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Agro-ecological management of the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) and other pests in brassicas

> A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

> > at Lincoln University by Sundar Tiwari

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Declaration

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Abstract

Agro-ecological management of the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) and other pests in brassicas

by

Sundar Tiwari

Modern agriculture offers a range of benefits including sufficient food production for a constantly increasing human population. Improved living standards, enhanced social stability and avoiding food insecurity are other advantages of agricultural intensification. Unfortunately, such agricultural intensification relies heavily on anthropogenic agricultural inputs such as high-yielding varieties, fertilizers and chemical pesticides. Some aspects of these practices are associated with human health problems, reduced biodiversity, degradation of soil fertility, air and water pollution, eutrophication of rivers and lakes, pollinator decline as well as impacts on atmospheric constituents and global warming.

In New Zealand, wheat bug, *Nysius huttoni*, is considered an economic pest of forage brassicas and many other cultivated crops such as cereals and vegetables. This bug damages forage brassicas; greater economic losses have been recorded at the germination/seedling stage (90% plant loss in extreme situations). Insecticides as seed coatings and sprays are frequently used to manage this and other New Zeland forage brassica pests. Although seed coatings represent selective placement of the toxin, it is still true that large quantities are applied. A high proportion of these compounds enters the soil and leads to pesticide resistance, and they impact beneficial arthropods and soil microorganisms creating an adverse effect on ecosystem services (ES).

This study developed a habitat management protocol using trap plant species in a 'sustainable intensification' approach, which is an alternative, more benign approach to pest management. Specifically, the study developed trap-crop technologies to draw *N. huttoni* away from kale seedlings. The use of less susceptible kale cultivars and integrating these into the trap cropping technology are important pest management strategies in integrated pest management (IPM) and

potentially reduce over-reliance on orthodox pesticides on brassicas. Flowering trap plants can improve conservation biological control (CBC) and improve multiple ES in and off-farm in brassicas. A range of laboratory, field-cage and open-field experiments were carried out at Lincoln University (43° 38' S; 172° 27' E), New Zealand, during 2016 and 2017, and at Chitwan (27° 37' N; 84° 22' E), Nepal, during 2018 to: 1) evaluate host plant selection by *N. huttoni*of a range of potential trap plant species; 2) evaluate the susceptibility of kale cultivars to *N. huttoni*; 3) assess the growth stage of alyssum (*Lobularia maritima*) preferred by *N. huttoni*; 4) evaluate potential trap plant species for the *N. huttoni* forage brassicas; and 5) improve CBC by using alyssum floral strips in a radish field.

A series of laboratory choice, no-choice and paired-choice tests were conducted to evaluate the preference of *N. huttoni* for seedlings of eight potential trap plant species: *L. maritima* (alyssum), Triticum aestivum (wheat), Phacelia tanacetifolia (phacelia), Fagopyrum esculentum (buckwheat), Coriandrum sativum (coriander), Trifolium repens (white clover) and Medicago sativa (alfalfa). These species were compared with *Brassica oleracea* (kale) as a potentially susceptible control. Alyssum and wheat were the most favoured potential trap plants for *N. huttoni*, with a significantly higher survival rate, earlier feeding damage and quicker settling time. Laboratory bioassays were performed to evaluate N. huttoni preference for a range of kale cultivars: Kestrel, Gruner, Sovereign, Regal, Corka and Colear. Kestrel and Coleor are the most popular kale cultivars used as forage brassicas in New Zealand but they are the most susceptible to N. huttoni. Corka and Regal were the least susceptible cultivars; the others showed medium susceptibility cultivars to the N. huttoni. However, farmers mostly consider other agronomic factors such as yield and disease resistance during cultivar selection. The less susceptible kale cultivars can be integrated into an IPM strategy with trap cropping, biological and microbial approaches, for future low-pesticide management of the bug. Laboratory bioassays of two growth stages of alyssum were performed to evaluate N. huttoni preference for the growth stages of alyssum. Flowering alyssum was significantly more suitable for *N. huttoni* than seedlings. Assessment of bug preference for the various growth stages of alyssum plants suggests appropriate planting times for the trap and main crop. Efficient trapping of N. huttoni in brassica fields can be achieved if flowering alyssum strips are maintained at the brassica seedling stage in fields.

Field cages and open-field experiments were established at the Biological Husbandry Unit (BHU), Lincoln University, to evaluate the performance of *L. maritima* and *T. aestivum* as a potential trap plants of *N. huttoni* compared with kale. In field cages, the most suitable trap plants, *L. maritima* and *T. aestivum*, were compared with the least suitable plants, *C. sativum* and *T. repens*, and all were compared with kale. In open field experiments, alyssum, wheat, 'alyssum plus wheat' and kale were used; other species were discarded based on their poor performance in the field-cage experiments. In field cages, alyssum was the most suitable trap plant of the bugs followed by wheat; this was also true in the open-field experiments. However, the 'alyssum plus wheat' trap strips have a greater potential to trap N. huttoni than wheat alone, but less potential than alyssum alone. In open fields, flowering, fruiting and senescent alyssum stages, and ripening and senescent wheat stages were significantly more suitable for trapping the bug than the vegetative stage. This information is important; it is necessary to maintain the flowering or fruiting stages of potential trap plants at the brassica seedling stage to reduce pest pressure in brassica fields. Nysius huttoni populations declined with distance from the edge trap strips. That significantly higher numbers of N. huttoni were intercepted at the edge trap strips suggests focussing N. huttoni management practices, such as 'soft 'chemicals, at the edges rather than other parts of fields, which would reduce pesticide cost. Less damage was recorded on kale seedlings next to wheat trap strips followed by alyssum, 'alyssum plus wheat' and kale strips. Flowering alyssum strips also provide habitat for many beneficial arthropods such as spiders, seven-spotted ladybirds (Coccinella septempunctata), and lacewings (Micromus tasmaniae), that could potentially kill N. huttoni and other brassica pests in forage brassicas.

A study in Nepal to test alyssum (*L. maritima*), as a potential trap plant for the *N. huttoni* in CBC of pests in radish fields. Alyssum in radish fields significantly increased beneficial arthropods such as hoverflies (Diptera: Syrphidae), ladybirds (*C. septempunctata*), and spiders and reduced the pest pressure of aphids (*Myzus persicae*) and other pests.

These findings are useful in developing a pest management protocol for *N. huttoni* using a 'pushpull' strategy in which less susceptible kale cultivars can be used as a 'push' component and alyssum plants as a 'pull' component. The less susceptible kale cultivars can also be used as a 'push' component and highly susceptible kale cultivars as a 'pull' component in a 'push-pull' strategy of pest management. Maintaining potential trap plant species at the flowering stage or growing highly susceptible kale cultivars at the edge of the main field can keep the wheat bugs away from the main crop and keep them from entering the main field. Flowering alyssum can also improve CBC and multiple ES in brassica fields and improve the quality of landscape.

Keywords: Wheat bug, *Nysius huttoni*, Hemiptera, Lygaeidae, trap cropping, forage brassicas, kale, choice, no-choice, paired-choice, *Lobularia maritima*, wheat, *Phacelia tanacetifolia*, preference, kale cultivars, radish pest, New Zealand, Nepal, conservation biological control, ecosystem services.

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Chapter 1 Introduction

A part of this chapter 'Biology and management of the New Zealand endemic wheat bug, *Nysius huttoni*, (Hemiptera: Lygaeidae)' was publihsed in November 2019 (doi.org/10.1093/jipm/pmz032) in the *Journal of Integrated Pest Management*; 'Habitat management for pest management: limitations and prospects' was published in June 2019 (doi: 10.1093/aesa/saz020) and 'Trap cropping in South Asia: concepts, limitation and

future strategy' was published in May 2019 (doi: 10.1093/aesa/saz003) in the Annals of the Entomological Society of America.

1.1 Global agriculture

The current world population is 7.5 billion, which is increasing daily; the growth rate has become 1.18 % per year or approximately 83 million people added annually (DESA, 2015; Lutz, Butz, & Samir, 2017). The population is projected to increase by one billion in the next 15 years, reaching 8.5 billion in 2030 and further increases are expected to 9.7 billion by 2050 and 11.2 billion by 2100 (DESA, 2015; Laurance, Sayer, & Cassman, 2014). The population growth rate is even higher in tropical developing nations (Laurance et al., 2014). About 1.0 billion people are under food insecurity (FAO, 2013) and 3.7 billion are malnourished (Pimentel & Pimentel, 2006).

To meet the global food demand by 2050, food production needs to increase by 70 - 110 % (Tilman et al., 2001) from the same land area, which is possible only by changing current agricultural systems and practices (Godfray et al., 2010). By that time, 10⁹ hectares of natural habitat would have been converted to agricultural land (Nelson et al., 2009). The 'green revolution' or agricultural intensification, using high yielding hybrid varieties, anthropogenic inputs such as chemical fertilizers and pesticides, and agricultural modernization, has doubled current food production (Bommarco, Kleijn, & Potts, 2013; Giller, Beare, Lavelle, Izac, & Swift, 1997; Laurance et al., 2014); these practices were not related to sustainable agricultural production systems (Pimentel & Pimentel, 2006). Modern results, such as nutrient losses, soil erosion, depletion of fresh water sources, pollution of waterways and marine environments, biodiversity loss, and disturbance to ecosystem services (ES), are detrimental to the environment (Geiger et al., 2010; Lichtfouse et al., 2009; McLaughlin & Mineau, 1995; Robinson & Sutherland, 2002), which further influences the production of food, fibre, pollination and natural pest control (Tscharntke et al., 2012; Tscharntke, Klein, Kruess, Steffan-Dewenter, & Thies, 2005). Biodiversity is a key driver of ecological farming

and sustainable crop production (Wratten, Sandhu, Cullen, & Costanza, 2013). The decline of biodiversity not only affects ecosystem functions (EF) but also increases the consequences of instability (Tilman, Reich, & Knops, 2006), crop productivity, (Letourneau & Altieri, 1999) as well as human well-being (Cardinale et al., 2012).

This global challenge to modern agriculture (Tscharntke et al., 2012) was originally emphasized in Rachel Carson's 1960s book '*Silent Spring*' (Carson, 1962). During the early 1940s to late 1960s, pesticides were considered a vital agricultural input commonly used to control pests and diseases in cultivated crops. These practices can be detrimental to human health, biodiversity loss and the surrounding environment (Dhaliwal, Jindal, & Dhawan, 2010; Karuppuchamy & Venugopal, 2016; Lou, Zhang, Zhang, Hu, & Zhang, 2013) and can increase farming production costs. Ecological or sustainable intensification is an alternative approach to meet future climatic, economic and social challenges of farming (Foley et al., 2005). For example, sustainable intensification (Godfray & Garnett, 2014) encourages agricultural practitioners to use more productive, stable and resilient agriculture without disturbing the environment (Foley et al., 2005), sustain ES, minimize environmental costs and maintain functional biodiversity (Pretty & Bharucha, 2014; Tscharntke et al., 2012).

1.2 Sustainable agriculture

Sustainable agriculture, as a concept, was born in the late 1950s to minimize the crisis from the overuse of harmful synthetic chemical pesticides (Brunner, 2009) and to produce well-functioning ES (Meyhöfer & Poehling, 2006; Wratten et al., 2013). Ecosystem services are defined as the benefits obtained from ecosystems for humans (Costanza et al., 1997). Different types of sustainable agriculture are currently practised in the world such as conservation agriculture (Kassam, Friedrich, Shaxson, & Pretty, 2009), organic agriculture (Niggli, 2015), permaculture (Lockyer & Veteto, 2013; Válek & Jašíková, 2013), biodynamic farming (Heckman, 2006; Tate, 1994), ecological agriculture (Ye, Wang, & Li, 2002), and integrated pest management (Kogan, 1998). These farming practices are also called 'ecosystem service-rich agriculture' (ESRA) (Power, 2010) and are considered an option to replace fossil fuel based inputs and improve ES at the farm level (Altieri, Nicholls, Henao, & Lana, 2015; Gurr, Wratten, Landis, & You, 2017; Wratten, Gillespie, Decourtye, Mader, & Desneux, 2012). Agricultural production systems depend highly on ES that help to improve conservation biological control (CBC) followed by pest control, enhanced pollination, carbon sequestration, soil fertility improvement, nutrient cycling and hydrological services (Altieri, 1999; Power, 2010). The quantification or valuation of ES is usually done by placing a monetary value on ecosystem functions (Costanza et al., 2014) but sometimes ethical, spiritual and aesthetic values are added during the valuation (Wratten et al., 2012). Recently, scientists have emphasized quantifying agricultural practices that promote ES. At the farm level, a service providing protocol (SPP) simplifies the application of ES. An SPP is a broad framework consisting of the detailed ecological, floral and seasonal characteristics of an ecosystem service provider (ESP) (Gurr et al., 2017) that support a service providing unit or organism (SPU). This organism can directly or indirectly contribute to agricultural production by delivering and/or supporting (e.g., soil fertility) and by regulating ES (e.g., pollination and pest control) (Bommarco et al., 2013). However, this SPP needs to be evaluated at the farm and landscape level to minimize potential ecosystem dis-services (DS). In this PhD study, habitat manipulation such as trap cropping, use of less suitable cultivars for the pest and the deployment of floral strips have been considered as important strategies in agro-ecological pest management and the promotion of sustainable agriculture in brassica fields (Shelton & Badenes-Perez, 2006).

1.3 Integrated pest management and habitat management

Integrated pest management (IPM) has been successfully implemented in many countries with the aim of reducing over-reliance on chemical pesticides and reducing the environmental impacts (Nicholls & Altieri, 2004; Pimentel & Peshin, 2014). IPM on farms generates an opportunity for sustainable agriculture. This management approach is popular because it is a whole-system approach using the ecological, social, and economic aspects of pest management (Barzman et al., 2015). It primarily focuses on the agro-ecological aspects of pest management such as regular scouting of pests and natural enemies; decisions on pest management take place based on the agro-ecological situation and recommends 'soft' pesticides on a 'needs' basis (Barzman et al., 2015; Pretty & Bharucha, 2015).

Habitat management is an important agroecology approach in IPM that can help to reduce pest pressure in agricultural fields (Gurr et al., 2017) and promote organic farming (Pimentel & Peshin, 2014). These management practices alone or integrating with other approaches to IPM can help to reduce pesticide use by regulating pest populations, reducing damage, improving multiple ES, and promoting CBC and sustainable agriculture (Gurr et al., 2017). Simple vegetative diversification on farms influences herbivore, predator and pollinator activities by visual or chemical cues (Hokkanen, 1991; Root, 1973), acts as a barrier to movement (Perrin & Phillips, 1978) or creates a different volatile profile in crop fields (Finch & Collier, 2000). Examples of habitat management in agricultural fields include trap cropping (Wan, Zhang, Huang, Ji, & Jiang, 2016), cover cropping (Storkey et al., 2015), and the use of the flower strips (Gurr et al., 2017; Westphal et al., 2015) that can facilitate habitat pest management activities in an agro-ecosystem. Two main hypotheses are associated here: the bottom-up approach or the 'resource concentration' hypothesis (acts on the second trophic level, i.e., herbivores) (Root, 1973) and the top-down approach or 'natural enemies' hypothesis (acts on the third trophic level i.e., natural enemies); (Russell, 1989); both can be involved in habitat pest management (Root, 1973; Russell, 1989).

Trap cropping, a form of sustainable agriculture, is common in traditional pest management in many crops (Pimentel & Peshin, 2014; Talekar & Shelton, 1993). It can help minimize or eliminate pesticides and conserve natural enemies that control pests (Hokkanen, 1991; Sarkar, Wang, Wu, & Lei, 2018). A trap crop is normally grown next to the main crop to attract the pest for oviposition and feeding (Shelton & Badenes-Perez, 2006; Badenes-Pérez, 2018). This strategy is based on the principle of using a relatively more preferred species to keep pests away from the main crop and so reduce pest damage (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). For example, buckwheat, Fagopyrum esculentum Moench (Polygonaceae), can be used as a trap crop in onion fields to suppress populations of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Buckland, Alston, Reeve, Nischwitz, & Drost, 2017). Some trap crops can also attract pest natural enemies (Naranjo, Ellsworth, & Frisvold, 2015) and ideally provide all or some component of SNAP (shelter, nectar, alternative hosts, and pollen), which has an additive or synergistic effect for pest natural enemies and pest suppression occurs (Gurr et al., 2017; Landis, Wratten, & Gurr, 2000; Westphal et al., 2015). Trap cropping, in the form of companion planting or habitat management in a monoculture, can attract both pests and many beneficial arthropods (Sarkar et al., 2018), which can improve CBC (Khan, Midega, Pittchar, Bruce, & Pickett, 2012). Such practices can improve the provision of ES (Gurr et al., 2017) and this is certainly needed for future farming. For example, Borage officinalis L. (Boraginaceae) in strawberry greenhouses simultaneously attracts both the green peach aphid, Myzus persicae (Sulzer), and its parasitoid, Aphidius colemani Viereck (Hymenoptera: Braconidae) and chrysopid predators (Fujinuma, Kainoh, & Nemoto, 2010; Mitchell, Hu, & Johanowicz, 2000) that can improve biological pest management (Sarkar et al., 2018). Semiochemicals produced by plants and herbivore-induced plant volatiles (HIPVs) can catalyse pest natural enemies and improve pest control. For example, maize, Zea mays L. (Poaceae), produces several types of semiochemicals that attract the generalist predator lacewing, Chrysoperla carnea Stephens (Neuroptera: Chrysopidae) (Zhu, Cossé, Obrycki, Boo, & Baker, 1999).

The 'push-pull' strategy is already established in pest management technology in Africa, where 20,000 farmers benefited from this approach using molasses grass (*Melinis minutiflora* Beauv.) and two species of desmodium (*Desmodium uncinatum* Jacq. and *D. intortum* Urb.) to repel maize stem borers, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), and *Busseola fusca* Fuller (Lepidoptera: Noctuidae). Napier (*Pennisetum purpureum* Schumuach) and sudan grass (*Sorghum vulgare sudanense* Stapf.) have been used as trap crops (pull) in maize fields to control maize stem borer (Khan, Pickett, Wadhams, & Muyekho, 2001).

However, the effectiveness of trap cropping varies based on the modalities used in the fields such as perimeter trap cropping (Boucher, Ashley, Durgy, Sciabarrasi, & Calderwood, 2003), sequential trap cropping (Srinivasan & Moorthy, 1991), multiple trap cropping (Hokkanen, 1989), push-pull trap cropping (Khan et al., 2001) and biological control (Landis et al., 2000). Taking all of the above into consideration, this study considers the opportunity to manage the brassica pest, *Nysius huttoni* White (Hemiptera: Lygaeidae), using a trap crop strategy, a form of habitat management. This strategy can be integrated into many other aspects of IPM such as potential brassica cultivars (see Section 1.4). Trap cropping has been considered as an important interest area in sustainable pest management (Shelton & Badenes-Perez, 2006), CBC (Gurr et al., 2017; Landis et al., 2000) and organic farming (Burgio et al., 2016; Gurr, Wratten, & Barbosa, 2000; Landis et al., 2000).

1.4 Forage brassicas and kale

From early summer to late winter, forage brassicas are widely grown as a supplement and an alternative vegetation source for cattle, sheep and deer in New Zealand (de Ruiter et al., 2009; PGG, 2009; Wilson, Zyskowski, Maley, & Pearson, 2004). The crop is popular in New Zealand for the following reasons: a) a high-quality feed (high protein and energy) at times in the year when the performance of ryegrass is limited because of the lack of moisture and higher temperatures (Speciality Seeds, 2016); b) the cost of production is lower than purchasing supplementary feed; and c) it reduces weeds, pests and diseases, and creates better soil conditions (de Ruiter et al., 2009). Kale (*Brassica oleracea* L.), rape (*Brassica napus* L. cv. *napus*), turnip (*Brassica rapa* L.) and swede (*Brassica napus* L. cv. *napo-brassica*) are four important forage brassicas grown for ruminant animals (Table 1.1) (PGG, 2009; Stewart, 2002; Westwood & Mulcock, 2012). About 400,000 hectares are grown annually in New Zealand (Horrocks, Horne, & Davidson, 2018) in New Zealand in a wide range of soils and climates and are cultivated throughout the year (Wilson et al., 2004).

Kale, *B. oleracea*, is used as the main crop in this study. It is the most popular traditional winter feed brassica crop (Brown, Maley, & Wilson, 2007) with a deep root system and good tolerance to clubroot and dry rot. It also has good potential to regrow after light grazing in February and March (de Ruiter et al., 2009). For a high yield and good quality, kale needs to be grown in fertile soil with good moisture. Tall or giant, medium, intermediate and short kale cultivars are common in New Zealand; the taller types are used for cattle feed, the shorter ones are suitable for sheep and deer grazing (Speciality Seeds, 2016). The most popular kale cultivars and their phenological characteristics are given in Table 1.2.

Common forage brassicas	Latin name	Phenological description	Production time	Cultivars
Rape	Brassica napus	Numerous leaves, fibrous stem and no – bulb	Summer to winter	Winfred, Rangi, Emerald, Giant, Maxima Plus, Titan, Goliath, Bonar, Greenland, Interval, Leafmore, Wairoa
Kale	Brassica oleracea	Numerous leaves, a large swollen stem, deep root system and no-bulb	Summer to winter	Kestrel, Sovereign, Regal, Gruner, Rawera, Caledonian, Burly, Colour, Keeper, Kestrel, Rawara
Swede	Brassica napus cv. napo brassica	Few leaves, no stem, a large fleshy bulb with an obvious neck	Summer to winter	Major Plus, Dominion, Aparima Gold, Keystone, Doon Major, Highlander, Invitation, Winton, Virtue
Leaf turnip	Brassica rapa	Numerous leaves, swollen tap root, no- bulb	Mid- summer to early winter	Hunter, Pasja, Barkant, Tyfon
Bulb turnip	Brassica rapa	Few leaves, no stem, a large fleshy bulb with no-neck.	Summer to late winter	New York, Barkant, Rival, Green Globe, York Globe, Appin, Dynamo, Green Resistant, Manga, Marco, White Star,

Table 1.1 The phenological description, including production time and cultivars, of common forage

brassicas in New Zealand

Sources: de Ruiter et al. (2009); PGG (2009); Speciality Seeds (2016)

Table 1.2 Descriptions of the kale cultivars commonly grown in New Zealand

Cultivar name	Height	Phenological descriptions
Coleor	Small to medium	High leaf-to-stem ratio, winter hardy, low to medium yield, used for feeding sheep and deer
Keeper	Medium	High leaf-to-stem ratio, palatable and digestible thick stems, low yield, used for sheep grazing
Kestrel KE35 TC	Medium	High leaf-to-stem ratio, palatable and digestible thick stems, low yield, used for sheep grazing
SF Fuel	Medium	High leaf-to-stem ratio, palatable and digestible thick stems, low yield, used for sheep grazing
Sovereign SOV 27 AC	Intermediate	High leaf-to-stem ratio and high yield, used for feeding cattle, sheep and deer
Regal KBG 01 AC	Intermediate	High leaf-to-stem ratio, winter hardy but low yield, used for feeding cattle, sheep and deer
Corka	Intermediate	High leaf-to-stem ratio, palatable, winter hardy and high yield, suitable for all stock types

Cultivar name	Height	Phenological descriptions	
Rawara	Intermediate	High leaf-to-stem ratio, palatable, winter hardy and	
		high yield, suitable for all stock types	
Crean	Intownodiato	Lick loof to store which we lately a winter bound, and	
Crosa	Intermediate	High leaf-to-stem ratio, palatable, winter hardy and	
		high yield, suitable for all stock types	
SovGold	Intermediate	High leaf-to-stem ratio, palatable, winter hardy and	
		high yield, suitable for all stock types	
Gruner	Giant	Tall and high yield, used for cattle	
D. J.	Circul	The state of the second s	
Burley	Giant	Tall and high yield, used for cattle	
Caledonian	Giant	Tall and high yield, used for cattle	
Sources: de Ruiter et al. (2009); PGG (2009); Speciality Seeds (2016)			

1.5 Common pest problems in forage brassicas

Forage brassicas are invaded by a wide range of insect pests, pathogens and weeds that limit crop production. Insects and pathogens damage almost all parts of plants including the roots (Dixelius, Bohman, & Wretblad, 2004) with few or no management options available all over the world (Granér, Persson, Meijer, & Alström, 2003). Clubroot, Plasmodiophora brassicae Woronin, dry root or blackleg, Leptosphaeria maculans (Sowerby) P. Karst., leaf spot, Alternaria brassicicola (Schwein) Wilt., ring spot, Mycosphaerea brassicicola (Duby) Lindau., downy mildew, Peronospora parasitica (Pers.) De Bary, white rust, Albugo candida (Pers.) Kuntze, black rot, Xanthomonas campestris (Pammel) Dowson, peppery leaf spot, Pseudomonas syringae pv. maculicola Mc Culloch, turnip mosaic virus (TuMV), and turnip yellows virus (TuYV) are economically important diseases of forage brassicas in New Zealand. Crop failure in forage brassicas is also associated with a number of insect pests such as wheat bug, N. huttoni, springtail, Bourletiella spp., diamondback moth, Plutella xylostella L., white butterfly, Pieris rapae L., cabbage grey aphid, Brevicoryne brassicae L., green peach aphid, M. persicae, and European leaf miner, Scaptomyza flava Fallen (de Ruiter et al., 2009). Springtail primarily attacks the cotyledons and emerging growing points, whereas diamondback moth (P. xylostella) feeds on the growing tips of plants and larva burrow into the leaves that may drop off in a severe infestation. White butterfly causes larger irregular holes in leaves. The aphids suck plant sap and cause yellowing and wilting of the plants. The leaf miner damages the plant by making the tunnels in the leaf tissue (de Ruiter et al., 2009). In this study, N. huttoni is used as the reference study insect with a focus on evaluating various agro-ecological aspects of IPM.

Nysius huttoni, is considered a major threat to forage brassicas and other cultivated crops such as wheat, barley, oats, clover and lettuce (Bejakovich, Pearson, & O'Donnell, 1998; He & Wang, 1999; Miller & Pike, 2002). In brassicas, it primarily attacks seedlings by sucking phloem; damage has

reached 90 % in direct drilled brassca seedlings (AgPest, 2016; Speciality Seeds, 2016). Various ecological aspects including the life cycle, habitat, damage potential, and pest management strategies are given in Section 1.6.

1.6 Nysius huttoni: the current state of knowledge

Wheat bug, *N. huttoni*, is a New Zealand endemic insect (Aukema, Bruers, & Viskens, 2005; Eyles, 1960a; He, Wang, & Carpenter, 2003) widely distributed in the North and South Islands (Eyles, 1960b; Eyles & Ashlock, 1969; Myers, 1926) from sea-shore to 1800 masl (metres above sea level) (Eyles & Ashlock, 1969). The species is also reported in the Chatham Islands and the Three Kings Islands (Woodward, 1954). In New Zealand, there are over 142 genera and 319 species belonging to 28 families of Hemiptera (Larivière & Larochelle, 2014); of them, two genera (*Nysius* and *Rhypodes*) with 32 species, belong to family Lygaeidae (Larivière & Larochelle, 2014). Four *Nysius* species, including three endemic and one adventive species, have been reported in New Zealand (Table 1.3): *Nysius huttoni; N. liliputanus* Eyles & Ashlock, 1969 and *N. convexus* (Usinger, 1942). *Nysius caledoniae* Distant, 1920 was accidentally introduced in 2006 from Lord Howe Island and Tasmania, Australia (Eyles & Malipatil, 2010); it was recorded for the first time in a lettuce crop in Auckland (Eyles & Malipatil, 2010; Rowe & Hill, 2015). *Nysius huttoni* was named by Buchanan White (1878) from the New Zealand collections of Hutton and Wakefield (Eyles, 1960b). More recently it has been recorded in The Netherlands and Belgium (Aukema et al., 2005; Bonte, Casteels, Maes, & Clercq, 2010).

Nysius huttoni are grey or black brown or sometimes creamy white. The apices of the femora and tibia are yellow. The body is covered by a long, thick and erect pubescence. Adult wings are translucent or transparent. The body is elongate oval and dorsally flattened. The head is triangular, slightly narrower than the pronotum, with prominent round eyes. There are four antennal segments, segments 1 and 4 are bigger than segments 2 and 3 (Fig. 1.1). The forelegs are thin with no spines (Eyles, 1960a). Nymphs and adults feed on at least 75 plant species belonging to > 25 plant families including vegetables, cereals, forage brassicas, and many weeds (Eyles, 1965; He et al., 2003; Yang & Wang, 2004) (Table 1.4).

