed up its development, helping it to escape predation or parasitism, or fit into a seasonal window (LoPresti, 2018). LoPresti (2018) demonstrated improved survival, growth rates or pupal masses for *Heliothis virescens* feeding on seven of eight 'intermediate quality hosts' when their diet was supplemented with dead *Drosophila*. Two of the host plant species could only be utilised when the supplementary flies were present; a similar result to that seen for *S. litura* on ryegrass in my Whole Plant Experiment. Two out of 25 *S. litura* were able to complete development to adulthood when provided with white clover after feeding on ryegrass for three weeks in my Ryegrass to Clover Diet-switching Experiment. A higher proportion, 18 of 25, survived to adulthood after feeding on ryegrass for two weeks. This, together with results from the Whole Plant Experiment, show that despite being a low-quality host *S. litura* can feed on perennial ryegrass and if alternative protein or higher quality host plant material is also available a small proportion will be able to complete development on it. *Spodoptera litura* larvae are relatively mobile and are likely to encounter alternative food sources in pasture. Therefore, perennial ryegrass in pasture may be vulnerable to *S. litura* despite being a poor host, particularly in outbreak years when numbers are high and alternative food sources are limited through feeding competition.

In all my experiments, *S. litura* individuals that successfully reached adulthood after feeding on ryegrass had longer larval phases — a well-known consequence of reduced consumption for insects in some circumstances (Kopec, 1924; Scriber & Slansky, 1981). Unfortunately, small sample sizes in the ryegrass-only treatment in the Whole Plant Experiment and the 'clover from 21 days' treatment in the Diet-switching Experiment prevented me from statistically analysing those data, and the increase in larval phase lengths in the ryegrass+clover Whole Plant treatment and the 'clover from 14 days' diet-switching treatment were not significantly different from those for larvae fed clover-only. However, these results indicate that while *S. litura* may be able to utilise ryegrass as a partial host, feeding on it is likely to increase the insect's development time and thereby restrict the number of generations per year. Phenological changes such as these have important implications for the population dynamics of this pest. Land use and agricultural practices that affect insect populations can either

exacerbate or mitigate against expected climate change increases (Boggs, 2016) and must be considered for a 'sleeper' pest such as *S. litura* in agricultural systems in New Zealand.

Larval age had a significant impact on the ability of *S. litura* to utilise perennial ryegrass. Larvae fed only ryegrass in the Diet-switching Experiments survived for an average of approximately three weeks; one individual survived for 32 days. In contrast, larvae that were switched from white clover to ryegrass when 14 or 21 days-old died within 15 and 11 days respectively. This was contrary to my hypothesis that more mature larvae would be able to switch to, and complete their development on, ryegrass if higher quality host plants were unavailable. To try to understand this result, I conducted a simple starvation experiment to determine if mature *S. litura* larvae die more quickly than young larvae when deprived of food. In fact, in my experiment older larvae survived for longer than younger larvae. A similar effect has been demonstrated for other insect species, for example fall webworm (*Hyphantria cunea*) (Zhang et al., 2025), Asian ladybird (*Harmonia axyridis*) (Reznik et al., 2019) and gypsy moth (*Lymantria dispar*) (Stockhoff, 1991). These results indicate that the shorter survival time for mature larvae on ryegrass was unlikely to have been solely due to feeding deterrence and starvation. In addition, I observed small amounts of feeding on ryegrass in all the age treatments, and larvae feeding on ryegrass lived longer on average than those that were completely deprived of food.

My finding that more mature *S. litura* larvae have a lower tolerance to ryegrass contrasts with the literature for many other species/host associations. Young insect larvae are often considered to be more selective and less able to tolerate low-quality hosts than older larvae (Scriber & Slansky, 1981; Zalucki et al., 2002). Studies have shown young larvae to be more susceptible to plant secondary chemicals, have lower detoxification enzyme activity and be less able to cope with physical defences (e.g. Jones & Despland, 2006; Kariyat et al., 2018; Quintero & Bowers, 2018; Vellau et al., 2013). Further research is required to explain the mechanism behind my results, but one possible explanation involves the feeding habits of the different aged/sized larvae. The smaller heads and mouthparts of neonate insects may allow them to feed more selectively on parts of the leaf that are low in fibre and easily digestible (Hochuli, 2001). As they become older, larvae often feed less discriminately, taking in lower quality food (Hochuli, 2001), and thus have reduced food assimilation efficiencies (Scriber & Slansky, 1981). In the case of *S. litura*, neonates skeletonise leaves whereas larger larvae eat through the whole leaf thickness and probably consume more vascular material. Furthermore, digestive physiology and nutrient requirements vary with larval age for many insects (Mitra et al., 2021; Simpson & Simpson, 1990). Young *S. litura* larvae feeding on a low-quality host like perennial ryegrass may

be able to ingest just enough nutrients to survive (although in this case, with reduced growth) while older larvae cannot.

An alternative hypothesis centres on larval responses to plant secondary metabolites responsible for deterrence or host recognition. Huang and Renwick (1995) showed that *Pieris rapae* neonate larvae could feed and grow normally on nasturtium (*Tropaeolum majus*) leaves but larger larvae that were transferred from cabbage (*Brassica oleracea*) or other host plants could not. Their work indicated that this was because the neonates were able to become habituated to chemical deterrents such as chlorogenic acid that were present in the nasturtium leaves. Yamamoto (1974) found that neonate *Manduca sexta* larvae could feed polyphagously, including on non-host plants, but once they had fed on solanaceous plants they became specialist feeders. They attributed this to induced feeding preference, later shown to be due to a tuning of taste receptors to a steroidal glycoside, indioside D (del Campo et al., 2001). In both cases, in common with my study, neonate larvae were more tolerant of alternative hosts than mature larvae.

Larvae and pupae caged on pots with both ryegrass+clover plants in my Whole Plant Experiment weighed significantly less than those in pots containing only clover. The clover plants were all large enough that larvae did not run out of herbage, so the reduction was not due to a lack of availability of the higher quality host food. Jensen et al. (2026) showed that neonate larvae may initially choose to remain and feed on a lesser quality host and this can have consequences for their growth through to pupation. In this study, however, larvae fed detached ryegrass leaves for two weeks and then switched to clover were a similar weight at pupation to those fed clover for the whole larval period. Growth rates of larvae caged on ryegrass+clover in the Whole Plant Experiment may have been negatively impacted by an increase in foraging time — an impact that would not have been evident in the no-choice Diet-switching Experiments. Bernays and Singer (1998) found that the polyphagous caterpillar *Grammia geneura* moved at regular intervals between plants, regardless of whether the plants were of the same or different species and independent of the relative quality of the plant host. Johnson and Zalucki (2005) found that *Helicoverpa armigera* similarly had periods of movement interspersed between feeding episodes, independent of the nutritional value of their food. For *S. litura* in pots with a poor host such as ryegrass, such movement would have reduced the time spent feeding on the higher quality host, white clover. However, as food intake was not measured in this experiment, I cannot confirm this theory. Plant quality factors such as nutrients, secondary metabolites and moisture content levels are likely to have varied between the detached herbage used in the Diet-switching Experiments and the Whole Plant Experiment. These cannot be discounted as potential

contributors to the demonstrated effects on *S. litura*. Regardless of the mechanism, my results indicate that larval *S. litura* growth in ryegrass-based pastures is likely to be reduced compared with larvae in crops with only their preferred, higher quality hosts such as white clover or plantain.

In New Zealand, pastures for grazing are usually sown with ryegrass and clover mixes (Ferguson et al., 2019) but additional forages, including broadleaf species, may be incorporated (Stewart et al., 2022). Broadleaf weed species such as dock (*Rumex obtusifolius*) and dandelion (*Taraxacum officinale*) are also commonly found in pasture (Blackwell et al., 2011; Tozer et al., 2011), and these may provide further high-quality food sources for *S. litura*. Impacts of perennial ryegrass on *S. litura* and thereby on pasture health and composition is likely to depend on the ratio of grasses to broadleaf species. In theory, a high proportion of broadleaf plants would result in higher *S. litura* numbers and therefore increased pasture damage. Conversely, a high proportion of grasses may reduce pest pressure. Field verification and modelling of impacts are required to confirm optimal planting ratios and weed control that balance pasture performance and resilience with reduced pest pressure.

My findings show that *S. litura* can utilise perennial ryegrass as a food source. However, it is a poor-quality host for this insect species, and its use will incur a cost to larval survival and growth and extend the length of time it takes to complete its lifecycle. At a population level these factors would reduce population sizes and decrease the number of generations per year. This may not be evident in non-outbreak years when *S. litura* numbers are low, or in mixed-sward pastures in which alternative food sources are abundant. I showed that mature larvae were less able to utilise ryegrass than younger larvae. As young larvae consume considerably less plant material, established ryegrass plants are unlikely to suffer substantial damage. However, young seedlings will still be vulnerable, as even small 'test-bites' from mature larvae can be significant to a very small plant. More, field-based research is needed to fully understand *S. litura* behaviour in mixed-sward pasture.

5.6. Acknowledgements

Thanks to Anne Barrington for supply of *S. litura* eggs.

5.7. Use of AI Statement

ChatGPT was used to translate the paper by Zhang et al. (2017).

6. General discussion

6.1. Summary of main objectives and outcomes

The objective of this work was to address key knowledge gaps concerning the New Zealand sleeper insect pest, *Spodoptera litura*. The aim was to:

- Investigate the availability and usefulness of existing sources of *S. litura* distribution data in New Zealand, and
- Conduct fundamental research into *S. litura* biology and interactions with forage plant hosts relevant to New Zealand pastoral systems.

In addition to a literature search and analysis of publicly available data, several laboratory experiments were conducted to address these objectives. In this chapter, I will review the work conducted, present main conclusions and discuss how the outcomes are interconnected. I will then address the limitations of the research and suggest future research directions.

6.1.1. How can the temporal and spatial distribution of insect sleeper pest populations be monitored with limited resources (Chapter 2)?

Monitoring the population dynamics of future pests such as *S. litura* is important for risk assessment and mitigation, but it can be expensive and time-consuming and there are often insufficient resources available for adequate surveying. Furthermore, published information can be sparse so data describing historical population sizes and geographical spread is often unavailable. I hypothesized that online tools such as iNaturalist and the Global Biodiversity Information Facility (GBIF) could provide useful information for monitoring sleeper pest species, in the absence of more targeted data collection.

To investigate this theory, I searched for suitable sources of such information on *S. litura* in New Zealand, including scientific journals, news articles, industry outputs and social media, in addition to data from two online platforms, iNaturalist and the Global Biodiversity Information Facility (GBIF). After appropriate quality checks and analyses, data from iNaturalist and GBIF indicated higher numbers and an extended geographical range for *S. litura* in 2018 and 2022-23. Furthermore, it confirmed that *S. litura* remains most prevalent in the upper North Island. The study suggests that these platforms can be a useful tool for future pest monitoring, thus confirming the hypothesis. However, the results also showed that caution must be taken with the analysis and interpretation of such data, and conducting targeted population surveys at multiple sites and across multiple years remains the preferred option where possible.

6.1.2. Are common broadleaf forage species vulnerable to feeding by *Spodoptera litura* and do they vary in quality as hosts (Chapter 3)?

Spodoptera litura is reported to have a very broad host range, however little is known about its host preferences in New Zealand, particularly regarding pasture plants species. I hypothesized that *S. litura* has the potential to cause considerable damage to New Zealand pastures and pasture crops through feeding on broadleaf pasture plants, such as plantain and clover, but that some plant host species will be higher quality than others. Lower quality hosts are likely to negatively affect *S. litura* behaviour, growth, reproduction.

The laboratory experiments described in Chapter 3 measured *S. litura* feeding, development, and reproduction on plantain, chicory, red clover and white clover, with the common weed species, dock, included as a positive control. The test forage species chosen for study are becoming increasingly favoured for pastures due to their stress tolerance, nitrogen-fixation, or leaching mitigation abilities and will therefore be important components of future New Zealand pastures.

Spodoptera litura larvae consumed more dock, plantain, and chicory per day than clovers, but more efficiently converted clover to body weight. Larvae reared on red clover from newly hatched took an average of 4 days longer to pupate, weighed 33% less at pupation and, as adults, laid 92% fewer eggs than those fed plantain. When given a choice, neonates initially settled equally on plantain and red clover. However, early feeding on red clover resulted in persistently lower larval weights, even after switching to better hosts. These effects did not carry through to pupation. This demonstrated that young *S. litura* larvae may choose to feed on a lower quality host rather than expend the energy or take the risk of moving to a higher quality host. However, they were later able to compensate for the early feeding choice.

These findings confirmed the hypothesis that some broadleaf pasture plants will be vulnerable to *S. litura* feeding damage. The results also demonstrated that some species are better quality hosts than others, as shown by changes to *S. litura* growth, feeding and reproduction. Identification of high- and low-quality host plants is a key step in mitigating the risks posed by this invasive and damaging pest and will inform future pasture species selection and breeding.

6.1.3. Does feeding on poor quality hosts have intergenerational effects on *Spodoptera litura* performance and gut microbiota (Chapter 4)?

Having identified red clover as a lower quality host than plantain for *S. litura*, I hypothesized that feeding on this forage species would impact the performance and gut microbiota of the offspring. I also speculated that offspring of *S. litura* fed red clover might be better able to tolerate the

poorer quality host than those whose parents had fed on plantain. In the laboratory experiments described in Chapter 4 I reared *S. litura* on red clover or plantain for two generations and compared the growth, feeding and mid-gut bacteria of second-generation larvae with that of individuals whose parents were raised on the same or the alternative plant host.

Parental host plant species significantly impacted the performance of the offspring *S. litura* generation. Individuals whose parents had fed on red clover were smaller and had reduced feeding efficiencies compared with those whose parents fed on plantain. This was true both for offspring feeding on plantain and for those on red clover. Parent host also affected offspring mid-gut microbiota. Offspring generation larvae feeding on red clover, and whose parent had fed on plantain, had significantly lower alpha (Shannon) diversity than other treatments. Feeding on red clover for two generations significantly reduced beta diversity, and larval mid-gut microbiota were strongly dominated by *Enterococcus*. This together with the lower larval weights and reduced survival when *S. litura* were fed red clover for two generations was contrary to the hypothesis that parental exposure to red clover would improve the tolerance of the offspring.

The results described in this chapter indicate the potential for red clover to reduce *S. litura* population diversity, resilience and adaptability through intergenerational reductions in growth and gut microbiota diversity.