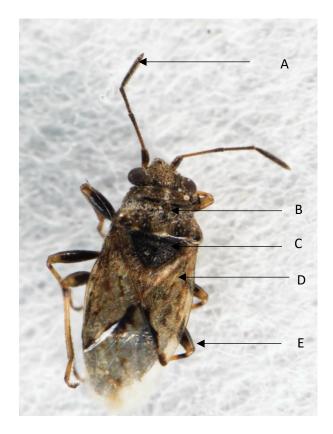


Figure 1.1 The key morphological features of *Nysius huttoni*: a) Four-segmented antenna, the last segment is larger than the others; b) Long dense hairs on the pronotum; c) Long dense hairs on the scutellum, and d) on the hemelytron; and e) the distal end of the femur is yellow. Photo: Sundar Tiwari

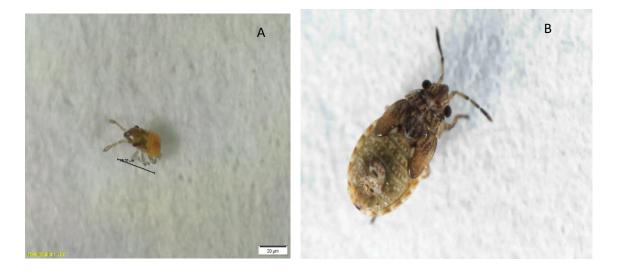


Figure 1.2 *Nysius huttoni* nymphs: a) newly emerged the first instar; b) the fourth instar. Photo: Sundar Tiwari

Table 1.3 The morphological and other biological features of the four Nysius species recorded from

New Zealand

Characteristics	Nysius	Nysius	Nysius	Nysius
Characteristics	huttoni	convexus	liliputanus	caledoniae
Morphological features	Have a complete double row of punctures following the claval suture (Eyles & Ashlock, 1969) with long, dense hairs on pronotum, scutellum and hemelytra	A single row of punctures along (the claval) the side of claval suture (Eyles & Ashlock, 1969) and long erect hairs on pronotum, scutellum and hemelytra	Punctures at claval suture absent and presence of short erect hairs on pronotum, scutellum and hemelytra	Punctures are absent at claval suture and scutellum are round
Origin	Endemic	Endemic	Endemic	Exotic
Wing type	Macropterous ¹ , sub- brachypterous ² and brachypterous ³ forms (Eyles & Ashlock, 1969)	Mainly sub- brachypterous forms (Eyles & Ashlock, 1969)	Sub- brachypterous to brachypterous (Eyles & Ashlock, 1969)	Macropterous (Rowe & Hill, 2015), sub- brachypterous and brachypterous (Eyles, 1960b)
Host	Common hosts are Brassicaceae, Polyngonaceae, Caryophyllaceae, Compositae and Leguminosae	Moss associations on glacial moraines and in river-bank vegetation (Larivière & Larochelle, 2004)	Moss associations on glacial moraines, dry river beds and also on ferns (at night) (Larivière & Larochelle, 2004)	Lettuce (Rowe & Hill, 2015)
Damage potential	Pest of a wide range of crops (Myers, 1926) including weed species	Damage not recorded in crops (Eyles & Ashlock, 1969)	Damage not recorded in crops (Larivière & Larochelle, 2004)	First recorded in lettuce but damage has not been recorded yet (Rowe & Hill, 2015)
Distribution in New Zealand	Widely distributed in both islands from seashore to 1800 masl (Eyles & Ashlock, 1969)	South Island (Eyles & Ashlock, 1969)	South Island (Eyles & Ashlock, 1969)	North Island (Auckland) (Rowe & Hill, 2015)

¹ Wings longer than abdomen (macropterous)

² Wings level with abdomen or slightly exceed (sub-brachypterous)

³ Wings are shorter than the abdomen (brachypterous)

Table 1.4 The host plant species of wheat bug, Nysius huttoni (Hemiptera: Lygaeidae)

Family	Host plants	References
Poaceae	Wheat (<i>Triticum aestivum</i> L.), ryegrass (<i>Lolium</i> spp.), barley (<i>Hordeum vulgare</i> L.; <i>Hordeum marinum</i> Huds.), bromegrass (<i>Bromus</i> spp.), oats (<i>Avena sativa</i> L.), rye (<i>Secale cereale</i> L.), Yorkshire fog (<i>Holcus lanatus</i> L.), paspalum (<i>Paspalum dilatatum</i> Poir.), tussock (<i>Nassella trichotoma</i> Hackel ex Arech.), browntop (<i>Agrostis tenuis</i> Sibth.; <i>Agrostis capillaris</i> L.), annual bluegrass (<i>Poa annua</i> L.), vulpia hair grass (<i>Vulpia megalura</i> Rydb.), perennial ryegrass (<i>Lolium perenne</i> L.)	(Bejakovich et al., 1998; CABI, 2011; Cressey, Farrell, & Stufkens, 1987; EPPO, 2006; Every, Farrell, & Stufkens, 1992; Every, Farrell, Stufkens, & Wallace, 1998; Every, Farrell, & Stufkens, 1990; Eyles, 1965; Eyles & Ashlock, 1969; Farrell & Stufkens, 1993; Gurr, 1952, 1957; Lorenz & Meredith, 1988; Morrison, 1938; Myers, 1926; Swallow & Cressey, 1987; Wise, Tucker, & Lamb, 2000)
Brassicaceae	Twin cress (<i>Coronopus didymus</i> L. Smith.), shepherd's purse (<i>Capsella bursa-pastoris</i> (L.) Medic.), swede (<i>Brassica napo-brassica</i> Mill.), turnip (<i>B. rapa</i> L.), rape (<i>B. napus</i> L), kale (<i>B. oleracea</i> var. <i>acephala</i> L.), cabbage (<i>B. oleracea</i> L.), narrow-leaved cress (<i>Lapidium</i> <i>pseudotasmanicum</i> Thell.), alyssum (<i>Lobularia</i> <i>maritima</i> (L.) Desv.), fodder beet (<i>Beta vulgaris</i> L.)	(EPPO, 2006; Eyles, 1963; Eyles, 1960b, 1965; Eyles & Ashlock, 1969; Farrell & Stufkens, 1993; Ferguson, 1994; Gurr, 1952, 1957; He & Wang, 1999; He, Wang, & Carpenter, 2002a; He et al., 2003; He & Wang, 2000; He, Wang, & Carpenter, 2002b, 2004; Pearson & Goldson, 1980; Wang, Yang, & Hedderley, 2008; Wei, 2001; Wei, 2008b, 2010; Wei, 2012; Yang & Wang, 2004)
Fabaceae	Lucerne (<i>Medicago sativa</i> L.), suckling clover (<i>Trifolium dubium</i> Sibth.), red clover (<i>T. pratense</i> L.), subterranean clover (<i>T. subterraneum</i> L.), white clover (<i>T. repens</i> L.), hare's foot clover (<i>T. arvense</i> L.), strawberry clover (<i>T. fragiferum</i> L.), clustered clover (<i>T. glomeratum</i> L.), gorse (<i>Ulex europaeus</i> L.), common broom (<i>Sarothamnus scoparius</i> L.)	(CABI, 2011; EPPO, 2006; Eyles, 1960b; Gurr, 1952, 1957; Myers, 1921; Myers, 1926; Schoreder, 1995; Schroeder & Chapman, 1995; Wei, 2001)
Asteraceae	Sunflower (<i>Helianthus annuus</i> L.), onehunga weed (<i>Soliva sessilis</i> Rulz & Pav.), tauhinu (<i>Cassinia leptophyla</i> (G. Frost) R. Br.), cat's ear (<i>Hypochaeris radicata</i> L.), hawkweed (<i>Hieracium</i> spp.), narrow-leaved ragwort (<i>Senecio</i> <i>inaequidens</i> DC.), Scotch thistle (<i>Cirsium vulgare</i> (Savi) Ten.), hawksbeard (<i>Crepis</i> sp.), Mexican daisy (<i>Erigeron karvinskianus</i> DC.), common fleabeane (<i>Pulicaria dysenterica</i> (L.) Bernh.), dandelion (<i>Taraxacum officinale</i> G. Weber.), buttonweed (<i>Cotula</i> spp.)	(EPPO, 2006; Eyles, 1965; Eyles & Ashlock, 1969; He et al., 2002b, 2004; Myers, 1921; Myers, 1926; Syrett, 1993; Wang et al., 2008; Wei, 2001; Yang & Wang, 2004)

Family	Host plants	References
Polygonaceae	Red clover (<i>Rumex acetosella</i> L.), wireweed (<i>Polygonum aviculare</i> L.), lady's thumb (<i>P. maculosa</i> S.F. Gray)	(EPPO, 2006; Eyles & Ashlock, 1969; Farrell & Stufkens, 1993; He et al., 2002b, 2004; Wang et al., 2008; Wei, 2001; Yang & Wang, 2004)
Caryophyllaceae	Catch fly (<i>Silene gallica</i> L.), chickweed (<i>Stellaria media</i> (L.) Vill.), red sand spurry (<i>Spergularia rubra</i> (L.) J. Presl & C. Presl.	(EPPO, 2006; Eyles & Ashlock, 1969; Myers, 1921; Myers, 1926)
Rosaceae	Strawberry (<i>Fragaria</i> spp.), raspberry (<i>Rubus</i> spp.), apple (<i>Malus</i> spp.)	(EPPO, 2006; Gurr, 1952, 1957; Wei, 2001)
Geraniaceae	Common storksbill (<i>Erodium cicutarium</i> (L.) L'Her.), cranesbill (<i>Geranium</i> sp.)	(Gurr, 1952; Wei, 2001)
Families	Flax (<i>Linum</i> spp: Linaceae), curnow's curse (<i>Calandrinia caulescens</i> Phil: Montiaceae), lamb's quarters (<i>Chenopodium album</i> L: Amaranthaceae), flowering kanuka (<i>Kunzea</i> <i>ericoides</i> (A. Rich) Joy Thomps: Myrtaceae), moss (<i>Triquetrella papillata</i> (Hook.f. & Wilson) Broth: Pottiaceae), red pimpernel (<i>Anagallis</i> <i>arvensis</i> L: Primulaceae), pine (<i>Pinus radiata</i> D. Don: Pinaceae), kapuka (<i>Griselinia littoralis</i> Raoul: Griseliniaceae), moss (<i>Gleichenia</i> <i>circinata</i> Swartz: Gleicheniaceae), pimelea (<i>Pimelea arenaria</i> A. Cunn: Thymelaeaceae), viper's bugloss (<i>Echium vulagre</i> L: raginaceae), mallow (<i>Malva</i> spp: Malvaceae), flannel leaf (<i>Verbascum thapsus</i> L: Scrophulariaceae), kiwifruit (<i>Actinidia</i> sp: Actinidiaceae), Monterey cypress (<i>Cupressus macrocarpa</i> Gordon: Cupressaceae), sleeping beauty (<i>Oxalis</i> <i>corniculata</i> L: Oxalidaceae), horokaka (<i>Disphyma</i> spp: Aizoaceae)	(EPPO, 2006; Eyles, 1965; Eyles & Ashlock, 1969; Farrell & Stufkens, 1993; Gurr, 1952, 1957; Myers, 1921; Myers, 1926; Wei, 2001)

1.6.1 Biology and ecology

Various aspects of the biology and ecology of *N. huttoni* have been studied by many authors (Birtles, Waddell, Maindonald, & Popay, 1992; Bonte et al., 2010; Eyles, 1963; Farrell & Stufkens, 1993; He & Wang, 2000; He et al., 2002b; Wei, 2008b, 2010). The bug has three life stages: egg, nymph and adult (Capinera, 2001; Wei, 2001) (Figs 1.2 and 1.3). *Nysius* normally lays its eggs in soil, particularly in groups, on host plant parts and on container walls in a laboratory study (Farrell & Stufkens, 1993; He & Wang, 2000). The egg is slightly concave ventrally, convex dorsally and laterally. A single female can lay 22 eggs per day at 35 °c and produce about 484 eggs in a total oviposition period of 70 to 90 days (Eyles, 1960a). Eggs are laid in groups or singly. Eggs when first

laid are 'creamy white' but turn deep orange at hatching. Eggs are 0.77 mm long and 0.28 mm wide; their duration ranges from 6 to12 days (Eyles, 1960 a).

Lygaeidae species usually have five nymph instars (Eyles, 1963; Eyles, 1965; Krinsky, 2002), but *N*. *huttoni* has 4-6 nymph instars (He et al., 2003; Wei, 2010) or 2-4 instars as suggested by Wei (2001). High temperature and long day length cause frequent five or six instars, and low temperature and short day length result in four instars (Wei, 2010). Duration ranges from 20 to 21 days for nymphs and 70-90 days for adults (Wei, 2008b). The small nymphs are dark orange but later instars are grey or brownish-grey with a reddish brown abdomen (Fig. 1.2). The average body lengths of first, second, third, fourth and fifth instars are 0.84 mm, 1.23 mm, 1.5 mm, 2.05 mm and 2.52 mm, respectively (Eyles 1960a). Higher mortality of *N. huttoni* has been observed in early instars than in the late instars and at lower temperatures (< 15 °c), higher temperatures (> 30 °c) and in a short photoperiod (8 h photoperiod) (Wei, 2001).

Nysius huttoni adults are small to medium insects (2.4 - 4.5 mm long). The bugs are highly mobile during summer, are polyphagous, lay eggs in the soil, can migrate to overwintering sites during the cold season; breeding takes place in open fallow land (Farrell & Stufkens, 1993). This bug mostly lives on the ground and feeds on dropping seeds and stems. It likes hot, dry environments with sparse vegetation where sunlight directly falls on the ground (Eyles, 1960b; Gurr, 1952, 1957). Crop field margins are more prone to infestation by *Nysius* than the inner parts of fields (Capinera, 2001).

Under Canterbury conditions, the bugs generally overwinter in mid-April to early May and emerge from overwintering sites from late August to early September (Wei, 2008b). In laboratory studies, an equal sex ratio has been reported (Wei, 2008a). However, low temperatures and short days influence a greater proportion of males; high temperatures and long days influence a greater proportion of females (Wei, 2008a). Reproductive diapause of *N. huttoni* has been reported (Farrell & Stufkens, 1993). Diapause is generally induced in the second generation when the third and fourth nymph stages of *N. huttoni* are transferred to short day length (12L: 12D) whereas long day length (16L: 8D) breaks diapause (Farrell & Stufkens, 1993). Similarly, when fifth instar nymphs are transferred from 16L: 8D photoperiod to 10L: 14D and 12L: 12D photoperiods, all females enter reproductive diapause (He et al., 2002a). In general, reproductive diapause occurs in *N. huttoni* for 30 days after oviposition (Farrell & Stufkens, 1993).

Nysius huttoni adults have various body forms on the basis of wing development: macropterous (M), sub-brachypterous (Sb) and brachypterous (B), called wing polymorphism (Eyles, 1960a). Larger individuals have only macropterous forms (wings longer than the abdomen), whereas the

13

medium and smaller individuals show the three different forms. In field conditions, macropterous forms predominate (94.1 %). It has been suggested that M (male) x M (female) is the predominant mating combination (80.9 %), with M (male) x Sb (female) the second combination (13.7 %) (Wei, 2010). Low (< 15 °c) and high (> 30 °c) temperatures, and short photoperiod (8 h photoperiod) lead to sub-brachypterous and brachypterous wing development. Temperatures ranging from 20 to 30 °c, and a long photoperiod (12 - 16 h photoperiod) produces macropterous forms (Eyles, 1960a).

For larger individuals (macropterous), male and female lengths range from 3.55 - 3.86 mm and 3.74 - 4.34 mm, respectively; male and female widths range from 1.32 - 1.39 mm and 1.61 - 1.75 mm, respectively. Medium size males and females lengths range from 3.00 - 3.48 mm and 3.36 - 3.74 mm, respectively and widths range from 1.15 - 1.32 mm and 1.44 - 1.53 mm, respectively. In the smallest size category, males and females lengths range from 2.38 - 3.00 mm and 2.47 - 3.19 mm, respectively, and male and female widths range from 0.94 - 1.15 mm and 1.20 - 1.32 mm, respectively (Eyles, 1960a).

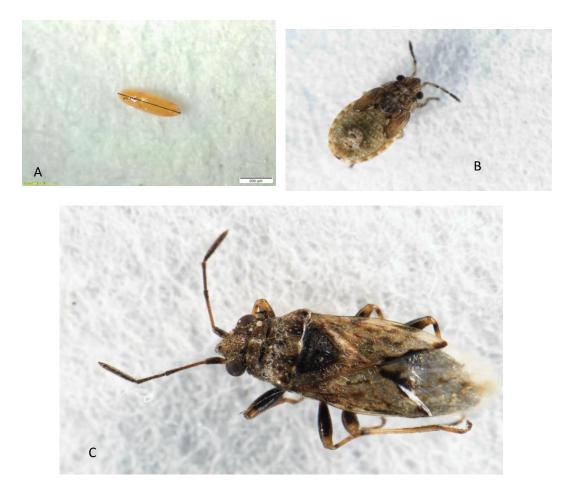


Figure 1.3 Life stages of Nysius huttoni a) Egg; b) Nymph; c) Adult. Photo: Sundar Tiwari

Macropterous and sub-brachypterous *N. huttoni* can fly when the air temperature reaches 27 °c and the ground temperature reaches 40 °c; brachypterous individuals are flightless (Wei, 2014).

Males generally prefer females with a thicker abdomen, more mature eggs and longer ovipositors (Yang & Wang, 2004) whereas females favour males with longer antennae and a larger genital structure (Yang & Wang, 2004). There are conflicting views on the number of generations per year: 1 per year (Myers, 1926); 2 per year (Every et al., 1992; Gurr, 1952); and 3 - 4 per year (Eyles, 1960a, 1963, 1965; Wei, 2001).

1.6.2 Damage potential of Nysius huttoni

The first New Zealand damage record of the *N. huttoni* was on a wheat crop in 1936 (Morrison, 1938). Two major *N. huttoni* outbreaks were recorded in between 1961 - 1986 (Swallow & Cressey, 1987). The worst outbreak was in 1970; it led to the loss of up to 10,000 tonnes of wheat (Swallow & Cressey, 1987). *Nysius huttoni* damages wheat grains during the milk-ripe stage by piercing through the glumes into the developing grains, which reduces the gluten protein so reducing baking quality (Cressey et al., 1987; Every et al., 1992; Every et al., 1998). During feeding, bugs inject toxic saliva that contains a potent enzyme responsible for the quality deterioration of bread (Lorenz & Meredith, 1988). Damaged wheat grains have a distinct feeding mark: a pale circular area with a dark puncture mark at the centre (Gurr, 1957; Miller & Pike, 2002). Injury to wheat varieties ranges from 10 to 100 % (Every, Farrell, & Stufkens, 1989); in mature kernels, injury ranges from 84 to 99 % (Every et al., 1990). Even a negligible infestation on wheat is enough to reduce the market and baking quality.



Figure 1.4 Nysius huttoni damage symptoms in the kale seedlings (*Brassica oleracea*) in a laboratory experiment at the Bio-Protection Research Centre (BPRC), Lincoln University, New Zealand. Photo: Sundar Tiwari

Brassica seedlings are readily attacked by this pest (Eyles, 1965). Both adults and nymphs *N. huttoni* damage brassica seedlings (Fig. 1.4); the damage potentially ranges up to 70 - 90 % (AgPest, 2016; Speciality Seeds, 2016). Damaged seedlings have feeding punctures at the base, resulting in cankerous tissue growth or ring barking (Eyles, 1965). This interferes with sap flow and either causes total loss of the plant or makes plants susceptible to breakage by wind or stock movement (He & Wang, 1999). Infestations in germinating seedlings can lead to plant death. He & Wang (1999) reported that the highest percentage of damage to swede seedlings was during a windy period.

1.7 Pest management practices in forage brassicas

In New Zealand, conventional pesticides are common for pest and disease management in forage brassicas (Trevor, 2010). Between 1999 and 2003, the use of pesticides in New Zealand increased by 27 %, with the sale of herbicides, insecticides and fungicides increasing by 25, 28 and 29 %, respectively (Manktelow et al., 2005). The highest pesticide consumption in New Zealand was recorded in the horticultural sector (13.2 kg a.i./ha) followed by the arable (2.4 kg a.i./ha), forestry (0.3 kg a.i./ha) and pastoral sectors (0.2 kg a.i./ha) (Manktelow et al., 2005). Seed dressing with imidacloprid (a neonicotinoid insecticide) and post-emergence insecticide sprays with chlorpyrifos and permethrin are used to control *N. huttoni* in forage brassicas (Goldson et al., 2015; Trevor, 2010; Young, 2018). These synthetic chemicals have been used to attract, reduce or kill the pest. However, these conventional synthetic pesticides have non-target effects on human health, the environment, and biodiversity. The registered insecticides to manage *N. huttoni* in forage brassicas in New Zealand are listed and described in Table 1.5.

Early scouting and field monitoring have been suggested before taking any pest management decisions (AgPest, 2016). However, preventive measures such as the use of less susceptible cultivars, clearing of weed hosts from fields and using potential trap crops are other important pest management options (Tiwari, Dickinson, Saville, & Wratten, 2018; Tiwari, Saville, & Wratten, 2019).

Chemical group	Active ingredient(s) (a.i.)	Products	a.i. per hectare	Dose / ha	Time of application	Withho Iding period (days)
Chloronicontinyl	600 g/litre imidacloprid	Acclaim	12-24 ml/kg of seed	12-24 ml/kg of seed	Seed treatment	42
Synthetic pyrethroid and organophosphate	475 g/litre pirimiphos - methyl and 25 g/litre permethrin	Ambush	750- 1000 ml/ha	100-300 litre water/ha	When damage becomes evident	7
Organophosphate	475g/litre pirimiphos-methyl and 25 g/litre permethrin	Attack	500- 1000 ml/ha	100-300 l water/ha	Apply when insect first appears and then at spray 2- 3 weeks intervals or as necessary	7
Organophosphate and synthetic pyrethroid	300 g/kg chlorpyrifos and 15.4 g/litre lambdacyhalothrin	Cobalt Advanced	667 ml/ha	500-litre water/ha	When damage becomes evident	14
Lambda-cyhalothrin and chlorantraniliprole	50 g/litre lambda- cyhalothrin and 100 g/litre chlorantraniliprole	Ampligo	100 ml/ha	200-litre water/ha	Apply when insect first appears and then every 2-3 weeks or as necessary	14
Organophosphate	Chlorpyrifos 50 EC	Chlorpyrif os EC	1.2 litre/ha	50-200 litre water	Apply to seedlings as soon as damage is evident or when <i>N.</i> <i>huttoni</i> nymphs found feeding on the plants	7
Organophosphate	200 g/kg terbufos	Counter 20G	3 kg/ha	3 kg/ha	In furrow treatment	42
Neonicotinoid	600 g/litre imidacloprid	Nuprid 600 ST	12 - 24 ml/kg seed	12-24 ml/kg seed	Seed treatment	42
Pyridine azomethine and anthranilic diamide	500 g/kg pymetrozine and 100 g/kg cyantraniliprole	Minecto Star	150 g/ha	200-litre water/ha	When the first sign of damage occur	28

Table 1.5 The insecticides registered for chemical control of Nysius huttoni in New Zealand

Chemical group	Active ingredient(s) (a.i.)	Products	a.i. per hectare	Dose / ha	Time of application	Withho Iding period (days)
Organophosphate	200 g/kg phorate	Phorate	5.0 kg/ha	5.0 kg/ha	Apply in furrow at sowing	42
Neonicotinod	600 g/litre imidacloprid	Pronto	12-24 ml/kg of seed	12-24 ml/kg of seed	Seed treatment	42
Synthetic pyrethroid and organophosphate	475 g/l pirimiphos- methyl and 25 g/litre permethrin	Ambush	1 litre/ha	700-litre water/ha	When damage becomes evident	3
Synthetic pyrethroid and organophosphate	475 g/litre pirimiphos-methyl and 25 g/litre permethrin	Attack	1 litre/ha	700-litre water/ha	Apply when insect first appears and then spray 2-3 week intervals or as necessary	3

Source: Young (2018)

1.8 Rationale for the study

The use of chemical pesticides is not sustainable because it increases insect resistance, eliminates beneficial arthropods and promotes ecosystem dis-services. The practice has been realized as a major cause of biodiversity loss and pollinator decline (Brittain, Vighi, Bommarco, Settele, & Potts, 2010). It also causes various negative effects such as secondary pest outbreaks and the emergence of new pest problems that further increase production costs (Dhaliwal et al., 2010; Karuppuchamy & Venugopal, 2016; Lou et al., 2013) and generates detrimental effects on biocontrol agents (Metcalf, 1994). The intensive use of pesticides and a high level of disturbance, including intensive crop production, simplify the landscape and results in a decline in biodiversity and ES (Lu et al., 2014). Hence, investigating a sustainable pest management approach to avoid environmental, economic and social problems of pesticides is necessary. In New Zealand, the dairy and meat industries provide a significant contribution to the national economy. Hence, the 'sustainability' concept has to be adopted in livestock feed production systems for long-term marketing. Currently, there is no relevant scientific information or IPM protocol for sustainable management of *N. huttoni* in forage brassicas.

This situation highlights the need to investigate alternative pest management strategies to reduce pest pressure and pesticide use in forage brassicas. An agro-ecological pest management approach or habitat management using a trap crop could be options (see Chapters 2 and 5). Available knowledge on the best suitable trap plants that can be used to attract (trap) *N. huttoni* from an area where the *N. huttoni* is a problem. Deployment of such a trap plant in brassica fields could

keep the pest away from the main crop. The strategy to achieve this objective is host plant selection by *N. huttoni* of a range of potential trap plant species in laboratory choice, no-choice and paired-choice tests (Chapter 2). Host plants were ranked based on the preference of *N. huttoni*under all test conditions. Alyssum, *Lobularia maritima* (L.) Desv., has been assessed as a potential trap plant for *N. huttoni*. However, that laboratory bioassay was done only with seedlings. Following that study, an experiment was hypothesised to see if there was a differential preference between two growth stages (flowering and vegetative) of the potential trap plant such as alyssum (*L. maritima*) by *N. huttoni*. That study can provide important information about how long alyssum can be left in the main field. The answer is given in Chapter 4. This information is useful from the perspectives of CBC and ES. In field-cage and open-field experiments, the most preferred plant(s) alyssum or wheat or alyssum plus wheat were compared with the kale (*B. oleracea*) to evaluate their efficacy in reducing *N. huttoni*populations and lowering the potential damage in the main kale crop (Chapter 5).

The second option is to use a kale cultivar less susceptible to *N. huttoni* (Chapter 3). Choice and nochoice tests were performed to select potential cultivars from the range of kale cultivars (Table 1.2). The use of a trap plant in brassica fields along with the use of less susceptible kale cultivars and encouraging farmers to integrate these with other IPM strategies such as biological, mechanical control and the use of 'soft' chemicals, can reduce pest pressure and pesticide use (Horrocks et al., 2018). These agro-ecological approaches to IPM can be used to develop a pest management protocol and used to manage other similar pests.

The purpose of using a flowering trap plant is not only to trap the *N. huttoni* but also, indirectly, to supply ES to pest natural enemies (NEs) by providing a better place in adverse conditions (shelter), food for natural enemies (nectar), alternative prey or host (alternative food) and pollen (SNAP) (Landis et al., 2000). For example, *Phacelia tanacetifolia* Bentham produces large quantities of pollen and nectar that contribute to an increased number of syrphids and lowers aphid populations in cabbages (White, Wratten, Berry, & Weigmann, 1995). In this study, alyssum has been assessed as a suitable trap plant for *N. huttoni* (Tiwari et al., 2018) (Chapters 2 and 5) and has been tested in CBC work on brassica pests in Nepal (Chapter 6). Alyssum is a well-known candidate plant to enhance CBC of insect pests by providing SNAP (Amorós-Jiménez, Pineda & Marcos-García, 2014; Barbir, Badenes-Pérez, Fernández-Quintanilla, & Dorado, 2015; Gillespie, Wratten, Sedcole, & Colfer, 2011; Laubertie, Wratten, & Hemptinne, 2012; Pineda & Marcos-García, 2008). Dissemination of such pest management ideas to the farmers of a developing country is necessary for wider use in sustainable pest management.

1.9 General objective

This PhD study aims to develop an ecologically-based integrated management strategy for the control of *N. huttoni* and other pests in brassica fields based on understanding of habitat manipulation

1.9.1 Specific objectives and hypotheses

The specific objectives of this study are to:

- 1. identify a potential host plant based on host selection by N. huttoni in a laboratory test;
- 2. identify susceptible kale cultivars based on host selection by N. huttoni;
- 3. identify the suitable growth stage of alyssum based on host selection by *N. huttoni;*
- 4. assess potential trap plants based on host selection by *N. huttoni* in field cages and open field conditions; and
- 5. investigate the effect of alyssum flowers on arthropod diversity and radish pest control.

The hypotheses tested under each objective were to:

1. Identify a potential host plant based on host selection by *N. huttoni* in a laboratory test.

Hypothesis 1: H₀ = All potential trap plants are equally preferred by *N. huttoni*.

2. Identify susceptible kale cultivars based on host selection by *N. huttoni*.

Hypothesis 1: H₀ = Kale cultivars are equally susceptible to bug damage.

3. Identify the suitable growth stages of alyssum based on the host selection by *N. huttoni*.

Hypothesis 1: H₀: Alyssum growth stages are equally preferred by *N. huttoni*.

4. Assess potential trap plants for *Nysius* based on host selection in field cages and open-field conditions.

Hypothesis 1: H_0 = There are no differences in the host plant preference by *N. huttoni* in field cages.

Hypothesis 2: H_0 = All potential trap plants are equally preferred by *N. huttoni* in an open brassica field.

5. Know the effect of alyssum flowers on arthropod density and radish pest control.

Hypothesis 1: H_0 = Alyssum flowers in radishes do not affect the arthropod populations.

Hypothesis 2: H_0 = Proximity to alyssum strips does not affect seven-spotted ladybird, syrphid and aphid populations.

Hypothesis 3: H_0 = Seven-spotted ladybird, syrphid and aphid populations are not influenced by alyssum strips in a radish field

1.10 Thesis structure

An outline of the thesis is presented in Table 1.6.

Title/Chapter	Purpose
Title page	The thesis title, author, degree submitted, date and university name
Abstract	A summary of the activities and key findings
Acknowledgments	Acknowledging the key contributors in this PhD journey
Contents	Thesis contents with page numbers
Tables	A list of tables with page numbers
Figures	A list of figures with page numbers
Chapter 1:	This chapter backgrounds the study. The human growth rate, the role of
Introduction	agriculture intensification and its consequences on the environment,
	biodiversity, ecosystem functions and habitat pest management are
	discussed. Sustainable agriculture and agro-ecological pest management
	are considered solutions for agricultural modernization and revitalizing
	ecosystem functions. The endemic New Zealand N. huttoni, a pest of
	brassica seedlings, is taken as a study case and possible agro-ecological
	and other integrated approaches to pest management such as trap
	cropping and suitable kale cultivars are considered as alternatives to
	chemical pesticides. Alyssum, a potential trap plant for N. huttoni, was
	also used to improve the conservation biological control of radish pests.
	The specific objectives and hypotheses to achieve the study's broad
	objectives for an ecologically-based IPM strategy are presented

Table 1.6 A detailed chapter by chapter outline of the thesis

Title/Chapter	Purpose
Chapters 2-6:	
Research sections	Chapters 2 to 6 have the structure: abstract, introduction, materials and
	methods, results, discussion and conclusions including references.
	Abstract: This summarises the research background, methodology,
	results and conclusion (s).
	Introduction: This presents the background to the study, reviews
	previous studies relevant to the topic and states the objectives and
	hypotheses tested.
	Materials and methods: This describes the detailed procedures followed
	to complete the study including data collection and statistical analysis.
	Results: This presents the study findings based on the hypotheses set.
	Discussion: The findings are discussed with the supporting literature and
	end with concluding remarks.
	The chapter topics are :
	Chapter 2: Host plant selection by the wheat bug, <i>N. huttoni</i> (Hemiptera: Lygaeidae) on a range of potential trap plant species.
	Chapter 3: Susceptibility of kale cultivars to the wheat bug, <i>N. huttoni</i> (Hemiptera: Lygaeidae) in New Zealand.
	Chapter 4: Preferences of the wheat bug (<i>N. huttoni</i>) for particular growth stages of the potential trap plant, alyssum (<i>L. maritima</i>).
	Chapter 5: Evaluation of potential trap plant species for the wheat bug, <i>N. huttoni</i> (Hemiptera: Lyageidae) in forage brassicas.
	Chapter 6: Alyssum flowers promote arthropod diversity and biological control of radish pests.
Chapter 7: Overall	This chapter broadly discusses the experiments, their key findings
discussion and	including implications. It also provides recommendations and
conclusions	suggestions.
References	The list of cited sources from which supporting relevant information and
	findings have been drawn.

Chapter 2

Host plant selection by the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) on a range of potential trap plant species

A version of this chapter was published on 27 February 2018: Tiwari, S., Dickinson, N., Saville, D. J., & Wratten, S. D. (2018). Host plant selection by the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) on a range of potential trap plant species. *Journal of Economic Entomology*, 111 (2), 586-594. https://doi: 10.1093/jee/toy017.

2.1 Abstract

Wheat bug, *Nysius huttoni*, is a native sap-sucking insect pest of wheat, other cultivated cereals, brassicas, and many weed species in New Zealand. Both adults and nymphs can cause damage to cultivated crops. Forage brassicas seedlings are highly susceptible to direct feeding damage by this insect, which can reduce plant establishment. In mature crucifers, spectacular damage symptoms are feeding punctures around the base of the stems, cankerous growth of the tissues, distortion of leaves, withered upper portions of the leaf and twisted petioles. On wheat, damage can be visually observed at the milky-ripe stage. 'Bug-damaged wheat' contains a salivary proteinase that significantly affects bread quality. Several insecticides and formulations are registered to manage *N. huttoni.* Seed dressing with imidacloprid, and chlorpyrifos and permethrin spray are the usual pesticide management practices in New Zealand. However, these practices have been linked to environmental pollution, biodiversity loss, and pollinator population declines in brassicas and other crops. Habitat management of the bug utilizing potential trap crops can be a better option for its management.

A series of choice, no-choice, and paired choice tests were conducted in a controlled-temperature room to evaluate the pest's preferences on seedlings of eight plant species. Seven non-kale plants such as *Lobularia maritima* (alyssum), *Triticum aestivum* (wheat), *Phacelia tanacetifolia* (phacelia), *Fagopyrum esculentum* (buckwheat), *Coriandrum sativum* (coriander), *Trifolium repens* (white clover), and *Medicago sativa* (lucerne) were used as potential non-kale trap plant species, all of which were compared with kale (*Brassica oleracea*), a potentially susceptible control. In choice tests, wheat was the most suitable followed by alyssum, buckwheat, and phacelia, all significantly more favoured than kale. In no-choice tests, alyssum was significantly more favoured than kale and the other plant species except wheat and phacelia. First feeding damage was recorded on alyssum

in both the above test conditions. For paired-choice tests including kale, wheat, and alyssum were significantly more suitable than other trap crop species.

These findings are important for developing agro-ecological management strategies. Alyssum followed by wheat were the most suitable trap plant species for *N. huttoni*. These two plant species can be deployed in and around brassica fields either independently or as in a multiple trapcropping systems to reduce bug damage, minimizing or avoiding pesticides, and delivering a range of ecosystem services.

Keywords: Wheat bug, Nysius huttoni, trap planting, kale, wheat, alyssum

2.2 Introduction

Nysius huttoni White (Hemiptera: Lygaeidae) is a widely distributed endemic New Zealand insect species (Aukema et al., 2005; Eyles, 1960b; He et al., 2003). It has also been reported in some European countries such as The Netherlands and Belgium (Aukema et al., 2005; Bonte et al., 2010). Nysius huttoni is commonly found in weedy fields, gardens, lawns, the bare ground between rows of fruit trees, sandy riverbeds, ornamental gardens, pasture lands, wastelands, sandy ground supporting a few weeds, and roadsides (Wei, 2001). This insect is considered a major pest for several reasons. It feeds on a wide range of plant species, > 75 plant species belonging to > 25 plant families. However, it strongly prefers forage brassicas, wheat and many other cereals, ornamentals, fruits, vegetables, and weeds (Bejakovich et al., 1998; Eden, Gerard, Wilson, & Bell, 2010; Eyles, 1965; He et al., 2003; Miller & Pike, 2002; USDA, 1962; Yang & Wang, 2004). The management of *N. huttoni* is difficult because of its polyphagous nature (feeding on a wide range of annual weeds and crops), its active dispersal, and its diapause behaviour (Farrell & Stufkens, 1993). Nysius huttoni is a piercing, sucking plant feeder. Adults and nymphs pierce plant parts with their pointed stylet and inject a salivary toxin during feeding (He & Wang, 1999). The plant population can be reduced as a result of feeding damage at the base of brassica seedlings, causing a cankerous growth of tissue and making them susceptible to breakage from the wind, which may finally kill the young plants (AgPest, 2016). The damage potential on crucifers in a severe situation ranges from 70 - 90 % (AgPest, 2016). The use of insecticides, particularly seed dressings with the imidacloprid is the current practice for N. huttoni management in forage brassica crops in New Zealand (PGG, 2009). These pesticides are broad-spectrum, have a long half-life in the soil, and are disruptive to ecosystem (nature's) services in agroecosystems (Holland & Rahman, 1999), including severe effects on potential natural enemies of pests and pollinators (Brittain et al., 2010; Goulson, Lye, & Darvill, 2008). Alternative management methods for N. huttoni will be important for the sustainable production of forage brassicas but such approaches have not been developed to date.

An understanding of the host preferences of *N. huttoni* adults may allow for the development of nonchemical insect management such as trap cropping, which can be important integrated pest management (IPM) tool. Alyssum, *Lobularia maritima* L. Desv., wheat, *Triticum aestivum* L., phacelia, *Phacelia tanacetifolia* Bentham, buckwheat, *Fagopyrum esculentum* Moench, coriander, *Coriandrum sativum* L., white clover, *Trifolium repens* L., and lucerne, *Medicago sativa* L., appear to be potential trap crops for *N. huttoni* (CABI, 2011; Eyles, 1960b; He et al., 2003; Yang & Wang, 2004). Flowering or nonflowering trap crops can potentially provide multiple ecosystem services (ES) in an agroecosystem, such as providing one or more of the components of SNAP (shelter, nectar, alternative hosts, and pollen) to enhance the 'fitness' of the pest's natural enemies and pollinators (Gurr et al., 2017). Host selection behaviour in phytophagous bugs has been examined for a range of species, including the cotton seed bug, *Oxycarenus hyalinipennis* Costa (Dimetry, 1971), the large milkweed bug, *Oncopeltus fasciatus* Dallas (Sweet, 1960), other tropical milkweed bugs, *Oncopeltus* spp. (Root & Chaplin, 1976), the chinch bug, *Blissus occiduus* Barber (Eickhoff, Baxendale, Heng-Moss, & Blankenship, 2004), and *Nysius vinitor* Bergroth (McDonald & Smith, 1988). However, this bug's host plant preferences are unknown.

The main focus of this study was to investigate the host selection behaviour of *N. huttoni* on potential trap plant species in the laboratory at seedling stages. Insects are attracted to, or recognize plants because of the latter's chemical (olfactory/gustatory and or physical (tactile/visual) stimuli (Badenes-Perez, Shelton, & Nault, 2005; Karuppuchamy & Venugopal, 2016). Here, the screening of a range of non-crop plant species may identify and rank their potential as trap plants for this pest in kale field and deliver other ES to the agroecosystem. For example, the production of pollen and nectar by phacelia can contribute to the fitness and efficacy of hover flies (Diptera: Syrphidae) and this can lead to lower aphid populations in cabbages (White et al., 1995). Hence, the current work aims to identify the potential trap plant species of the *N. huttoni*; those identified may offer other ES as well, although that was not part of this work.

2.3 Materials and methods

Plant preparation and *N. huttoni* colony management experiments were conducted in a controlled temperature room at the Bio-Protection Research Centre (BPRC) at Lincoln University, New Zealand (https://www. bioprotection.org.nz). Kale was used as a potentially susceptible host for *N. huttoni* (Eyles, 1965; He et al., 2003), and seven other non-kale plant species (Table 2.1) were chosen for evaluation as potential trap plants (CABI, 2011; Eyles, 1960b; Wei, 2001).

Table 2.1 Host plant species used in Nysius huttoni choice tests

Host species	Common name	Family	Cultivar
Coriandrum sativum	Coriander	Apiaceae	Santo
Phacelia tanacetifolia	Phacelia	Boraginaceae	Lacy
Brassica oleracea	Kale	Brassicaceae	Kestrel KE35TC
Lobularia maritima	Alyssum	Brassicaceae	Benthamii White
Trifolium repens	White clover	Fabaceae	Nomad NMD01AC
Medicago sativa	Lucerne	Fabaceae	Kaituna FS6147
Triticum aestivum	Wheat	Poaceae	Morph
Fagopyrum esculentum	Buckwheat	Polygonaceae	Katowase

2.3.1 Nursery management

The plants were maintained in a glasshouse with a temperature range of 18 0 c (night) to 30 0 c (day) and 40 % relative humidity (RH). The faster germinating plant species (kale, alyssum, wheat, coriander, and buckwheat) and the slower ones (lucerne, clover, and phacelia) were planted 5 and 8 d before the start of the experiment, respectively. Eleven-day-old seedlings of kale, alyssum, wheat, buckwheat, and coriander and the others (lucerne, clover, and phacelia) at 14 d were used for all experiments. Two seedlings of each plant species for choice and paired-choice and one seedling for the no-choice tests were grown in potting mix (400-litre composted bark, 100-litre pumice [1.0 - 7.0 mm], 1500 g Osmocote [3 - to 4 - month release], 500 g horticultural lime, 500 g Hydraflo) in a glasshouse. The sizes (diameter and depth) of pots used in no-choice and paired-choice tests were eapproximately the same size and height during bioassays. The seedlings grown in the glasshouse were transferred to a controlled temperature (CT) room (temperature 22 0 C, photoperiod 16L: 8D h, and 60 ± 10 % RH). The light intensity in the room was maintained by using 22 discharge lamps (Philips TL - D 30W/865, 77 Im/W).

2.3.2 Laboratory culture of Nysius huttoni

The bugs were collected by using a suction machine (Shred n Vac Plus, Stihl BG 75, USA, 80.0 cm length × 12.0 cm inlet diameter) on shepherd's purse, *Capsella bursa-pastoris* (L.) Medic.), at Lincoln University. Laboratory colonies were maintained in circular Petri dishes (13.5 cm diameter) with 100 bugs per dish with slightly moistened filter paper in the base of the dishes. Twin cress, *Coronopus didymus* (L.) Smith. (Brassicaceae) and hulled organic sunflower seed (*Helianthus annuus* L. cv. Golden Toasted) were used as the bugs' food materials, which were replaced daily.

The ambient temperature, humidity, and photoperiod of the culture room were as previously mentioned (Farrell & Stufkens, 1993; He & Wang, 1999; He et al., 2002a; He et al., 2003; Wang et

al., 2008). *Nysius huttoni* adults were obtained from the laboratory culture and maintained with various cohorts of the same age and used in bioassays.

2.3.3 Laboratory bioassay experiments

Twenty-five newly-emerged *N. huttoni* adults (13 males and 12 females) for each choice test and 10 (five males and five females) for each no-choice and paired-choice test were released in the centre of a plant pot (see the previous section) which was covered by a cylindrical sleeve. The sleeve was made of flexible transparent PVC sheet (1 mm) and was used above for choice tests. The dimension of each sleeve was 23.5 cm diameter × 14 cm depth for choice test and 7 cm diameter × 12 cm depth for no-choice and paired-choice tests, respectively. The tops of the sleeves were tightly covered by fine white mesh and Fluon (BioQuip, fluoropolymer resin, PTFE-30) was used on the inner surface as a *Nysius* barrier. All *N. huttoni* were starved for 12 h before the release into the study arena. The study comprised a randomized block design, with 10 replicates for choice and no-choice and six replicates for paired-choice tests. Similar choice designs to those used here were involved in host preference work on the bagrada bug, *Bagrada hilaris* Burmeister (Hemiptera: Pentatomidae) (Huang, Reed, Perring, & Palumbo, 2014), the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Badenes-Perez et al., 2004), the African stem borer, *Busseloa fusca* Fuller (Lepidoptera: Noctuidae) (Khan, Midega, Wadhams, Pickett, & Mumuni, 2007) , and the seed bug, *Nysius natalensis* Evans (Hemiptera: Orsillidae) (du Plessis, Byrne, & van den Berg, 2012).

2.3.4 Choice tests

Two seedlings of each of eight plant species were grown together in pairs, spacing 2.8 cm apart within each pair was mainted based on the size of choice pots, with the eight pairs arranged in a circular fashion (on a circle of radius 6.5 cm) in 23 cm-diameter pots - see the previous section. Each of the 10 blocks comprised two of these pots, each with a cylinder (see the previous section), one with *Nysius* (Treatment) and one without (Control). The ordering of eight plant species was independently randomized within each pot, as was ordering of the pots (Treatment and Control) within each of the 10 blocks (Fig. 2.1).

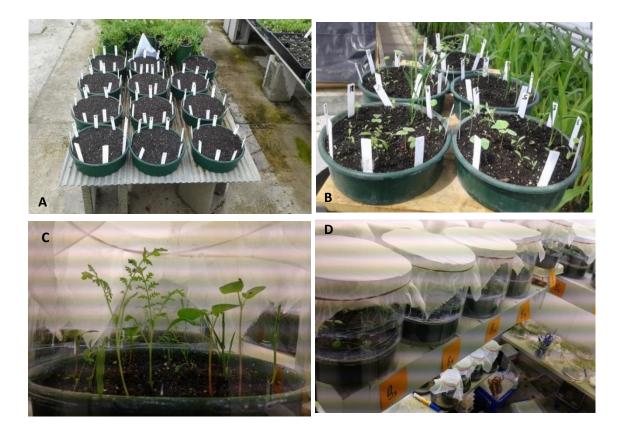


Figure 2.1 Choice experiments maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (BPRC), Lincoln University. a) Potential trap plants grown in choice pots in a glasshouse; b) Trap plants in choice pots in a glasshouse; c) Plant species in a choice pot; d) Choice test experiment arranged in a randomized block design in the CT room.

2.3.5 No-choice tests

In these tests, eight cylinders as above with 10 newly emerged (five males and five females) *N. huttoni* adults (Treatment) and eight without (Control) for eight plant species were used in one block with one seedling/cylinder and a total of 10 replicates (Fig. 2.2). The treatments were randomized in each block, and the blocks were replicated three times and set up on each of three dates (October 14, October 29, and November 3, 2016).

2.3.6 Paired-choice tests with kale

For the paired-choice tests, two seedlings of kale were positioned in one half of each pot and two each of one of the other plant species were in the other half (i.e., kale vs kale, kale vs alyssum, and with buckwheat, lucerne, wheat, coriander, clover, and phacelia, respectively) (Fig. 2.3). Paired-choice tests involving two plant species are useful in ranking organisms' relative preferences from one to another (Raffa, Havill, & Nordheim, 2002). This is realistic as in all but the most intensive agriculture, monocultures may not be the norm.



Figure 2.2 No-choice experiments maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (BPRC), Lincoln University. a) No-choice pot, alyssum plant and *Nysius huttoni*; b) No-choice experiment arranged in a randomized block design in a CT room.



Figure 2.3 Paired-choice experiments maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (BPRC), Lincoln University. a) Plant preparation for the pair

choice-test; b) Kale and alyssum pairs grown in pots to use in the pair-choice tests; c) Buckwheat and kale grown in pots to use in the pair-choice tests.

2.3.7 Paired-choice tests with non-kale plant species

Based on the results of choice and no-choice experiments, the two most favoured plant species (alyssum and wheat) were selected and paired with other potential trap-plant species (i.e., alyssum vs alyssum, buckwheat, coriander, lucerne and wheat, respectively). Two other potentially favoured pairs (wheat vs buckwheat and buckwheat vs coriander) were also used and examined in paired-choice experiments, with six replications for each treatment. The latter two pairs were chosen on the basis of the bugs' second group of potential preference plant species (Tables 2.2 and 2.4; Figs 2.4, 2.6, 2.7, 2.8, 2.9 and 2.10).

The parameters recorded were: time to first settlement (mins.); number of bugs recorded on each plant species after the introduction of *N. huttoni* at different time intervals (0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h); time to first obvious feeding damage; survival rate at 120 h after the bugs' introduction, and % reduction in plant weight and height.

2.3.8 Statistical analysis

Data were analysed by using two-way (treatments and blocks) analysis of variance (ANOVA), using the GenStat statistical package (GenStat 16, VSN International, Hemel Hempstead, Hertfordshire HP1 1ES, United Kingdom), to test the effect of plant species on time to first settling (mins.), number of bugs recorded on each plant species, time to first obvious feeding damage, and survival rate at 120 h after bug introduction. To ensure an adequate level of normality and homogeneity of variance, variables were transformed as follows. Time to first settling and time to first obvious feeding damage were both logarithm transformed. The number of N. huttoni recorded on each plant species at different time intervals was square-root transformed before integration over time by the area under the curve (AUC) method (Hanley & McNeil, 1983), with the resulting AUC divided by the total time period to derive a (weighted) average over time. Survival rates were averaged over 10 or 25 N. huttoni, so approximately followed a normal distribution by the Central Limit Theorem (Wood & Saville, 2013). The percentage reduction in dry weight and height of plant species over the control plants (no N. huttoni) were calculated as (control – treated)/ control × 100, where 'treated' refers to the presence of N. huttoni. As a further check, the residuals from each ANOVA were inspected to check for normality and homogeneity of variance. Means of treatments were separated by the unprotected least significance difference (LSD) at p < 0.05 (Saville, 2015). For each non-kale pair, the mean number of bugs on each plant species was first calculated by the

AUC method (using V data) over 120 h and then tested for significance using a two-tailed paired samples *t*-test at p < 0.05.

2.4 Results

2.4.1 Choice tests

In the choice tests, *N. huttoni* settled significantly more promptly on wheat than on clover and buckwheat but was not significantly different to other plant species in settlement time. There were no significant differences between kale and other plant species. Overall, *Nysius* required approximately one-third of the time to first settlement on wheat ($\overline{x} = 5.98$ min) than on clover ($\overline{x} = 19.90$ min) (Table 2.2).

Table 2.2 Mean time (\log_{10} transformed) required for first settlement and first obvious feeding damage by *Nysius huttoni* adults on different plant species in choice tests. Backtransformed means are given in brackets. Means within the same column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 10).

Plant Species	Choice t	est
	Settlement time	Feeding damage
	(log ₁₀ minutes ± SEM ⁴)	(log ₁₀ hours ± SEM)
Kale cv Kestrel	1.035 abc ± 0.157 (10.83)	2.079 abc ± 0.000 (120)
Phacelia cv Lacy	1.035 abc ± 0.110 (10.83)	2.069 ab ± 0.009 (117)
Alyssum cv Benthamii White	0.975 abc ± 0.105 (9.44)	2.052 a ± 0.021 (113)
Buckwheat cv Katowase	1.161 bc ± 0.161 (14.48)	2.158 d ± 0.000 (144)
Clover cv Nomad	1.299 c ± 0.170 (19.90)	2.091 bc ± 0.021 (123)
Wheat cv Morph	0.777 a ± 0.054 (5.98)	2.079 abc ± 0.000 (120)
Lucerne cv Kaituna	1.053 abc ± 0.167 (11.29)	2.111 c ± 0.0120 (129)
Coriander cv Santo	0.915 ab ± 0.125 (8.22)	2.206 e ± 0.005 (161)
LSD (5 %)	0.371	0.034
SEM	0.131	0.012
Significance	*	***

(*) *p* < 0.05, significant; (***) *p* < 0.001, extremely significant.

The time to first feeding damage by *N. huttoni* varied significantly between the plant species (*p* < 0.001). The first feeding damage by *N. huttoni* occurred on alyssum (113 h) followed by phacelia (117 h), kale (120 h), and wheat (120 h), none of which were significantly different from each

⁴ Standard error or mean

other. However, feeding damage on alyssum was significantly earlier than on buckwheat, clover, lucerne, and coriander. Coriander required the highest time (161 h), and this was significantly later than on all other host plants. Finally, kale damage occurred significantly earlier than that for either buckwheat or coriander (Table 2.2).

The numbers of *Nysius* observed over 120 h varied significantly between plant species (p < 0.001) tests (Fig. 2.4 and Table 2.3). The number of individuals on wheat was significantly higher than on phacelia, lucerne, kale, coriander, and clover. Kale and lucerne were not significantly different from each other, but each was significantly higher than clover and coriander (Fig. 2.4).

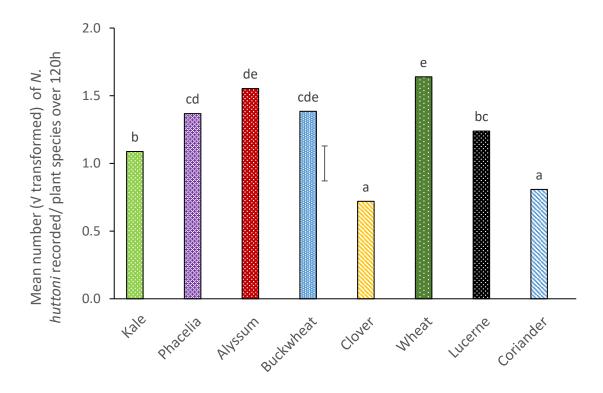


Figure 2.4 Choice tests. Mean numbers (V transformed) over 120 h of *Nysius huttoni* adults recorded on each of eight plant species. The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; *p* < 0.05).

Table 2.3 For the choice tests, the mean numbers (V transformed) of *Nysius huttoni* adults recorded on each of eight plant species at times 0.5 h to 120 h plus the overall AUC mean.

Plant Species			Ν	/lean num	nbers (V tra	ansformed) of adult <i>N</i> y	vsius hutto	ni			AUC
	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	Mean
Kale cv Kestrel	0.44a	0.51a	0.91bc	0.72ab	0.68ab	0.76abc	0.79abc	1.06abc	0.87abc	1.15bc	0.68a	1.09b
Phacelia cv Lacy	0.71a	1.03ab	1.05bcd	0.92ab	1.16bc	1.07cd	1.03bcd	1.24bc	1.17bcd	1.73d	1.06ab	1.37cd
Alyssum cv Benthamii White	0.87a	1.24b	1.51cd	1.17b	1.43c	1.33d	1.29cde	1.11abc	1.54d	1.75d	1.44b	1.55de
Buckwheat cv Katowase	0.92ab	0.64a	0.86ab	0.92ab	0.93abc	1.20cd	1.45de	1.24bc	1.29cd	0.89ab	1.38b	1.39cde
Clover cv Nomad	0.34a	0.59a	0.66ab	0.64ab	0.40a	0.50ab	0.54ab	0.52a	0.54a	0.48a	0.69a	0.72a
Wheat cv Morph	1.48b	1.37b	1.65d	1.85c	2.16d	1.36d	1.72e	1.54c	1.36cd	1.70cd	1.19ab	1.64e
Lucerne cv Kaituna	0.79a	1.01ab	1.20bcd	0.79ab	0.66ab	0.94bcd	1.12bcde	0.87ab	1.37cd	1.13bc	1.07ab	1.24bc
Coriander cv Santo	0.54a	0.54a	0.30a	0.50a	0.67ab	0.24a	0.27a	0.57a	0.64ab	0.85ab	0.71a	0.81a
LSD (5 %)	0.585	0.571	0.607	0.649	0.649	0.540	0.602	0.625	0.554	0.581	0.527	0.258
SEM ⁵	0.207	0.212	0.207	0.230	0.231	0.198	0.218	0.231	0.207	0.213	0.225	0.091
Significance	*	*	***	**	***	***	***	*	**	***	*	***

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant and arena, the 120 - hour weighted mean was obtained by calculating the area under the curve (AUC) for a graph of \vee count against time (h) using the trapezoid rule, then dividing by the time period (120 h) (n = 10). (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.

Table 2.4 For the no-choice tests, the mean numbers (V transformed) of *Nysius huttoni* adults recorded on each of eight plant species at times 0.5 h to 120 h plus the overall AUC mean.

Plant Species			Μ	lean numbe	rs (V tran	sformed) c	of adult Nys	sius hutto	ni			AUC
	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	Mean
Kale cv Kestrel	0.48ab	0.98ab	1.17cd	1.04abcd	1.23cd	0.94bcd	1.19cd	0.71ab	0.20a	0.34a	0.10a	0.84ab
Phacelia cv Lacy	0.48ab	0.82ab	0.71abc	1.28cd	0.90bc	1.19cd	0.83abc	1.15bc	1.08cd	0.34a	0.47ab	1.09bc
Alyssum cv Benthamii White	1.53d	1.84c	1.39d	1.53d	1.32cd	1.28d	1.54d	1.47c	1.28d	1.01b	0.72b	1.35c
Buckwheat cv Katowase	0.76abc	0.74ab	1.00bcd	0.71ab	1.09cd	0.98cd	0.74abc	0.38a	0.56ab	0.84ab	0.72b	0.94ab
Clover cv Nomad	0.20a	0.40a	0.38a	0.48a	0.44ab	0.44ab	0.47a	0.78ab	0.72bc	0.58ab	0.30ab	0.70a
Wheat cv Morph	1.12cd	1.29bc	1.37d	1.06bcd	1.41d	0.76abc	1.11bcd	1.16bc	0.81bcd	0.69ab	0.51ab	1.11bc
Lucerne cv Kaituna	0.87bc	0.50a	0.51ab	0.48a	0.34a	0.40a	0.40a	0.82ab	0.52ab	0.52ab	0.69ab	0.69a
Coriander cv Santo	0.91bc	0.97ab	1.03bcd	0.77abc	0.30a	0.34a	0.54ab	0.38a	0.30ab	0.38a	0.34ab	0.62a
LSD (5 %)	0.560	0.599	0.559	0.564	0.498	0.512	0.594	0.549	0.510	0.606	0.595	0.326
SEM	0.198	0.212	0.198	0.199	0.176	0.181	0.210	0.194	0.181	0.214	0.211	0.113
Significance	***	***	**	**	***	***	**	**	***	*	*	***

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 10). For each plant and arena, the 120 - hour weighted mean was obtained by calculating the area under the curve (AUC) for a graph of Vcount against time (h) using the trapezoid rule, then dividing by the time period (120 h) (n = 10). (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.

The overall plant dry weight reduction (%) compared with control was significantly different across plant species (*p* < 0.001) (Fig. 2.5 a). The dry weight reductions on alyssum, wheat, and lucerne were not significantly different from each other, but the first two had significantly greater reduction than kale, phacelia, buckwheat, clover, and coriander. Maximum dry weight reduction (%) was recorded on alyssum, followed by wheat and lucerne. The lowest weight reduction (%) was on buckwheat. Height reductions (%) compared with control were also significantly different across plant species (Fig. 2.5 b). The reduction was significantly higher on wheat (29.55 %) than on kale (14.17 %) and buckwheat (11.73 %). Those on alyssum, buckwheat, kale, coriander, phacelia, lucerne, and clover were not significantly different (Fig. 2.5 b).

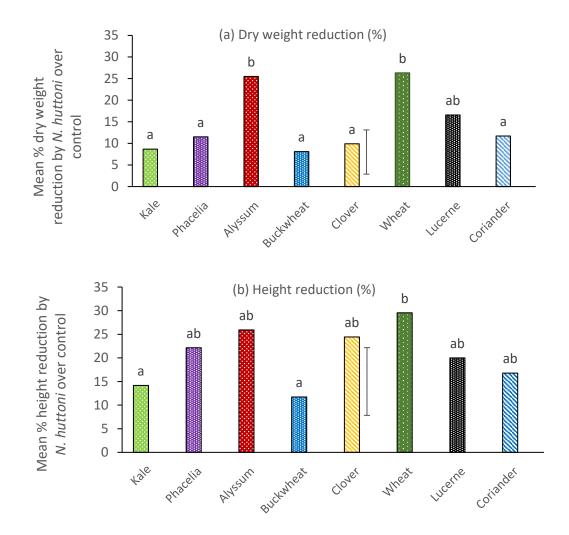


Figure 2.5 Choice tests. Mean (a) dry weight reduction (%) and (b) height reduction (%) from *Nysius huttoni* adult feeding on eight plant species. The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 10).

2.4.2 No-choice tests

In the no-choice tests, *N. huttoni* settled significantly sooner on wheat than on kale, coriander, buckwheat, and phacelia (p < 0.05). First settlement times of the bug on wheat, lucerne, alyssum, and clover were not significantly different. Phacelia, buckwheat, and coriander were less suitable for *N. huttoni* than kale, although not significantly so. Overall, *N. huttoni* required approximately 75 % less time ($\overline{x} = 5.05$ min) for first settlement on wheat than on kale, which took $\overline{x} = 20.74$ min (Table 2.5).

The time to first feeding damage by *N. huttoni* varied significantly between the plant species (*p* < 0.001) (Table 2.5). The time to first *N. huttoni* feeding damage was the shortest on alyssum (55 h), which was significantly shorter than on coriander, phacelia, buckwheat, kale, lucerne, and wheat. Kale was a significantly more susceptible host than coriander but was significantly less susceptible than alyssum, clover and wheat (Table 2.5).

Table 2.5 Mean time (log_{10} transformed) required for first settlement and first obvious feeding damage by *Nysius huttoni* adults on different plant species in no-choice tests. Backtransformed means are given in brackets. Means within the same column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 10).

Plant Species	No-choi	ce test
	Settlement time	Feeding damage
	(log ₁₀ minutes ± SEM)	(log ₁₀ hours ± SEM)
Kale cv Kestrel	1.317 bc ± 0.187 (20.74)	1.979 c ± 0.056 (95)
Phacelia cv Lacy	1.502 c ± 0.204 (31.76)	2.010 c ± 0.039 (102)
Alyssum cv Benthamii White	0.942 ab ± 0.110 (8.74)	1.740 a ± 0.067 (55)
Buckwheat cv Katowase	1.375 bc ± 0.126 (23.71)	1.993 c ± 0.044 (98)
Clover cv Nomad	0.943 ab ± 0.260 (8.77)	1.790 ab ± 0.053 (62)
Wheat cv Morph	0.704 a ± 0.074 (5.05)	1.797 b ± 0.054 (63)
Lucerne cv Kaituna	0.905 ab ± 0.163 (8.03)	1.906 bc ± 0.054 (81)
Coriander cv Santo	1.369 bc ± 0.136 (23.38)	2.348 d ± 0.000 (223)
LSD (5 %)	0.487	0.139
SEM	0.157	0.049
Significance	*	***

(*) p < 0.05, significant; (***) p < 0.001, extremely significant.

The numbers of *Nysius* observed over 120 h also varied significantly between plant species (*p* < 0.001) (Fig. 2.6 and Table 2.4). The number of *Nysius* observed on alyssum was significantly higher

than on buckwheat, kale, clover, lucerne, and coriander but not significantly different to phacelia and wheat. Kale, buckwheat, clover, lucerne, and coriander did not differ significantly from one another (Fig. 2.6).

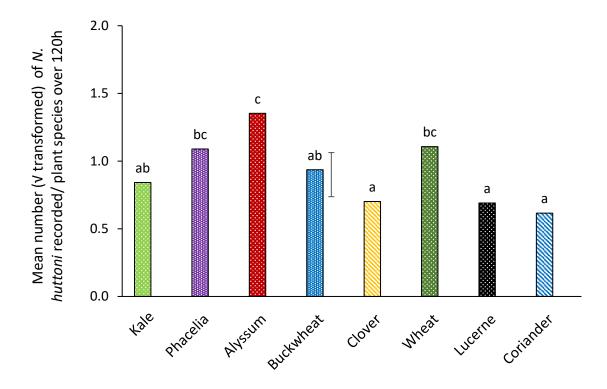


Figure 2.6 Mean numbers (V transformed) over 120 h of *Nysius huttoni* adults recorded in each of eight plant species in no-choice tests (n = 10). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05).

There were significant bug survival differences between the plant species (p < 0.001) (Fig. 2.7). Maximum survival was recorded on phacelia followed by clover, and both were significantly higher than all other plant species except coriander. The survival rates on phacelia and clover were more than five times higher than on kale plants. The lowest survival was recorded on kale which was significantly different from all other plant species. Alyssum, buckwheat, wheat, and lucerne did not differ significantly, but their survival rate was much higher than that on kale.

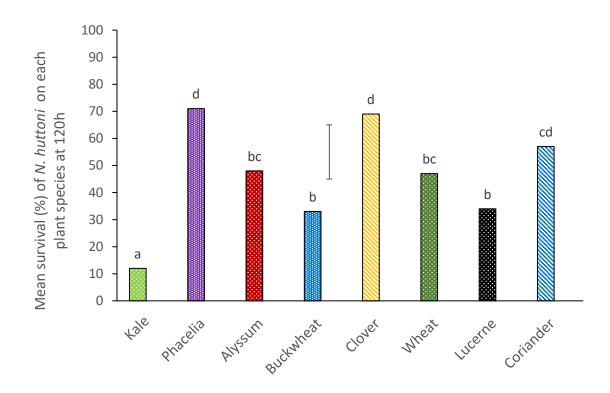


Figure 2.7 Mean survival (%) of *Nysius huttoni* adults on eight plant species at 120 h in no-choice tests (n = 10). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05).

2.4.3 Paired-choice tests with kale

In paired-choice tests involving kale and a second species, the numbers of *Nysius* observed over 120 h varied significantly between the non-kale plant species (p < 0.05) (Fig. 2.8 and Table 2.6). Wheat and alyssum did not differ significantly. Wheat was significantly more preferred by the bug than was kale, clover, coriander, and lucerne but did not differ in this respect from alyssum, buckwheat, and phacelia (Fig. 2.8). Comparing the numbers of *Nysius* observed over 120 h on the kale component of the pairs, numbers on kale were significantly higher when the kale was paired with buckwheat than when it was paired with lucerne, while numbers on the kale were intermediate on all other pairs (Fig. 2.9 and Table 2.7).