6.1.4. Can *Spodoptera litura* use a poor quality/non-host species like perennial ryegrass as a host for part of its development and does the age of the insect affect this ability (Chapter 5)?

Spodoptera litura is a known pest of broadleaf plant species, and the work described in this thesis has identified several important forage species that will be vulnerable to feeding by this pest. Given its highly polyphagous nature I hypothesized that during population outbreaks, when numbers are high and food sources become scarce, *S. litura* will also be able to utilise the dominant pasture species in New Zealand, perennial ryegrass, as a host for at least part of its development. Furthermore, I speculated that this ability would be age-dependent, with more mature larvae being better able to digest ryegrass than younger larvae. I investigated *S. litura*'s ability to utilise perennial ryegrass for all or part of its development by comparing its growth and survival when caged on potted perennial ryegrass plants, white clover plants or both together. I then tested the effect of larval age by switching *S. litura* larvae from one host to the other at each of three different ages.

The study confirmed that perennial ryegrass is a poor host for *S. litura*. Survival to adulthood was contingent on access to additional food sources. In the potted plant experiment 6% (1/26) of individuals survived to adulthood when caged on perennial ryegrass, and this was

probably aided by the presence of aphids. Larvae were able to complete development after feeding on ryegrass for 14 or 21 days (72% and 8% survival respectively) if they were subsequently fed with white clover. Interestingly larvae and pupae caged on both ryegrass and clover were lighter than those on only white clover.

Larval age affected the ability of *S. litura* to utilise ryegrass but contrary to the hypothesis larvae fed ryegrass from neonate survived for longer than those that were switched from clover to ryegrass when older. I measured starvation resistance of larvae at each of three ages to test whether the longer survival time of young larvae was due to a decrease in starvation resistance with age. However, starvation resistance increased with age, thus the impact of larval age on the ability to use ryegrass was not solely due to deterrence and starvation.

The study results indicate that perennial ryegrass may be vulnerable to damage from *S. litura* during outbreaks, despite being a poor host, but also that ryegrass may help mitigate for predicted climate change-related increases in population sizes in New Zealand pastures.

6.2. Synthesis and discussion of key conclusions

Interactions between herbivorous insects and their plant hosts are highly complex. The studies reported in this thesis provide a basis for understanding the potential risk that *S. litura* poses to future New Zealand pasture under climate change scenarios through its interactions with forage plant species. Here I discuss key and overarching conclusions from these studies. A summary of some of the key outcomes and their potential pathways to population level effects are shown in Figure 6-1.

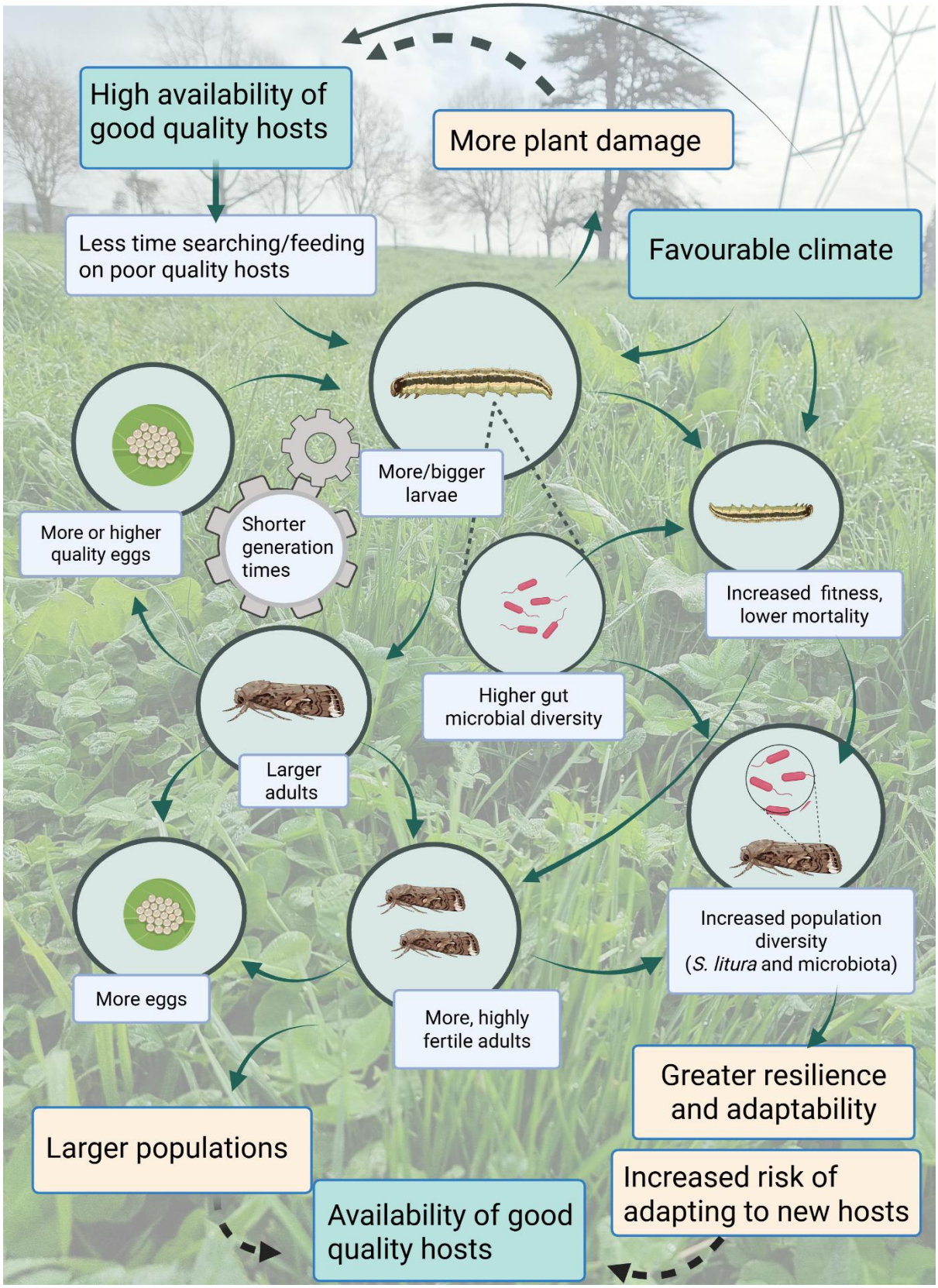


Figure 6-1 – Interacting effects of climate and forage plant host on Spodoptera litura *individuals and potential pathways to population level effects. Solid arrows represent positive influence, and dashed arrows represent negative influence. (Image created in https://BioRender.com).*

6.2.1. Population monitoring and climate

Climatic conditions are a key driver of *S. litura* population size and geographical spread (Bragard et al., 2019). With optimal temperature and humidity ranges for development of 30–35°C and 60–83% for this species (Fu et al., 2025), climatic conditions in New Zealand are marginal for its survival. Anecdotally, *S. litura* populations currently become large enough to cause significant harm only in some years and in some locations in New Zealand, however robust population survey data is lacking. In its absence, the online tools iNaturalist and GBIF provided useful data about *S. litura* numbers and location in New Zealand from 2014 (better quality data from 2018) (Chapter 2; Jensen et al., 2025). While potential biases in reporting mean that the data must be used with caution, I was able to relate anecdotally reported outbreaks in 2018 and 2022-23 with increased relative observations in iNaturalist. Observed geographical spread correlated well to its expected distribution in New Zealand based on the temperatures required for its survival and development.

Incorporation of iNaturalist *S. litura* observations from 2025 into the analysis presented in Jensen et al. (2025; Chapter 3) confirms that 2022-23 represented *S. litura* 'outbreak' years rather than a trend upwards (Figure 6-2). This aligns with predictions based on CLIMEX models (Figure 1-2 and Figure 1-4) that *S. litura* population sizes and ranges are likely to increase by 2060. Continued monitoring of online data and, ideally, confirmation through physical surveys, would allow us to verify if *S. litura* is in fact a sleeper pest in New Zealand, and if so, provide an early warning of its awakening.

I was unable to link *S. litura* outbreaks in 2018 and 2022-23 to any specific climate conditions. Gerard et al. (2011) attributed a 2011 outbreak in New Zealand to a mild and wet winter in 2010 followed by above average spring and summer temperatures and near record summer rainfall. Kean et al. (2015) and Peacock et al. (2006) suggest that warm summer and autumn temperatures are key as they allow for additional generations to develop. In fact, it is likely that *S. litura* populations are impacted by more than one seasonally defined climate variable. As comprehensive *S. litura* presence/absence data and site-specific climate data was unavailable, accurate modelling was beyond the scope of the study. Such models would help to identify important climate predictors and thereby predict outbreak years and locations.

In addition to *S. litura*, six further invertebrates were listed by Orre-Gordon and Jensen (2025) as potential New Zealand sleeper pests. Some of these are good candidates for monitoring using iNaturalist/GBIF while others are less so. Migratory locusts (*Locusta migratoria*) are large (35 mm – 75 mm in length), striking in appearance, mobile and therefore more likely to be encountered than more sedentary species, and novel enough to attract attention. Research

grade observations in iNaturalist range in number from 24 to 86 per year since 2016. Tropical grass webworm (*Herpetogramma licarsisalis*) and Flores weevil (*Floresianus sordidus*) to date have been reported in smaller numbers, perhaps reflecting their smaller size and, for tropical grass webworm, more limited geographical range. However, for all these species, continued monitoring via this online tool is likely to provide a useful warning of increasing numbers. In contrast, blue oat mite (*Penthaleus* spp.) and red legged earth mite (*Halotydeus destructor*), while visually striking, are small (approximately 1 mm) and therefore difficult to photograph and to distinguish with the naked eye. Inspection of iNaturalist observations for these two species revealed low numbers of observations, only 39 in total since 2016, and only 26% of these were 'research grade'. There are no recorded iNaturalist observations of the sixth putative sleeper species, spotted alfalfa aphid (*Therioaphis trifolii* f. *maculata*), in New Zealand.

6.2.2. Host plant quality

Across multiple laboratory experiments, described in Chapters 3 to 5, I demonstrated that *S. litura* can utilise several common forage plant species, but some species are higher quality hosts than others. Growth, feeding, behaviour, gut microbiota and reproduction were affected by plant host species, confirming the original hypotheses. A high-level summary of main effects is shown in Figure 6-3.

Chicory, dock, plantain and white clover were all shown to be good hosts for *S. litura*. Larvae feeding on these broadleaf species had equal or greater growth, feeding and reproduction than those feeding on red clover. Chicory, dock, plantain and white clover will therefore be vulnerable to attack in pasture. Interestingly, larvae feeding on plantain had lower feeding efficiencies and therefore needed to eat more herbage (wet weight) while the opposite was true for larvae on white clover. This has implications for levels of damage to these plants in the field, an aspect that warrants further investigation.

In comparison to the other broadleaf species tested, red clover was a poor host. Although *S. litura* was able to complete development on red clover, all performance measures other than feeding efficiency were reduced relative to the other species. This has interesting implications for the use of red clover in future New Zealand pastures. It may prove more resilient than other broadleaf species to attack by *S. litura* through reduced feeding and survival, and *S. litura* populations sizes may be limited in pastures dominated by red clover, however this theory requires field verification.

I was unable to identify the mechanisms behind red clover's negative effect on *S. litura* performance. Although the results demonstrated an association between feeding on red clover and reduced gut microbiota diversity the study could not show whether this was a causal

relationship or a result of lower rates of *S. litura* survival. Physical and chemical plant leaf characteristics such as water content, leaf toughness and secondary metabolites are likely to contribute to red clover's low host quality. Identification of specific mechanisms would be a valuable step towards breeding more resilient cultivars.

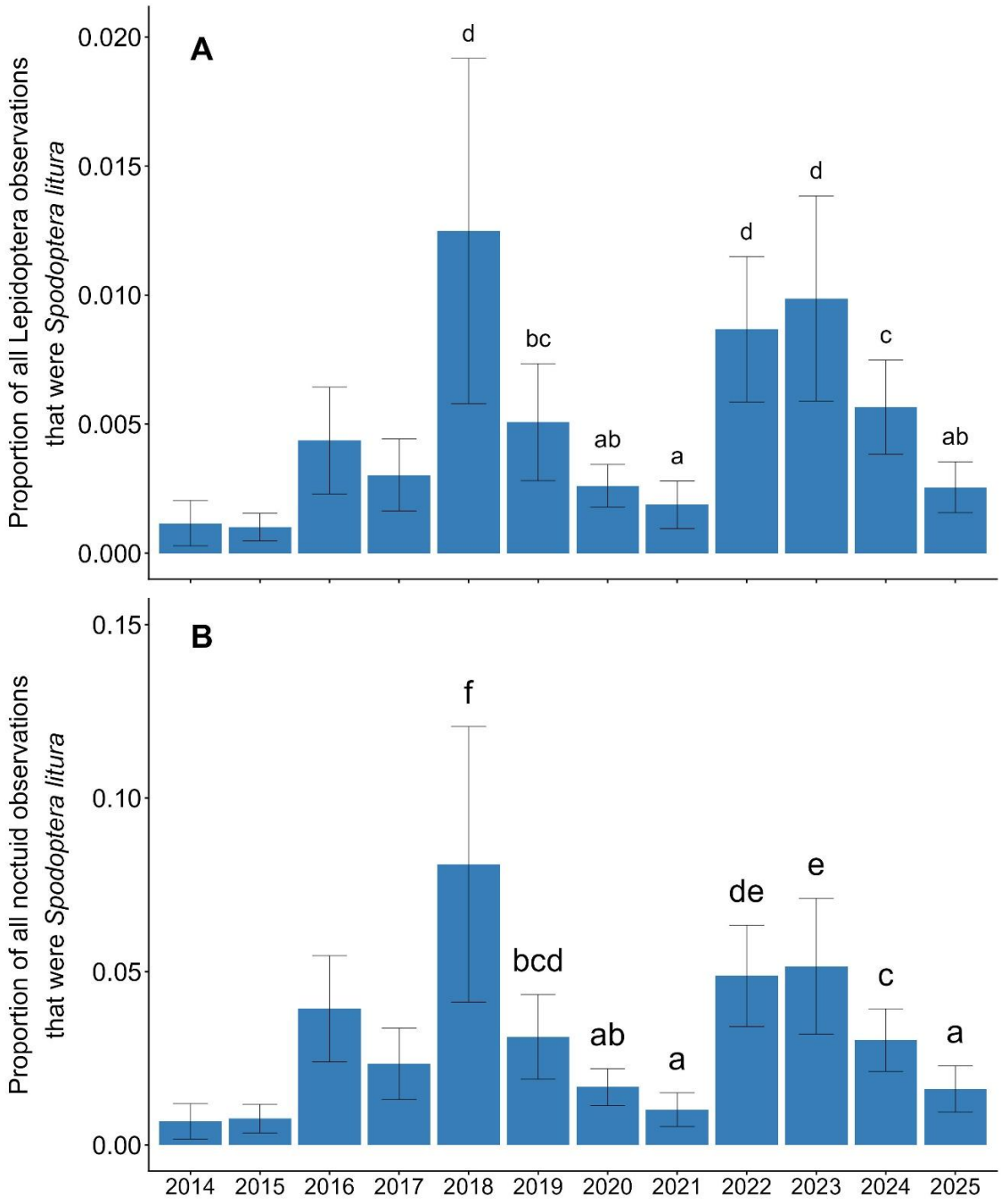


Figure 6-2 – Annual means of Spodoptera litura *observations reported each month in iNaturalistNZ for mainland New Zealand from January 2014 to December 2025 as a proportion of (a) Lepidoptera and (b) Noctuidae observations. Error bars are ± one standard error of the mean. Observations from 2014 to 2017 were omitted from the statistical analysis model due to low observation numbers. Means with different letters are significantly different ($p<0.05$). Figure adapted from (Jensen et al., 2025; Chapter 2).*

Perennial ryegrass was confirmed to be a very poor host for *S. litura*. This was not a surprising finding for this broadleaf feeding insect. The high silica and lignin content and lower nutritive value in grasses compared with broadleaf species makes it physiologically challenging for herbivorous insects to switch between the two (Jonas & Joern, 2008). Despite its poor quality as a host, *S. litura* were able to feed and survive solely on perennial ryegrass for up to 24 days (from neonate). When *S. litura* populations are large and preferred host food plants are scarce larvae shift to non-favoured host plant species and plant parts (Ahmad et al., 2013). Therefore, perennial ryegrass may still be vulnerable to this pest in some circumstances, but this will incur a cost to larval survival and performance.