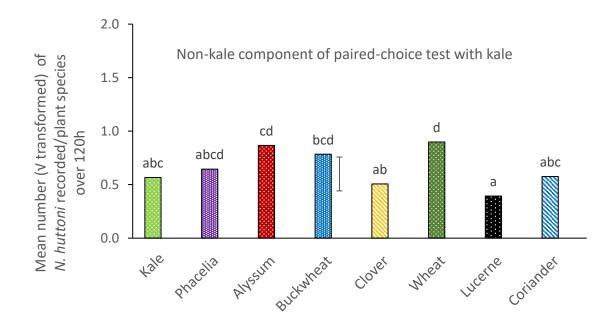


Figure 2.8 Mean numbers (√ transformed) over 120 h of *Nysius huttoni* adults recorded on each of eight plant species in paired-choice tests (Non-kale component of paired-choice test with kale) (n = 6). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05).</p>

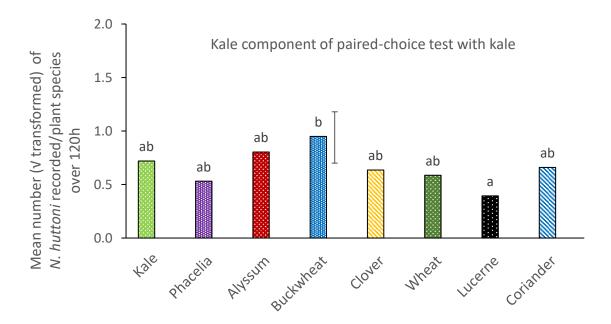


Figure 2.9 Mean numbers (√ transformed) over 120 h of *Nysius huttoni* adults recorded on each of eight plant species in paired-choice tests (kale component of paired-choice test with kale)
(n = 6). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; *p* < 0.05).

Table 2.6 For the non-kale component of paired-choice test with kale, mean numbers (V transformed) of *Nysius huttoni* adults recorded on each of eight plant species at times 0.5 h to 120 h plus the overall AUC mean.

Plant Species			M	ean numbe	ers (V trans	sformed) o	of adult Ny	sius huttoi	ni			AUC Mean
	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	-
Kale cv Kestrel	0.33ab	0.57bc	0.17a	0.50ab	0.33a	0.00a	0.57ab	0.33a	0.33a	0.33ab	0.57b	0.57abc
Phacelia cv Lacy	0.57ab	0.00a	0.33ab	0.57abc	1.12b	0.74b	0.87b	0.40a	0.40ab	0.00a	0.00a	0.64abcd
Alyssum cv Benthamii White	0.74ab	0.64bcd	1.04c	0.71bc	1.04b	0.74b	0.97b	1.14b	0.64ab	0.33ab	1.28c	0.87cd
Buckwheat cv Katowase	0.90b	0.90cd	0.74bc	0.57abc	0.40a	0.57ab	0.50ab	0.90ab	0.40ab	0.80b	0.40ab	0.78bcd
Clover cv Nomad	0.17a	0.00a	0.33ab	0.33ab	0.67ab	0.17ab	0.17a	0.33a	0.33a	0.50ab	0.00a	0.51ab
Wheat cv Morph	0.64ab	1.14d	1.02c	1.21c	1.08b	0.67b	0.83ab	0.80ab	1.04b	0.24a	0.40ab	0.90d
Lucerne cv Kaituna	0.33ab	0.00a	0.17a	0.17ab	0.33a	0.33ab	0.33ab	0.50a	0.17a	0.00a	0.17ab	0.39a
Coriander cv Santo	0.33ab	0.33ab	0.17a	0.00a	0.33a	0.33ab	0.33ab	0.50a	0.50ab	0.50ab	0.40ab	0.58abc
LSD (5 %)	0.677	0.512	0.567	0.643	0.613	0.574	0.668	0.625	0.656	0.536	0.522	0.316
SEM	0.236	0.178	0.197	0.224	0.213	0.200	0.233	0.218	0.229	0.187	0.182	0.110
Significance	*	***	**	*	*	*	*	*	*	*	* * *	*

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 6). For each plant and arena, the 120 - hour weighted mean was obtained by calculating the area under the curve (AUC) for a graph of Vcount against time (h) using the trapezoid rule, then dividing by the time period (120 h) (n = 6). (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.

Table 2.7 For the kale component of paired-choice test with kale, mean numbers (V transformed) of *Nysius huttoni* adults recorded on each of eight plant species at times 0.5 h to 120 h plus the overall AUC mean.

Plant Species			N	/lean numb	ers (V tran	sformed)	of adult N	ysius hutto	oni			AUC Mean
	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	-
Kale cv Kestrel	0.40a	0.17ab	0.40ab	0.57ab	0.91bc	0.00a	0.46a	0.57a	0.62ab	0.46abc	0.69a	0.71ab
Phacelia cv Lacy	0.46a	0.00a	0.33ab	0.00a	0.17a	0.33a	0.40a	0.33a	0.33ab	0.17ab	0.64ab	0.53ab
Alyssum cv Benthamii White	1.02a	0.67bc	0.71ab	0.67bc	0.67abc	0.50ab	0.40a	0.33a	0.33ab	1.11c	0.33ab	0.80ab
Buckwheat cv Katowase	0.50a	0.50abc	0.57ab	0.74bc	1.22c	0.97b	0.74a	0.40a	1.00b	0.86bc	0.40ab	0.94b
Clover cv Nomad	0.87a	0.86c	0.74ab	0.50ab	0.17a	0.17a	0.40a	0.74a	0.47ab	0.57abc	0.33ab	0.63ab
Wheat cv Morph	0.79a	0.80c	1.07b	1.28c	0.57abc	1.02b	0.40a	0.57a	0.17a	0.00a	0.00a	0.58ab
Lucerne cv Kaituna	0.33a	0.00a	0.24a	0.24ab	0.50ab	0.24a	0.17a	0.29a	0.17a	0.17ab	0.24ab	0.39a
Coriander cv Santo	0.40a	0.57abc	0.64ab	0.74bc	0.40ab	0.46ab	0.80a	0.57a	0.24a	0.64abc	0.47ab	0.65ab
LSD (5 %)	0.764	0.580	0.774	0.659	0.672	0.619	0.683	0.717	0.742	0.693	0.665	0.480
SEM	0.266	0.202	0.269	0.230	0.234	0.216	0.238	0.250	0.258	0.241	0.232	0.167
Significance	ns	*	*	*	*	*	ns	ns	*	*	*	*

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 6). For each plant and arena, the 120 - hour weighted mean was obtained by calculating the area under the curve (AUC) for a graph of Vcount against time (h) using the trapezoid rule, then dividing by the time period (120 h) (n = 6). (ns) non - significant; (*) p < 0.05, significant.

Moreover, the mean survival of the bugs on the pairs of kale and non-kale plants varied significantly (p < 0.001) (Fig. 2.10). Survival was significantly higher with the kale plus alyssum pair (78.33 %) than with all other pairs (kale plus non-kale) of plants. The second highest survival was recorded on the kale plus wheat pair (46.67 %), which was significantly higher than kale plus coriander (23.33 %), kale plus clover (8.33 %), and kale plus phacelia (6.67 %) but not significantly different from kale plus buckwheat (41.67 %), kale plus lucerne (30 %), and kale plus kale (26.67 %). The lowest survival was recorded on the pairs of kale plus clover (8.33 %) and kale plus phacelia (6.67 %), which were not significantly different from each other (Fig. 2.10).

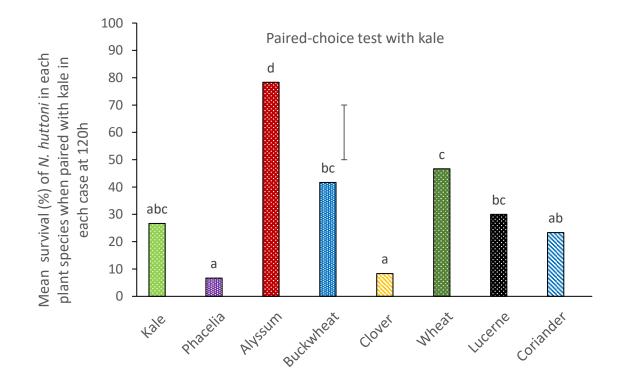


Figure 2.10 Mean survival (%) of *Nysius huttoni* adults on eight plant species at 120 h in pairedchoice tests with kale (when paired with kale in each case) (n = 6). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05).

2.4.4 Paired-choice tests with non-kale plant species

In experiments involving pairs of seedlings of non-kale plant species, the numbers of *N. huttoni* individuals recorded on alyssum were significantly higher than on lucerne (t = 4.308; df = 5; p = 0.007) but not significantly different from buckwheat (t = -0.169; df = 5; p = 0.869), coriander (t = -1.296; df = 5; p = 0.226), and wheat (t = -0.641; df = 5; p = 0.544) (Fig. 2.11). There was a suggestion that wheat seedlings were more attractive than seedlings of buckwheat (t = 1.936; df = 5; p = 0.081), although not significantly so (Fig. 2.11).

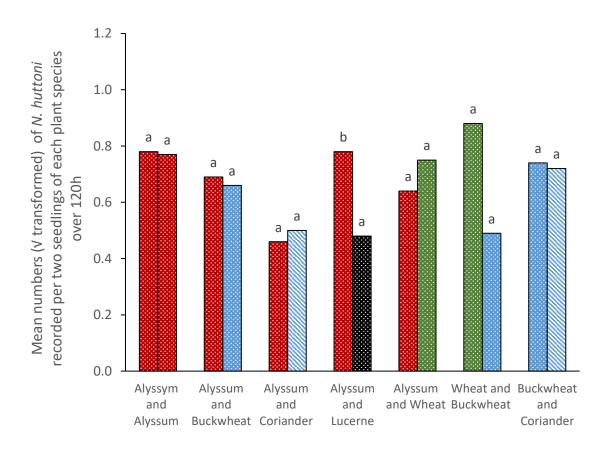


Figure 2.11 Mean numbers (V transformed) over 120 h of *Nysius huttoni* recorded in paired-choice tests between non-kale plants (where the first plant species mentioned in the left-hand bar in each pair). Within each pair, plant species were statistically compared using a paired sample *t*-test (p < 0.05) (n = 6). Within each pair, means with no letters in common are significantly different (p < 0.05).

The survival rates of *N. huttoni* between pairs of non-kale plants varied significantly (p < 0.001) (Fig. 2.12). The maximum survival was recorded on the alyssum and buckwheat pair (71.67 %), which was significantly higher than all other non-kale pairs. Next in survival rate were alyssum and wheat (45 %) and wheat and buckwheat (40 %), which were not statistically different from each other, but both were significantly higher than all other non-kale pairs (Fig. 2.12).

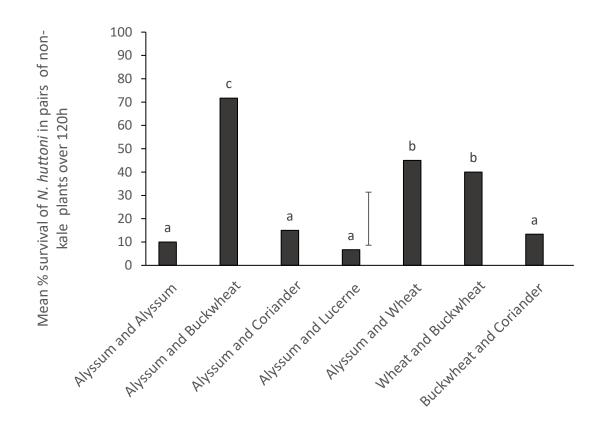


Figure 2.12 Mean survival (%) of *Nysius huttoni* adults on different plant species pairs of non-kale plants at 120h after the introduction of the wheat bugs. The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 6).

2.5 Discussion

The study examined the host plant selection of the wheat bug, *N. huttoni*, on a range of potential trap plant species. The parameters were the bug's time to first settling on the plant, the mean number of bugs settling over time, time to first obvious feeding damage, survival rate, and % reduction in plant weight and height. There was a wide range of susceptibility to the bug between kale and other tested plant species and cultivars. The results provided the first information on the host selection behaviour of *N. huttoni* on potential host trap plants and can potentially inform future decisions on trap cropping protocols. The current work demonstrated that no one plant species was overwhelmingly preferred by the *N. huttoni*, but the seedlings of alyssum and wheat were more suitable and acceptable hosts of *N. huttoni* than were other tested plant species. *Nysius huttoni* settled more promptly on wheat and alyssum in all experiments. For the choice, no-choice, and paired-choice tests, the mean number of individuals on wheat and alyssum was higher than on kale and all other plant species, although not always significantly so. Furthermore, on alyssum and wheat, the bug's time to first feeding damage was very short. The survival rates of the bugs in no-choice tests over a period of 120 h on phacelia (71.0 %), clover (69.0 %), alyssum (48.0 %), wheat

(47 %), and the other three non-kale plant species were all significantly higher than on kale seedlings. Higher survival rates were recorded in pairs of kale plus alyssum (78.3 %) (Fig. 2.10), alyssum plus buckwheat (71.68 %), alyssum plus wheat (45 %), and wheat plus buckwheat (40 %) compared to the other pairs of plant species (Fig. 2.12).

Based on the results in this work, alyssum is potentially the most suitable host for *Nysius*. The work of Wei (2001) supports the fact that *Nysius* uses alyssum in summer. It also overwinters on alyssum in New Zealand. It is a potentially good candidate trap crop for oviposition by the diamondback moth (*P. xylostella*), but alyssum is relatively unsuitable for larval development of this insect (De Groot, Winkler, & Potting, 2005). However, alyssum is a suitable feeding host of *B. hilaris* (Reed, Newman, Perring, Bethke, & Kabashima, 2013).

The significant weight loss of alyssum caused by *N. huttoni* feeding could have negative consequences if this plant were to be deployed as a trap crop in the field. This could also limit these plants potential for its delivering of multiple ES, such as nectar and pollen for beneficial insects (Gurr et al., 2017). Alyssum can potentially deliver multiple ES to agroecosystems. For example, the use of this plant and buckwheat increases the impact of natural enemies of leaf rollers (Tortricidae) in apple orchards (Irvin et al., 2006). Hoverflies (Diptera: Syrphidae) are more attracted to alyssum compared with other flowering plants such as coriander (C. sativum), yarrow, Achillea millefolium (L.), fennel, Foeniculum vulgare Miller, and Korean licorice mint, Agastache rugosa Kuntze (Fischer & C. A. Meyer) (Colley & Luna, 2000). Based on the laboratory findings here, wheat was the second most important potential trap crop of this pest. Several other authors also showed that wheat is a potential host of N. huttoni (Eyles, 1965; He et al., 2003; PGG, 2009; Yang & Wang, 2004). In fact, *N. huttoni* uses wheat during its panicle and milk-ripe stages at a time when the usual annual host plants have desiccated or died (Bejakovich et al., 1998; Every et al., 1998; Farrell & Stufkens, 1993; Swallow & Cressey, 1987). Relatively high populations of N. huttoni on wheat can potentially damage this crop, which could lead to the death of plants. In a commercial crop, this potential damage may lead to insecticide use, increasing the management cost needed for bug control.

The dead tillers of wheat plants also can be a good breeding habitat and a winter hibernation site for the bugs, as well as other insect pests, which can increase potential pest problems for the next season's crops (Hokkanen, 1991). However, this is unlikely to be a problem if a very small proportion of a kale field is planted as a wheat trap crop, the latter is not intended to be harvested and its residues do not remain over winter. In summary, the results have the potential to help in a better understanding of the host selection behaviour of the *N. huttoni*, which may contribute to the design of trap cropping protocols. This could take the form of drilling or planting the most preferred trap crops (alyssum, wheat, or both) before kale germination.

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The pest may destroy these trap plants but if that occurs after the vulnerable seedling stage, it is unlikely to be important. However, the effectiveness of trap crops depends on the relative attractiveness of the plant species (Badenes-Perez et al., 2004), the proportion of the field occupied by the trap crop (Banks & Ekbom, 1999), the duration of the trapping effect (Cook et al., 2006), insect migratory and host-finding behaviours (Shelton & Badenes-Perez, 2006), and the relative planting times of trap plants and kale etc. Trap plant species at the field edge can prevent the pest from reaching the crop (Rea et al., 2002) or may concentrate it in a certain part of the field. If the bugs remain on the trap plant species for around 4 wks in the current situation, the seedlings of the main crop can potentially be risk-free. However, other potential pests such as Lepidoptera, Coleoptera may still need to be managed. This was the case when alyssum was used to enhance aphid CBC in lettuce in California, United States. Flea beetles (Coleoptera: Chrysomelidae) became a pest of alyssum, nearly destroying it. So in that case, flowering buckwheat (Polygonaceae) and phacelia (Boraginaceae) were substituted (Ramy Colfer, Mission Organics, pers. comm.). The use of trap cropping in agriculture is not well established because this approach is more knowledge-intensive than is pesticide use. However, in the least developed countries, pesticides can represent an unacceptable extra cost (Gurr et al., 2017; Khan et al., 2001) and generally lead to many environmental problems.

2.6 Conclusions

The results suggest that the garden plant alyssum (L. maritima) and wheat (T. aestivum) are the most preferred species by N. huttoni over phacelia (P. tanacetifolia), buckwheat (F. esculentum), coriander (C. sativum), white clover (T. repens), lurcerne (M. sativa) and kale (B. olearacea). These non-kale species such as alyssum and wheat can be used as a trap crop in an IPM strategy to attract ('pull') bugs away from the brassicas to reduce the damage in brassica seedlings. Low preference non-kale plant species such as P. tanacetifolia, F. esculentum, C. sativum, can be used to drive the pest away ('push') from the main crops. These two categories of non-kale flowering plants in brassica fields can be integrated in a 'push-pull' strategy of pest management as suggested by Khan et al. (2001). Potential trap crops such as alyssum and wheat attract ('pull') N. huttoni, and, when combined with the repellent species such as phacelia, coriander or buckwheat, can divert ('push') the bug away from the main crop. The mechanisms behind this behaviour change of *N. huttoni* by the 'push-pull' plants was not evaluated. It has been suggested that visual or plant volatiles (olfactory cues) of host plants might be mediating the bugs' behaviour (Badenes-Perez et al., 2004). The attractant or repellent effect of plants for N. huttoni have never been previously suggested, so the results presented here provide a new opportunity for further study to understand insect behaviour on a wide range of host plants. There is an opportunity to develop a 'push-pull' strategy

for *N. huttoni* management in brassica fields. The deployment of such flowering non-kale species in brassica fields not only improves CBC but also enhances multiple ES that are needed for future farming. Further experiments such as the proportion of main crop and trap crop, location of the trap crop, growth stages, and cultivars for both, are needed to develop a suitable pest management protocol for sustainable pest management in brassica crops.

Chapter 3

Susceptibility of kale cultivars to the wheat bug, (*Nysius huttoni*) (Hemiptera: Lygaeidae) in New Zealand

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3.1 Abstract

Seedlings of kale cultivars in New Zealand are highly susceptible to direct feeding by the wheat bug *Nysius huttoni,* an endemic insect pest.

Two assays (choice and no-choice) were conducted to compare the relative susceptibility of seedlings of the six most popular kale cultivars in New Zealand (Kestrel, Gruner, Sovereign, Regal, Corka and Coleor). The earliest occurrence of feeding damage in the choice assay was on cv. Kestrel, significantly earlier than on Corka and Gruner. In the no-choice assay, significantly more *N. huttoni* were found on Kestrel than on Corka. Damage to Kestrel occurred significantly earlier than on all the other cultivars except Corka. Reduction in plant dry weight was significantly higher on Coleor and Kestrel. These results are important for developing integrated pest management protocols for kale pests.

Keywords: Integrated pest management, wheat bug, *Nysius huttoni*, susceptibility cultivar, Kestrel, Coleor

3.2 Introduction

Nysius huttoni White (Hemiptera: Lygaeidae), is an endemic New Zealand insect (Eyles, 1960b) widely distributed in both the North and South Islands (Eyles, 1960b; Eyles & Ashlock, 1969; Myers, 1926). Three other *Nysius* species belonging to the family Lygaeidae (Hemiptera) are recorded in New Zealand. *Nysius convexus* Usinger 1942 and *N. liliputanus* Eyles and Ashlock 1969 are endemic, whereas *N. caledoniae* Distant 1920 is an adventive species from Australia (Eyles & Ashlock, 1969; Gurr, 1957). *Nysius huttoni* has a wide host range comprising almost all brassicas, other cultivated crops and a wide variety of weeds, but *N. convexus* and *N. liliputanus* are recorded only in moss habitats.

Kale (*Brassica oleracea* L.), rape (*B. napus* L.var. *napus*), turnip (*B. campestris* L.) and swede (*B. napus* L. var. *napo-brassica*) are common, widely grown forage brassicas in New Zealand (Speciality Seeds, 2016). These crops are normally drilled in summer (November to December) (AgPest, 2016) at which time the bug's populations are at peak levels (Wei, 2001). In New Zealand, about 400,000 ha of brassicas are grown annually (Horrocks et al., 2018). Forage brassicas have a high feeding value for ruminants, grow rapidly and have a high dry matter content (Speciality Seeds, 2016). *Nysius huttoni* is a threat to 4- to 6-week-old brassica seedlings and plant populations can be reduced as a result of feeding damage at the base of the plants (Eyles, 1965), causing a cankerous growth of tissue that can kill them or make them susceptible to breakage from wind and stock movement (Fig. 3.1) (AgPest, 2016). There has been up to 90 % damage in brassica crops in severe situations (AgPest, 2016; Speciality Seeds, 2016).

Forage brassicas may require several insecticide sprays/season to prevent damage (PGG, 2009). Effective management of this pest mostly relies on seed treatment with neonicotinoids and foliar application of chlorpyrifos and permethrin insecticides (Goldson et al., 2015; Young, 2018). All these pesticides are a broad spectrum in nature (AgPest, 2016; Horrocks et al., 2018). Neonicotinoids are systemic and traces of them have been found in pollen and nectar, which impact pollinators including wild bees (Goulson et al., 2008; Pook & Gritcan, 2017), bumble bees (Whitehorn, O'Connor, Wackers, & Goulson, 2012) and many other insect pollinators (Godfray et al., 2014). Hence, these pesticides are under increasing environmental pressure in Europe and many other countries (Cressey, 2017; Woodcock et al., 2018). Furthermore, concern exists about non-target effects on insect natural enemies (Goulson et al., 2008; Goulson, 2013), as well as birds and many fish species (Gibbons, Morrissey, & Mineau, 2015). Hence, these pesticides need to be replaced by environmentally friendly and agro-ecological pest management strategies.

There is increasing pressure from consumers, media and governments to reduce pesticide use, but no practical alternatives are currently being offered to manage the *N. huttoni* in New Zealand. It is therefore necessary to develop a cost-effective and sustainable method of pest management. Encouraging farmers to use integrated pest management (IPM) strategies that combine biological, cultural and chemical approaches in a compatible way could be one strategy to reduce pest damage and pesticide use in forage brassicas (Horrocks et al., 2018). Pest-resistant/tolerant cultivars are vital component of many IPM programmes. The present study was undertaken to screen kale cultivars on the basis of susceptibility to *N. huttoni* and the results could guide the development of a future IPM programme for kale.



Figure 3.1 Damage symptoms from Nysius huttoni attacks on kale seedlings

3.3 Materials and methods

3.3.1 Nysius huttoni collection and identification

Adult *N. huttoni*were collected from the weed shepherd's purse *C. bursa-pastoris* (L.) Medik. (Brassicaceae) from the Iversen Field Plant Science Research Unit (43° 38' 50.4" S, 172° 27' 29.9" E) at Lincoln University in the spring of 2016. The collected bugs were preserved in 1.7 ml microtubes (MCT-175-C) that contained 70 % ethanol and delivered to a Hemiptera taxonomist (Dr Marie-Claude Larivière, Landcare Research, Auckland, New Zealand) for confirmation and preserved in the Lincoln University Entomology Research Museum. After the confirmation of the species, *N. huttoni* were collected from the same field (see above) and used in laboratory rearing.

3.3.2 Nysius huttoni cultures

Nysius huttoni breeding colony was established following methods based on those of Burgess and Weegar (1986) and He and Wang (2000). Field-collected *N. huttoni* were released inside a transparent rectangular plastic container (29 x 19 x 10 cm) that contained a mesh-covered lid for air circulation. Fifty mating pairs were transferred to individual 50 ml polypropylene centrifuge tubes (11.0 cm length x 2.0 cm diameter) using a fine-hair brush. Food for *Nysius* consisted of fruiting twin cress, *Coronopus didymus* (L.) Smith. (Brassicaceae) and hulled organic sunflower seeds (*Helianthus annuus* L. cv. Golden Toasted) (Asteraceae) (BioGro organic certified) which were replaced daily. Males were removed from the pair when the female began to lay eggs on the

moistened cotton dental roll (10 mm x 38 mm) that was also included (Yang & Wang, 2004). The tubes were checked daily and freshly laid eggs were removed using the brush and transferred to Petri dishes (5 cm diameter). The newly emerged nymphs along with the cotton dental rolls were transferred to another Petri dish (14 cm diameter) in which partially moistened filter papers had been placed. The colony was maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (https://www.bioprotection.org.nz), Lincoln University, New Zealand (Fig. 3.2). The ambient temperature, humidity and photoperiod were 23 °c with a 4 °C range, 65 % relative humidity and 16L: 8D.

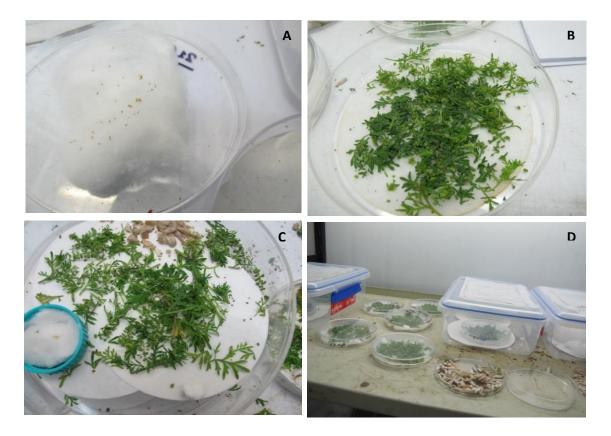


Figure 3.2 Laboratory culture of *Nysius huttoni* maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (BPRC), Lincoln University. a) *Nysius huttoni* eggs in a cotton swab; b) Twin cress (*Coronopus didymus*); c) *Nysius huttoni* nymphs in a Petri dish with twin cress and sunflower seeds; d) *Nysius huttoni* cohorts in the CT room.

3.3.3 Plant selection and cultivation

The uncoated seeds of the six most commonly used kale cultivars (Table 3.1) were obtained from PGG Wrightson (https://www.pggwrightsonseeds.com) and Speciality Seeds (https://www.specseed.co.nz). Their growth habits are described in Table 3.1.

The seedlings were grown in a glasshouse with a mean temperature of 22 ^oC and relative humidity of 40 %. Seeds were direct-seeded into pots containing a potting mix made by mixing 400 L

composted bark, 100 L pumice (1.0–7.0 mm), 1500 g Osmocote (slow, 3- to 4-month release plant food), 500 g horticultural lime and 500 g HydraFLO (wetting agent,

https://www.solutions4earth.com). The seedlings were 9 days old and approximately 3.5 cm high when used in the bioassays. Those grown in the glasshouse were transferred to a CT room with a temperature of 21 °C with a 4 °C range and a day length of 16 hours.

SN	Cultivar	Height	Phenological characteristics
1	Kestrel KE35 TC	Medium	High leaf-to-stem ratio, palatable and digestible
			thick stems
2	Coleor	Small-medium	High leaf-to-stem ratio, winter hardy and high
			yield potential
3	Sovereign SOV 27 AC	Intermediate	High leaf-to-stem ratio and high yield potential
4	Regal KBG 01 AC	Intermediate	High leaf-to-stem ratio, winter hardy but low
			yield potential
5	Gruner	Giant	Tall and high yield potential
6	Corka	Intermediate	High leaf-to-stem ratio, palatable, winter hardy
			and high yield potential

Table 3.1 Phenological characteristics of the six most popular kale cultivars in New Zealand

Sources: www.pggwrightsonseeds.com and www.specseed.co.nz

3.3.4 Choice tests

Two seedlings of each kale cultivar were arranged in a circular fashion around the perimeter of a 23.0 cm diameter x 5.0 cm depth pot with cultivars arranged approximately 5.0 cm apart and 5.0 cm from the pot wall. Each kale cultivar with *N. huttoni* (treatment) being compared with a control with no bugs, so that two adjacent cylindrical sleeves (/pot) served as a block, with a total of ten blocks. Each cultivar was 'marked' on the outer wall of the choice pots. The pots were enclosed in cylindrical sleeves made of flexible transparent PVC sheets (1 mm thickness). The dimension (diameter x height) of the cylinders were 23.5 cm x 14 cm. The tops of the sleeves were covered with fine white mesh and Fluon[®] (BioQuip, fluoropolymer resin, PTFE-30) was used on the inner surface of the sleeves to prevent *Nysius* from climbing (Fig. 3.3 a)

The study comprised a randomised block design, with ten replicates. Twenty adult *N. huttoni* of the same age were starved for 12 h, then introduced into the centre of each cylinder. The times to first settlement (mins) and first obvious feeding damage on a seedling for each pot were recorded by visually. Feeding damage was assessed by recording the presence or absence of girdling of the stem and/or discolouration of the leaf. These were the most common damage symptoms along with leaf distortion, twisted leaf veins and petiole, and finally collapse of the seedlings. Then the number of bugs on seedlings within each cylinder were counted at different time intervals

following introduction (0.5, 1, 2, 4, 8 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h). At the conclusion of the assay the survival rate of the bugs was assessed.

3.3.5 No-choice tests

Two seedlings of each cultivar were grown per 6.5 cm diameter x 5.0 cm depth pot, giving a total of six treatments. There were twenty replicates of each treatment, ten with *N. huttoni* and ten as controls. The pots were covered with 7 cm x 12 cm cylindrical sleeves constructed as above and seven bugs were introduced into the treatment cylinders. Treatments were randomised and assessed as above. At the completion of the assay, the dry weights of the seedlings including roots were measured and the percentage weight change calculated (Fig. 3.3 b).

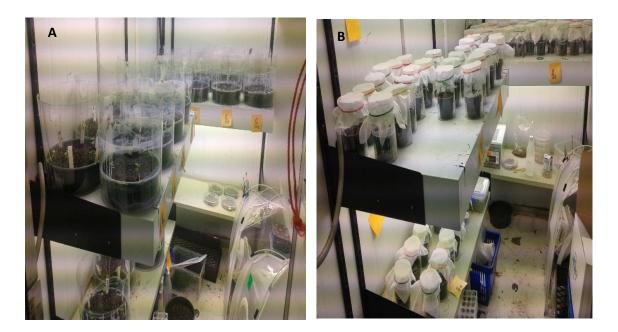


Figure 3.3 Laboratory kale choice and no-choice test maintained in a randomized block design in a controlled temperature (CT) room at Bio-Protection Research Centre (BPRC), Lincoln University a) Choice tests b) No-choice tests.