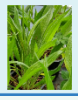
	Chicory, Dock 	Plantain 	White clover 	Red clover 	Ryegrass 
Eggs  • number	=	↑	=	↓	too few adults to test
Larvae  • size	↑	↑	↑	↓	↓↓
• feeding amount	↑	↑	↓	↓	↓↓
• feeding efficiency	=	↓	↑	↑	not tested
• gut microbiota diversity	not tested	↑	not tested	↓	not tested
Pupae  • size	↑	↑	↑	↓	↓
Development rate	=	↑	↑	↓	↓
Survival 	=	↑	↑	↓	↓↓

Figure 6-3 – Relative forage host plant effects on Spodoptera litura. *Vertical arrows represent a statistically significant increase (↑) or decrease (↓) relative to another host plant species in at least one experiment. Multiple arrows show a large effect. Equals sign (=) represents no statistically significant difference. Chicory and dock were not significantly different from each other for any of the variables measured. Overall, red clover and ryegrass were poorer hosts for* S. litura *than the other plant species assessed. (Image created in https://BioRender.com).*

6.2.3. Insect responses

New Zealand pastures are traditionally based on ryegrasses and clovers (Ferguson et al., 2019) but famers are increasingly turning to more diverse forage mixes, including alternative broadleaf

forages such as plantain and chicory. Ingress of broadleaf weeds such as dock, dandelion (*Taraxacum officinale*) and buttercup (*Ranunculus repens*) is also common (Blackwell et al., 2011; Tozer et al., 2011) and many of these weed species are likely hosts for *S. litura*. It is therefore likely that a relatively mobile insect such as *S. litura* will encounter more than one potential host plant species during its larval phase. Several mechanisms are available to polyphagous herbivores trying to counter poor host quality. These include compensatory feeding, food selection and physiological adjustments (Behmer, 2009; Jonas & Joern, 2008; Simpson & Simpson, 1990). Eating more of a poor quality food to obtain enough of a limiting nutrient can result in over-consumption of non-limiting or deterrent chemicals, so where possible, diet-switching may be a better option (Jonas & Joern, 2008). Results presented here showed that very young larvae do not necessarily move to and feed on a preferred host over a lower quality one, and that feeding on the lower quality host could have lasting consequences for larval growth. Larvae offered red clover and plantain were initially equally as likely to feed on the lower quality red clover as on the higher quality plantain (Chapter 3; Jensen et al., 2026). The larvae that fed on red clover for the first two days had lower weights up to but not including pupation. This demonstrates that the presence of high-quality host species such as plantain can allow *S. litura* to compensate for early feeding on low-quality hosts. However, conversely, the finding in Chapter 5 that *S. litura* caged on both perennial ryegrass and white clover as neonates weighed less at pupation than those on only white clover shows that the negative effects of low-quality hosts, if great enough, could impact *S. litura* at a population level. Although I was unable to show the mechanism for this it may have been because larvae were unable to compensate for the lower growth rates and unfavourable chemical and physical properties of perennial ryegrass, or it could reflect the cost of spending more time foraging. Regardless of the mechanism, results from these studies show that plant diversity, when it includes less favourable species such as red clover or perennial ryegrass, and when availability of higher quality hosts is limited, could have a negative impact on *S. litura*.

Several experiments showed direct effects of poor-quality hosts on *S. litura* growth and reproductive capacity through decreased adult weights, lower egg numbers and/or lower survival. These effects all demonstrate potential pathways by which poor-quality diet could impact *S. litura* intergenerationally, through changes in population size and the number of generations per year (Figure 6-1). Results in Chapter 4 also showed that poor-quality diet can cause a reduction in the performance of the subsequent generation; larvae whose parents had fed on a low-quality host were smaller, had lower feeding efficiencies and decreased gut microbial diversity. Over-dominance and low diversity in larval gut microbiota is likely to reduce

larval resilience and adaptability (Henry et al., 2021; Lange et al., 2023), thereby representing a further mechanism by which feeding on a poor host can negatively impact *S. litura* at a population level.

Neonate *S. litura* larvae offered both red clover and plantain did not immediately move to the higher quality host (Chapter 3). This may be a behavioural strategy where the potential risks and costs associated with moving are greater than the benefits. Mitra et al. (2021) noted that adult *S. litura* chose to oviposit and neonate larvae performed best on castor (*Ricinus communis*), but larvae migrated to eggplant (*Solanum melongena*) at 3rd–4th instar. The authors suggested a change in larval digestive physiology as a driver for this behaviour. The experiments described in Chapter 5 demonstrated a difference with larval age in the ability of *S. litura* to tolerate perennial ryegrass as a food source. Larvae feeding on perennial ryegrass from neonate survived for longer than mature larvae that were switched from white clover to perennial ryegrass. I was unable to explain the mechanism behind this.

6.3. Study limitations

The experiments reported here were all conducted in a laboratory under controlled temperature conditions and in the absence of natural enemies and interacting plant stressors (e.g. other herbivores, plant disease). Most were also conducted in the absence of the additional food sources that would be available in pasture. Field verification is required to determine whether the effects observed in the laboratory would have biologically significant consequences for *S. litura* populations and how this would impact the levels of damage imposed on their plant hosts. Conducting field trials can be expensive and time-consuming, and they are at greater risk of unexpected impacts including weather events and insufficient numbers of the pest species being studied (O'Callaghan & Mansfield, 2026). Caging and augmentation of insect numbers may be appropriate to ensure adequate pest pressure when populations sizes are low or unpredictable as done, for example, in a field trial with *Heteronychus arator* (Mansfield et al., 2020). An intermediate step would be to use potted plants in mini swards. Whole plant experiments mitigate the potential loss of nutrients, allelochemicals and/or moisture content that may occur when using excised leaf material, provide a more natural environment for the test insect, and account for any potential impacts of plant structure (e.g. woody stems, variable plant density). Mini sward or field experiments would provide an opportunity to assess the impact of varying the proportions of high- and low-quality hosts, thereby enabling the calculation of optimum planting ratios, and to determine economic damage thresholds.

The experiments described in Chapter 4 showed that feeding on a poor-quality host such as red clover can negatively affect the subsequent *S. litura* generation. This was contradictory to the hypothesis that offspring might be better able to tolerate red clover through inheritance of an altered gut microbiota. However, the experiments only ran for two generations of *S. litura* and it is possible that selection for improved performance on red clover would only become evident after more generations. The study also did not distinguish between offspring of different parent pairs. This would have allowed a better understanding of whether there was a direct effect of red clover on gut microbiota or if the effects were due to decreased survival in the parental generation and thereby population diversity. Given more time and resources, a more robust experiment would include separation of individual larvae from each other at hatching, monitoring and feeding each one separately throughout its lifespan, and more extensive DNA sequencing. Sequencing of the microbiota of the eggs and/or unfed neonate larvae in addition to that of later instar larvae and the herbage they were feeding on would help further clarify host *S. litura*–plant–microbial interactions and whether gut microbiota are acquired vertically or horizontally. Brinkmann et al. (2008) demonstrated a likely pathway for vertical inheritance of *Enterococcus* through DNA-based profiling of eggs, larval guts and faeces of *Manduca sexta* together with their diet plant, tobacco leaves (*Nicotiana tabacum*). The gut microbiota study described in this thesis was restricted to *S. litura* mid-gut bacteria. Fungal gut communities in Lepidoptera are less well studied than bacterial communities, despite their potential role in insecticide resistance and immune responses (Zhang et al., 2022). Furthermore, bacterial and fungal microbial communities may vary along the alimentary canal (e.g. Tang et al., 2012). Comparison of bacterial and fungal microbiota through both 16S and ITS sequencing in fore-, mid- and hind-gut sections would therefore add to our understanding of *S. litura*-plant-microbiota interactions.

Understanding the mechanisms behind the poor host quality of red clover and perennial ryegrass for *S. litura* would aid future work on identification of less susceptible forage species, cultivars or genotypes. Chemical analysis of allelochemicals such as formononetin *in planta* may provide guidance. A comparison of *S. litura*'s performance on plant cultivars with low and high levels of any putative active compounds or bioassays in which it is exposed to the pure compound should then be conducted to confirm the compound's effects. Plant physical characteristics also contribute to a plant's host quality. Cibils-Stewart et al. (2023) showed that higher levels of silicon in tall fescue (*Festuca arundinacea*) reduced the performance of *Helicoverpa armigera* larvae. Scanning electron microscopy showed that mineralised silica cells in the leaf epidermis caused significant wear to larval mandibles. The authors also suggested that the high abrasiveness of silicon-filled cells may physically damage the gut and reduce

nutrient extraction. Plant physical characteristics and insect responses such as these are worth further examination in *S. litura* and may help explain the age-related differences in the ability of this species to utilise perennial ryegrass (Chapter 5).

6.4. Future research

6.4.1. *Spodoptera litura*

The studies reported in this thesis have paved the way for understanding interactions between *S. litura* and future forages in New Zealand and reveal several important next steps. Tolerance factors identified under constant conditions in laboratory experiments such as these need to be verified with field experiments. These would allow us to understand population-level effects for both the plant species and *S. litura*, the influence of mixed-species planting, and help to calculate planting ratios that would maintain an optimal balance between improved climate resilience and the potential for increased pest pressure. These are important steps towards the development of a suitable climate mitigation strategy for agricultural systems. Continued research into *S. litura* in New Zealand pastoral systems should include:

- Monitoring *S. litura* populations through targeted surveys
- Modelling of population data and site-specific climate variables to enable risk prediction
- Public education to enable more reliable reporting and to help farmers understand best practice mitigation strategies
- Field verification of laboratory results
- Identification of physical and/or chemical properties that reduce host quality for *S. litura*
- Identification of cultivars or plant genotypes with greater tolerance to *S. litura* feeding
- Establishing economic damage thresholds for different plant species
- Calculation of optimum planting ratios.

6.4.2. Other future pests

Several other sleeper or current pest species in New Zealand may become increasingly widespread and damaging under climate change (Orre-Gordon & Jensen, 2025) and, as with *S. litura*, these warrant further study. One listed species, Flores weevil, is not considered a serious pest in other countries (Chadwick, 1978) but has been noted as causing significant damage to pasture in New Zealand (Esson, 1975). It has a below-ground feeding larval stage, a long generation time and no established methods for laboratory rearing or experimentation. The species' geographic distribution in New Zealand, host range and trophic level interactions are

therefore poorly understood. Flores weevil may to be a good candidate for monitoring through iNaturalist (Section 6.2.1), and identification of its favoured host plant species would be an important first step towards predicting its pest potential.

As with *S. litura*, some other species listed by Orre-Gordon and Jensen (2025) have known host plant ranges that include both agricultural and horticultural crops. For example, black beetle (*H. arator*) causes extensive damage to pasture in the northern North Island and can also feed on crops such as potatoes, kumara, cabbage and kiwifruit vines. In its solitary phase, the migratory locust is graminivorous but during outbreaks, swarming locusts are less discriminating and can completely devastate vegetation (Lecoq, 2022). In New Zealand, migratory locusts currently do not swarm however they may begin to do so as temperatures warm (Orre-Gordon & Jensen, 2025). Although more is known globally about these pests than is the case for Flores weevil, the risk that species such as black beetle and migratory locusts pose to multiple, economically important industries mean that monitoring their geographical spread and population sizes is vital for risk assessment and mitigation.

6.5. Overall conclusions

Spodoptera litura is likely to become more widespread and damaging in New Zealand under predicted climate change scenarios. Public observations of *S. litura* registered on the online platform iNaturalist showed that population outbreaks occurred in 2018 and again in 2022-23 but indicate that the species remains a 'sleeper pest' at present. However, as an extremely polyphagous and invasive herbivore, it poses a serious risk to pastoral agriculture. This research has demonstrated that several plant species commonly found in pasture, plantain, chicory, dock and white clover, are good-quality hosts for *S. litura*. These will be vulnerable to attack and may need protection during outbreak years or in regions where *S. litura* is prevalent. Red clover, however, is a significantly poorer host, and was shown to negatively impact *S. litura* growth, survival, reproductive output and mid-gut microbiota. Furthermore, these negative effects had intergenerational consequences through decreased performance and gut microbiota diversity of the offspring generation. The research confirmed that perennial ryegrass is a very poor host for *S. litura*, but it can be vulnerable to attack. Although feeding on perennial ryegrass severely reduces survival and growth, larvae can feed and survive on this plant, particularly at earlier instars and if additional, higher quality food sources are also available. Together, these results indicate the potential value of focussing on developing mixed pastures, with optimised combinations of good and poor hosts, rather than more traditional low diversity or monoculture pastures. Monitoring surveys, field verification and identification of resistant cultivars are

important next steps towards mitigating the risks posed by *S. litura* to future New Zealand agriculture. Similar studies focusing on other sleeper pests in New Zealand would also be beneficial.