3.3.6 Statistical analysis

The mean numbers recorded in each cultivar at different time intervals were integrated over the 216 h period by the area under the curve (AUC) method (Hanley & McNeil 1983). Time data (mins) obtained from the experiments were first normalised by using the log_{10} - transformation, and count data were normalised by using a square root (V) transformation. The percentage reduction in plant dry weight (compared with the control) was not transformed. After normality checking, data were subjected to two-way (treatments and blocks) analysis of variance (ANOVA) and means were separated by unprotected least significance difference (LSD) at *p* < 0.05 (Saville, 2015).

3.4 Results

3.4.1 Choice tests

For choice tests, the settling time of the *N. huttoni* on seedlings did not differ significantly between cultivars (Table 3.2) (p > 0.05). The time to first-feeding damage by the bugs across the kale cultivars varied significantly for the choice tests (p < 0.05). First-feeding damage occurred on Kestrel followed by Coleor, Sovereign and Regal, respectively, all of which were not significantly different from one another. However, feeding damage on Kestrel was significantly earlier than on Gruner and Corka (Table 3.2).

Table 3.2 For the choice tests, mean time (Log_{10} transformed) required for settling and first-feeding damage on different kale cultivars (n = 10). Back-transformed means are given in brackets.

Kale cultivars	Settling time	First feeding damage
	(Log ₁₀ minutes ± SEM)	(Log ₁₀ hours ± SEM)
Kestrel	0.93 a ± 0.106 (8.5)	1.98 a ± 0.038 (96.2)
Coleor	1.13 a ± 0.116 (13.2)	1.99 ab ± 0.043 (99.3)
Sovereign	1.16 a ± 0.170 (14.5)	2.10 abc ± 0.063 (125.0)
Regal	1.30 a ± 0.200 (19.9)	2.12abc ± 0.038 (131.2)
Gruner	1.16 a ± 0.167 (14.5)	2.12 bc ± 0.043 (131.5)
Corka	1.22 a ± 0.159 (16.6)	2.15 c ± 0.045 (141.3)
LSD (5 %)	0.406	0.134
SEM	0.143	0.047
Significance	Ns	*

Means within a column with no letters in common are significantly different (Unprotected LSD; p < 0.05). (ns) non - significant; (*) p < 0.05, significant.

The numbers of *N. huttoni* on seedlings across kale cultivars over 216 h were not significantly different in choice tests (p > 0.05) (Fig. 3.4). However, *Nysius* numbers were significantly different across kale cultivars at 12 h and 216 h of bug introduction (Table 3.3). The largest number of bugs was recorded on Coleor followed by Gruner with the lowest on Sovereign (Fig. 3.4). The mean survival rate in the choice test was about 53 %, averaging 10 *N. huttoni/cylinder*.

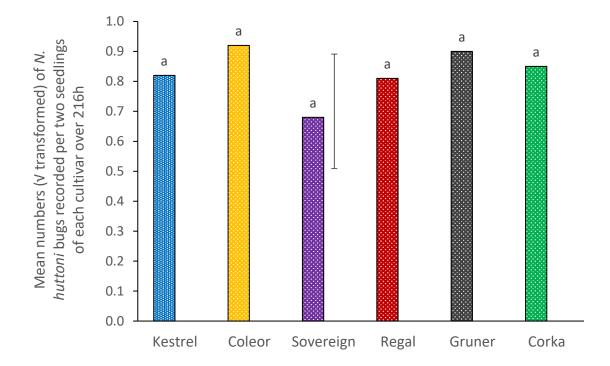


Figure 3.4 Choice tests. Mean numbers (√ transformed) of adult Nysius huttoni recorded in each of six kale cultivars over 216 h. The vertical bar is the least significant difference, LSD (5 %) (n = 10).

Table 3.3 For the choice tests, mean numbers (V transformed) of adult *Nysius huttoni* recorded on each of six kale cultivars at times 0.5 h to 216 h plus 216 h weighted mean calculated by the area under the curve (AUC) method.

Kale					Me	an numbe	ers (V tra	nsforme	d) of adı	ult <i>Nysiu</i>	s hutton	i				AUC
cultivars	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	Mean
Kestrel	0.60a	1.16a	0.99a	1.23a	1.41a	1.28ab	1.21a	1.06a	0.92a	0.87a	0.87a	0.87a	0.81a	0.72a	0.41a	0.82a
Coleor	0.58a	1.06a	0.93a	0.86a	1.20a	1.49b	1.25a	1.25a	1.19a	0.99a	0.89a	1.07a	1.03a	0.72a	0.34a	0.92a
Sovereign	0.48a	0.76a	0.95a	0.82a	0.88a	0.73a	0.71a	0.71a	0.72a	0.83a	0.50a	1.22a	0.76a	0.79a	0.44a	0.68a
Regal	0.44a	0.82a	1.10a	0.78a	0.82a	1.06ab	1.09a	0.90a	0.92a	1.30a	1.10a	0.60a	0.62a	0.83a	0.47ab	0.81a
Gruner	0.34a	0.94a	1.13a	0.93a	1.37a	1.46ab	1.15a	1.33a	1.18a	0.78a	0.98a	0.77a	0.78a	0.76a	0.97b	0.90a
Corka	0.52a	1.32a	1.17a	1.10a	1.27a	1.15ab	0.96a	0.89a	1.16a	0.97a	0.87a	0.70a	0.97a	1.15a	0.79ab	0.85a
LSD (5 %)	0.524	0.594	0.712	0.697	0.733	0.734	0.764	0.813	0.733	0.693	0.654	0.599	0.706	0.583	0.511	0.382
SEM	0.184	0.209	0.250	0.245	0.257	0.258	0.268	0.285	0.257	0.243	0.230	0.210	0.248	0.205	0.180	0.134
Significance	ns	ns	ns	Ns	ns	*	ns	ns	ns	Ns	ns	ns	ns	ns	*	ns

Means within a column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each kale cultivar and pot, the 216 h weighted mean was obtained by calculating AUC for a graph of $\sqrt{216}$ count against time (h) using the trapezoid rule, then dividing by the time period (216 h) (n = 10). (ns) non - significant; (*) p < 0.05, significant.

Table 3.4 For the no-choice tests, mean numbers (V transformed) of adult *Nysius huttoni* recorded on each of six kale cultivars at times 0.5 h to 216 h plus 216 h weighted mean calculated by the area under the curve (AUC) method.

Kale cultivars						Mean nu	umbers (√ transfor	med) of a	adult Nys	sius hutto	oni				AUC Mean
Cultivars	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	
Kestrel	0.72a	0.73a	1.05a	0.71a	0.24a	0.54ab	0.80a	0.92b	1.00b	0.61a	0.61a	0.38a	0.37a	0.86b	0.30ab	0.89b
Coleor	0.45a	0.58a	1.10a	0.57a	0.14a	0.73b	0.70a	0.62ab	0.78ab	0.55a	0.57a	0.34a	0.34a	0.35ab	0.28ab	0.69ab
Sovereign	0.56a	0.62a	1.00a	0.79a	0.56a	0.66b	0.89a	0.72ab	0.81ab	0.66a	0.61a	0.40a	0.27a	0.17a	0.00a	0.73ab
Regal	0.74a	0.77a	0.66a	0.49a	0.14a	0.14a	0.67a	0.61ab	0.55ab	0.41a	0.28a	0.10a	0.10a	0.41ab	0.24ab	0.58ab
Gruner	0.30a	0.71a	0.83a	0.55a	0.44a	0.48ab	0.67a	0.75ab	0.85ab	0.79a	0.61a	0.24a	0.41a	0.52ab	0.59b	0.77ab
Corka	0.54a	0.51a	0.71a	0.47a	0.37a	0.57b	0.67a	0.20a	0.34a	0.48a	0.40a	0.00a	0.14a	0.30ab	0.40ab	0.52a
LSD (5 %)	0.560	0.646	0.624	0.504	0.487	0.423	0.665	0.604	0.594	0.616	0.573	0.422	0.491	0.572	0.485	0.310
SEM	0.197	0.227	0.219	0.177	0.171	0.149	0.233	0.212	0.208	0.216	0.201	0.148	0.172	0.201	0.170	0.109
Significance	ns	ns	ns	Ns	ns	*	ns	*	*	ns	ns	ns	ns	*	*	*

Means within a column with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 10). For each kale cultivar and pot, the 216 h weighted mean was obtained by calculating AUC for a graph of Vcount against time (h) using the trapezoid rule, then dividing by the time period (216 h) (n = 10). (ns) non - significant; (*) p < 0.05, significant.

3.4.2 No-choice tests

Settling time of the *N. huttoni* did not differ significantly between cultivars (p > 0.05) (Table 3.5). In no-choice tests, feeding damage was detected earliest on Kestrel followed by Corka, both of which were significantly more susceptible than Gruner, Sovereign and Regal. Coleor was the third earliest for feeding damage and differed significantly only from Kestrel (Table 3.5).

Table 3.5 For the no-choice tests, mean time (Log₁₀ transformed) required for settling and first feeding damage on different kale cultivars (n = 10). Back-transformed means are given in brackets.

Kale cultivars	Settling time	First feeding damage
	(Log ₁₀ minutes ± SEM ⁶)	(Log ₁₀ hours ± SEM)
Kestrel	1.19 a ± 0.036 (15.5)	1.99 a ± 0.028 (98.2)
Coleor	1.29 a ± 0.113 (19.3)	2.08 bc ± 0.020 (120.5)
Sovereign	1.27 a ± 0.137 (18.6)	2.12 c ± 0.037 (132.4)
Regal	1.38 a ± 0.126 (24.2)	2.13 c ± 0.022 (136.1)
Gruner	1.21 a ± 0.072 (16.1)	2.11 c ± 0.031 (130.0)
Corka	1.33 a ± 0.131 (21.2)	2.01 ab ± 0.018 (102.3)
LSD (5 %)	0.300	0.074
SEM	0.110	0.026
Significance	ns	**

Means within a column with no letters in common are significantly different (Unprotected LSD; p < 0.05). (ns) non - significant; (**) p < 0.01, very significant.

In the no-choice tests, the number of *N. huttoni* observed on Kestrel seedlings was significantly higher than on Corka but not significantly different from Gruner, Sovereign, Coleor or Regal (Fig. 3.5). Kestrel, Gruner, Sovereign, Regal and Coleor were not significantly different from each other. However, *Nysius* numbers were not significantly different at 0.5 h, 1 h, 2 h, 4 h, 8 h, 24 h, 96 h, 120 h, 144 h, and 168 h of bug introduction (Table 3.4)

⁶ Standard error of mean

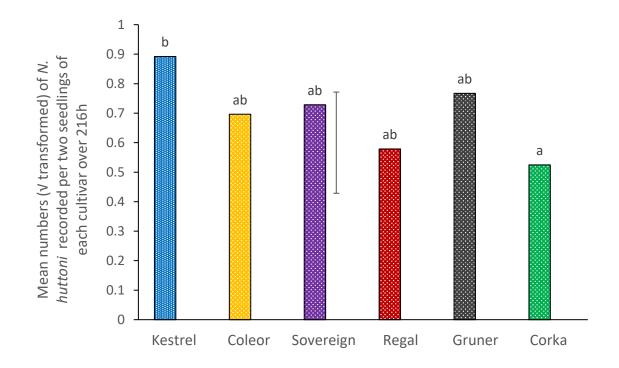
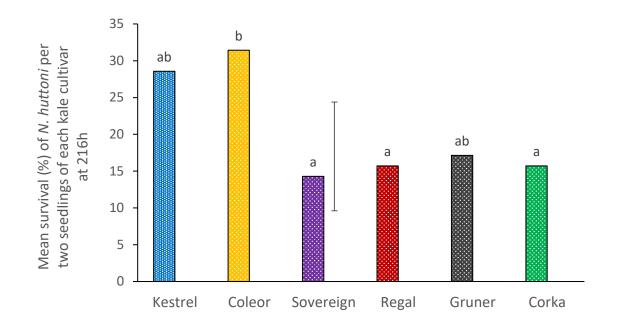
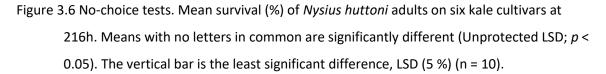


Figure 3.5 No-choice tests. Mean numbers (V transformed) of adult *Nysius huttoni* recorded on each of six kale cultivars over 216 h. Means with no letters in common are significantly different (Unprotected LSD; p < 0.05). The vertical bar is the least significant difference, LSD (5 %) (n = 10).

Survival rate was low, averaging between one and two bugs/cylinder. The highest survival rate occurred on Coleor, followed by Kestrel and Gruner, all of which were not significantly different from one another. Also, the survival on Kestrel, Gruner, Sovereign, Regal and Corka did not differ significantly. Furthermore, the survival rate on the latter three cultivars was significantly lower than on Coleor (Fig. 3.6).

Seedling dry weight reduction by the bug, compared with controls was significantly higher in Kestrel and Coleor than on the other four cultivars. The lowest reduction was recorded on Corka which was not significantly different from that on Sovereign, Gruner and Regal, respectively (Fig. 3.7).





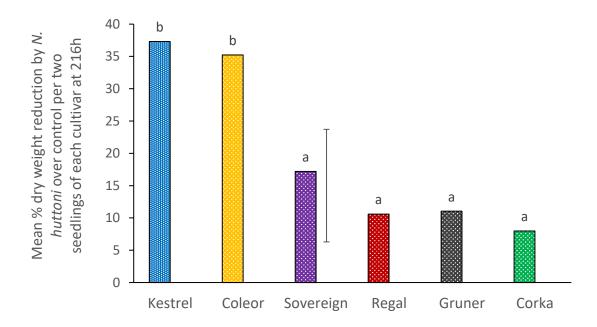


Figure 3.7 Mean percentage dry weight reduction over control from adult *Nysius huttoni* on six kale cultivars in no-choice tests. Means with no letters in common are significantly different (Unprotected LSD; p < 0.05). The vertical bar is the least significant difference, LSD (5 %) (n = 10).

3.5 Discussion

Kale is an important forage crop for ruminants (cattle and sheep), being drilled during the summer for winter feeding in New Zealand (Speciality Seeds, 2016). Damage from N. huttoni is obvious during the seedling stage of that crop (AgPest, 2016; PGG, 2009). The aim of this work was to examine the susceptibility of this bug on a range of commercial kale cultivars. The results confirmed that, in order of preference, N. huttoni favoured the kale cultivars Kestrel, Coleor and Gruner over Sovereign, Corka and Regal (Tables 3.2, 3.5 and Fig. 3.5). Significantly higher survival of the bug was recorded on Coleor and Kestrel than on Regal, Corka and Sovereign, respectively. The bug's preference for Kestrel and Coleor could be partly caused by various cultivar characteristics such as digestibility, palatability, leaf to stem ratio, growth vigour, and concentrations of S-methyl cysteine sulphoxide (SMCO) compared with the other cultivars (PGG, 2009). Damage to the bugs' favoured cultivars can later lead to the death of the seedlings and further reduce their seedling number per unit area in brassica fields. Hence, this pest sometimes called a crop establishment pest (AgPest, 2016). The cultivars growth rate in both choice and no-choice tests were similar. However, the first obvious damage was noticed on Kestrel in both choice and no-choice tests. This could be the result of the more prompt settling and higher numbers of *N. huttoni* on Kestrel. The damage was slowest to appear on Corka in choice tests, and on Regal in no-choice tests. However, high mortality (70 – 80 %) of the bugs was recorded on all the cultivars, perhaps due to the limited availability of food in these experiments (Wei, 2001). The greatest reduction in plant dry weight occurred on Kestrel and Coleor. Higher numbers of bugs settled over time with a high survival rate on these cultivars (Figs 3.4, 3.5, 3.6 and 3.7). Gruner was the medium category of cultivar in terms of preference by the bugs. Although these cultivar rankings imply that Kestrel and Coleor could be avoided by growers, other more important agronomic factors such as yield and diseases resistance can be the main criteria for cultivar selection. For example, past studies on forage brassicas have mostly focused on varietal screening for resistance to clubroot disease and other aspects of varietal improvements (Asrat, Yesuf, Carlsson, & Wale, 2010; Bradshaw & Wilson, 2012) but not resistance to the N. huttoni. However, there is evidence that disease resistance in brassicas may be negatively correlated with insect resistance (Rostás & Hilker, 2002).

Bug preference for some cultivars may be affected by cues comprising volatile plant chemicals or by visual cues (Finch & Collier, 2000). Among plant chemicals, glucosinolates have been widely studied in crucifers and they can have feeding deterrent or stimulatory properties on generalist or specialist insects, respectively (Renwick, 2002). Further, the variation in glucosinolate profile

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between cultivars can also affect the host-plant preference (Poelman, Dam, Loon, Vet, & Dicke, 2009). However, the chemical basis of resistance to *N. huttoni* on forage brassicas is not known.

Global agriculture is beginning to adopt 'sustainable intensification' approaches (Pretty et al., 2018). Reasons for this include insecticide resistance, along with a decline in the rate at which new insecticide molecules are developed (Hawkins, Bass, Dixon, & Neve, 2018; Nauen & Denholm, 2005). There is also increasing consumer resistance to pesticides in some markets (Wollaeger, Getter, & Behe, 2015).

An IPM strategy developed with farmer input has been suggested to reduce reliance on insecticides (Horrocks et al., 2018). While the results in this study show cultivar differences in *N. huttoni* susceptibility, further research is needed to investigate if the best cultivars in this study are also less susceptible to other potential insect pests of kale crops such as aphids, beetles and caterpillars. In the future, insecticide use in kale crops, and the potential environmental impacts could be minimised by incorporating less susceptible cultivars, such as Corka or Regal, into an integrated pest management programme with other management tools such as biological control and the use of 'soft' chemicals (Dent, 2000).

3.6 Conclusions

Integrated pest management uses a wide range of plant protection methods such as cultural, mechanical, biological, use of resistant cultivars, and subsequent integration of these measures can help reduce pest densities, and thereby reduce crop damage. The work presented here has focussed on finding an effective way to control the N. huttoni by using the potentially resistant/tolerant kale cultivars. It is recommended that kale cultivars such as Corka or Regal are used because they are relatively less susceptible to the N. huttoni. Susceptible kale cultivars such as Kestrel, Gruner, Sovereign, and Coleor should be avoided by farmers. The current work can help farmers choose appropriate crop cultivars to reduce pesticide costs. It is suggested that less susceptible kale cultivars such as Corka or Regal be integrated into other pest management approaches such as trap cropping, biological control and the use 'soft' chemicals for sustainable results. A pest management protocol can be developed using the two kale cultivars in a 'pushpull' pest management strategy. First, deployment of highly susceptible kale cultivars at field edges can attract ('pull') the bugs from the main crop and prevent bugs from entering the main field from outside the field boundary. Low preference kale cultivars in a main field can protect the bugs from landing, which works as a 'push' component. Secondly, the use of potential trap crops such as alyssum (Lobularia maritima) or wheat (Triticum aestivum) at field edges works as a 'pull' component and the less susceptible kale cultivars in the main field act as a 'push'

component to keep *N. huttoni* away from the main crop. However, when a cultivar has a lower degree of tolerance, other pest management approaches must be used to achieve a successful level of pest control to attain a desired profit level. Particular cultivars can be less susceptible to a particular insect or group of insects, but the crop can still be damaged by other insects and diseases. Current cultivar selection by farmers mostly depends on yield potential and disease resistant.

Chapter 4

Preferences of the wheat bug (*Nysius huttoni*) for particular growth stages of the potential trap plant, alyssum (*Lobularia maritima*)

A version of this chapter was published in 22 July 2019: Tiwari, S., Saville, D. J., & Wratten, S.D. (2019). Preferences of the wheat bug (*Nysius huttoni*) for particular growth stages of the potential trap plant, alyssum (*Lobularia maritima*). *New Zealand Plant Protection*. 72: 237-244. https://doi.org/10.30843/nzpp.2019.72.269.

4.1 Abstract

Wheat bug, *Nysius huttoni*, is a pest of brassica seedlings. This pest normally sucks sap from leaf veins, petioles and stems of young forage brassicas that become twisted and show withered leaves. *Nysius huttoni* preferences for brassicas varies within species and their phenologies. The non-crop brassica alyssum (*Lobularia maritima*) is a potential trap plant of *N. huttoni*, having the potential to keep the bugs away from seedlings.

Laboratory no-choice and choice tests evaluated the relative preference of *N. huttoni* for two major growth stages of alyssum – vegetative and flowering. In both bioassays, *N. huttoni* adults settled significantly more promptly on the flowering than on the vegetative stage. The same preference was evident for adult numbers settling. Survival was higher on the flowering (38 %) than on the vegetative stage (28 %), although this was not significant.

The implications of these findings are important in the design of trap cropping protocols for *N. huttoni* management. Flowering alyssum in brassica fields can also potentially improve pest biological control and provide other ES that can contribute to mitigating diminished ecosystem functions in agriculture.

Keywords: Alyssum, wheat bug, trap plant, choice test, ecosystem services

4.2 Introduction

The primary pest of forage brassicas is wheat bug, *Nysius huttoni* White 1878 (Hemiptera: Lygaeidae) (Eyles, 1965; He et al., 2003; Yang & Wang, 2004), an endemic New Zealand insect

(Aukema et al., 2005; Eyles, 1960b; He et al., 2003). This pest is widely distributed in New Zealand's South and North Islands from sea-level to 1800 m (Eyles, 1960b; Eyles & Ashlock, 1969; Myers, 1926). Wheat and forage brassicas are the primary hosts of this pest but its associations cover > 75 plant species belonging to > 25 plant families (Wei, 2001). Although it is a seed feeder, it also feeds on plant host stems, petioles, leaves and fruits (He & Wang, 1999). The damage is most obvious in seedling brassicas; 70 – 90 % damage has been reported in New Zealand (AgPest, 2016; Speciality Seeds, 2016). Bug damaged wheat grains can reduce flour's baking quality (Every et al., 1998). Insecticide use is the usual practice for N. huttoni management in New Zealand although specialised trap plants is a potential alternative management option (AgPest, 2016; Tiwari et al., 2019; Wei, 2001). However, a number of cultural practices such as field sanitation, and the use of less susceptible cultivars, are recommended to reduce bug populations in brassica fields (AgPest, 2016; Tiwari et al., 2019; Wei, 2001). A series of previous laboratory, field cage and open field experiments studied a range of potential trap plant species by this bug. The results showed that the popular garden plant alyssum, Lobularia maritima L. Desv. (Brassicaceae), has a greater potential to trap this bug than other plants such as Triticum aestivum L. (wheat), Phacelia tanacetifolia Bentham (phacelia), Fagopyrum esculentum Moench (buckwheat), Coriandrum sativum L. (coriander), Trifolium repens L. (white clover), Medicago sativa L. (lucerne) and kale (Brassica oleracea L.) (Tiwari et al., 2018). That work was the first study evaluating potential trap plant species for this pest. Benign methods to protect the crop can be achieved either by preventing the pest from crawling into the crop or by concentrating the bug in a particular part of the field where it can be economically managed by mechanical, biological or any 'soft' chemical pesticides (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). However, this bug, like other herbivores, shows potentially strong preferences for particular plant parts. Therefore, the current study involved an experiment to evaluate the host selection behaviour of N. huttoni between two growth stages of alyssum in laboratory no-choice and choice bioassays. The second important potential wheat trap plant was not considered in this study because of its non-significant role in conservation biological control.

The experiment evaluated the host selection behaviour of *N. huttoni* between two alyssum growth stages. The parameters considered were: time for the first insects to settle; rate of colonisation of the plant and survival rate of the insect. The results presented here can help inform decisions on effective trap cropping for the *N. huttoni*.

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4.3 Materials and methods

4.3.1 Insect and plant preparation

An experiment was conducted in a controlled temperature (CT) room at the Bio-Protection Research Centre, Lincoln University, New Zealand, to evaluate the most suitable growth stage of alyssum for the *N. huttoni*. Seeds of alyssum (*L. maritima* cv. Benthamii White) were sown in 144 - cell trays in a glasshouse using a Dalton organic potting mix (composted bark, coco fibre, NuFert and pumice) at weekly intervals from 1 November to 20 December, 2017, to ensure a regular supply of specific growth stages of alyssum for the experiments. Plants were watered regularly. Alyssum seeds were obtained from PGG Wrightson, Canterbury, New Zealand. Seedlings were grown in cell trays for 13 days after sowing then transplanted into pots (6.5 cm diameter and 5.0 cm high) with two seedlings/pot. Two cohorts of the plants were grown for 21 days (vegetative stage) and 42 days (flowering stage) respectively in a glasshouse and transferred to a CT room for bioassays. The temperature, photoperiod and RH of the CT room were maintained at 22 °C with a 4 °C range, 16L: 8D h, and 60% (with a 10 °C range) humidity.

4.3.2 No-choice and choice assays

For the no-choice tests, the two seedlings of one stage of alyssum (see above) were planted in the centre of each pot. In the choice tests, two seedlings of each stage were planted in a single pot. In 'choice' pots, stages were 2.5 cm apart and 0.5 cm away from the pot margin. No-choice tests were carried out from 12 to 24 December 2017 and choice tests from 11 to 22 January 2018. A randomised block design, with 14 replicates for the no-choice tests and 12 replicates for the choice tests was used. Twenty newly-emerged N. huttoni adults for each test were released in the centre of each pot, which was covered by a cylindrical sleeve (flexible transparent PVC sheet, 1 mm thick). The sleeve was 7 cm in diameter and 18 cm high and was used in both types of tests. The sleeve tops were covered by a fine white mesh and Fluon (BioQuip, fluoropolymer resin, PTFE - 30) was used on the inner surface of the sleeves to prevent N. huttoni climbing. In the no-choice tests, the mean alyssum height was 6.7 ± 0.27 (SEM ⁷) cm for the vegetative stage and 13.3 ± 0.37 (SEM) cm for the flowering cohort. In the choice tests, the mean height was $7.1 \pm$ 0.29 (SEM) cm for the vegetative stage and 13.0 cm ± 0.42 (SEM) cm for the flowering stage. The N. huttoni colony was maintained in a controlled temperature (CT) room as above to provide a regular supply of the bug for the experiment (Fig. 4.1 a). *Nysius huttoni* numbers settling at each growth stage of alyssum were counted at 2 h, 4 h, 17 h, 21 h, 41 h, 45 h, 65 h, 93 h, 108 h, 141 h,

⁷ Standard error of mean

156 h, 165 h, 189 h, 204 h, 213 h, 228 h, 252 h and 261 h after release of the bug. Time to first settlement (mins) and survival rate at 261 h (no-choice tests only) were also quantified.

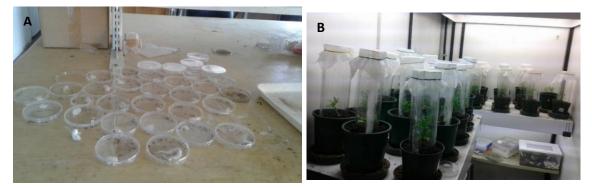




Figure 4.1 Alyssum stage choice and no-choice experiments maintained in a controlled temperature (CT) room at the Bio-Protection Research Centre (BPRC), Lincoln University.
a) *Nysius huttoni* starved for 12 h before release into the choice and no-choice pots; b) Choice tests arranged in a randomized block design in a CT room; c) No-choice flowering alyssum plant inside the cylindrical polythene sleeve.

4.3.3 Data analysis

The mean number of *N. huttoni* recorded on each alyssum stage over 261 h was calculated by using the area under the curve method (AUC) (Hanley & McNeil, 1983). These data were first square-root transformed to achieve adequate normality before AUC averaging. First settlement times (mins) were logarithm transformed (log₁₀). The number of insects settling over time and first settlement time (h) for each stage were compared by using a paired sample *t*-test using the GenStat statistical package (GenStat 16, VSN International, Hemel Hempstead, Hertfordshire HP1 1ES, United Kindom). The survival rate (%) at 261 h at each stage followed an approximately

normal distribution by the Central Limit Theorem (Wood & Saville, 2013), so a paired sample *t*-test was used for the comparison of the means.

4.4 Results

4.4.1 First settlement time

Nysius huttoni settled significantly earlier on the flowering than on the vegetative stage in nochoice tests (t = -2.5; df = 13; p = 0.026) and choice tests (t = -5.6; df = 11; p < 0.001) (Fig. 4.2). In the latter tests, the bug took a mean at 14.07 (log₁₀ transformed = 1.27) minutes for first settlement on the vegetative stage and 9.92 (log₁₀ transformed = 0.95) minutes on the flowering stage of alyssum. In the choice tests, the bug took approximately 12.5 (log₁₀ transformed = 1.15) minutes for the first settlement on the vegetative stage and 5.25 (log₁₀ transformed = 0.49) minutes on the flowering stage (Fig. 4.2).

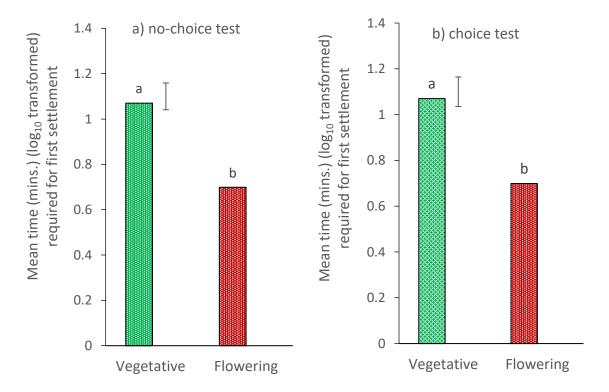


Figure 4.2 Mean times (log_{10} transformed) required for first settlement on the two growth stages of alyssum plant in no-choice (n = 14) and choice tests (n = 12). The vertical bar is the least significant difference, LSD (5 %). In each test, plant stages were compared using a

paired samples *t*-test (p < 0.05). Means with no letters in common are significantly different (p < 0.05).

4.4.2 Nysius huttoni populations over time

The time spent of *Nysius huttoni* on each of the two alyssum stages over the 261 h of the experiment varied significantly (p < 0.05). Numbers were significantly higher at the flowering stage than on the vegetative one in the no-choice (t = 3.39; df = 13; p = 0.004) (Fig. 4.3 a) and choice tests (t = 12.4; df = 11; p < 0.001) (Fig. 4.3 b). In the no-choice tests, the numbers of *N. huttoni* counted on each stage were not significantly different at 2 h, 41 h, 45 h, 65 h, 93 h, and 261 h (Table 4.1). By comparison, in the choice tests, the numbers collected on each stage differed significantly at each sampling time from 2 h to 261 h (Table 4.2).

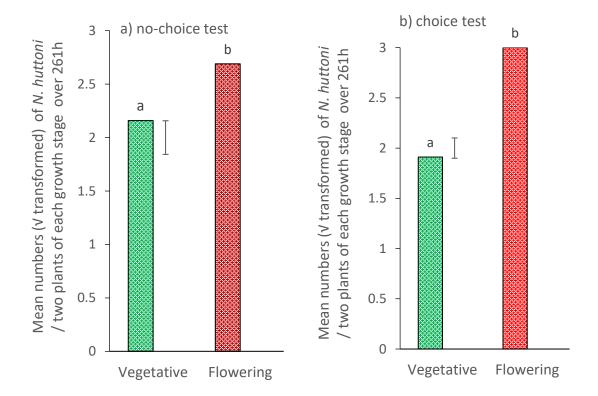


Figure 4.3 (a) a no-choice and (b) a choice tests in the laboratory. Mean numbers (V transformed) of *Nysius huttoni* adults on two alyssum growth stages over 261 h in no-choice (n = 14) and choice tests (n = 12). The vertical bars are the least significant differences, LSD (5 %). In each test, plant stages were statistically compared using a paired sample *t*-test (p < 0.05) (n = 14 and 12, respectively). For each figure, means with no letters in common are significantly different (p < 0.05).