7. References

Abbas, A., Wei-Bo, Q., Hafeez, F., Hasnain, A., Shoukat, K., Ali, J., Xiao, F., Liu, J.-L., Ghramh, H. A., Khan, K. A., Ullah, F., & Ri-Zhao, C. (2025). Unveiling the feeding response of *Spodoptera litura* to natural host and artificial diet. *Entomologia Experimentalis et Applicata*, *173*(3), 218–231. https://doi.org/10.1111/eea.13537

Aceituno-Medina, M., Hernández, E., Rincón-Betancurt, O., García-Fajardo, L. V., & Diego-García, E. (2024). Larval nutritional effects on male and female survival and fecundity in *Anastrepha ludens*. *Entomologia Experimentalis et Applicata*, *172*(1), 2–14. https://doi.org/10.1111/eea.13383

AgPest. (2026). *Tropical armyworm*. Retrieved 4/5/26 from https://agpest.co.nz/?pesttypes=tropical-armyworm

Agricom. (2025). *Farmer's forage guide*. *https://www.agricom.co.nz/Files/Files/Public/Agricom/Guides/Farmers%20Forage%20Guide.pdf*.

Ahmad, M., Ghaffar, A., & Muhammad, A. (2013). Host plants of leafworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. *Asian J Agri. Biology*, *1*, 1–6.

Alfeus, M., Irish, J., & Birkhofer, K. (2025). Recognition and completeness metrics from iNaturalist and GBIF can inform future citizen science and research projects: a case study on arthropods in Namibia. *Biodiversity and Conservation*, *34*(2), 467–480. https://doi.org/10.1007/s10531-024-02981-z

Angelo, M. J., & Slansky, F. (1984). Body building by insects: Trade-offs in resource allocation with particular reference to migratory species. *The Florida Entomologist*, *67*(1), 22–41. https://doi.org/10.2307/3494102

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, *47*, 817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300

Badyaev, A. V., & Uller, T. (2009). Parental effects in ecology and evolution: mechanisms, processes and implications. *Philos Trans R Soc Lond B Biol Sci*, *364*(1520), 1169–1177. https://doi.org/10.1098/rstb.2008.0302

Bale, J. S. (1991). Insects at low temperature: A predictable relationship? *Functional Ecology*, *5*(2), 291–298. https://doi.org/10.2307/2389267

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x

Bapatla, K. G., Singh, A., Yeddula, S., & Patil, R. H. (2018). Annotation of gut bacterial taxonomic and functional diversity in *Spodoptera litura* and *Spilosoma obliqua*. *Journal of Basic Microbiology*, *58*(3), 217–226. https://doi.org/10.1002/jobm.201700462

Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annual Review of Entomology*, *54*(1), 165–187. https://doi.org/10.1146/annurev.ento.54.110807.090537

Bernays, E., & Singer, M. (1998). A rhythm underlying feeding behaviour in a highly polyphagous caterpillar. *Physiological Entomology*, *23*(4), 295–302. https://doi.org/10.1046/j.1365-3032.1998.234103.x

Bernays, E. A. (1998). Evolution of feeding behavior in insect herbivores: success seen as different ways to eat without being eaten. *BioScience*, *48*(1), 35–44. https://doi.org/https://doi.org/10.2307/1313226

Bernays, E. A., Bright, K. L., Gonzalez, N., & Angel, J. (1994). Dietary mixing in a generalist herbivore: Tests of two hypotheses. *Ecology*, *75*(7), 1997. https://doi.org/10.2307/1941604

Berry, J. A., & Walker, G. P. (2004). *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae: Euphorinae): an exotic polyphagous parasitoid in New Zealand. *New Zealand Journal of Zoology*, *31*, 33–44. https://doi.org/10.1080/03014223.2004.9518357

Biere, A., Marak, H. B., & van Damme, J. M. M. (2004). Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? *Oecologia*, *140*(3), 430–441. https://doi.org/10.1007/s00442-004-1603-6

Blackwell, G., Lucock, D., Moller, H., Hill, R., Manhire, J., & Emanuelsson, M. (2011). Abundance and diversity of herbaceous weeds in sheep/beef pastures, South Island, New Zealand. *New Zealand Journal of Agricultural Research*, *54*(1), 53–69. https://doi.org/10.1080/00288233.2010.535493

Boakes, E. H., Gliozzo, G., Seymour, V., Harvey, M., Smith, C., Roy, D. B., & Haklay, M. (2016). Patterns of contribution to citizen science biodiversity projects increase understanding of volunteers' recording behaviour. *Scientific Reports*, *6*(1), 33051. https://doi.org/10.1038/srep33051

Boardman, L. A. (1977). Insectary culture of *Spodoptera litura* (Lepidoptera: Noctuidae). *The New Zealand Entomologist*, *6*, 316–318. https://doi.org/10.1080/00779962.1977.9722276

Bodeker, G., Cullen, N., Katurji, M., McDonald, A., Morgenstern, O., Noone, D., Renwick, J., Revell, L., & Tait, A. (2022). *Aotearoa New Zealand climate change projections guidance: Interpreting the latest IPCC WG1 report findings* (Prepared for the Ministry for the environment. Report number CR 501, 51p).

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, *23*(1), 27–37. https://doi.org/10.1111/j.1365-2435.2009.01527.x

Boggs, C. L. (2016). The fingerprints of global climate change on insect populations. *Current Opinion in Insect Science*, *17*, 69–73. https://doi.org/10.1016/j.cois.2016.07.004

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, *144*(3), 353–361. https://doi.org/10.1007/s00442-005-0076-6

Bradley, B., Beaury, E., Fusco, E. J., Laginhas, B., & Pasquarella, V. (2018). Regional invasive species and climate change management challenge: Preparing for sleeper pests. *Northeast Climate Adaptation Science Center. Environmental Conservation Educational Materials*. https://doi.org/10.7275/R5F18WXT

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.-A., Jaques Miret, J. A., Justesen, A. F., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Reignault, P. L., Thulke, H.-H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., Malumphy, C.,...MacLeod, A. (2019). Pest categorisation of *Spodoptera litura*. *EFSA Journal*, *17*(7), e05765. https://doi.org/10.2903/j.efsa.2019.5765

Brambila, J. (2013). *Identification notes for Spodoptera litura and Spodoptera littoralis (Lepidoptera: Noctuidae) and some native Spodoptera moths*. https://www.invasive.org/publications/aphis/Handout_Spodoptera_Wings_2013.pdf

Bright, M., & Bulgheresi, S. (2010). A complex journey: transmission of microbial symbionts. *Nature Reviews Microbiology*, *8*(3), 218–230. https://doi.org/10.1038/nrmicro2262

Brinkmann, N., Martens, R., & Tebbe, C. C. (2008). Origin and diversity of metabolically active gut bacteria from laboratory-bred larvae of *Manduca sexta* (Sphingidae, Lepidoptera, Insecta). *Appl Environ Microbiol*, *74*(23), 7189–7196. https://doi.org/10.1128/aem.01464-08

Brock, J. L., Hyslop, M. G., & Widdup, K. H. (2003). A review of red and white clovers in the dryland environment. *Grassland Research and Practice Series No. 11: Legumes for dryland pastures*, 101–107. https://doi.org/10.33584/rps.11.2003.3001

Brockerhoff, E. G., Barratt, B. I. P., Beggs, J. R., Fagan, L. L., Kay, M. K., Phillips, C. B., & Vink, C. J. (2010). Impacts of exotic invertebrates on New Zealand's indigenous species and ecosystems. *New Zealand Journal of Ecology*, *34*(1), 158–174.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*, 378–400. https://doi.org/10.32614/RJ-2017-066

Brown, H. E., Moot, D. J., & Pollock, K. M. (2003). Long term growth rates and water extraction patterns of dryland chicory, lucerne and red clover. *Grassland Research and Practice Series No. 11: Legumes for dryland pastures*, 91–99. https://doi.org/10.33584/rps.11.2003.2991

Bryant, R. H., Snow, V. O., Shorten, P. R., & Welten, B. G. (2020). Can alternative forages substantially reduce N leaching? findings from a review and associated modelling. *New Zealand Journal of Agricultural Research*, *63*(1), 3–28. https://doi.org/10.1080/00288233.2019.1680395

CABI. (2014). *Spodoptera litura* (taro caterpillar): Technical factsheet. *CABI Compendium*. https://doi.org/10.1079/cabicompendium.44520

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M.,...Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Caradus, J. R., Bouton, J. H., Brummer, E. C., Faville, M. J., George, R. M., Hume, D. E., Inch, C., Kerr, G. A., Montel, S., Stewart, A., & Woodfield, D. R. (2021). Plant breeding for resilient pastures. *Grassland Research and Practice Series No. 17: Resilient pastures symposium*, 81–103. https://doi.org/10.33584/rps.17.2021.3441

Caradus, J. R., & Johnson, L. J. (2020). *Epichloë* fungal endophytes—From a biological curiosity in wild grasses to an essential component of resilient high performing ryegrass and fescue pastures. *Journal of Fungi*, *6*, 322. https://doi.org/10.3390/jof6040322

Caradus, J. R., Lovatt, S., & Belgrave, B. (2013). Adoption of forage technologies by New Zealand farmers - case studies. *Proceedings of the 22nd International Grassland Congress*, 1843–1845. https://doi.org/10.33584/jnzg.2013.75.2917

Chadwick, C. E. (1978). Distribution and food plants of certain Curculionoidea (Coleoptera) with special reference to New South Wales. *General and Applied Entomology*, *10*, 3–38.

Chapman, D. F. (2021). Resilient pastures. *Grassland Research and Practice Series No. 17: Resilient pastures symposium*, 7–14. https://doi.org/10.33584/rps.17.2021.3522

Chapman, R. B. (1984). Pasture pests. In R. R. Scott (Ed.), *New Zealand Pests and Beneficial Insects* (pp. 119–167). Lincoln University College of Agriculture.

Chen, B., Mason, C. J., Peiffer, M., Zhang, D., Shao, Y., & Felton, G. W. (2022). Enterococcal symbionts of caterpillars facilitate the utilization of a suboptimal diet. *Journal of Insect Physiology*, *138*, 104369. https://doi.org/10.1016/j.jinsphys.2022.104369

Chen, B., Teh, B. S., Sun, C., Hu, S., Lu, X., Boland, W., & Shao, Y. (2016). Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci Rep*, *6*, 29505. https://doi.org/10.1038/srep29505

Chen, Y.-J., Cheah, B. H., Lin, C.-Y., Ku, Y.-T., Kuo, C.-H., Zhang, Y.-Y., Chen, B.-R., Nean, O., Hsieh, C.-H., Yeh, P.-M., Yeo, F. K. S., Lin, Y.-P., Chuang, W.-P., Lee, C.-R., & Ting, H.-M. (2023). Inducible chemical defenses in wild mungbean confer resistance to *Spodoptera litura* and possibly at the expense of drought tolerance. *Environmental and Experimental Botany*, *205*, 105100. https://doi.org/10.1016/j.envexpbot.2022.105100

Choudhary, A. K., & Shrivastava, S. K. (2007). Efficacy and economics of some neem based products against tobacco caterpillar, *Spodoptera litura* F. on soybean in Madhya Pradesh, India. *International Journal of Agricultural Science*, *3*, 15–17.

Chu, C.-C., Spencer, J. L., Curzi, M. J., Zavala, J. A., & Seufferheld, M. J. (2013). Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm. *Proceedings of the National Academy of Sciences*, *110*(29), 11917–11922. https://doi.org/10.1073/pnas.1301886110

Cibils-Stewart, X., Putra, R., Islam, T., Fanna, D. J., Wuhrer, R., Mace, W. J., Hartley, S. E., Popay, A. J., & Johnson, S. N. (2023). Silicon and *Epichloë*-endophyte defences in a model temperate grass diminish feeding efficiency and immunity of an insect folivore. *Functional Ecology*, *37*(12), 3177–3192. https://doi.org/10.1111/1365-2435.14453

Clissold, F. J., Sanson, G. D., Read, J., & Simpson, S. J. (2009). Gross vs. net income: How plant toughness affects performance of an insect herbivore. *Ecology*, *90*(12), 3393–3405. https://doi.org/10.1890/09-0130.1

Cooper, B. M., Crush, J. R., Woodfield, D. R., Willoughby, B. E., & Panckhurst, K. A. (2003). Red clover - a legume showing tolerance to clover root weevil. *Proceedings of the New Zealand Grassland Association*, *65*, 153–156. https://doi.org/10.33584/jnzg.2003.65.2490

Cottier, W., & Gourlay, E. S. (1955). New horticultural pest found on Nelson tobacco. *New Zealand Journal of Agriculture*, *91*, 349–351.

Couture, J. J., Mason, C. J., Habeck, C. W., & Lindroth, R. L. (2016). Behavioral and morphological responses of an insect herbivore to low nutrient quality are inhibited by plant chemical defenses. *Arthropod-Plant Interactions*, *10*(4), 341–349. https://doi.org/10.1007/s11829-016-9439-7

Crosby, T. K., S., D. J., & and Watt, J. C. (1998). Area codes for recording specimen localities in the New Zealand subregion. *New Zealand Journal of Zoology*, *25*(2), 175–183. https://doi.org/10.1080/03014223.1998.9518148

Cull, B. (2021). Potential for online crowdsourced biological recording data to complement surveillance for arthropod vectors. *PLOS ONE*, *16*(4), e0250382. https://doi.org/10.1371/journal.pone.0250382

Cunningham, J. P., West, S. A., & Zalucki, M. P. (2001). Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos*, *95*(3), 537–543. https://doi.org/10.1034/j.1600-0706.2001.950319.x

Cunningham, J. P., & Zalucki, M. P. (2014). Understanding Heliothine (Lepidoptera: Heliothinae) pests: What is a host plant? *Journal of Economic Entomology*, *107*(3), 881–896. https://doi.org/10.1603/ec14036

Dale, P. S., Hayes, J. C., & Johannesson, J. (1976). New records of plant pests in New Zealand. *New Zealand Journal of Agricultural Research*, *19*(2), 265–269. https://doi.org/10.1080/00288233.1976.10426777

Deguenon, J. M., Dhammi, A., Ponnusamy, L., Travanty, N. V., Cave, G., Lawrie, R., Mott, D., Reisig, D., Kurtz, R., & Roe, R. M. (2021). Bacterial microbiota of field-collected *Helicoverpa zea* (Lepidoptera: Noctuidae) from transgenic Bt and non-Bt cotton. *Microorganisms*, *9*(4). https://doi.org/10.3390/microorganisms9040878

del Campo, M. L., Miles, C. I., Schroeder, F. C., Mueller, C., Booker, R., & Renwick, J. A. (2001). Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature*, *411*(6834), 186–189. https://doi.org/10.1038/35075559

DeLucia, E. H., Nabity, P. D., Zavala, J. A., & Berenbaum, M. R. (2012). Climate change: resetting plant-insect interactions. *Plant Physiology*, *160*(4), 1677–1685. https://doi.org/10.1104/pp.112.204750

Dhayalan, A., Sumathi, M., Balasubramani, G., & PD, K. J. (2025). Symbiotic gut bacteria found capable of producing detoxification enzymes aiding their host, a polyphagous herbivore, *Spodoptera litura* (Fab.) in its xenobiotic adaptation. *International Journal of Pest Management*, 1–19. https://doi.org/10.1080/09670874.2025.2502362

Dhir, B. C., Mohapatra, H. K., & Senapati, B. (1992). Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). *Indian Journal of Plant Protection*, *20*, 215–217.