Table 4.1 In no-choice tests, the mean number (V transformed) of adult *Nysius huttoni* recorded on two alyssum stages at 2 h to 261 h plus the 261 h weighted mean calculated by the area under the curve (AUC) method.

Alyssum							Mean	numbers	(V transf	ormed) o	f Nysius h	nuttoni							AUC
stages																			Mean
	2 h	4 h	17 h	21 h	41 h	45 h	65 h	93 h	108 h	141 h	156 h	165 h	189 h	204 h	213 h	228 h	252 h	261 h	
Vegetative	2.24a	2.01a	2.15a	2.57a	2.11a	2.46a	2.01a	2.48a	2.27a	1.85a	2.02a	2.06a	2.12a	1.55a	1.96a	1.94a	1.86a	1.42a	2.09a
Flowering	2.68a	2.73b	2.66b	2.97b	2.49a	2.82a	2.30a	2.82a	2.81b	2.57b	2.60b	2.56b	2.76b	2.21b	2.73b	2.62b	2.52b	2.03a	2.65b
LSD (5 %)	0.49	0.37	0.47	0.37	0.06	0.44	0.46	0.49	0.42	0.44	0.49	0.36	0.50	0.52	0.55	0.47	0.38	0.68	0.32
<i>p</i> - value	0.080	< 0.001	0.036	0.034	0.401	0.107	0.219	0.157	0.014	0.003	0.020	0.008	0.014	0.014	0.008	0.006	0.001	0.079	0.002
Significance	ns	***	*	*	ns	ns	ns	ns	*	**	*	**	*	*	**	**	**	ns	**

Means within a column with no letters in common are significantly different (a paired sample t-test; p < 0.05) (n = 14). For each alyssum stage and pot, the 261 h weighted mean was obtained by calculating AUC for a graph of V count against time (h) using the trapezoid rule, then dividing by the time period (261 h). (ns) non - significant; (*) p < 0.05, significant; (**) p < 0.01, very significant; (**) p < 0.001, extremely significant.

Table 4.2 For the choice tests, mean numbers (V transformed) of adult *Nysius huttoni* recorded on two alyssum stages at times 2 h to 261 h plus 261 h weighted mean calculated by the area under the curve (AUC) method.

Alyssum							Mean	numbers	(V transf	ormed) o	of Nysius	huttoni							AUC
stages _																			Mean
	2 h	4 h	17 h	21 h	41 h	45 h	65 h	93 h	108 h	141 h	156 h	165 h	189 h	204 h	213 h	228 h	252 h	261 h	
Vegetative	1.54a	2.09a	1.90a	2.15a	1.72a	2.14a	1.79a	2.10a	1.92a	1.77a	1.54a	1.98a	2.06a	1.74a	2.00a	1.63a	1.62a	1.34a	1.84a
Flowering	3.15b	3.05b	3.18b	3.23b	2.94b	2.76b	2.72b	2.93b	3.13b	2.89b	3.15b	2.90b	3.12b	2.98b	3.04b	2.93b	2.78b	2.68b	2.98b
LSD (5 %)	0.29	0.44	0.40	0.29	0.54	0.49	0.54	0.48	0.48	0.55	0.53	0.42	0.31	0.52	0.45	0.42	0.37	0.59	0.20
<i>p</i> - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.011	0.002	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Significance	***	***	***	***	***	*	**	**	***	***	***	***	***	***	***	***	***	***	***

4.4.3 Survival rate

The survival rate of *N. huttoni* did not differ significantly between the two alyssum stages. Only nochoice tests were carried out for this parameter (t = 1.121; df = 13; p = 0.282). Survival at flowering was 38 % and at the vegetative stage was 28 % (Fig. 4.4).

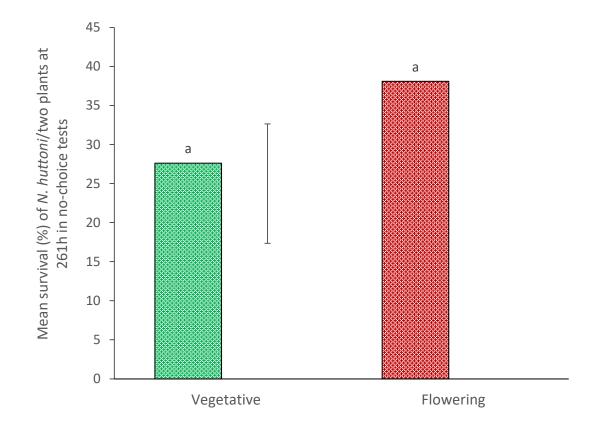


Figure 4.4 No-choice laboratory tests. Mean survival (%) of *Nysius huttoni* adults on two growth stages of alyssum plant at 261 h after release of *Nysius huttoni* (n = 14). The vertical bar is the least significant difference, LSD (5 %). Mean survival was statistically compared using a paired samples t-test (p < 0.05). Means with no letters in common are significantly different (p < 0.05).

4.5 Discussion

The study examined the host plant selection of *N. huttoni* in choice and/or no-choice tests between two growth stages (vegetative and flowering) of alyssum. This study provided evidence that the flowering stage of alyssum is more attractive to *N. huttoni* than the vegetative stage so the flowering stage is potentially more suitable as a trap crop than the vegetative stage of the plant. This result was similar to the conclusions of Yang, Hu, van Santen & Zeng (2017) for the kudza bug *Megacopta cribraria* Fab. (Heteroptera: Plataspidae) in soybean (*Glycine max* L.) in which preference of *M. cribraria* on the flowering stage of soybean was higher than on the vegetative, pod or seed stages of that crop. Several authors have also demonstrated similar preferences to the flowering stage by other species of insect. For example, Apolygus lucorum Meyer-Dür (Hemiptera: Miridae) was shown to prefer the flowering stages of Vigna radiata (L.) Wilczek (Leguminosae), Gossypium hirsutum L. (Malvaceae), Helianthus annuus L. (Compositae) and Chrysanthemum coronarium L. (Compositae) over the vegetative stages of these plants (Pan, Lu, Wyckhuys, & Wu, 2013). In cotton (G. hirsutum) crops, Lygus hesperus Knight was more attracted to the flowering stage of the trap-crop species, alfalfa (M. sativa) and Russian thistle (Salsola iberica L.), than it was to flowering stages of sunflower (H. annuus) and pigweed (Amaranthus palmeri L.) (Barman, Parajulee, & Carroll, 2010). In another study, flowering sunflower and seed-head stage of sorghum, Sorghum bicolor L. Moench, were used as trap crops for the brown marmorated stink bug, Halyomorpha halys Stål (Hemiptera: Pentatomidae), in an organic pepper field (Mathews et al., 2017). Preferences of this pest were also shown to be for the reproductive structure of other vegetables, especially those that have extended fruiting periods, such as sweet corn (Zea mays saccarata Sturt), okra (Abelmoschus esculentus L.) and bell pepper (Capsicum annuum L.) (Zobel, Hooks, & Dively, 2016). Flowering sunflower (H. annuus) or flowering lucerne (M. sativa) were more attractive to the European tarnished plant bug, Lygus rugulipennis (Heteroptera: Miridae), than to flowering cucumber (Cucumis sativa L.) (Ondiaka et al., 2016). The green stink bug (Nezara viridula L.) can be trapped in the panicles of sorghum and at the seed stage of sunflower rather than at their vegetative stages (Gordon, Haseeb, Kanga, & Legaspi, 2017). It was also trapped by the fruits of beans (Phaseolus vulgaris L.) around sweetcorn (Z. mays) fields in New Zealand (Rea et al. 2002). Flowering host plants, such as tobacco (Nicotiana tabacum L.) and sunflower increased the oviposition preference and larval performance of Helicoverpa armigera L. (Lepidoptera: Noctuidae) compared with their vegetative stage (Liu, Schiers, & Heckel, 2010).

Physical, nutritional and chemical cues are responsible for the attraction of herbivores to host plants (Bernays & Chapman, 2007; Hokkanen, 1991; Lucas-Barbosa, van Loon, & Dicke, 2011; Shelton & Badenes-Perez, 2006). In general, the flowering stage of a plant releases more volatile chemicals (Ceballos, Fernández, Zúñiga, & Zapata, 2015) and provides nutritional rewards to many generalist herbivores than other stages (Wäckers, Romeis, & Rijin, 2007). However, the type of volatiles and their concentrations can vary between growth stages (Silva, Carrao-Panizzi, Blassioli-Moraes, & Panizzi, 2013). Such volatiles emitted by alyssum flowers could be extracted and artificially produced in a laboratory. Exogenous application of such volatiles to alyssum flowering strips could potentially increase *N. huttoni* trapping efficacy (Bruce, Wadhams, & Woodcock, 2005). However, this idea needs to be verified by, for example, beginning with Y-tube olfactometer tests in the laboratory.

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Habitat manipulation with the provision of floral resources may increase the fitness of natural enemies (NEs) (Gurr et al., 2017; Lichtenberg et al., 2017) and reduce pest populations (Tscharnkte et al., 2005; Gurr et al., 2016). Added flowering plants in forage brassicas potentially increase the population of natural enemies of *N. huttoni* and may reduce *N. huttoni* populations and other pests in brassica fields (Wei, 2001). Appropriate trap plants added to an agro-ecosystem can provide shelter, nectar, alternative food and pollen (SNAP) for beneficial arthropods such as predators and parasitoids, which can increase their fitness and efficacy and potentially improve the provision of multiple ES in and outside the farm (Gurr et al., 2017). For example, the use of flowering alyssum and buckwheat in apple orchards increased the populations and parasitism rates of the parasitoid Dolichogenidea tasmanica Cameron (Hymenoptera: Braconidae) on the larvae of the light-brown apple moth, Epiphyas postvittana Walker (Lepidoptera: Tortricidae) (Irvin et al., 2006). Furthermore, alyssum flowers in laboratory studies increased the longevity, fecundity and sex ratio of the above parasitoid (Berndt & Wratten, 2005) and also increased the activity of hover flies (Diptera: Syrphidae) (Colley & Luna, 2000). In some cases, those flowering plants in an agroecosystem promote ecosystem dis-services (benefiting pest's more than natural enemies) (Baggen & Gurr, 1998; Gurr et al., 2017). For example, the soybean hairy caterpillar, Spilarctia casigneta Rothschild (Lepidoptera: Erebidae), a pest of soybean and other brassica crops damaged the alyssum flowers which was planted to improve the CBC of radish pests in radish field (Tiwari et al. unpublished data).

The use of appropriate plant phenology is an important parameter for efficient trapping of insect pests (Hokkanen 1991; Shelton & Badenes-Perez, 2006). In the current system, alyssum trap plants should be cultivated so that they flower when kale plants are at the seedling stage to maximise their effectiveness as a trap plant (Shelton & Badenes-Perez, 2006). However, careful attention should be given to the beneficial arthropods and pollinators while using pesticides to manage the trapped *N. huttoni* in flowering alyssum plants in brassica fields (Hokkanen, 1991). In summary, 'push-pull' bug management protocol (Khan et al., 2001) can be developed by using a less susceptible kale cultivar in brassica fields as a 'push' factor (keep the bugs away from main crop) (Tiwari et al., 2019) and potential trap plant 'alyssum' (Tiwari et al., 2018) and their preferred growth stages 'flowering stage' as a 'pull' factor to attract *N. huttoni* from the main crop that can also support CBC and enhancement of natural enemies.

4.6 Conclusions

Alyssum (*L. maritima*) is a potential trap plant for the *N. huttoni*. The laboratory study examining the seedling stage of potential trap plant species (alyssum, wheat, buckwheat, phacelia, clover,

lucerne, coriander, and kale) suggests that the seedling alyssum is more preferred by *N. huttoni* than other tested plant species. In open field conditions, information regarding the duration of alyssum plant establishment is lacking. This study suggests that alyssum at its flowering stage is more preferred by *N. huttoni* than seedling stages. The reasons why the flowering stage of alyssum plant is more preferred by the *N. huttoni* are unclear, but the information is important for designing a trap cropping protocol for *N. huttoni* management in brassica fields.

It is concluded that maintaining alyssum plants in their flowering stage at the seedling stage of forage kale can trap the *N. huttoni* more effectively and thereby reduce damage on kale seedlings. Flowering alyssum can also improve CBC in brassica fields by providing SNAP for beneficial natural predators and parasitoids. This is a good example of multiple ES provided by the flowering plants in an agro-ecosystem.

Chapter 5

Evaluation of potential trap plant species for the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) in forage brassicas

A version of this chapter has been accepted for publication: Tiwari, S., Saville, D.J., & Wratten, S.D. (accepted on 19 August 2019). Evaluation of potential trap plant species for the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) in forage brassicas. *Agricultural and Forest Entomology*.

5.1 Abstract

The wheat bug, *Nysius huttoni* is a major pest of brassica seedlings. Management of this insect currently relies on seed treatment with neonicotinoids and spraying with chlorpyrifos and pyrethroid insecticides. These practices can generate severe external costs, including human health, the environment and biodiversity. Trap cropping is one alternative option to protect brassica seedlings from the bug's damage.

Experiments were established in field cages and open fields at Lincoln University, New Zealand, to evaluate potential trap plant species for *N. huttoni*. Species evaluated in field-cage experiments were: alyssum (*Lobularia maritima*), wheat (*Triticum aestivum*), coriander (*Coriandrum sativum* L) and clover (*Trifolium repens*). These were compared with kale (*Brassica oleracea*). In open-field experiments, some of the above treatments were used. Those discarded were clover (*T. repens*) and coriander (*C. sativum*), because of the previous poor performance of these two species.

The main aim of study is to select suitable trap plants of the *N. huttoni* from a wide range of potential trap plants. Alyssum and wheat were the most favoured potential trap plants for the bugs, with a significantly higher survival rate on those plants in field-cage experiments compared with clover, coriander and kale. This was also the case in the open-field experiments, resulting in lower numbers of bugs and less damage in kale plots next to the wheat, alyssum and 'alyssum plus wheat' strips compared with those for kale strips. Results indicated that two treatments: alyssum (used as a single trap crop) or 'alyssum plus wheat' (a multiple trap crop) may be useful in and around brassica fields to protect the seedlings from the bugs' damage. The effects of the best trap-crop species or combinations of them on *Nysius* numbers declined with distance from those strips.

Such a trap-cropping protocol potentially reduces pesticide use in forage brassicas, and can also deliver multiple ecosystem services (ES) such as biological control of insect pests.

Keywords: Trap cropping, forage brassica, alyssum, wheat bug, Nysius huttoni, ecosystem service

5.2 Introduction

Wheat bug, *Nysius huttoni* White 1878 (Hemiptera: Lygaeidae), is a pest of many New Zealand, crops, reducing the yield of forage brassicas by 70 - 90 % (AgPest, 2016; Eyles, 1965) and wheat yields by 90 % in some cultivars (Every et al., 1998). It is an endemic New Zealand insect (Eyles, 1960a) and is considered a major threat to cultivated crops such as barley, oats, clover, lettuce and is found on many weed species (Bejakovich et al., 1998; He & Wang, 1999; Miller & Pike, 2002). It damages plants by sucking the phloem liquid from the leaves, stems and seeds (Aukema et al., 2005; He et al., 2003). 'Bug-damaged wheat' contains salivary enzymes that reduce the baking quality of flour (Every et al., 1992). This pest has been accidentally introduced to The Netherlands and Belgium during apple transport from New Zealand (Aukema et al., 2005; Bonte et al., 2010).

The management of N. huttoni is difficult because of its high mobility, and wide host range (Farrell & Stufkens, 1993). Partly because of this, prophylactic use insecticides is the primary means of managing this bug (AgPest, 2016). Typically, neonicotinoid insecticides are mixed with other agrochemicals in seed dressings for N. huttoni management (Young, 2018). Chlorpyrifos and permethrin sprays are also used when the first sign of N. huttoni damage is detected in the field (Chapman, 2010). These practices have negatively influenced agroecosystem functional biodiversity such as the actions of natural enemies and pollinators (Heard et al., 2017; Pamminger, Botías, Goulson, & Hughes, 2018). The loss of functional biodiversity further exacerbates the on-farm costs of production as well as external costs such as human and environmental health (Becker, 2017; Carvalho, 2017; Ramankutty et al., 2018; Rayl, Shields, Tiwari, & Wratten, 2018; Williams, 2015). Ecological pest management such as trap cropping (Gurr et al., 2017; Reddy, 2017; Shelton & Badenes-Perez, 2006), cover cropping (Storkey et al., 2015), use of flower strips (Westphal et al., 2015) etc. have been recognized as a core concept of habitat manipulation of pests in integrated pest management (IPM) (Evans, 2005). Trap cropping was a common cultural pest management practice in several agro-ecosystems before the invention of synthetic chemical insecticides (Hokkanen, 1991; Talekar & Shelton, 1993). Growing one or more trap plant species adjacent to or within the main crop, and at farm or landscape scales (Gurr et al., 2017; Landis et al., 2000) can reduce the pest population density in the main crop (Shelton & Nault, 2004). Hokkanen (1991) and Shelton and Badenes-Perez (2006) confirmed that trap cropping had the potential to manage the pests in field crops. The idea was that when pests used the trap crops, the main crops would be

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protected from this pest (Hokkanen, 1991). The main crop can be protected either by preventing the pests from reaching it (Rea et al., 2002) or by leading them to a certain part of the field where the insect can be economically managed, either by removal of the trap refuges and pest together or using insecticides locally (Shelton & Nault, 2004). Recent laboratory studies on *N. huttoni* hostplant selection confirmed that alyssum, *Lobularia maritima* L. Desv. (Brassicaceae) and wheat, *Triticum aestivum* L. (Poaceae) were potential trap plants for that pest (Tiwari et al., 2018). However, in order to develop an IPM strategy using these plants in trap cropping, they need to be evaluated under field conditions.

Field cages and open-field experiments were conducted at Lincoln University, New Zealand to evaluate potential trap plants of *N. huttoni*. Flowering or non-flowering trap plants potentially provide multiple ecosystem services (ES) in an agro-ecosystem, such as weed suppression and enhanced biocontrol through SNAP (shelter, nectar, alternative hosts and pollen) to enhance the 'fitness' of natural enemies and also pollinators (Gurr et al., 2017).

5.3 Materials and methods

5.3.1 Colony management

In Spring 2016, *N. huttoni* was collected at Lincoln University (43° 38' S; 172° 27' E), New Zealand by using a suction machine (Shred n Vac Plus[™], Stihl BG 75, USA, 80 cm length x 12 cm inlet diameter) from shepherd's purse (*Capsella bursa-pastoris* L. Medik : Brassicaceae). Laboratory colonies were maintained in a controlled temperature (CT) room in circular Petri dishes (13.5 cm diameter) and the insect was provided with twin cress, *Coronopus didymus* (L.) Smith (Brassicaceae), and seeds of sunflower (*Helianthus annuus* L. cv. Golden Toasted) as their food materials. The temperature, photoperiod and humidity in the CT room were maintained at 22 °C with a 4 °C range, 16L: 8D h, and 60 % RH, respectively.

5.3.2 Field-cage experiment

In 2017, field-cage experiments were established from February to April at the organic Biological Husbandry Unit (BHU) (https://www.bhu.org.nz), at Lincoln University, New Zealand. Potential trap plant species evaluated were alyssum, *L. maritima* cv. Benthamii White, wheat, *T. aestivum* cv. Morph, coriander, *Coriandrum sativum* L. cv. Santo and clover, *Trifolium repens* L. cv. Nomad, all of which were compared to kale (control) (*Brassica oleracea* L. cv. Kestrel). Cylindrical field cages were made from an iron frame (50 cm height and 30 cm diameter) and covered by fine high-density polyethylene (HDPE) mesh (0.6 x 0.6 mm).

Seedlings of potential trap plants (see above) were grown in a glasshouse in cell trays using organic potting mix composed of composted bark, coco fibre, NuFert and pumice and were irrigated daily. Five seedlings of each trap plant species (18 days old, 3 - 5 true leaves and no reproductive buds) were transplanted inside each cage on 20 February 2017. Each cage was fixed into the soil with bifurcated aluminium fixers, with 1 m between adjacent cages. The mean height of seedlings at transplanting was 6.4 cm for alyssum, 7.7 cm for wheat, 5.5 cm for clover, 8 cm for coriander and 7 cm for kale (control).

The cages were arranged in a randomized complete block design (RCBD). Ten field cages (five cages with *Nysius* and five without) were randomly allocated in two rows for each block, five cages in each row (Fig. 5.1). Each treatment cage was compared with its control cage to confirm the extent to which seedlings were discoloured, leaf distorted, wilted or dead because of *N. huttoni*. On 5 March 2017, each *N. huttoni* designated cage received ten pairs (male and female) of *N. huttoni*. The cages were covered by a transparent polythene sheet (600 mm x 900 mm) on 11 March 2017 to protect them from rain and wind. An additional ten pairs of *N. huttoni* were released on 17 March 2017 into each treatment. Aphid, *Myzus persicae* (Sulzer) populations began to develop in some of the cages and these were managed by releasing parasitoid wasps (*Aphidius colemani* Viereck), obtained from Bioforce (https://www.bioforce.co.nz), into each cage. Weeds were manually removed.

The numbers of *N. huttoni* colonising on each plant species were observed over 29 days after release. The numbers of *N. huttoni* settled on each plant species were counted on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 21, 22, 26 and 29 days after introduction. The mean numbers of *N. huttoni* settled over time were calculated by the area under the curve (AUC) method (Hanley & McNeil, 1983). The overall survival of *N. huttoni* in each cage was recorded at 55 days after *N. huttoni* release on 28 April 2017.



Figure 5.1 Field cage experiments at the Biological Husbandry Unit (BHU), Lincoln University. a)
Field cages arranged in a randomized block design; b) Alyssum plants inside a field cage; c)
Kale (control) plants inside a field cage; d) Petri dishes and soil preparation from each field
cage to measure the soil moisture; e) Digital stem thermometer to measure the soil
temperature.

5.3.3 Open-field experiment

A field experiment was established at the Biological Husbandry Unit (BHU) (see above) and ran until 15 January 2018. Trap plant treatments were: alyssum, wheat, alyssum plus wheat, with all trap plant species compared to kale (control). All seeds were purchased from PGG Wrightson (<u>https://www.pggwrightson.co.nz</u>) and complied with BioGro NZ (https://www.biogro.co.nz) organic certification standard.



Figure 5.2 Open field experiments at the Biological Husbandry Unit (BHU), Lincoln University. a) Alyssum seedlings grown in cell trays in a glasshouse; b) Open field experiment arranged in a randomized block design; c) Weeds collection and displayed in a table for the identification; d) Kale plants were taken to measure the damage by *Nysius huttoni*

There were five blocks and four replicate plots in each block. Plots were 7 m x 4 m, with 1 m between them and 4 m between the blocks. Each block measured 147 m² (7 m x 21 m). Trap plant species were arranged in a randomized complete block design (RCBD). Three blocks ran parallel to a shelter belt (7 m wide x 71 m long), with the other two alongside (7 m wide and 46 m long), further 22 m out from the shelter belt. The field was bordered on the east, west and north sides by weedy vegetation, and to the south by poplar trees (*Populus* spp.: Salicaceae), c. 25 m in height (Fig. 5.2).

The trap strips (1 m wide and 4 m long) were established at the edges of each kale plot (6 m wide and 4 m long) on 21 October, resulting in a 7 m wide and 4 m long plot. Kale seeds were drilled in each kale plot using hand drill on 20 October 2017 and thinned when mean kale seedling height was 8 cm to establish a spacing between the plant of 25 cm x 12.5 cm. The plant spacing for alyssum in the trap strips was 10 cm x 10 cm, in a total of 10 rows and 40 seedlings/row (400 seedlings / (4 m²)). Wheat and kale seeds were sown at 10 cm x 10 cm and 25 cm x 12.5 cm spacing, respectively, i.e., 40 wheat seedlings / row (400 wheat seedlings /(4 m²)) and 32 kale seedlings / row (128 seedlings / (4 m²)), respectively. In 'alyssum plus wheat' strips, the two species were established in alternate rows (5 rows for wheat and 5 rows for alyssum) at a 10 cm x 10 cm spacing (200 alyssum seedlings and 200 wheat seedlings / (4 m²)). The wheat seeds were resown on 30 October 2017 due to bird damage and were then covered by a bird net.

Kale plots and trap strips were scouted daily for the arrival of local populations of *N. huttoni* (Fig. 5.3) and when adults were first observed, *N. huttoni* densities were sampled using a quadrat (0.5 m x 0.5 m) and a suction machine (see above). *Nysius huttoni* (adults and nymphs) samples were recorded approximately weekly from 12 November 2017 to 15 January 2018 on days 29 (12 Nov. 2017), 40 (23 Nov), 47 (30 Nov.), 51(4 Dec.), 54 (7 Dec.), 58 (11 Dec.), 62 (15 Dec.), 65 (18 Dec.), 68 (21 Dec.), 77 (30 Dec.), 82 (04 Jan. 2018), 89 (11 Jan.) and 93 (15 Jan.) days after planting.

Nysius huttoni samples (0.5 m x 0.5 m quadrat) were also collected from the adjoining weedy vegetations from shelterbelt (east) and cultivated field (west) sides of the research field from five locations and the common weed species were identified (Table 5.1). The phenological stages such as vegetative, flowering, fruiting, and senescence of alyssum plants and vegetative, flowering, seed ripening and senescence of wheat plants were recorded on each sampling date on days from 29 to 93 days after planting. Nysius huttoni samples were collected from each kale plot next to each trap plant species over the sampling periods from 29 to 93 days after planting. Damaged percentage of kale seedlings by the N. huttoni was recorded in 0.5 m x 0.5m quadrat on 11 Dec. 2017 (at 58 days after planting). Nysius huttoni normally damage forage brassicas at their seedling stages (aged 4 - 6 week). Percentage feeding damage on seedlings was measured by recording the presence or absence of girdling of the stem and/or discolouration of the leaf. Other common damage symptoms are leaf distortion, twisted leaf veins and petiole, and finally collapse of the seedlings. These symptoms are typical of Nysius. This bug greatly dominated catches of other Hemiptera in all samples. The abundance of *N. huttoni* was recorded at increasing distances from the edges trap strips (0 m) and to 2 m, 5 m, and 7 m distances from trap strips. The number of potential N. huttoni predators such as spiders, seven-spotted ladybirds (Coleoptera: Coccinellidae) and the lacewing, Micromus tasmaniae Walker (Neuroptera: Hemerobiidae) (Wei, 2001), was also counted (0.5 m x 0.5m quadrat) on each trap plant species and each sampling date (29 - 93 days after planting).

Flowering or non-flowering trap plants can provide shelter (e.g., refugia for overwintering), nectar, alternative food, and prey (SNAP) to the predators and parasitoids that can improve CBC and

declines in pest 'fitness' in an agro-ecosystem. Hence the purpose of recording beneficial arthropods in potential trap plants is to evaluate their suitability for the pest natural enemies.

Weed species	Common name	Family
Lolium multiflorum Lam.	Ryegrass	Poaceae
Bromus willdenowii Kunth	Prairie grass	Poaceae
Bromus hordeaceus L.	Soft brome	Poaceae
Avena fatuwa L.	Oat grass	Poaceae
Elytrigia repens (I.) Nevski	Couch grass	Poaceae
Stellaria media (L.) Vill.	Chickweed	Caryophyllaceae
Coronopus didymus (L.) Smith	Twin cress	Brassicaceae
Rumex obtusifolius L.	Dock	Polygonaceae
Plantago lanceolata L.	Plantain	Plantaginaceae
Cirsium arvense L. Scop.	Californian thistle	Asteraceae
Taraxacum officinale L. Weber ex F.H. Wigg.	Dandelion	Asteraceae
Trifolium repens L.	Clover	Fabaceae
Symphytum officinale L.	Comfrey	Boraginaceae
Dactylis glomerata L.	Cocksfoot	Poaceae
Althea officinalis L.	Marshmallow	Malvaceae
Capsella bursa-pastoris (L.) Medik.	Shepherd's purse	Brassicaceae
<i>Soliva sessilis</i> Ruiz & Pav.	Onehunga Weed	Asteraceae
Amaranthus powellii S. Wats.	Red root	Amaranthaceae
Beta vulgaris L.	Weed beet	Amaranthaceae
Pennisetum macrourum Trin.	Feather grass	Poaceae
Amaranthus graecizans L.	Pigweed	Amaranthaceae
Chenopodium album L.	lamb's quarters	Amaranthaceae
Veronica sps. L.	Gypsyweed	Plantaginaceae
Solanum nigrum L.	Black Nightshade	Solanaceae
Lolium perenne L.	Ryegrass	Poaceae
Polygonum aviculare L.	Common knotgrass	Polygonaceae
Marrubium vulgare L.	Horehound	Lamiaceae

Table 5.1 A list of weeds found in the open-field experiments at Lincoln University



Figure 5.3 Open field experiments at the Biological Husbandry Unit (BHU), Lincoln University. a) Open field experiments arranged in a randomized block design; b) *Nysius huttoni* samples taken by a suction machine in a kale field.

5.3.4 Statistical analysis

Field-cage experiment

The mean number of *N. huttoni* recorded on each plant species over the above period was statistically analysed by two - way analysis of variance (ANOVA). Data were square-root transformed prior to the area under the curve (AUC) analysis to meet the normality assumptions of the ANOVA. The percentage survival data at 55 days after *N. huttoni* introduction were analysed

using two-way ANOVA. Mean numbers and survival data were separated by the unprotected least significance difference (LSD) at p < 0.05 (Saville, 2015).

Open-field experiment

The mean numbers of *N. huttoni* recorded in each trap strip were calculated by the AUC method (Hanley & McNeil, 1983). Data were transformed to meet normality assumptions by using the square-root transformation prior to the AUC calculation. Data were then subjected to two-way ANOVA and multiple comparisons were done using unprotected LSD at p < 0.05 (Saville 2015). *Nysius* number collected from each trap strip on each sampling date were plotted with their standard error value for each date. Samples collected from each trap strip (alyssum, 'alyssum plus wheat' and wheat) were compared to kale (control) strips and analysed using a paired samples *t*-test at p < 0.05.

The numbers of *N. huttoni* (square-root transformed) in kale plots next to trap strips were first averaged by the AUC method and analysed by two - way ANOVA, with mean separation by unprotected LSD at p < 0.05. Damage percentage in the kale plots at 53 days after planting (DAP) was analysed similarly. For the density of *N. huttoni* from each trap strip (0 m) to 2 m, 5 m and 7 m distances, data were analysed as above.

The numbers of *N. huttoni* collected from alyssum and wheat strips were categorized into four phenological stages such as vegetative, flowering, fruiting, and senescence for alyssum, and vegetative, heading/flowering, seed ripening and senescence for the wheat. Data were square-root transformed prior to the AUC calculation, analysed by two-way ANOVA and means were separated by unprotected LSD at p < 0.05 (see above). A paired sample *t*-test at p < 0.05 was also used to assess any differences between the two treatments.

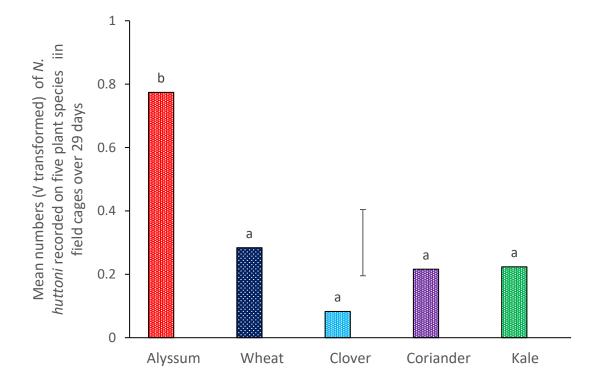
The numbers of predatory arthropods (square-root transformed) such as spiders, seven-spotted ladybirds and lacewings (*M. tasmaniae*) were averaged by the AUC method, used in a two-way ANOVA and tested for differences as above.