Di Cecco, G. J., Barve, V., Belitz, M. W., Stucky, B. J., Guralnick, R. P., & Hurlbert, A. H. (2021). Observing the observers: How participants contribute data to iNaturalist and implications for biodiversity science. *BioScience*, *71*(11), 1179–1188. https://doi.org/10.1093/biosci/biab093

Dillon, R. J., & Dillon, V. M. (2004). The gut bacteria of insects: Nonpathogenic interactions. *Annual Review of Entomology*, *49*, 71–92. https://doi.org/10.1146/annurev.ento.49.061802.123416

dos Santos, L. F. C., Ruiz-Sánchez, E., Andueza-Noh, R. H., Garruña-Hernández, R., Latournerie-Moreno, L., & Mijangos-Cortés, J. O. (2020). Leaf damage by *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) and its relation to leaf morphological traits in maize landraces and commercial cultivars. *Journal of Plant Diseases and Protection*, *127*(1), 103–109. https://doi.org/10.1007/s41348-019-00276-y

Douglas, A. E. (2009). The microbial dimension in insect nutritional ecology. *Functional Ecology*, *23*, 38–47. https://doi.org/10.1111/j.1365-2435.2008.01442.x

Drury, J. P., Barnes, M., Finneran, A. E., Harris, M., & Grether, G. F. (2019). Continent-scale phenotype mapping using citizen scientists' photographs. *Ecography*, *42*(8), 1436–1445. https://doi.org/10.1111/ecog.04469

Du, X.-X., Cao, S.-K., Xiao, H.-Y., Yang, C.-J., Zeng, A.-P., Chen, G., & Yu, H. (2023). Feeding *Spodoptera exigua* larvae with gut-derived *Escherichia* sp. increases larval juvenile hormone levels inhibiting cannibalism. *Communications Biology*, *6*(1), 1086. https://doi.org/10.1038/s42003-023-05466-x

Duke, S. O., & Dayan, F. E. (2022). The search for new herbicide mechanisms of action: Is there a 'holy grail'? *Pest Management Science*, *78*(4), 1303–1313. https://doi.org/10.1002/ps.6726

Enders, L., & Begcy, K. (2021). Unconventional routes to developing insect-resistant crops. *Molecular Plant*, *14*(9), 1439–1453. https://doi.org/10.1016/j.molp.2021.06.029

Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - diversity in structure and function. *FEMS Microbiology Reviews*, *37*(5), 699–735. https://doi.org/10.1111/1574-6976.12025

EPPO. (2015). *PM 7/124 (1) Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania* (EPPO Bulletin, Issue 45).

Esson, M. J. (1975). Notes on the biology and distribution of three recently discovered weevils in Hawkes Bay. *Proceedings of the 28th New Zealand Weed and Pest Control Conference*, 208–212.

Fand, B. B., Sul, N. T., Bal, S. K., & Minhas, P. S. (2015). Temperature impacts the development and survival of common cutworm (*Spodoptera litura*): simulation and visualization of potential

population growth in India under warmer temperatures through life cycle modelling and spatial mapping. *PLOS ONE*, *10*(4), e0124682. https://doi.org/10.1371/journal.pone.0124682

Faville, M. J., Griffiths, A. G., Baten, A., Cao, M., Ashby, R., Ghamkhar, K., Hong, W., Larking, A., Williamson, M., & Webber, Z. (2020). Genomic assessment of white clover and perennial ryegrass genetic resources. *Journal of New Zealand Grasslands*, *82*, 27–34. https://doi.org/10.33584/jnzg.2020.82.437

Ferguson, C. M., Barratt, B. I. P., Bell, N. L., Goldson, S. L., Hardwick, S., Jackson, M., Jackson, T. A., Phillips, C. B., Popay, A. J., Rennie, G., Sinclair, S., Townsend, R., & Wilson, M. (2019). Quantifying the economic cost of invertebrate pests to New Zealand's pastoral industry. *New Zealand Journal of Agricultural Research*, *62*(3), 255–315. https://doi.org/10.1080/00288233.2018.1478860

Ferrari, S., & Cribari-Neto, F. (2004). Beta regression for modelling rates and proportions. *Journal of Applied Statistics*, *31*, 799–815. https://doi.org/10.1080/0266476042000214501

Firth, D. (1993). Bias reduction of maximum likelihood estimates. *Biometrika*, *80*(1), 27–38. https://doi.org/10.2307/2336755

Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T., Cizek, L., Coley, P. D., Dem, F., Diniz, I. R., Drozd, P., Fox, M., Glassmire, A. E., Hazen, R., Hrcek, J., Jahner, J. P., Kaman, O., Kozubowski, T. J., Kursar, T. A.,...Dyer, L. A. (2015). The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences*, *112*(2), 442–447. https://doi.org/10.1073/pnas.1423042112

Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression*. SAGE Publications.

Fox, K. J. (1970). More records of migrant Lepidoptera in Taranaki and the South Island. *New Zealand Entomologist*, *4*, 63–66. https://doi.org/10.1080/00779962.1970.9723076

Fox, K. J. (1971). Migrant Lepidoptera in New Zealand 1970-1971. *The New Zealand Entomologist*, *5*, 59–62. https://doi.org/10.1080/00779962.1971.9722959

Fox, K. J. (1973a). Migrant Lepidoptera in New Zealand 1971-1972. *The New Zealand Entomologist*, *5*, 143–146. https://doi.org/10.1080/00779962.1973.9722984

Fox, K. J. (1973b). Migrant Lepidoptera in New Zealand 1972-1973. *The New Zealand Entomologist*, *5*, 268–271. https://doi.org/10.1080/00779962.1973.9723017

Fox, K. J. (1975). Migrant Lepidoptera in New Zealand 1973-1974. *The New Zealand Entomologist*, *6*, 66–69. https://doi.org/10.1080/00779962.1975.9723103

Fox, K. J. (1976). Migrant Lepidoptera in New Zealand. *New Zealand Entomologist*, *6*, 199–200. https://doi.org/10.1080/00779962.1976.9722247

Frank, S. D., & Just, M. G. (2020). Can cities activate sleeper species and predict future forest pests? A case study of scale insects. *Insects*, *11*(3), 142. https://doi.org/10.3390/insects11030142

Fu, C., Liu, Z., Xu, D., Gan, T., Deng, X., Zhang, H., & Zhuo, Z. (2025). Influence of temperature, humidity, and photophase on the developmental stages of *Spodoptera litura* (Lepidoptera: Noctuidae) and prediction of its population dynamics. *Insects*, *16*(4), 355. https://doi.org/10.3390/insects16040355

Fu, X., Zhao, X., Xie, B., Ali, A., & Wu, K. (2015). Seasonal pattern of *Spodoptera litura* (Lepidoptera: Noctuidae) migration across the Bohai Strait in Northern China. *Journal of Economic Entomoly*, *108*(2), 525–538. https://doi.org/10.1093/jee/tov019

Gayatri Priya, N., Ojha, A., Kajla, M. K., Raj, A., & Rajagopal, R. (2012). Host plant induced variation in gut bacteria of *Helicoverpa armigera*. *PLOS ONE*, *7*(1), e30768. https://doi.org/10.1371/journal.pone.0030768

Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., & Rubin, D. B. (2013). *Bayesian data analysis*. Chapman and Hall/CRC. https://doi.org/10.1201/b16018

Gerard, P. J., Addison, P. J., Hedley, P., Bell, N. L., & Vink, C. J. (2011). Outbreak of armyworms in eastern Bay of Plenty. *New Zealand Plant Protection*, *64*, 285–285. https://doi.org/10.30843/nzpp.2011.64.5989

Gerard, P. J., Barringer, J. R. F., Charles, J. G., Fowler, S. V., Kean, J. M., Phillips, C. B., A.B., T., & Walker, G. P. (2013). Potential effects of climate change on biological control systems: case studies from New Zealand. *BioControl*, *58*, 149–162. https://doi.org/10.1007/s10526-012-9480-0

Gerard, P. J., Crush, J. R., & Hackell, D. L. (2005). Interaction between *Sitona lepidus* and red clover lines selected for formononetin content. *Annals of Applied Biology*, *147*, 173–181. https://doi.org/10.1111/j.1744-7348.2005.00022.x

Gerard, P. J., Ferguson, C. M., & van Amsterdam, S. (2017). Comparison of New Zealand perennial clovers for resilience against common pasture pests. *New Zealand Plant Protection*, *70*, 241–249. https://doi.org/10.30843/nzpp.2017.70.57

Gerard, P. J., Wilson, D. J., Barker, G. M., & Addison, P. J. (2024). The New Zealand spread and distribution of the South American weevils *Floresianus sordidus* Hustache and *Atrichonotus taeniatulus* (Berg). *New Zealand Entomologist*, 1–9. https://doi.org/10.1080/00779962.2024.2417465

Giron, D., Dedeine, F., Dubreuil, G., Huguet, E., Mouton, L., Outreman, Y., Vavre, F., & Simon, J. C. (2017). Influence of microbial symbionts on plant–insect interactions. *Advances in Botanical Research*, *81*, 225–257. https://doi.org/10.1016/bs.abr.2016.09.007

Giron, D., Dubreuil, G., Bennett, A., Dedeine, F., Dicke, M., Dyer, L. A., Erb, M., Harris, M. O., Huguet, E., Kaloshian, I., Kawakita, A., Lopez-Vaamonde, C., Palmer, T. M., Petanidou, T., Poulsen, M., Sallé, A., Simon, J.-C., Terblanche, J. S., Thiéry, D.,...Pincebourde, S. (2018). Promises and challenges in insect–plant interactions. *Entomologia Experimentalis et Applicata*, *166*(5), 319–343. https://doi.org/10.1111/eea.12679

Goldson, S. L., Bourdôt, G. W., Brockerhoff, E. G., Byrom, A. E., Clout, M. N., McGlone, M. S., Nelson, W. A., Popay, A. J., Suckling, D. M., & Templeton, M. D. (2015). New Zealand pest management: current and future challenges. *Journal of the Royal Society of New Zealand*, *45*(1), 31–58. https://doi.org/10.1080/03036758.2014.1000343

Gougherty, A. V., & Davies, T. J. (2022). Host phylogenetic diversity predicts the global extent and composition of tree pests. *Ecology letters*, *25*(1), 101–112. https://doi.org/10.1111/ele.13908

Hamann, E., Blevins, C., Franks, S. J., Jameel, M. I., & Anderson, J. T. (2021). Climate change alters plant–herbivore interactions. *New Phytologist*, *229*(4), 1894–1910. https://doi.org/10.1111/nph.17036

Hanife, G. (2006). General principles of insect nutritional ecology. *Trakya University Journal of Natural Sciences*, *7*(1), 53–57.

Hansen, A. K., Pers, D., & Russell, J. A. (2020). Symbiotic solutions to nitrogen limitation and amino acid imbalance in insect diets. In K. M. Oliver & J. A. Russell (Eds.), *Advances in Insect Physiology* (Vol. 58, pp. 161–205). Academic Press. https://doi.org/10.1016/bs.aiip.2020.03.001

Hartig, F. (2016). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *CRAN: Contributed Packages*.

Harvey, J. A., Van Nouhuys, S., & Biere, A. (2005). Effects of quantitative variation in allelochemicals in *Plantago lanceolata* on development of a generalist and a specialist herbivore and their endoparasitoids. *Journal of Chemical Ecology*, *31*(2), 287–302. https://doi.org/10.1007/s10886-005-1341-1

Henry, L. P., Bruijning, M., Forsberg, S. K. G., & Ayroles, J. F. (2021). The microbiome extends host evolutionary potential. *Nature Communications*, *12*(1), 5141. https://doi.org/10.1038/s41467-021-25315-x

Herrero, M. I., Dami, L. C., Fogliata, S. V., Casmuz, A. S., GÓMEZ, D. R., Gastaminza, G. A., & Murua, M. G. (2018). Fertility life table, population parameters and biotic potential of *Helicoverpa gelotopoeon* (Dyar)(Lepidoptera: Noctuidae). *Anais da Academia Brasileira de Ciências*, *90*, 3831–3838. https://doi.org/10.1590/0001-3765201820180318

Hochmair, H. H., Scheffrahn, R. H., Basille, M., & Boone, M. (2020). Evaluating the data quality of iNaturalist termite records. *PLOS ONE*, *15*(5), e0226534. https://doi.org/10.1371/journal.pone.0226534

Hochuli, D. F. (2001). Insect herbivory and ontogeny: How do growth and development influence feeding behaviour, morphology and host use? *Austral Ecology*, *26*(5), 563–570. https://doi.org/10.1046/j.1442-9993.2001.01135.x

Howden, S. M., Soussana, J.-F., Tubiello, F. N., Chhetri, N., Dunlop, M., & Meinke, H. (2007). Adapting agriculture to climate change. *Proceedings of the National Academy of Sciences*, *104*(50), 19691–19696. https://doi.org/10.1073/pnas.0701890104

Hu, L., Sun, Z., Xu, C., Wang, J., Mallik, A. U., Gu, C., Chen, D., Lu, L., Zeng, R., & Song, Y. (2022). High nitrogen in maize enriches gut microbiota conferring insecticide tolerance in lepidopteran pest *Spodoptera litura*. *iScience*, *25*(1), 103726. https://doi.org/10.1016/j.isci.2021.103726

Huang, D., Xia, J., Liu, H., Wang, W., Ji, Z., & Wang, Q. (2008). Effects of three leafy turfgrasses on the development and nutrition utilization of *Spodoptera litura* (Lepidoptera: Noctuidae). *Acta Entomologica Sinica*, *51*, 155–160.