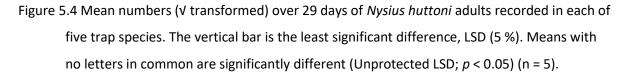
5.4 Results

5.4.1 Field-cage experiment

The mean numbers of *N. huttoni* adults on different trap plant species over 29 days differed significantly between plant species (p < 0.001). Except at 6 days, *N. huttoni* numbers on five potential trap plant species were not significantly different from 24 hours to 12 days after *N*.

huttoni release. However, their numbers were significantly different from 15 to 29 days of bugs released (Table 5.2). Significantly more *N. huttoni* adults were recorded on alyssum than on any other trap species (Fig. 5.4 and Table 5.2). The mean numbers of *N. huttoni* recorded on wheat, clover, coriander and kale were not significantly different to each other.





At 55 days, the survival rates of *N. huttoni* adults differed significantly between trap plant species (Fig. 5.5). Highest survival (16 %) was recorded on alyssum followed by wheat (10 %). These two plant species were significantly different from each other and from the others (p < 0.05). The survival on clover, coriander and kale did not differ significantly.

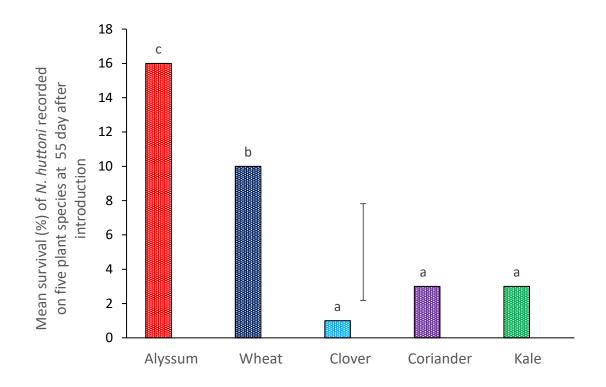


Figure 5.5 Mean survival (%) of *Nysius huttoni* adults on five plant species in field cages at 55 days after *N. huttoni* introduction. The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 5).

5.4.2 Open-field experiment

Nysius huttoni on the trap plants

The highest numbers of *N. huttoni* (adults and nymphs) were recorded in alyssum strips and were significantly higher than on the wheat and kale strips, respectively (Table 5.3). There were no significant differences between wheat and kale strips for both adult and nymphal populations (Figs 5.6 a and 5.6 b). Within the trap plant species, alyssum trapped significantly higher numbers of *N. huttoni* adults than did any other plant species evaluated (p < 0.001) (Fig. 5.6 a and Table 5.4). 'Alyssum plus wheat' strips were the second most effective followed by wheat and kale, respectively. The same trend of results were also recorded for *N. huttoni* nymphs (Fig. 5.6 b and Table 5.5). This study suggests that alyssum has the highest potential as a trap plant for *N. huttoni*. Furthermore, 'alyssum plus wheat' had the second highest potential to trap *N. huttoni* in brassica fields. The highest densities of *N. huttoni* were recorded at the cultivated field side (west, $\bar{x} = 8.015 \pm 0.846$, n = 5) of the research site than in the shelter belt side (east, $\bar{x} = 4.841 \pm 1.272$, n = 5), suggesting that *N. huttoni* had migrated from the cultivated field sides (west) into the research plots.

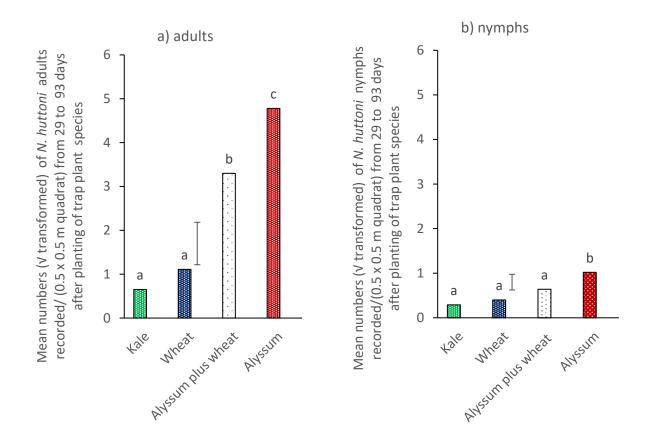


Figure 5.6 Mean numbers (V transformed) of *Nysius huttoni* / (0.5 m x 0.5 m quadrat) recorded in trap plants from 29 to 93 days after planting of trap species (n = 5). A = adults, B = nymphs. The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 5). For each plant species, the day 64 weighted mean was obtained by calculating the area under the curve (AUC) for a graph of V count against time (days) using the trapezoid rule, then dividing by the time period (64 days). Table 5.2 For the field-cage tests, the mean numbers (V transformed) of *Nysius huttoni* adults recorded on each of five plant species after 24h to 29 days plus the overall AUC mean.

Plant					Mean	numbers	(V trans	formed)	of adult	Nysius	huttoni					AUC
Species	1 d ⁸	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	12 d	15 d	21 d	22 d	26 d	29 d	Mean
Alyssum	0.20ab	0.20a	0.20a	0.20a	0.68a	0.60bc	0.60a	0.00a	0.88a	0.40a	0.68b	1.18b	1.21b	1.33b	1.29b	0.77b
Wheat	0.60b	0.20a	0.40a	0.40a	0.40a	0.80c	0.40a	0.00a	0.97a	0.40a	0.00a	0.00a	0.40a	0.20a	0.20a	0.28a
Clover	0.00a	0.00a	0.20a	0.40a	0.00a	0.00a	0.20a	0.00a	0.40a	0.20a	0.00a	0.00a	0.00a	0.00a	0.00a	0.08a
Coriander	0.00a	0.40a	0.20a	0.00a	0.20a	0.20ab	0.20a	0.20a	0.68a	0.20a	0.20ab	0.40a	0.20a	0.00a	0.20a	0.21a
Kale	0.60b	0.40a	0.00a	0.00a	0.68a	0.20ab	0.60a	0.20a	0.80a	0.20a	0.00a	0.20a	0.00a	0.20a	0.20a	0.22a
LSD (5%)	0.536	0.656	0.636	0.561	0.767	0.545	0.764	0.328	0.622	0.650	0.502	0.569	0.538	0.715	0.678	0.209
SEM	0.179	0.219	0.212	0.187	0.256	0.182	0.255	0.110	0.207	0.217	0.168	0.190	0.179	0.238	0.226	0.070
Significance	*	ns	ns	Ns	ns	*	ns	ns	ns	Ns	*	ns	ns	**	**	* * *

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant species and arena, the 696h - hour weighted mean was obtained by calculating the area under the curve (AUC) for a graph of Vcount against time (h) using the trapezoid rule, then dividing by the time period (696 h) (n = 5). (ns) non - significant; (*) p < 0.05, significant; (**) p < 0.01, very significant; (**) p < 0.001, extremely significant.

Table 5.3 For the open field experiments, the mean numbers (V transformed) /(0.5 m x 0.5 m quadrat) of *Nysius huttoni* adults and nymphs recorded in each trap plant species after 29 to 93 days after planting (DAP) plus the overall AUC mean.

Plant species	Days after planting (DAP) plus overall AUC mean													AUC
										02.1	_ Mean			
	29 d	40 d	47 d	51 d	54 d	58 d	62 d	65 d	68 d	77 d	82 d	89 d	93 d	
Alyssum	0.97b	0.97b	1.34a	3.40b	1.52a	8.17c	7.17b	7.89b	7.67b	11.36c	11.17b	7.43c	7.70b	5.80c
Alyssum plus Wheat	0.00a	0.00a	0.97a	2.76ab	0.55a	4.50b	5.67b	6.64b	7.34b	5.70b	9.64b	4.98b	4.13ab	3.40b
Wheat	0.20a	0.20a	0.45a	1.18a	0.00a	0.60a	0.48a	1.57a	1.98a	1.96a	4.55a	3.06a	4.54ab	1.51a
Kale	0.20a	0.20a	0.89a	0.68a	0.28a	0.20a	0.95a	1.16a	1.34a	1.20a	1.89a	1.85a	1.87a	0.94a
LSD (5%)	0.662	0.662	1.764	2.268	1.731	3.342	2.681	1.519	2.494	2.030	2.802	1.651	3.697	0.993
SEM	0.215	0.215	0.573	0.736	0.562	1.085	0.870	0.493	0.809	0.659	0.909	0.536	1.200	0.322
Significance	*	*	Ns	*	Ns	***	***	***	***	***	***	***	*	***

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant species, the day 64 weighted mean was obtained by calculating the area under the curve (AUC) for a graph of V count against time (days) using the trapezoid rule, then dividing by the time period (64 days) (n = 5). (ns) non - significant; (*) p < 0.05, significant; (***) p < 0.001, extremely significant.

Table 5.4 For the open field experiments, the mean numbers (V transformed) /(0.5 m x 0.5 m quadrat) of *Nysius huttoni* adults recorded in each trap plant species after 29 to 93 days after planting (DAP) plus the overall AUC mean.

Plant species	Days after planting (DAP) plus overall AUC mean													AUC
												Mean		
	29 d	40 d	47 d	51 d	54 d	58 d	62 d	65 d	68 d	77 d	82 d	89 d	93 d	
Alyssum	0.97b	0.97a	0.85a	3.27b	1.52a	6.98c	0.62b	7.34b	0.87b	8.86c	8.49b	5.77c	5.57b	4.78c
Alyssum plus Wheat	0.00a	0.00a	0.77a	2.15ab	0.55a	4.21b	5.46b	6.64b	6.79b	4.34b	7.08b	4.01b	2.87ab	3.30b
Wheat	0.00a	0.00a	0.00a	0.69a	0.00a	0.60a	0.48a	1.37a	1.98a	1.47a	3.62a	1.85a	3.26ab	1.11a
Kale	0.00a	0.00a	0.48a	0.68a	0.28a	0.20a	0.74a	1.16a	1.35a	0.60a	1.54a	0.88a	1.24a	0.65a
LSD (5%)	0.398	0.398	0.1277	1.919	1.552	2.620	2.018	1.424	2.843	2.015	3.337	1.271	2.904	0.967
SEM	0.129	0.129	0.414	0.623	0.504	0.850	0.655	0.462	0.923	0.654	1.083	0.412	0.943	0.314
Significance	***	ns	Ns	*	Ns	***	* * *	***	* * *	***	* * *	* * *	*	* * *

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant species, the day 64 weighted mean was obtained by calculating the area under the curve (AUC) for a graph of V count against time (days) using the trapezoid rule, then dividing by the time period (64 days) (n = 5). (ns) non - significant; (*) p < 0.05, significant; (***) p < 0.001, extremely significant.

Table 5.5 For the open field experiments, the mean numbers (V transformed) /(0.5 m x 0.5 m quadrat) of *Nysius huttoni* nymphs recorded in each trap plant species after 29 to 93 days after planting (DAP) plus the overall AUC mean.

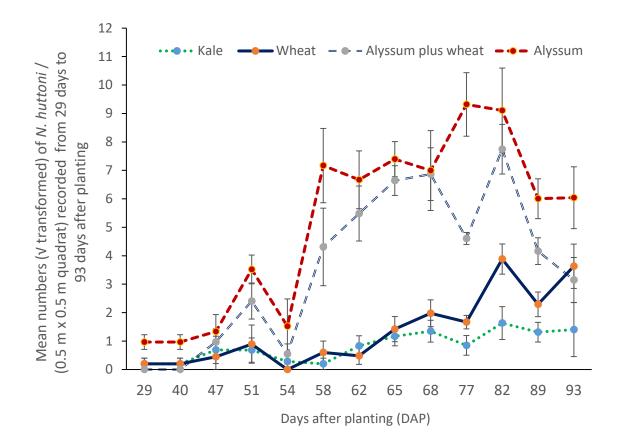
Plant species	Days after planting (DAP) plus overall AUC mean												AUC	
	29 d	40 d	47 d	51 d	54 d	58 d	62 d	65 d	68 d	77 d	82 d	89 d	93 d	- Mean
Alyssum	0.00a	0.00a	0.49a	0.68a	0.00a	1.18b	0.55a	0.55a	0.79b	2.50b	2.67b	1.67a	2.14b	1.02b
Alyssum plus Wheat	0.00a	0.00a	0.20a	0.60a	0.00a	0.28a	0.20a	0.00a	0.55ab	1.36ab	2.56b	0.97a	1.26ab	0.64a
Wheat	0.20a	0.20a	0.45a	0.49a	0.00a	0.00a	0.00a	0.20a	0.00a	0.48a	0.93a	1.21a	1.28ab	0.40a
Kale	0.20a	0.20a	0.49a	0.00a	0.00a	0.00a	0.20a	0.00a	0.00a	0.60a	0.35a	0.97a	0.63a	0.29a
LSD (5%)	0.454	0.454	0.395	0.841	0.000	0.850	0.673	0.597	0.775	1.361	1.018	0.754	1.025	0.350
SEM	0.147	0.147	0.128	0.273	0.000	0.276	0.218	0.194	0.252	0.442	0.331	0.245	0.333	0.114
Significance	ns	ns	Ns	ns	Ns	ns	ns	ns	*	*	***	ns	*	**

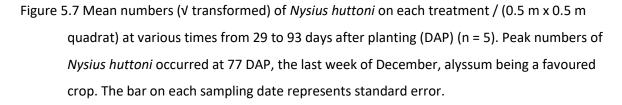
Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant species, the day 64 weighted mean was obtained by calculating the area under the curve (AUC) for a graph of V count against time (days) using the trapezoid rule, then dividing by the time period (64 days) (n = 5). (ns) non - significant; (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.

Table 5.6 For the open field experiments, the mean numbers (V transformed) of *Nysius huttoni* /(0.5 m x 0.5 m quadrat) in kale next to the trap plants from 29 to 93 days after planting plus the overall AUC mean.

Plant species		Days after planting (DAP) plus overall AUC mean												
	29 d	40 d	47 d	51 d	54 d	58 d	62 d	65 d	68 d	77 d	82 d	89 d	93 d	_ Mean
	25 u	40 U	47 U	510	54 U	58 U	02 U	05 U	08 U	// u	02 U	85 U	55 U	
Alyssum	0.00a	0.00a	0.85a	0.00a	0.00a	0.00a	1.28b	1.17b	0.00a	0.48a	1.17a	0.00a	0.00a	0.38a
Alyssum plus Wheat	0.48ab	0.60b	0.49a	0.00a	0.00a	0.00a	0.48a	0.00a	0.20a	0.60a	1.00a	0.60a	0.00a	0.42a
Wheat	0.20ab	0.20ab	0.28a	0.00a	0.00a	0.00a	0.20a	0.20a	0.00a	0.20a	1.17a	0.68a	0.00a	0.28a
Kale	0.60b	0.20ab	0.94a	0.00a	0.00a	0.00a	0.28a	0.00a	0.00a	0.00a	0.69a	0.68a	2.40b	0.44a
LSD (5%)	0.523	0.534	0.991	0.000	0.000	0.000	0.580	0.316	0.308	0.810	0.916	0.765	0.119	0.219
SEM	0.170	0.173	0.322	0.000	0.000	0.000	0.188	0.102	0.100	0.263	0.297	0.248	0.039	0.071
Significance	*	*	Ns	ns	Ns	ns	**	* * *	ns	ns	ns	ns	* * *	ns

Means within a same column with no letters in common are significantly different (Unprotected LSD; p < 0.05). For each plant species, the day 64 weighted mean was obtained by calculating the area under the curve (AUC) for a graph of V count against time (days) using the trapezoid rule, then dividing by the time period (64 days) (n = 5). (ns) non - significant; (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.





During each of the 13 sampling periods (12 November 2017 (29 DAP) to 15 January 2018 (93 DAP)) (Fig. 5.7), alyssum strips and 'alyssum plus wheat' strips supported more *N. huttoni*, with significantly higher numbers than on wheat and kale strips at 51 DAP (December 4), 58 DAP (December 11) and onwards until 89 DAP (p < 0.05). Overall, numbers of *N. huttoni* gradually increased on trap plant species from 29 to 68 DAP (12 November to 21 December 2017), sharply increasing on kale, wheat and 'alyssum plus wheat' strips from 77 to 82 DAP (30 December 2017 to 4 January 2018). Numbers remained almost steady on alyssum strips, declining rapidly on all trap species from 82 DAP (January 4) to 89 DAP (January 11) (Fig. 5.7). However, the bug population sharply increased on wheat from 89 to 93 DAP (January 11 to 14). The peak population of *N. huttoni* was reached at 77 DAP (December 30) in alyssum strips ($\overline{x} = 87/(0.5 \text{ m x } 0.5 \text{ m quadrat})$ which was significantly higher (p < 0.001) than on the other trap plants. However, on 82 DAP (January 4), numbers of *N. huttoni* were at peak level, being approximately $\overline{x} = 60/(0.5 \text{ m x } 0.5 \text{ m}$ quadrat) on 'alyssum plus wheat' strips, and these numbers were significantly higher (p < 0.001) than on the wheat alone ($\overline{x} = 15/(0.5 \text{ m x } 0.5 \text{ m quadrat})$) and on kale ($\overline{x} = 3/(0.5 \text{ m x } 0.5 \text{ m}$ quadrat)). Numbers remained lower on wheat and kale strips throughout the sampling period (Fig. 5.7) than on alyssum and 'alyssum plus wheat' strips. However, the numbers of *N. huttoni* on wheat at 82 DAP (January 4) were significantly higher than on kale (p < 0.05). The abundance of *N. huttoni* on each trap plant species increased through the months with November < December < January (Fig. 5.7). In summary, these data exhibit a 'preference' for alyssum over wheat over time.

Nysius huttoni individuals and damage to kale plants next to each trap crop

Nysius huttoni numbers in between kale plots next to the trap strips were not significantly different (p > 0.05) (Fig. 5.8). However, their numbers on 29, 40, 62 and 65 days after planting were significantly different (Table 5.6). The population of *N. huttoni* tended to be highest in kale plots next to the kale strips. In contrast, the lowest bug numbers were recorded on kale next to the wheat strips followed by alyssum, and 'alyssum plus wheat' strips, respectively. However, none of these differences was statistically significant (Fig. 5.8 and Table 5.6).

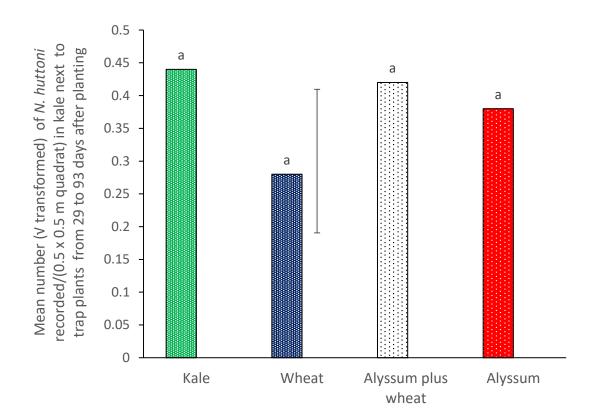


Figure 5.8 Mean numbers (V transformed) of *Nysius huttoni* (adults and nymphs) / (0.5 m x 0.5 m quadrat) in kale plot next to each trap plant species from 29 to 93 days after planting (n =

5). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 5).

Damage to kale plants next to the trap strips over the sampling period was also not significantly different between trap plants (p > 0.05) (Fig. 5.9). However, damage was the highest in kale next to kale strips (15.32 %), followed by kale next to 'alyssum plus wheat' (11.32 %), alyssum (11.30 %) and wheat (9.98 %), respectively.

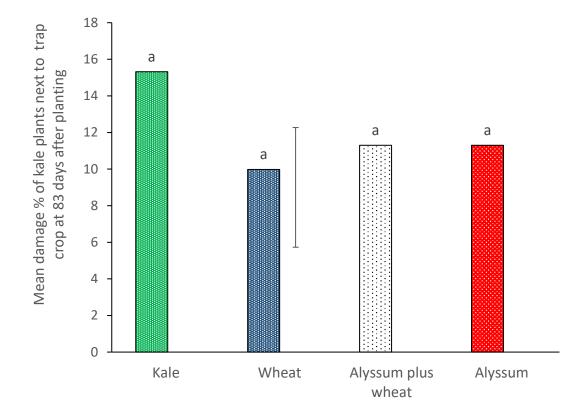


Figure 5.9 Mean damage (%) of kale plants next to trap strips at 53 days after sowing (n = 5). The vertical bar is the least significant difference, LSD (5 %). Means with no letters in common are significantly different (Unprotected LSD; P < 0.05) (n = 5).</p>

The effect of edge trap strips on Nysius huttoni numbers

The density of *N. huttoni* declined away from the plot edge in all treatments including the kale (p < 0.001). Numbers were highest in the alyssum strips. Beyond 2 m, bug numbers were virtually zero in all treatments (Fig. 5.10).

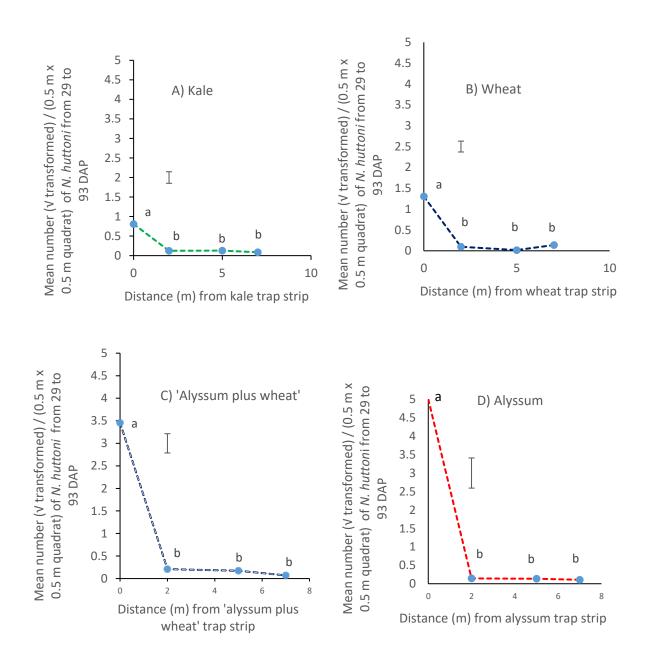


Figure 5.10 Mean numbers (V transformed) of Nysius huttoni / (0.5 m x 0.5 m quadrat) from 29 to
93 days after planting (DAP) at various distances (m) from each trap strip. Samples were
collected at 0 m from trap strips and at 2 m, 5 m and 7 m distances from each trap strip to
the kale plots. The vertical bar is the least significant difference, LSD (5 %). Within each trap

strip, treatment means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 5).

Effect of alyssum and wheat growth stages on Nysius huttoni numbers

There was a significant effect of alyssum and wheat plant phenology on the abundance of *N*. huttoni (p < 0.001). In alyssum, numbers of *N*. huttoni (adults and nymphs) were at peak levels during the fruiting stage ($\overline{x} = 63 / 0.5 \text{ m x } 0.5 \text{ m quadrat}$) and these were higher than at flowering (\overline{x} = 23 / 0.5 m x 0.5 m quadrat) (t = 3.162; p = 0.034; n = 5) and vegetative ($\overline{x} = 1 / 0.5 \text{ m x } 0.5 \text{ m}$ quadrat) (t = 5.305; p = 0.006; n = 5). The density of the bug at the senescence stage was not significantly different to the fruiting stage (t = -2.553; p = 0.063; n = 5) and flowering (t = 2.523; p =0.065; n = 5). The density of *N*. huttoni was significantly lower at the vegetative stage of the alyssum than at the senescence stage (t = -5.844; p = 0.004; n = 5) (Fig. 5.11 a).

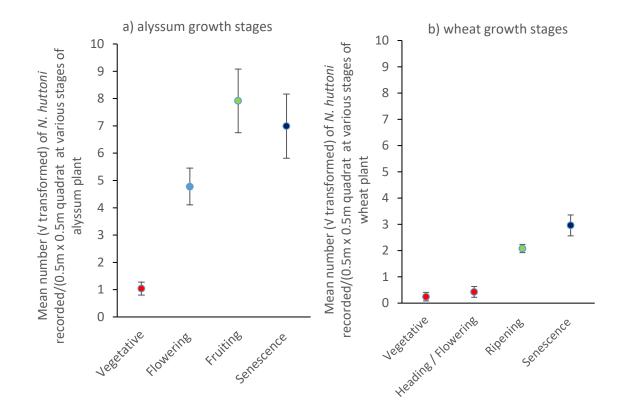


Figure 5.11 Mean numbers (\vee transformed) of *Nysius huttoni* (adults and nymphs) / (0.5 m x 0.5 m quadrat) at various phenological stages of alyssum and wheat plants (n = 5). Error bar in each stage represents standard error. Paired sample *t* -tests were performed to compare the phenological stages.

In wheat plants, *N. huttoni* numbers were at peak levels during senescence ($\overline{x} = 9 / (0.5 \text{ m x } 0.5 \text{ m}$ quadrat)) followed by those at the ripening stage (approx. $\overline{x} = 4 / 0.5 \text{ m x } 0.5 \text{ m}$ quadrat), both of

them being significanty different to each other (t = 2.966; p = 0.041, n = 5), but significantly higher than vegetative and heading/flowering stages. Vegetative stage and heading/flowering stage were not significanly different each other (t = -0.922; p = 0.408; n = 5) (Fig. 5.11 b).

5.4.3 Beneficial arthropods

Significantly more spiders (p < 0.001) were collected/(0.5 m x 0.5 m quadrat) on alyssum which had significantly higher than seven-spotted ladybirds and the lacewing adults, *M. tasmaniae* in each trap plant except in kale (control) (Fig. 5.12). Spider numbers were significantly higher (p < 0.05) on alyssum strips ($\overline{x} = 3$ / (0.5 m x 0.5 m quadrat) (n = 5)) than were those of *M. tasmaniae* and seven-spotted ladybirds (approx. $\overline{x} < 1$ for each (0.5 m x 0.5 m quadrat) (n = 5), respectively. The second highest numbers of spiders were recorded in 'alyssum plus wheat' (approx. $\overline{x} = 2$ / (0.5 m x 0.5 m quadrat) (n = 5)), followed by wheat (approx. $\overline{x} = 1$ / (0.5 m x 0.5 m quadrat) (n = 5)). *Micromus tasmaniae* numbers in kale were not significantly different from those of spiders and seven-spotted ladybirds (Fig. 5.12).

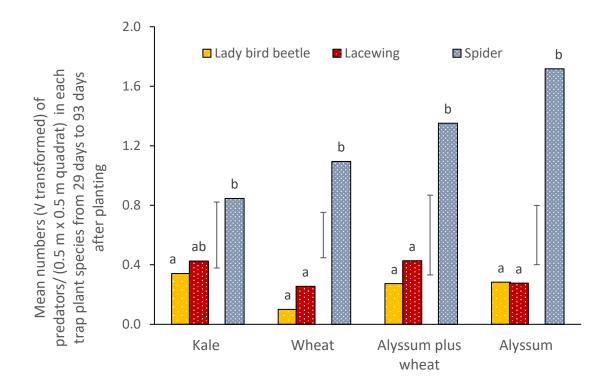


Figure 5.12 Mean numbers (V transformed) of natural enemies in each trap strip / (0.5 m x 0.5 m quadrat) recorded from 29 to 93 days after planting. Vertical bars are the least significant

difference, LSD (5 %). Within each trap strips, means with no letters in common are significantly different (Unprotected LSD; p < 0.05) (n = 5).

5.5 Discussion

Trap plants provide an opportunity to manipulate *N. huttoni* populations to reduce their abundance in brassica fields (Tiwari et al., 2018). Understanding *N. huttoni* host-plant 'preferences' in the field is essential to design trap cropping protocols for this pest.

5.5.1 Nysius huttoni on the trap strips

The field-cage results presented here suggest that alyssum has the greatest potential as a trap crop for N. huttoni in forage brassicas followed by wheat (Fig. 5.4). However, in the open-field experiment, alyssum was significantly the most effective trap plant evaluated ($\overline{x} = 34$ individuals / $0.5 \text{ m} \times 0.5 \text{ m}$ quadrat) (n = 5), followed by 'alyssum plus wheat' (16 individuals / $0.5 \text{ m} \times 0.5 \text{ m}$ quadrat) (n = 5) (p < 0.001) (Fig. 5.6). Not only does alyssum have high potential to trap N. huttoni in brassica fields (Tiwari, et al., 2018; Wei, 2008b), but it also traps the diamondback moth, Plutella xylostella L. (Lepidoptera: Plutellidae) in cabbage fields (De Groot et al., 2005). Multiple species trap cropping has the potential to be used in *N. huttoni* management as indicated by the 'alyssum plus wheat' treatment being the second most affected trap plant tested in the open-field experiments (Fig. 5.6). There are similar examples in which multiple trap plant species such as Chinese cabbage (Brassica rapa L.), rape (B. napus L.), marigold (Tagetes spp. L.), and sunflower (H. annuus) together have been used to trap pollen beetle (Coleoptera: Nitidulidae) in cauliflower (B. oleracea L.) (Hokkanen, 1989), corn (Zea mays L.) and potato (Solanum tuberosum L.) for wireworms (Coleoptera: Elateridae) control in potato fields (Seal et al., 1992). However, multiple trap cropping may increase the cost of production because of the potentially complex management practices involved and can be labour intensive, which may not be practical for most farmers (Shelton & Badenes-Perez, 2006). Also, it would help to know the relative contributions from the individual plant species.

Nysius huttoni nymphs were much less abundant in all trap plant species compared to adults (Fig. 5.6 b) suggesting that the latter are the colonising population and using these trap crop habitats for short time (Wei, 2001). Badenes-Perez et al., (2004) hypothesised that a low nymph population was due to the plants' acting as 'dead-end' trap-crops. However, the studies here did not cover their oviposition preferences and sex ratios. Anyway, *L. maritima* is a member of the Brassicaceae, as is kale.

A trend of *N. huttoni* populations increasing from 12 November 2017 (29 DAP) to 4 January 2018 (82 DAP) was an indication of late migration into the trap crops (Fig. 5.7). There were higher densities of *N. huttoni* from 21 December 2017 (68 DAP) to 11 January 2018 (89 DAP) probably because the trap crops were in reproductive stages, which *N. huttoni* prefers. The rapid decline of *N. huttoni* at senescence of alyssum could be a result of movement of bugs from the trap crop into the main field (Easterbrook & Tooley, 1999). Hence, control measures of *N. huttoni* should be attempted in trap crops before any such migration occurs. However, in the current study regular sampling of *N. huttoni* in weedy habitats adjacent to the study site indicated that movement of *N. huttoni* took place from trap crops (alyssum or wheat or both) into the weedy vegetation (Mensah & Khan, 1997). This vegetation is also thought to be the original source of the pest. In any case, if *N. huttoni* numbers have increased rapidly in the trap plant which is near to senescence, the trap crops, including *N. huttoni* and nearby weedy vegetations should be immediately removed or sprayed together. This is because management of *N. huttoni* in the trap crop at this time would be less expensive than pesticides use in the entire brassicas fields (Cook et al., 2006).