Huang, X. P., & Renwick, J. A. A. (1995). Chemical and experiential basis for rejection of *Tropaeolum majus* by *Pieris rapae* larvae. *Journal of Chemical Ecology*, *21*(10), 1601–1617. https://doi.org/10.1007/BF02035155

Hulme, P. E. (2017). Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews*, *92*(3), 1297–1313. https://doi.org/10.1111/brv.12282

Isaac, N. J. B., van Strien, A. J., August, T. A., de Zeeuw, M. P., & Roy, D. B. (2014). Statistics for citizen science: extracting signals of change from noisy ecological data. *Methods in Ecology and Evolution*, *5*(10), 1052–1060. https://doi.org/10.1111/2041-210X.12254

Jaramillo, D. M., Sheridan, H., Soder, K., & Dubeux, J. C. B. (2021). Enhancing the sustainability of temperate pasture systems through more diverse swards. *Agronomy*, *11*(10), 1912. https://doi.org/10.3390/agronomy11101912

Javal, M., Terblanche, J. S., Benoit, L., Conlong, D. E., Lloyd, J. R., Smit, C., & Chapuis, M.-P. (2023). Does host plant drive variation in microbial gut communities in a recently shifted pest? *Microbial ecology*, *86*(1), 636–646. https://doi.org/10.1007/s00248-022-02100-x

Jensen, J., Clearwater, M., Barnes, A., & Orre-Gordon, S. (2025). The challenges of monitoring insect sleeper pests: the example of *Spodoptera litura* in New Zealand. *Journal of New Zealand Grasslands*, *87*, 95–106. https://doi.org/10.33584/jnzg.2025.87.3738

Jensen, J. G. (2026). *Supplementary material for 'Forage host species has intergenerational effects on Spodoptera litura'* (https://doi.org/10.57935/AGR.32331300

Jensen, J. G., Somchit, C., Clearwater, M. J., Barnes, A. D., & Orre-Gordon, S. (2026). Forage plant host and early feeding choices affect *Spodoptera litura* feeding, development and egg-Laying. *Entomologia Experimentalis et Applicata*, *174*, 670–684. https://doi.org/10.1111/eea.70097

Johnson, M.-L., & Zalucki, M. P. (2005). Foraging behaviour of *Helicoverpa armigera* first instar larvae on crop plants of different developmental stages. *Journal of Applied Entomology*, *129*(5), 239–245. https://doi.org/10.1111/j.1439-0418.2005.00958.x

Jonas, J. L., & Joern, A. (2008). Host-plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). *Ecological Entomology*, *33*(4), 546–554. https://doi.org/10.1111/j.1365-2311.2008.01004.x

Jones, A. G., Mason, C. J., Felton, G. W., & Hoover, K. (2019). Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. *Scientific Reports*, *9*(1), 2792. https://doi.org/10.1038/s41598-019-39163-9

Jones, B. C., & Despland, E. (2006). Effects of synchronization with host plant phenology occur early in the larval development of a spring folivore. *Canadian Journal of Zoology*, *84*(4), 628–633. https://doi.org/10.1139/z06-025

Jung, J.-M., Byeon, D.-h., Jung, S., & Lee, W.-H. (2019). Effect of climate change on the potential distribution of the common cutworm (*Spodoptera litura*) in South Korea. *Entomological Research*, *49*(12), 519–528. https://doi.org/10.1111/1748-5967.12398

Kariyat, R. R., Hardison, S. B., Ryan, A. B., Stephenson, A. G., De Moraes, C. M., & Mescher, M. C. (2018). Leaf trichomes affect caterpillar feeding in an instar-specific manner. *Communicative & Integrative Biology*, *11*(3), 1–6. https://doi.org/10.1080/19420889.2018.1486653

Karowe, D. N., & Martin, M. M. (1989). The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). *Journal of Insect Physiology*, *35*(9), 699–708. https://doi.org/10.1016/0022-1910(89)90089-9

Kean, J. M., Brockerhoff, E. G., Fowler, S. V., Gerard, P. J., Logan, D. P., Mullan, A. B., Sood, A., Tompkins, D. M., & Ward, D. F. (2015). Effects of climate change on current and potential biosecurity pests and diseases in New Zealand. *MPI Technical Paper No: 2015/25*, 96pp.

Keena, M. A., Odell, T. M., & Tanner, J. A. (1998). Environmentally based maternal effects are the primary factor in determining the developmental response of Gypsy Moth (Lepidoptera: Lymantriidae) to dietary iron deficiency. *Annals of the Entomological Society of America*, *91*(5), 710–718. https://doi.org/10.1093/aesa/91.5.710

Knowles, I. M., Fraser, T. J., & Daly, M. J. (2003). White clover: loss in drought and subsequent recovery. *Grassland Research and Practice Series No. 11: Legumes for dryland pastures*, 37–42. https://doi.org/10.33584/rps.11.2003.2997

Kopec, S. (1924). Studies on the influence of inanition on the development and the duration of life in insects. *The Biological Bulletin*, *46*(1), 1–21.

Krams, I. A., Kecko, S., Jõers, P., Trakimas, G., Elferts, D., Krams, R., Luoto, S., Rantala, M. J., Inashkina, I., Gudrā, D., Fridmanis, D., Contreras-Garduño, J., Grantiņa-Ieviņa, L., & Krama, T. (2017). Microbiome symbionts and diet diversity incur costs on the immune system of insect larvae. *Journal of Experimental Biology*, *220*(22), 4204–4212. https://doi.org/10.1242/jeb.169227

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26. https://doi.org/10.18637/jss.v082.i13

Lange, C., Boyer, S., Bezemer, T. M., Lefort, M.-C., Dhami, M. K., Biggs, E., Groenteman, R., Fowler, S. V., Paynter, Q., Verdecia Mogena, A. M., & Kaltenpoth, M. (2023). Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *The ISME Journal*, *17*(11), 1798–1807. https://doi.org/10.1038/s41396-023-01500-2

Lavola, A., Julkunen-Tiitto, R., Roininen, H., & Aphalo, P. (1998). Host-plant preference of an insect herbivore mediated by UV-B and CO2 in relation to plant secondary metabolites. *Biochemical Systematics and Ecology*, *26*(1), 1–12. https://doi.org/10.1016/S0305-1978(97)00104-X

Lawrence, J., Mackey, B., Chiew, F., Costello, M. J., Hennessy, K., Lansbury, N., Nidumolu, U. B., Pecl, G., Rickards, L., Tapper, N., Woodward, A., & Wreford, A. (2022). Chapter 11: Australasia. In D. C. R. H.-O. Portner, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegria, M. Craig, S. Langsdorf, S. Loschke, V. Moller, A. Okem, B. Rama (Ed.), *Climate Change 2022: Impacts, adaptation, and vulnerability. Contribution of working group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. (pp. 1581–1688). Cambridge, UK.

Le Gall, M., & Behmer, S. T. (2014). Effects of protein and carbohydrate on an insect herbivore: The vista from a fitness landscape. *Integrative and Comparative Biology*, *54*(5), 942–954. https://doi.org/10.1093/icb/icu102

Lecoq, M. (2022). *Locusta migratoria* (migratory locust). *CABI Compendium*, 31151. https://doi.org/10.1079/cabicompendium.31151

Lee, K. P. (2007). The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore. *Journal of Experimental Biology*, *210*(18), 3236–3244. https://doi.org/10.1242/jeb.008060

Lee, K. P., Kwon, S.-T., & Roh, C. (2012). Caterpillars use developmental plasticity and diet choice to overcome the early life experience of nutritional imbalance. *Animal Behaviour*, *84*(4), 785–793. https://doi.org/10.1016/j.anbehav.2012.06.033

Leite-Mondin, M., DiLegge, M. J., Manter, D. K., Weir, T. L., Silva-Filho, M. C., & Vivanco, J. M. (2021). The gut microbiota composition of *Trichoplusia ni* is altered by diet and may influence its polyphagous behavior. *Scientific Reports*, *11*(1), 5786. https://doi.org/10.1038/s41598-021-85057-0

Lenth, R., Singman, H., Love, J., Buerkner, P., & Herve, M. (2022). Emmeans: Estimated marginal means, aka least-squares means. *R package version 1.7.3*.

Li, Y., Yu, J., Lu, Q., SXu, J., & Ye, H. (2014). Developmental and emergence patterns of the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctuidae). *GSTF Journal of BioSciences*, *3*, 18–20. https://doi.org/10.5176/2251-3140_3.1.46

Lilburne, L., Ausseil, A.-G., Sood, A., Guo, J., Teixeira, E., Vetharaniam, I., van der Weerden, T., Smith, H., Neverman, A., Cichota, R., Phillips, C., Johnson, P., Thomas, S., & Dynes, R. (2025). Modelling to identify direct risks for New Zealand agriculture due to climate change. *Journal of the Royal Society of New Zealand*, *55*(6), 1683–1700. https://doi.org/10.1080/03036758.2024.2393295

Lindroth, R. L., Hofman, R. W., Campbell, B. D., McNabb, W. C., & Hunt, D. Y. (2000). Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: foliar chemistry and consequences for two lepidopteran herbivores. *Oecologia*, *122*(1), 20–28. https://doi.org/10.1007/PL00008831

LoPresti, E. F. (2018). Polyphagy by omnivory: Scavenging improves performance of a polyphagous caterpillar on marginal hosts. *Oecologia*, *186*(4), 1007–1015. https://doi.org/10.1007/s00442-018-4057-y

Lu, M., Hulcr, J., & Sun, J. (2016). The role of symbiotic microbes in insect invasions. *Annual Review of Ecology, Evolution, and Systematics*, *47*(Volume 47, 2016), 487–505. https://doi.org/10.1146/annurev-ecolsys-121415-032050

Ma, C.-S., Wang, B.-X., Wang, X.-J., Lin, Q.-C., Zhang, W., Yang, X.-F., van Baaren, J., Bebber, D. P., Eigenbrode, S. D., Zalucki, M. P., Zeng, J., & Ma, G. (2025). Crop pest responses to global changes in climate and land management. *Nature Reviews Earth & Environment*, *6*(4), 264–283. https://doi.org/10.1038/s43017-025-00652-3

Maes, K. (2022). *Spodoptera litura* (taro caterpillar). *CABI Compendium*. https://doi.org/10.1079/cabicompendium.44520

Mansfield, S., Ferguson, C. M., Gerard, P. J., Hodges, D., Kean, J. M., Phillips, C. B., Hardwick, S., & Zydenbos, S. M. (2021). Climate change impacts on pest ecology and risks to pasture resilience. *Grassland Research and Practice Series*, *17*, 123–138. https://doi.org/10.33584/rps.17.2021.3477

Mansfield, S., Wilson, M. J., Gerard, P. J., Wilson, D. J., Swaminathan, J., Wright, D. A., van Koten, C., & Hurst, M. R. (2020). Potential for a biopesticide bait to control black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae). *Pest Management Science*, *76*(12), 4150–4158. https://doi.org/10.1002/ps.5973

Martin, B. D., Witten, D., & Willis, A. D. (2020). Modeling microbial abundances and dysbiosis with beta-binomial regression. *Annals of Applied Statistics*, *14*(1), 94–115. https://doi.org/10.1214/19-aoas1283

Mason, C. J., Peiffer, M., Chen, B., Hoover, K., & Felton, G. W. (2022). Opposing growth responses of lepidopteran larvae to the establishment of gut microbiota. *Microbiology Spectrum*, *10*(4), e0194122. https://doi.org/10.1128/spectrum.01941-22

Mason, C. J., St. Clair, A., Peiffer, M., Gomez, E., Jones, A. G., Felton, G. W., & Hoover, K. (2020). Diet influences proliferation and stability of gut bacterial populations in herbivorous lepidopteran larvae. *PLOS ONE*, *15*(3), e0229848. https://doi.org/10.1371/journal.pone.0229848

Mattson, W. J., Jr. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, *11*, 119–161. https://doi.org/jstor.org/stable/2096905

McCahon, A., & Dale, T. (2025). Future Northland Pastures: 1. Introduction and temperate forages. *Journal of New Zealand Grasslands*, *87*, 37–41. https://doi.org/10.33584/jnzg.2025.87.3730

McCahon, K., McCahon, A., & Ussher, G. (2021). Diversified pastures at the front line of climate change in Northland: farmers experiences, new directions and wider implications for other parts of the country. *NZGA: Research and Practice Series*, *17*, 213–224. https://doi.org/10.33584/rps.17.2021.3474

McKinney, M. L., & Lockwood, J. L. (1999). Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology & Evolution*, *14*(11), 450–453. https://doi.org/10.1016/S0169-5347(99)01679-1

Minard, G., Tikhonov, G., Ovaskainen, O., & Saastamoinen, M. (2019). The microbiome of the *Melitaea cinxia* butterfly shows marked variation but is only little explained by the traits of the butterfly or its host plant. *Environ Microbiol*, *21*(11), 4253–4269. https://doi.org/10.1111/1462-2920.14786

Minnee, E. M. K., Waghorn, G. C., Lee, J. M., & Clark, C. E. F. (2017). Including chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production and rumen digestion. *Animal Feed Science and Technology*, *227*, 52–61. https://doi.org/10.1016/j.anifeedsci.2017.03.008

Mitra, S., Firake, D. M., Umesh, K. P., Pandey, P. P., & Pandit, S. (2021). Polyphagous caterpillars of *Spodoptera litura* switch from a trap crop to the main crop, improve fitness, and shorten generation time. *Journal of Pest Science*, *94*(4), 1091–1103. https://doi.org/10.1007/s10340-021-01351-w

Mousseau, T. A., & Dingle, H. (1991). Maternal effects in insect life histories. *Annual Review of Entomology*, *36*, 511–534. https://doi.org/10.1146/annurev.en.36.010191.002455

MPI New Zealand. (2025). *Situation and outlook for primary industries*. *https://www.mpi.govt.nz/dmsdocument/70984-Situation-and-Outlook-for-Primary-Industries-SOPI-December-2025*.

Murata, M., & Tojo, S. (2004). Flight capability and fatty acid level in triacylglycerol of long-distance migratory adults of the common cutworm, *Spodoptera litura*. *Zoological Science*, *21*(2), 181–188. https://doi.org/doi.org/10.2108/zsj.21.181

Naser, S. M., Vancanneyt, M., Hoste, B., Snauwaert, C., Vandemeulebroecke, K., & Swings, J. (2006). Reclassification of *Enterococcus flavescens* Pompei *et al*. 1992 as a later synonym of *Enterococcus casseliflavus* (*ex* Vaughan *et al.* 1979) Collins *et al.* 1984 and *Enterococcus saccharominimus* Vancanneyt *et al*. 2004 as a later synonym of *Enterococcus italicus* Fortina *et al*. 2004. *International Journal of Systematic and Evolutionary Microbiology*, *56*(Pt 2), 413–416. https://doi.org/10.1099/ijs.0.63891-0

Nestel, D., Papadopoulos, N. T., Pascacio-Villafán, C., Righini, N., Altuzar-Molina, A. R., & Aluja, M. (2016). Resource allocation and compensation during development in holometabolous insects. *Journal of Insect Physiology*, *95*, 78–88. https://doi.org/10.1016/j.jinsphys.2016.09.010

Nguyen, T. T., Navarrete, S., Horne, D. J., Donaghy, D. J., & Kemp, P. D. (2022). Incorporating plantain with perennial ryegrass-white clover in a dairy grazing system: Dry matter yield, botanical composition, and nutritive value response to sowing rate, plantain content and season. *Agronomy*, *12*(11), 2789. https://doi.org/10.3390/agronomy12112789

NIWA. (2000–2025, 20/3/25). *Climate and weather summaries*. Retrieved 29/12/25 from https://niwa.co.nz/climate-and-weather/climate-summaries-0

O'Callaghan, M., & Mansfield, S. (2026). Evaluating the field efficacy of microbial products – important technical matters to consider. *New Zealand Plant Protection*, *79*, 17–32. https://doi.org/10.30843/nzpp.2026.79.11798

Orre-Gordon, S., & Jensen, J. (2025). Future Northland Pastures 4. Northland arthropod pests and 'sleeper pests' that will increase in response to climate change. *Journal of New Zealand Grasslands*, *87*, 61–69. https://doi.org/10.33584/jnzg.2025.87.3728

Ørsted, M., Yashiro, E., Hoffmann, A. A., & Kristensen, T. N. (2022). Population bottlenecks constrain host microbiome diversity and genetic variation impeding fitness. *PLOS Genetics*, *18*(5), e1010206. https://doi.org/10.1371/journal.pgen.1010206

Ortiz-Carreón, F. R., Bernal, J. S., Rojas, J. C., Cruz-López, L., Kolomiets, M. V., & Malo, E. A. (2025). Fall armyworm females choose host plants that are detrimental to their offspring's performance but young larvae amend their mother's choices. *Entomologia Experimentalis et Applicata*, *173*, 21–31. https://doi.org/10.1111/eea.13512

Paniagua Voirol, L. R., Frago, E., Kaltenpoth, M., Hilker, M., & Fatouros, N. E. (2018). Bacterial symbionts in Lepidoptera: Their diversity, transmission, and impact on the host. *Frontiers in Microbiology*, *9*, 556. https://doi.org/10.3389/fmicb.2018.00556

Paul, S. C., Singh, P., Dennis, A. B., & Müller, C. (2022). Intergenerational effects of early-life starvation on life history, consumption, and transcriptome of a holometabolous insect. *The American Naturalist*, *199*(6), E229–E243. https://doi.org/10.1086/719397

Peacock, L., Worner, S., & Sedcole, R. (2006). Climate variables and their role in site discrimination of invasive insect species distributions. *Environmental Entomology*, *35*(4), 958–963. https://doi.org/10.1603/0046-225x-35.4.958

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3), 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Popay, I., Champion, P., & James, T. (2010). *An illustrated guide to common weeds of New Zealand*. New Zealand Plant Protection Society. https://doi.org/10.1080/01140671.2010.535499

Qi, J., Xiao, F., Liu, X., Li, J., Wang, H., Li, S., Yu, H., Xu, Y., & Wang, H. (2024). The fall armyworm converts maize endophytes into its own probiotics to detoxify benzoxazinoids and promote caterpillar growth. *Microbiome*, *12*(1), 240. https://doi.org/10.1186/s40168-024-01957-z

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*, *41*, D590–596. https://doi.org/10.1093/nar/gks1219

Quintero, C., & Bowers, M. D. (2018). Plant and herbivore ontogeny interact to shape the preference, performance and chemical defense of a specialist herbivore. *Oecologia*, *187*(2), 401–412. https://doi.org/10.1007/s00442-018-4068-8

Quiroz, A., Mendez, L., Mutis, A., Hormazabal, E., Ortega, F., Birkett, M. A., & Parra, L. (2017). Antifeedant activity of red clover root isoflavonoids on *Hylastinus obscurus*. *Journal of Soil Science and Plant Nutrition*, *17*. https://doi.org/10.4067/S0718-95162017005000018

R Core Team. (2024). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*.

R Core Team. (2025). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*.

Rafter, M. A., & Walter, G. H. (2020). Generalising about generalists? A perspective on the role of pattern and process in investigating herbivorous insects that use multiple host species. *Arthropod-Plant Interactions*, *14*(1), 1–20. https://doi.org/10.1007/s11829-019-09737-5

Ranga Rao, G. V., Wightman, J. A., & Ranga Rao, D. V. (1993). World review of the natural enemies and diseases of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). *Insect Science and its Application*, *14*, 273–284. https://doi.org/10.1017/S1742758400014764

Ratnadas, A., Fernandes, P., Avelino, J., & Habib, R. (2012). Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development*, *32*, 273–303.

Reznik, S. Y., Ovchinnikov, A. N., Belyakova, N. A., & Ovchinnikova, A. A. (2019). Larval starvation resistance in invasive and native populations of the multicolored Asian ladybird, *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae). *Entomological Review*, *99*(1), 13–23. https://doi.org/10.1134/S0013873819010020

Roeder, K. A., & Behmer, S. T. (2014). Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Functional Ecology*, *28*(5), 1135–1143. https://doi.org/10.1111/1365-2435.12262

Roigé, M., & Phillips, C. B. (2021). Validation and uncertainty analysis of the match climates regional algorithm (CLIMEX) for pest risk analysis. *Ecological Informatics*, *61*, 101196. https://doi.org/10.1016/j.ecoinf.2020.101196

Rooney, T. P., Smith, A. T., & Hurd, L. E. (1996). Global warming and the regional persistence of a temperate-zone insect (*Tenodera sinensis*). *The American Midland Naturalist*, *136*(1), 84–93. https://doi.org/10.2307/2426633

Rotem, K., Agrawal, A. A., & Kott, L. (2003). Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? *Ecological Entomology*, *28*(2), 211–218. https://doi.org/10.1046/j.1365-2311.2003.00507.x

Salazar, D., & Marquis, R. J. (2022). Testing the role of local plant chemical diversity on plant–herbivore interactions and plant species coexistence. *Ecology*, *103*(9), e3765. https://doi.org/10.1002/ecy.3765

Salem, H., Kirsch, R., Pauchet, Y., Berasategui, A., Fukumori, K., Moriyama, M., Cripps, M., Windsor, D., Fukatsu, T., & Gerardo, N. M. (2020). Symbiont digestive range reflects host plant breadth in herbivorous beetles. *Current Biology*, *30*(15), 2875–2886.e2874. https://doi.org/10.1016/j.cub.2020.05.043

Schofield, R. C. (1979). Pasture and field crop pest survey. *Ministry of Agriculture and Fisheries: Advisory Services Division Report*.

Scriber, J. M. (1977). Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of *Hyalophora cecropia* (Lepidoptera: Saturniidae). *Oecologia*, *28*(3), 269–287. https://doi.org/10.1007/bf00751605

Scriber, J. M., & Slansky, F. (1981). The nutritional ecology of immature insects. *Annual Review of Entomology*, *26*, 183–211. https://doi.org/10.1146/annurev.en.26.010181.001151

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. *Trends in Ecology & Evolution*, *31*(7), 539–549. https://doi.org/10.1016/j.tree.2016.03.006

Sharma, H. C., & Dhillon, M. K. (2020). Climate change effects on arthropod diversity and its implications for pest management and sustainable crop production. In J. L. Hatfield, M. V. K. Sivakumar, & J. H. Prueger (Eds.), *Agroclimatology: Linking agriculture to climate* (Vol. 60, pp. 595–619). https://doi.org/10.2134/agronmonogr60.2016.0019

Sharma, S., Mathpal, S., & Jyoti, S. (2025). Life cycle and biology of *Spodoptera litura* (Noctuidae: Lepidoptera) on castor (*Ricinus communis*). *International Journal of Fauna and Biological Studies*, *12*, 1–6. https://doi.org/10.22271/23940522.2025.v12.i2a.1093

Shekhawat, S. S., Shafiq Ansari, M., & Basri, R. (2018). Effect of host plants on life table parameters of *Spodoptera litura*. *International Journal of Pure and Applied Bioscience*, *6*, 324–332.

Short, C. A., & Hahn, D. A. (2023). Fat enough for the winter? Does nutritional status affect diapause? *Journal of Insect Physiology*, *145*, 104488. https://doi.org/10.1016/j.jinsphys.2023.104488

Simpson, S., & Simpson, C. (1990). The mechanisms of nutritional compensation by phytophagous insects. In E. A. Bernays (Ed.), *Insect-Plant Interactions* (Vol. II, pp. 111–160). CRC Press.

Simpson, W. R., Schmid, J., Singh, J., Faville, M. J., & Johnson, R. D. (2012). A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. *Fungal biology*, *116*(2), 234–240. https://doi.org/10.1016/j.funbio.2011.11.006

Singh, I. K., Ragesh, P. R., Ganta, S., & Singh, A. K. (2015). Oviposition behaviour of tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) on different host plants. *Journal of*

Entomology and Zoology Studies, *3*(3), 50–54. https://doi.org/api.semanticscholar.org/CorpusID:90227992

Singh, P., & Moore, R. F. (1985). *Handbook of Insect Rearing*. Elsevier.

Slansky Jr., F., & Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs*, *47*(2), 209–228. https://doi.org/10.2307/1942617

Stamp, N. E., & Yang, Y. (1996). Response of insect herbivores to multiple allelochemicals under different thermal regimes. *Ecology*, *77*(4), 1088–1102. https://doi.org/10.2307/2265578

Staudacher, H., Kaltenpoth, M., Breeuwer, J. A. J., Menken, S. B. J., Heckel, D. G., & Groot, A. T. (2016). Variability of bacterial communities in the moth *Heliothis virescens* indicates transient association with the host. *PLOS ONE*, *11*(5), e0154514. https://doi.org/10.1371/journal.pone.0154514

Stewart, A., Kerr, G., Rowarth, J., & Stevens, D. (2022). Pasture and forage plants for New Zealand. *Grassland Research and Practice Series*, *8*, 165pp.

Stockhoff, B. A. (1991). Starvation resistance of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae): tradeoffs among growth, body size, and survival. *Oecologia*, *88*(3), 422–429. https://doi.org/10.1007/BF00317588

Stuff. (2022). Plague of black worms, 'like they fell out of the sky', chomping Bay of Plenty gardens. *https://www.stuff.co.nz/bay-of-plenty/300574302/plague-of-black-worms-like-they-fell-out-of-the-sky-chomping-bay-of-plenty-gardens?rm=a*.

Stuff. (2023). Q&A: How do I stop the march of the armyworm? *https://www.stuff.co.nz/life-style/homed/garden/132051327/qa--how-do-i-stop-the-march-of-the-armyworm*.

Subramanian, K., Raja, N., & Ignacimuthu, S. (2006). Feeding performance of *Spodoptera litura* on different cotton cultivars. *Journal of Entomological Research*, *30*, 125–129.

Sushilkumar, & Ray, P. (2007). Biology of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on some of its major weed hosts. *Entomon*, *32*, 287–290.

Sushilkumar, & Ray, P. (2018). Host plant preference of army worm (*Spodoptera litura*) on crops and weeds. *Indian Journal of Weed Science*, *50*(1), 100–102. https://doi.org/10.5958/0974-8164.2018.00025.4

Tang, X., Freitak, D., Vogel, H., Ping, L., Shao, Y., Cordero, E. A., Andersen, G., Westermann, M., Heckel, D. G., & Boland, W. (2012). Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. *PLOS ONE*, *7*(7), e36978. https://doi.org/10.1371/journal.pone.0036978

Tava, A., Biazzi, E., Ronga, D., Pecetti, L., & Avato, P. (2022). Biologically active compounds from forage plants. *Phytochemistry Reviews*, *21*(2), 471–501. https://doi.org/10.1007/s11101-021-09779-9

Taylor, M. F. J. (1989). Compensation for variable dietary nitrogen by larvae of the Salvinia moth. *Functional Ecology*, *3*(4), 407–416. https://doi.org/10.2307/2389614

Thakur, A., Dhammi, P., Saini, H. S., & Kaur, S. (2015). Pathogenicity of bacteria isolated from gut of *Spodoptera litura* (Lepidoptera: Noctuidae) and fitness costs of insect associated with consumption of bacteria. *Journal of Invertebrate Pathology*, *127*, 38–46. https://doi.org/10.1016/j.jip.2015.02.007

Thakur, A., Dhammi, P., Saini, H. S., & Kaur, S. (2016). Effect of antibiotic on survival and development of *Spodoptera litura* (Lepidoptera: Noctuidae) and its gut microbial diversity. *Bulletin of Entomological Research*, *106*(3), 387–394. https://doi.org/10.1017/s0007485316000031

Thakur, N., Gotyal, B. S., Selvaraj, K., & Satpathy, S. (2017). Effect on biology of jute indigo caterpillar, *Spodoptera litura* (Fabricius) under five different constant temperatures. *Journal of Entomology and Zoology Studies*, *5*, 102–106.

Timeanddate.com. (1995–2025). *Hamilton, New Zealand - Sunrise, Sunset, and Daylength*. https://www.timeanddate.com/sun/new-zealand/hamilton. Retrieved 29 December 2025 from https://www.timeanddate.com/sun/new-zealand/hamilton

Tong, H., Su, Q., Zhou, X., & Bai, L. (2013). Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *Journal of Pest Science*, *86*(3), 599–609. https://doi.org/10.1007/s10340-013-0505-y

Tozer, K. N., Cameron, C. A., & Thom, E. R. (2011). Weed ingress and pasture persistence in Bay of Plenty dairy farms field observations and farmer perceptions. *New Zealand Plant Protection*, *64*, 68–74. https://doi.org/10.30843/nzpp.2011.64.5964

Tu, Y.-G., Wu, K.-M., Xue, F.-S., & Lu, Y.-H. (2010). Laboratory evaluation of flight activity of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Insect Science*, *17*(1), 53–59. https://doi.org/10.1111/j.1744-7917.2009.01281.x

Tuan, S.-J., Li, N.-J., & Yeh, C.-C. (2015). Growth performance and biometric characteristics of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on different host plants. *Journal of Economic Entomology*, *108*(5), 2242–2249. https://doi.org/10.1093/jee/tov192

Vellau, H., Sandre, S.-L., & Tammaru, T. (2013). Effect of host species on larval growth differs between instars: The case of a geometrid moth (Lepidoptera: Geometridae). *European Journal of Entomology*, *110*(4), 599–604.

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S. Fourth edition*. Springer.

Vilanova, C., Baixeras, J., Latorre, A., & Porcar, M. (2016). The generalist inside the specialist: gut bacterial communities of two insect species feeding on toxic plants are dominated by *Enterococcus* sp. *Front Microbiol*, *7*, 1005. https://doi.org/10.3389/fmicb.2016.01005

Waldbauer, G. P. (1968). The consumption and utilization of food by insects. In J. W. L. Beament, J. E. Treherne, & V. B. Wigglesworth (Eds.), *Advances in Insect Physiology* (Vol. 5, pp. 229–288). Academic Press. https://doi.org/10.1016/S0065-2806(08)60230-1

Wan, P., Wu, K., Huang, M., Yu, D., & Wu, J. (2008). Population dynamics of *Spodoptera litura* (Lepidoptera: Noctuidae) on Bt cotton in the Yangtze River Valley of China. *Environ Entomol*, *37*(4), 1043–1048. https://doi.org/10.1603/0046-225x(2008)37[1043:pdosll]2.0.co;2

Wang, L., Hui, C., Sandhu, H. S., Li, Z., & Zhao, Z. (2015). Population dynamics and associated factors of cereal aphids and armyworms under global change. *Scientific Reports*, *5*(1), 18801. https://doi.org/10.1038/srep18801

Wang, S. F., Ridsdill Smith, T. J., & Ghisalberti, E. L. (1998). Role of isoflavonoids in resistance of subterranean clover trifoliates to the redlegged earth mite *Halotydeus destructor*. *Journal of Chemical Ecology*, *24*, 2089–2100. https://doi.org/10.1023/A:1020789710706

Wang, Y., Huang, X., Chang, B. H., & Zhang, Z. (2020). The survival, growth, and detoxifying enzyme activities of grasshoppers *Oedaleus asiaticus* (Orthoptera: Acrididae) exposed to toxic rutin. *Applied Entomology and Zoology*, *55*(4), 385–393. https://doi.org/10.1007/s13355-020-00694-7

Ward, D. F. (2008). Lucerne for the future. *Report prepared for the New Zealand Farm Environment Trust and MAF*.

Ward, L. (1988). The validity and interpretation of insect foodplant records. *British Journal of Entomology and Natural History*, *1*, 153–162. https://doi.org/10.5281/zenodo.16662007

Werenkraut, V., Baudino, F., & Roy, H. E. (2020). Citizen science reveals the distribution of the invasive harlequin ladybird (*Harmonia axyridis* Pallas) in Argentina. *Biological Invasions*, *22*(10), 2915–2921. https://doi.org/10.1007/s10530-020-02312-7

Wilkins, P. W., & Humphreys, M. O. (2003). Progress in breeding perennial forage grasses for temperate agriculture. *The Journal of Agricultural Science*, *140*(2), 129–150. https://doi.org/10.1017/S0021859603003058

Williams, C. M. (1959). Hormonal regulation of insect metamorphosis. In W. D. McElroy & B. Glass (Eds.), *The Chemical Basis of Development* (pp. 794). Johns Hopkins University Press.

Williams, W. M., Easton, H. S., & Jones, C. S. (2007). Future options and targets for pasture plant breeding in New Zealand. *New Zealand Journal of Agricultural Research*, *50*(2), 223–248. https://doi.org/10.1080/00288230709510292

Wilman, D., & Altimimi, M. A. K. (1984). The in-vitro digestibility and chemical composition of plant parts in white clover, red clover and lucerne during primary growth. *Journal of the Science of Food and Agriculture*, *35*(2), 133–138. https://doi.org/10.1002/jsfa.2740350203

Wilson, J. K., Ruiz, L., Duarte, J., & Davidowitz, G. (2019). The nutritional landscape of host plants for a specialist insect herbivore. *Ecology and Evolution*, *9*(23), 13104–13113. https://doi.org/10.1002/ece3.5730

Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann, C. F., Forchhammer, M. C., Grytnes, J.-A., Guisan, A., Heikkinen, R. K., Høye, T. T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.-C., Normand, S., Öckinger, E., Schmidt, N. M.,...Svenning, J.-C. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological Reviews*, *88*(1), 15–30. https://doi.org/10.1111/j.1469-185X.2012.00235.x

Woodfield, D. R., & Caradus, J. R. (1996). Factors affecting white clover persistence in New Zealand pastures. *Proceedings of the New Zealand Grassland Association*, *58*, 229–235. https://doi.org/10.33584/jnzg.1996.58.2196

Wu, Q., Wang, M., & Simon, J. E. (2003). Determination of isoflavones in red clover and related species by high-performance liquid chromatography combined with ultraviolet and mass spectrometric detection. *Journal of Chromatography A*, *1016*(2), 195–209. https://doi.org/10.1016/j.chroma.2003.08.001

Xia, X., Lan, B., Tao, X., Lin, J., & You, M. (2020). Characterization of *Spodoptera litura* gut bacteria and their role in feeding and growth of the host. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01492

Xia, X., Sun, B., Gurr, G. M., Vasseur, L., Xue, M., & You, M. (2018). Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). *Frontiers in Microbiology*, *Volume 9 - 2018*. https://doi.org/10.3389/fmicb.2018.00025

Xue, M., Pang, Y.-H., Wang, H.-T., Li, Q.-L., & Liu, T.-X. (2010). Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. *Journal of Insect Science*, *10*(1). https://doi.org/10.1673/031.010.2201

Yamamoto, R. T. (1974). Induction of hostplant specificity in the tobacco hormworm, *Manduca sexta*. *Journal of Insect Physiology*, *20*, 641–650. https://doi.org/10.1016/0022-1910(74)90185-1

Ye, L.-M., Di, X.-Y., Yan, B., Liu, J.-F., Wang, X.-Q., & Yang, M.-F. (2022). Population parameters and feeding preference of *Spodoptera litura* (Lepidoptera: Noctuidae) on different *Asparagus officinalis* tissues. *Insects*, *13*(12), 1149. https://doi.org/10.3390/insects13121149

Yinghua, S., Yan, D., Jin, C., Jiaxi, W., & Jianwu, W. (2017). Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two Bt corn hybrids expressing Cry1Ab. *Scientific Reports*, *7*(1), 41577. https://doi.org/10.1038/srep41577

Yuning, L., Luyang, L., Xueming, C., Xianmei, Y., Jintian, L., & Benshui, S. (2022). The bacterial and fungal communities of the larval midgut of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) varied by feeding on two cruciferous vegetables. *Scientific Reports*, *12*(1), 13063. https://doi.org/10.1038/s41598-022-17278-w

Zakria, M., Zaib, M. S., Abbas, K., Sarmad, M., Zaka, S. M., & Noor-ul-Ane, M. (2022). Influence of temperature on the development and reproduction of *Spodoptera litura* (Fabricius) on castor bean: implications for its use as a trap crop. *Arthropod-Plant Interactions*, *16*(5), 505–515. https://doi.org/10.1007/s11829-022-09913-0

Zalucki, M. P., Clarke, A. R., & Malcolm, S. B. (2002). Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology*, *47*, 361–393. https://doi.org/10.1146/annurev.ento.47.091201.145220

Zhang, A., Liang, K., Yuan, L., Li, T., Jiang, D., & Yan, S. (2024). Insect adaptation: unveiling the physiology of digestion in challenging environments. *Chemical and Biological Technologies in Agriculture*, *11*(1), 129. https://doi.org/10.1186/s40538-024-00642-5

Zhang, L., Su, S., Lv, Z., Zhou, Y., & Meng, Z. (2017). Insect species investigation of *Lolium perenne* artificial grassland in Guizhou province. *Guizhou Agricultural Sciences*, *45*(1), 59–61.

Zhang, X., Zhang, F., & Lu, X. (2022). Diversity and functional roles of the gut microbiota in lepidopteran insects. *Microorganisms*, *10*(6). https://doi.org/10.3390/microorganisms10061234

Zhang, Y., Zhang, L., Zheng, J., Li, T., & Zhao, L. (2025). Effects of larval starvation stress on the life history and adult fitness of fall webworm, *Hyphantria cunea*. *Insects*, *16*(4), 410. https://doi.org/10.3390/insects16040410

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, A. M. (2009). *Mixed effects models and extensions in ecology with R* (Vol. 574).

8. Supplementary data

Table 8-1 – Mean weight (mg) (and standard error) of herbage eaten and frass produced over a 24-hour period for 2-week-old Spodoptera litura *larvae that had been fed either plantain or red clover (offspring diet) and whose parents had fed on either plantain or red clover (parent diet) in a Larval Performance Experiment. Letters in common within a column show no statistically significant difference ($p > 0.05$).*

Parent diet	Offspring diet	Wet weight of herbage eaten (mg)	Dry weight* of herbage eaten (mg)	Frass weight (mg) (wet)
Plantain	Plantain	349.4 (16.0) a	63.2 (3.7) a	180.0 (10.7) a
Red clover	Plantain	345.3 (16.0) a	61.4 (3.6) a	182.8 (10.9) a
Plantain	Red clover	222.0 (16.5) b	46.9 (2.8) b	58.1 (3.6) b
Red clover	Red clover	190.5 (17.5) b	39.4 (2.5) c	54.1 (3.7) b
Test statistics:				
Parent diet		$F_{1,52}$=2.48; p=0.121	$F_{1,52}$=5.80; p=0.020	$F_{1,53}$=0.331; p=0.567
Offspring diet		$F_{1,19}$=45.3; p<0.001	$F_{1,19}$=23.87; p<0.001	$F_{1,19}$=131.3; p<0.001
Parent x offspring diet		$F_{1,52}$=0.92; p=0.200	$F_{1,52}$=3.41; p=0.070	$F_{1,53}$=0.04; p=0.844

**Calculated using wet weight:dry weight ratios of herbage samples from control (without larvae) vials*

Table 8-2 – Mean time (days) (and standard error) from hatch to pupation or adulthood for Spodoptera litura individuals that had been fed either plantain or red clover (offspring diet) and whose parents had fed on either plantain or red clover (parent diet) in a Larval Performance Experiment.

Parent diet	Offspring diet	Time (days) to pupation	Time (days) to adulthood
Plantain	Plantain	24.1 (1.1)	35.6 (1.4)
Red clover	Plantain	23.6 1.1)	35.5 (1.3)
Plantain	Red clover	24.2 (1.2)	36.2 (1.4)
Red clover	Red clover	24.9 (1.3)	35.7 (1.5)
Test statistics:		df=1, N=74	df=1, N=71
Parent diet		X^2=0.002; p=0.961	X^2=0.041; p=0.840
Offspring diet		X^2=0.328; p=0.567	X^2=0.088; p=0.767
Parent x offspring		X^2=0.230; p=0.632	X^2=0.009; p=0.925

Table 8-3 – Wet weight herbage consumed (mg) and feeding conversion efficiencies (%) calculated over a 24-hour period for 16-day old Spodoptera litura larvae fed either plantain or red clover and whose parents fed on plantain or red clover in a Mid-gut Microbiota Experiment. ECI = Efficiency of conversion of ingested food, ECD = Efficiency of conversion of digested food, AD = Approximate digestibility. Letters in common within a column show no statistically significant difference ($p > 0.05$).

Parent diet	**Offspring diet**	**No larvae**	**Wet weight herbage eaten (mg)**	**ECI**	**ECD**	**AD**
Plantain	Plantain	19	523.7 (69.3) a	42.1 (3.2) a	79.4 (2.9) a	51.6 (13.1) a
Red clover	Plantain	18	475.9 (61.4) a	40.8 (3.0) b	75.3 (3.0) a	49.8 (3.0) a
Plantain	Red clover	5	86.1 (22.5) b	47.1 (5.5) a	68.6 (6.3) a	69.8 (5.1) b
Red clover	Red clover	15	136.8 (20.1) b	49.2 (3.4) a	71.3 (3.5) a	64.1 (3.1) b
Test statistics:						
Parent diet			$F_{1,5}$=0.14; p=0.723	X^2=0.01; p=0.93	X^2 =0.40; p=0.528	X^2 =0.61; p=0.437
Offspring diet			$F_{1,57}$ =85.01; p<0.001	X^2 =4.91; p=0.027	X^2 =3.19; p=0.074	X^2 =20.55; p<0.001
Parent x offspring			$F_{1,56}$ =2.81; p=0.099	X^2 =0.24; p=0.627	X^2 =0.88; p=0.347	X^2 =0.34; p=0.558

9. Co-Authorship Forms

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Nature of contribution by PhD candidate	Conceptualization (lead), data curation (lead); formal analysis (lead), investigation (lead), methodology (lead), writing – original draft, review and editing (lead)
Extent of contribution by PhD candidate (%)	95%

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Andrew Barnes	Supervision (equal), writing – original draft, review and editing (supporting)
Sofia Orre-Gordon	Funding acquisition (lead), supervision (equal), writing – original draft, review and editing (supporting)

Certification by Co-Authors

The undersigned hereby certify that:

❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and ❖ that the candidate wrote all or the majority of the text.

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Nature of contribution by PhD candidate	Conceptualization (equal), data curation (lead); formal analysis (supporting), investigation (lead), methodology (lead), project admin (equal), writing – original draft, review and editing (lead)
Extent of contribution by PhD candidate (%)	90%

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Nature of contribution by PhD candidate	Conceptualization (lead), data curation (lead); formal analysis (equal), investigation (lead), methodology (lead), writing – original draft, review and editing (lead)
Extent of contribution by PhD candidate (%)	90%

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Chapter 5: Jensen, J. G., Clearwater, M. J., Barnes, A. D., & Orre-Gordon, S. (2026). Larval age affects the ability of *S litura* to survive on a low-quality host. Intended for submission to a scientific journal for publication

Nature of contribution by PhD candidate	Conceptualization (lead), data curation (lead); formal analysis (lead), investigation (lead), methodology (lead), writing – original draft, review and editing (lead)
Extent of contribution by PhD candidate (%)	95%

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