5.5.2 Nysius huttoni individuals and damage to kale crops next to each trap plant

The abundance of *N. huttoni* and its feeding damage on kale seedlings next to the trap species were significantly lower than in the trap crops but did not differ between trap species. This could be explained by two possibilities: a) *N. huttoni* were immediately intercepted by the trap strips and arrested them into each trap strip, and did not make damage on the kale seedlings (Badenes-Perez, Shelton, & Nault, 2004); b) *N. huttoni* entered the kale fields only after the kale matured and did not demonstrate obvious damage on the mature kale plants. *Nysius huttoni* populations occasionally increased in the kale plots which could be drying off of edges trap strips or migrated population from weedy vegetations (Wei, 2001). Furthermore, these results (i.e., fewer populations of *N. huttoni* and low damage on kale plants) would reduce insecticide use in brassica fields.

5.5.3 Edge trap strips effect on Nysius huttoni numbers

Nysius huttoni numbers were significantly higher in the trap strips only at the edge of the main crop (Fig. 5.10). Similar findings were made by Badenes-Perez et al., (2005) while using yellow rocket, *Barbarea vulgaris* (R. Br.) cv. *arcuata* to trap the diamondback moth, *P. xylostella* in cabbage (*B. oleracea* cv. *capitata*) fields (Jackai & Singh, 1983). There are many possibilities for the higher densities of *N. huttoni* at the edges, as appropriate perimeter plantings can be the first ones intercepting the pest moving into the field and may limit its further dispersal (Badenes-Perez et al., 2005; Boucher et al., 2003; Shelton & Badenes-Perez, 2006). Other major factors that influence *N*. *huttoni* individuals at the edges of trap crops are: the relative attractiveness of trap plant species (Badenes-Perez et al., 2004), its height (Fereres, 2000), the area covered by the trap plants (Shelton & Badenes-Perez, 2006), planting time relative to that of the main and trap plants (Hokkanen, 1991), plant phenology (Smyth, Hoffmann, & Shelton, 2003), physical and chemical cues provided by trap plants (Fenemore, 1988), trap crops' proximity to the main crops (Tscharntke & Brandl, 2004) and behaviour of the target pest (Hokkanen, 1991).

5.5.4 Effect of alyssum and wheat growth stages on Nysius huttoni

Significantly, the highest numbers of *N. huttoni* were collected at the reproductive stages of alyssum and wheat plants compared with their vegetative stages (p < 0.001) (Fig. 5.11). A laboratory choice experiment on growth stage preferences by N. huttoni showed that it favoured the flowering over the vegetative stage of alyssum (Tiwari et al., unpublished data). Farrell & Stufkens (1993) reported that N. huttoni used the wheat plants at the milky-ripe stage. These findings emphasise the importance of plant phenology when considering deployment time of trap crops in crop fields (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Hence, alyssum plants should be deployed in such a way that they must have sufficient flowers and fruits (Fig. 5.11 a) at the seedling stage of kale in fields, and wheat plants should be at the milky or seed-ripening stage (Fig. 5.11 b) to protect brassica seedlings from N. huttoni damage. Nysius huttoni is mainly a seedling pest (AgPest 2016) and damages 4- to 6 - week old brassica seedlings (PGG, 2009). In such a situation, if N. huttoni is trapped/remains for 4- to - 6 week on their preferred stages, the brassica seedlings can be risk-free (Tiwari et al., 2018). However, other potential pests such as the cabbage grey aphids, Brevicoryne brassicae, the green peach aphids, M. persicae, the springtail, Bourletiella hortensis Fitch, the diamondback moth, P. xylostella, the leaf miners, Scaptomyza flava Fallen, the white butterfly, Pieris rapae L. still need to be managed in brassica fields by other safe management practices (Speciality Seeds, 2016).

Adding trap crops may also create ecosystem dis-services such as benefiting pests other than the target one (Gurr et al., 2017). The high density of *N. huttoni* on alyssum could potentially kill the plant which may result *N. huttoni* dispersing into the brassica field where it could damage the crop's seedlings. In such a situation, additional measures of management such as selective insecticides or other cultural management practices could be recommended before the bug damage occurs on kale seedlings. For example, the soybean hairy caterpillar, *Spilarctia casigneta* Rothschild (Lepidoptera: Erebidae) and flea beetles (Coleoptera: Chrysomelidae) damaged alyssum flowers, which were deployed with the aim of improving CBC of radish pests in radish fields (Tiwari et al, unpublished data). Similarly, the painted bug, *Bagrada hilaris* (Hemiptera: Pentatomidae) also damages the alyssum flowers in vegetable nurseries (Reed et al., 2013). The dead tillers of wheat in

brassica fields may also provide overwintering habitat for other insect pests that damage next season's crops (Hokkanen, 1991; Ludwig & Kok, 1998).

5.5.5 Beneficial arthropods in trap strips

Flowering alyssum plants potentially provide the resources such as shelter, nectar, alternative food and pollen (SNAP) for beneficial arthropods and pollinators, which enhance multiple ES on and off the farm (Gurr et al., 2017). In this study, alyssum and 'alyssum plus wheat' strips harboured significant numbers of spiders compared to the wheat and the kale plants (Fig. 5.12). Such noncrop habitats such as flowering strips, banker plants and hedgerows potentially provide shelter and habitat for beneficial arthropods (Pywell et al., 2005) and reduce their mortality during migration from one field to another (Rusch et al., 2016). These beneficial arthropods may also reduce *N. huttoni* and other insect pest numbers in brassica fields (Wei, 2001). However, the population dynamics of such predators and their effectiveness to manage *N. huttoni* in brassica field has not been assessed.

This study was conducted in small plots and there is the possibility of *N. huttoni* migration between plots and further larger scale trials are recommended before commercially used (Badenes-Perez et al., 2005; Shelton & Badenes-Perez, 2006). However, results demonstrated the potential, even if inter-plot interactions did occur at the scales used here. The fact that in this work, *Nysius* populations were concentrated at the crop edges (i.e., restricted to a 2-m strip), suggests the potential practical applicability of this management approach at commercial scales.

Overall, this work demonstrates the potential for trap cropping for *N. huttoni*, but inexpensive (excluding external costs) insecticides are readily available. Also, although clear delivery systems of practical knowledge may be available (leaflets, videos etc.), pathways to implementation (e.g. farmer field schools) are often not provided (Arnés, 2018; Warner, 2007).

5.6 Conclusions

The study's results clearly demonstrate that alyssum and wheat are two potential trap plants for the *N. huttoni*. The fruiting and flowering stages of alyssum, and the seed ripening and senescence stages of wheat were significantly more suitable for the bug than the other stages of those plants. The result also shows that *Nysius* numbers were declined with distance from the edges trap strips. This information is important in designing a trap cropping protocol for *N. huttoni* management in brassica fields. Two potential trap plants such as alyssum (e.g., single trap cropping) or alyssum plus wheat (e.g., as multiple trap cropping) should be deployed at the edge of fields at their appropriate

growth stages for effective trapping of *N. huttoni*. It is also strongly recommended that the control strategies should focus on the edge of the brassica fields to reduce bug populations. However, careful attention should also be given to beneficial organisms, such as predators and pollinators, in flowering trap crops when adopting pesticide management strategies. Flowering alyssum plants in brassica fields can potentially provide resources such as SNAP to beneficial predators, parasitoids and pollinators, which can improve CBC and enhance ecosystem services. Work of this type can be applied to most cropping systems globally. However, in many countries, the basic concept of enhancing functional agricultural biodiversity is not well understood. Nepalese agriculture is a good example of where practical demonstrations of this are badly needed. For this reason, it was decided to undertake trials in the plains of Inner-Terai (Chitwan, Nepal). This involved farmer participatory techniques (see Chapter 6). Other key issues such as the planting time of the trap and main crop, other agronomic and economic characteristics of both, coverage area of the trap crop (s) and their placement, pest insect traits, including their dispersal rates and distribution could affect the control outcomes.

Chapter 6

Alyssum flowers promote arthropod diversity and biological control of radish pests

A version of this chapter has been accepted for publication: Tiwari, S, Sharma, S & Wratten S.D. (accepted on 20 January 2020). Flowering alyssum (*Lobularia maritima*) promote arthropod diversity and biological control of *Myzus persicae* and other radish pest. *Journal of Asia-Pacific Entomology*.

6.1 Abstract

Radish, Raphanus sativus, is an important vegetable crop worldwide. In winter, It is the second most important vegetable after cabbage (January to March) in Nepal. This crop is damaged by various herbivores such as green peach aphid, Myzus persicae, soybean hairy caterpillar, Spilarctia casigneta and flea beetle, Monolepta signata. Prophylactic pesticide use is a part of the common pest management practice in Nepal. Habitat manipulation using non-crop floral resources in an agro-ecosystem can provide shelter, nectar, alternative food and prey (SNAP) to natural enemies and improve pest biological control by restoring lost ecosystem functions such as predation and parasitism, thereby reducing pesticide consumption and adding value to the product. The candidate floral plant, alyssum, Lobularia maritima, was deployed in a radish field to improve pest biological control. Beneficial arthropods such as Carabidae, Staphylinidae, Coccinellidae, Formicidae, Syrphidae, Lycosidae, Apidae and Ichneumonidae were significantly more abundant in flowering alyssum plots than the control (non-flowering) plots. The flea beetle was the most frequently encountered insect pest in flowering alyssum plots. The populations of syrphids and C. septempunctata were significantly higher near flowering strips and their numbers declined away from those strips. These results provide evidence of the alyssum's ability to increase the abundance of predators and support the suppression of aphids and other pests in radishes. In yellow water trap samples, the number of syrphids and seven-spotted ladybird, Coccinella septempunctata, was significantly higher in alyssum plots than in control plots. In visual sampling, syrphid populations were significantly higher in flowering plots than control plots. The aphid population was significantly lower in flowering alyssum plots than the control plots. This information is useful in developing an integrated pest management protocol with flowering strips in a radish field. Habitat manipulation in radish fields by maintaining flower strips can improve pest biological control and support the provision of multiple ecosystem services that restore diminished ecosystem functions in agriculture.

Keywords: Radish, Nepal, habitat management, conservation biological control, alyssum, ecosystem functions

6.2 Introduction

Radish (Raphanus sativus L.) (Brassicales: Brassicaceae) (of the Diakon type) is an economically important vegetable crop in Nepal. It covers 16.47 % of the total vegetable area with a production 268,119.6 MT and a productivity of 15.85 MT/ha (MOALD, 2015/16). The crop is damaged by many insect herbivores such as green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae), soybean hairy caterpillar, Spilarctia casigneta Rothschild (Lepidoptera: Erebidae), flea beetle, Monolepta signata Olivier (Coleoptera: Chrysomelidae) and other minor insect pests such as leafhoppers (Hemiptera: Cicadellidae), red pumpkin beetle (Coleoptera: Chrysomelidae), white grub (Coleoptera: Scarabaeidae) (Kunjwal & Srivastava, 2018; Neupane, 2011). Damage in radishes has led to up to 30 % plant loss in Chitwan, Nepal (MOALD, 2015/16). Unfortunately, agricultural production heavily relies on anthropogenic fossil fuel derived inputs such as chemical pesticides as part of vegetable farming in Nepal (Gyenwali et al., 2017) that may affect human health, biodiversity and the environment (Vaidya, Gyenwali, Tiwari, Pande, & Jørs, 2017). Modern agriculture is directly linked to the destruction of non-crop habitats and a decline in plant biodiversity (Wade, Gurr, & Wratten, 2008) followed by a reduction in crop productivity and sustainability (Letourneau & Altieri, 1999). These practices also limit the supply of food resources to beneficial predators and parasitoids (Gurr et al., 2017) by affecting the 'fitness' of natural enemies (Gurr et al., 2000; Gurr et al., 2017) and limiting the efficiency of CBC and provision of multiple ES (Gurr et al., 2017; Robinson, Jonsson, Wratten, Wade, & Buckley, 2008).

Habitat management, a form of CBC (Landis et al., 2000), has been promulgated as a core concept of integrated pest management (IPM) for over many years in sustainable pest management (Meyhöfer & Poehling, 2006; Wratten et al., 2013) and for improving the provision of multiple ES needed for future farming (Gurr et al., 2017). Habitat can be managed within-crop, within-farm or at the landscape level (Landis et al., 2000) to share favourable environments and floral rewards to the natural enemies so improving the 'fitness' of biocontrol agents (Buchanan, Grieshop, & Szendrei, 2018; Gurr et al., 2017; Landis et al., 2000; Zehnder et al., 2007). Conservation biological control practices reduce herbivore populations in agricultural fields (Gurr, van Emden, & Wratten, 1998; Gurr et al., 2017; Landis et al., 2000), manage agricultural pests (Norris & Kogan, 2017), decrease dependency on chemical pesticides (Gurr et al., 2016), and increase farmers' profits (Gurr et al., 2017). There are two popular mechanisms of pest management in diversified crop fields that act on pests by the top-down approach ('natural enemy hypothesis') (Russell, 1989) and the bottom-up approach ('resource concentration hypothesis') (Root, 1973). However, floral habitat in a crop field can directly affect insect pest populations by increasing the fitness of natural enemies (Landis et al., 2000). For example, flowering alyssum in a farming system provides suitable floral resources to syrphids (Diptera: Syrphidae) and leads to more efficient CBC of aphids (Amorós-Jiménez et al., 2014; Barbir et al., 2015; Gillespie et al., 2011; Hickman & Wratten, 1996; Laubertie et al., 2012; Pineda & Marcos-García, 2008). However, sometimes, simply increasing diversity in agricultural fields cannot manage pests and suppress crop damage (Andow & Risch, 1985; Baggen & Gurr, 1998). Hence, the important elements of diversity need to be considered and verified before deciding to manipulate them in an agro-ecosystem (Way, 1966).

A popular garden plant, alyssum, Lobularia maritima L. Desv. (Brassicales: Brassicaceae), has been used worldwide in CBC work (Berndt & Wratten, 2005; Fiedler, Landis, & Wratten, 2008; Haseeb, Gordon, Kanga, & Legaspi, 2018; Landis et al., 2000; Ribeiro & Gontijo, 2017) and has also been evaluated as a potential trap crop for wheat bug (Nysius huttoni White: Lygaeidae) in New Zealand (Tiwari et al., 2018). This plant is the most popular used in CBC because of its high potential as an insectary plant (Hogg, Bugg, & Daane, 2011; Landis et al., 2000; Pease & Zalom, 2010), its long flowering duration (Xavier Picó & Retana, 2001), permanent shelter habitat for natural enemies (Buchanan et al., 2018) and is an easy fit in organic vegetable production (Norris & Kogan, 2017). For example, alyssum flowers increase the fitness (longevity, fecundity, and sex ratio) of the parasitoid, Dolichogenidea tasmanica Cameron (Hymenoptera: Braconidae) that potentially manages light brown apple moth, Epiphyas postvittana Walker (Lepidoptera: Tortricidae), in apple orchards (Berndt & Wratten, 2005). Alyssum flowers also significantly increase the performance of the generalist predator, Jalysus wickhami VanDuzee (Hemiptera: Berytidae) of the two bugs, Euschistus conspersus Uhler and Thyanta pallidovirens Stal, in tomato fields (Pease & Zalom, 2010). In Brazil, a study confirmed that alyssum flowers increased the abundance of spiders, Coccinellidae, Syrphidae and Orius sp. in collard fields and increased collard biological pest control (Ribeiro & Gontijo, 2017). In contrast, alyssum also increased the abundance of herbivores such as mirids, lygaeids, leafhoppers, aphids and many other crop pests compared with buckwheat, Faba bean, vetch and oats in an organic vineyard in Northern Italy (Burgio et al., 2016) and potentially increased ecosystem dis-services (Gurr et al., 2017; Zhang, Ricketts, Kremen, Carney, & Swinton, 2007).

Alyssum flowers primarily supply nectar and pollen to the predators and parasitoids of agricultural insect pests (Barbosa & Wratten, 1998; Landis et al., 2000). Nectar is an important source of

carbohydrates for the arthropods that used them to maintain their activity and metabolism (Jonsson, Wratten, Landis, & Gurr, 2008). For example, non-web-building spiders such as jumping spiders (Saliticidae), crab spiders (Thomisidae), and other fast-moving spiders such as Miturgidae, Anyphaenidae and Corinnidae, use flower nectar as their food source (Taylor & Pfannenstiel, 2008). A laboratory experiment with an ant, *Myrmica rubra* L. (Hymenoptera: Formicidae), confirmed that ants used flower sucrose to increase their fecundity, larval size and worker activity (Brian, 1973). Pollen is the main source of protein, minerals and vitamins for beneficial arthropods; it increases longevity, fecundity and the physiological functions of these arthropods. For example, in a laboratory study, *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) exhibited a better performance when feeding on alfalfa and maize pollen than on a control (no-pollen) (Ostrom, Colunga-Garcia, & Gage, 1996).

However, negligible work on habitat management and CBC has been done in South Asia, including Nepal (Sharma, Kafle & Tiwari, 2017). Arthropod-mediated ecosystem services (AMES) using flowering strips in a wide range of crops including radish have been expected to reduce pest pressure and reduce pesticide use (Isaacs, Tuell, Fiedler, Gardiner, & Landis, 2009). There is very little information about suitable candidate plant species useful for CBC, their geographical distribution, landscape effect on natural enemies and pest control. All this information should be evaluated and verified before deployment of flowering plants on agricultural farms for CBC (Isaacs et al., 2009). This study hypothesizes that flowering alyssum increases the abundance of generalist beneficial arthropods such as spiders, staphylinids, carabids, syrphids, seven-spotted ladybirds (*Coccinella septempunctata* L.) and many other beneficial predators and parasitoids, thereby reducing pest populations in radish fields. Such work could contribute to reducing pest pressure on radish crops and reducing pesticide consumption. Integrated pest management by habitat manipulation using flower strips can be useful for researchers, extension workers, policymakers and small farmers who cannot afford pesticides, and it can be exploited in organic farming and sustainable agriculture in developing countries.

6.3 Materials and methods

6.3.1 Field site

The study was carried out at Shivanagar (27 ° 37' N; 84 ° 22' E), Bharatpur, Nepal, from February to May 2018. The area for the experiment was 1710 m² (95 m long by 18 m wide), with the long side running North-South. The plot was divided into two 95 m x 7 m strips, separated by a 95 m x 4 m buffer strip. There were five sub-plots within each of the two strips. Each sub-plot was 17 m long and 7 m wide, with a 2 m gap between them (Fig. 6.1 a). The field was bordered on the east, south

and north by cultivated fallow land and on the west by a road. All flowering vegetation for approximately 15 m on all sides of the plots was removed to minimize the cross effect of adjoining flowering vegetation. There were two beehives, *Apis mellifera* L. (Hymenoptera: Apidae), near the study plots. The average daily temperature range and RH were $18 - 25^{\circ}c$ and 70 %, respectively.



Figure 6.1 Alyssum fused in radish fields for pest biological control at Shivanagar, Chitwan, Nepal.a) Field layout in a randomized block design; b) Alyssum flowers at the edge of a radish plot; c) Yellow water traps for arthropods sampling; d) Manual weeding

On 14 February 2018, the research field was thoroughly and finely tilled using bullocks; 2 kg of chicken manure plus 1 kg compost per m² was thoroughly incorporated into the soil. After consultation with the farmers, radish, *R. sativus* cv. Mino Early Long White, was selected as a main crop because farmers have intensively used conventional pesticides to control *M. persicae* and other insect pests in this crop. This crop has a better market value than other winter season vegetables such as cabbage and cauliflower at the site and in Nepal, in general. Alyssum was selected on the basis of the following criteria: 1) it is widely used in CBC and is a popular insectary plant (Berndt & Wratten, 2005; Berndt, Wratten, & Scarratt, 2006; Pease & Zalom, 2010); 2) it has a long flowering duration (Xavier Picó & Retana, 2001); and 3) can fit into organic vegetable production (Norris & Kogan, 2017).

The ridge beds (7 m long, 70 cm wide and 20 cm above the furrow bottom) and a 30 cm wide furrow along the width of each plot, were prepared with a total of 17 ridges per 17 m long and 7 m wide plot. The experiment had two treatments: i) non-flowering control plots (radish plots were bordered by 1 m wide radish strips) and ii) flowering treatment plots (radish plots were bordered by 1 m wide alyssum strips). The experiment was a randomized block design with five replicates. Each block consisted of two plots, one for each treatment, in east-west pairs (Fig. 6.2). The alyssum treatment plot was on the right-hand side (east) in block 1 (Fig. 6.2), and on the left, right, left and right-hand side in blocks 2, 3, 4 and 5, respectively (i.e., alternating east and west). On 25 February 2018, radish seeds were directly sown 3 cm below the soil surface in each plot (see above), with a crop geometry of 20 (row to row) X 20 (plant to plant) cm spacing.

-	7m	4m	7m	
	Radish		Alyssum	1m
	Radish (Control plot)		Radish (Treated plot)	16m
	gap		gap	2m
	next plot		next plot	

Figure 6.2 Field layout showing the dimensions of the pair of plots in Block 1. For each "treated" plot, a 1m-wide strip of alyssum was sown at the north end.

Alyssum seeds (received from <u>https://www.pggwrightsonseeds.com</u>) were broadcast at the northern edge (1-m wide) of each treatment plot along its width (7 m) on 26 February 2018; later, seedlings were manually thinned to maintain approximately 10 cm x 10 cm spacing. The treatment plots (with 1-m wide alyssum strips) were compared with the control plots (with 1-m wide radish

strips). The field was irrigated by furrow irrigation and weeds were manually removed on an "as needed" basis. First alyssum flowering occurred on 28 March 2018.

6.3.2 Arthropods in flowering alyssum and control plots

Yellow water traps (17 cm diameter, 12 cm deep and 0.5 cm thick) were used to sample the arthropods in the research plots (flowering alyssum and control plots) (Fig. 6.1 c). In each flowering alyssum plot, three traps were placed at the centre (cross-ways) and along the length (17 m) of the top half of each plot, at 1, 5 and 7 m distance from the centre of the flower strips. The same procedure was followed in the control plots (i.e., 1, 5 and 7 m from the centre of the radish strips). After the empty traps had been positioned in the plots, they were two-thirds filled with water and 5 ml dish soap was added. On April 3 in the morning (7:00-11:00 a.m.), after two days of deployment, arthropod samples were taken at 37 days after alyssum planting (DAP), then at weekly intervals on April 10 (44 DAP), April 17 (51 DAP), April 24 (58 DAP), May 3 (67 DAP) and May 11 (75 DAP)). The trap contents were strained through a transparent muslin cloth and transferred to a labelled plastic container (4 cm diameter and 4 cm deep) containing 70 % ethanol. The containers were taken the same day to the nearby entomology laboratory of the Agriculture and Forestry University (https://www.afu.edu.np) for arthropod identification and counting. The arthropods were categorized into the following five orders: Coleoptera, Diptera, Araneae, Hemiptera and Hymenoptera.

6.3.3 Arthropods in flowering alyssum strips

Beneficial and harmful insects encountered in the 1-m wide flowering alyssum strips were collected using yellow water traps (see above). Two water traps (4 m apart) were placed in the centre of each flower strip of treatment plots. The traps were two - thirds filled with water and 5 ml dish soap was added. Samples were taken the day after the traps were deployed on April 5 (39 DAP), and on April 19 (53 DAP), April 26 (60 DAP) and April 30 (64 DAP). The collected arthropods were strained through a white, transparent muslin cloth and spread in a white tray (32 cm long and 24 cm wide) for identification and enumeration.

6.3.4 Arthropod abundance (syrphids, *Coccinella septempunctata* and winged *Myzus persicae*) next to flowering alyssum strips and the control strips

Yellow water traps (see above) were placed at 0 m, 2 m, 3 m, 5m and 7 m along the length (17 m) of the top half of the plots near to flowering alyssum strips. The same distances were maintained in the control plots near the radish strips. Arthropods (syrphids, *C. septempunctata* and winged *M. persicae*) samples were collected 24 h after trap set-up. Samples were collected on April 7 (41 DAP), April 14 (48 DAP), April 29 (63 DAP), May 1 (65 DAP) and May 6 (70 DAP). Each yellow water

trap was filled with water (see above), the collected samples were strained (see above), transferred to a Petri dish (6 cm diameter), labelled and brought to the entomology laboratory of the Agriculture and Forestry University for identification and counting. Except for syrphids, sevenspotted ladybirds and green peach aphids, other insects were discarded.

6.3.5 Visual observations

Five radish plants from the half closer to the 1-m wide flowering alyssum strips and control strips in each plot were randomly selected and carefully inspected using a hand-lens (10X) when necessary to count larval and adult syrphids, larval and adult seven-spotted ladybirds and green peach aphids. Counts were taken four times: March 31 (34 DAP), April 6 (40 DAP), April 15 (49 DAP) and April 25 (59 DAP).

6.3.6 Statistical analysis

Arthropods in flowering alyssum and control plots

The arthropod counts for each plot and date were averaged by the area under the curve (AUC) method over time as suggested by Hanley & McNeil (1983) and a paired samples *t*-test was used to compare means of the two treatments. To ensure an adequate level of normality and homogeneity of variance, count variables were square-root transformed before AUC calculation. Note that analysis using a paired samples *t*-test is statistically identical to using an analysis of variance on five blocks of a randomised block design with two treatments and is identical to summarising the statistical results using a least significant difference (LSD). The abundance of the orders was compared at each time using a paired sample *t*-test for each of the flowering alyssum and control plots separately (Fig. 6.4).

Arthropods in flowering alyssum strips

The numbers of beneficial and harmful arthropods collected in the flowering alyssum strips were first square-root transformed to achieve adequate normality and homogeneity of variance; the mean number over time was calculated by the AUC method. A paired sample *t*-test was used for pairwise comparisons of the abundance of any two arthropod groups in the flowering alyssum treatment.

Arthropod abundance (syrphids, *Coccinella septempunctata* and winged *Myzus persicae*) next to flowering alyssum strips and the control strips

The numbers of insect species (syrphids, *C. septempunctata* and *M. persicae*) collected at various distances from the flowering alyssum and control strips were first square-root transformed to achieve normality and homogeneity, averaged by the area under the curve (AUC) method, and a paired sample *t*-test at p < 0.05 was used to compare means. A paired sample *t*-test was used to compare the abundance of species between any two distances from the 1 m - strip within either the flowering alyssum or control treatments.

Visual observations

Visually counted insects (larval and adult syrphids, larval and adult *C. septempunctata* and *M. persicae* in each plot at each time were square-root transformed to achieve normality, averaged by the area under the curve (AUC) method and a paired sample *t*-test at p < 0.05 was used to compare means between treatments.

6.4 Results

6.4.1 Arthropods in flowering alyssum and control plots

The mean number of arthropods (averaged over samples at 1 m, 5 m and 7 m from the strips at the northern end of each plot and sampling times) significantly increased in the presence of flowering alyssum in the plots (p < 0.05) (Table 6.1). The numbers of carabids, rove beetles, seven-spotted ladybirds, syrphids, wolf spiders, ants, honeybees and ichneumonids were significantly higher in flowering alyssum plots than in the control plots (Table 6.1). Overall, the populations of beneficial arthropods were significantly higher in flowering alyssum plots than in the control plots strongly influence the numbers of weevils, *M. signata*, leafhoppers, planthoppers, fruit flies, *M. persicae* and other pests (Table 6.1).

Table 6.1 The mean number (V transformed) of arthropods averaged over three samples at 1 m, 5 m, and 7 m from the strip of each plot, and over six samplings from 37 to 75 days after planting (DAP) in flowering alyssum and control plots.

Arthropods	Flowering alyssum plots	Control plots	Paired samples t- test (d.f. = 4) at p < 0.05	Signific ance
COLEOPTERA				
Carabidae (carabids)	1.61	0.94	<i>t</i> = 6.827; <i>p</i> = 0.002	**
Staphylinidae (rove beetles)	1.61	1.17	<i>t</i> = 4.593; <i>p</i> = 0.010	*
Coccinellidae (seven-spotted ladybirds	2.03	0.74	<i>t</i> = 6.479; <i>p</i> = 0.002	**
Curculionidae (weevils)	0.45	0.48	t = - 0.190; p = 0.858	ns
Chrysomelidae (flea beetles)	1.62	1.46	t = 0.711; p = 0.516	ns
Unidentified	1.47	1.56	t = - 0.699; p = 0.522	ns
Total	8.79	6.35	<i>t</i> = 5.415; <i>p</i> = 0.008	**
DIPTERA				
Culicidae (mosquitoes)	1.64	1.62	<i>t</i> = 0.086; <i>p</i> = 0.935	ns
Muscidae (flesh flies)	1.58	1.27	t = 1.525; p = 0.201	ns
Syrphidae (syrphids)	1.74	0.69	<i>t</i> = 7.710; <i>p</i> = 0.001	**
Tephritidae (fruit flies)	0.87	0.81	t = 0.431; p = 0.688	ns
Unidentified	1.47	1.41	t = 0.628; p = 0.563	ns
Total	7.31	5.80	<i>t</i> = 5.130; <i>p</i> = 0.006	**
ARANEAE				
Lycosidae (wolf spiders)	1.64	1.09	<i>t</i> = 4.278; <i>p</i> = 0.012	*
Salticidae (jumping spiders)	1.68	1.36	<i>t</i> = 0.964; <i>p</i> = 0.389	ns
Unidentified	1.30	1.05	t = 1.849; p = 0.138	ns
Total	4.62	3.51	<i>t</i> = 2.541; <i>p</i> = 0.063	ns
HEMIPTERA			-	
Pentatomidae (green bugs)	1.04	1.03	<i>t</i> = 0.006; <i>p</i> = 0.995	ns
Miridae (mirids)	0.79	0.86	<i>t</i> = - 0.421; <i>p</i> = 0.695	ns
Lygaeidae (lygaeids)	1.09	0.98	<i>t</i> = 0.433; <i>p</i> = 0.687	ns
Cicadellidae (leafhoppers)	1.28	1.17	<i>t</i> = 1.049; <i>p</i> = 0.353	ns
Delphacidae (planthoppers)	1.60	1.50	<i>t</i> = 0.179; <i>p</i> = 0.866	ns
Aphididae (green peach aphids)	1.25	1.18	<i>t</i> = 0.591; <i>p</i> = 0.585	ns
Unidentified	1.34	1.66	<i>t</i> = - 1.873; <i>p</i> = 0.134	ns
Total	8.39	8.38	t = - 0.066; <i>p</i> = 0.950	ns
HYMENOPTERA				
Vespidae (wasps)	1.15	1.22	t = - 1.447; p = 0.221	ns
Formicidae (ants)	1.18	0.87	<i>t</i> = 3.064; <i>p</i> = 0.037	*
Apidae (honey bees)	1.85	0.83	<i>t</i> = 9.636; <i>p</i> = < 0.001	***
Ichneumonidae (Ichneumonids)	1.91	0.88	<i>t</i> = 9.557; <i>p</i> = < 0.001	* * *
Unidentified	0.96	1.04	<i>t</i> = - 1.147; <i>p</i> = 0.305	ns
Total	7.05	4.84	<i>t</i> = 14.206; <i>p</i> = < 0.001	***
Grand Total	36.16	28.89	<i>t</i> = 5.370; <i>p</i> = 0.005	**
			· •	

For each flowering alyssum and control plot, the 38-day weighted mean was obtained by calculating AUC for a graph of V count against time (day) using the trapezoid rule, then dividing by the time period (38 days) (n = 5); (ns) non-significant; (*) p < 0.05, significant; (**) p < 0.01, very significant; (***) p < 0.001, extremely significant.

The abundance of Coleoptera (p < 0.01), Diptera (p < 0.01) and Hymenoptera (p < 0.001) was significantly higher in flowering alyssum plots than in control plots. However, the abundance of Araneae (p > 0.05) and Hemiptera (p > 0.05) was not significantly different in the flowering alyssum plots than in control plots (Fig. 6.3).

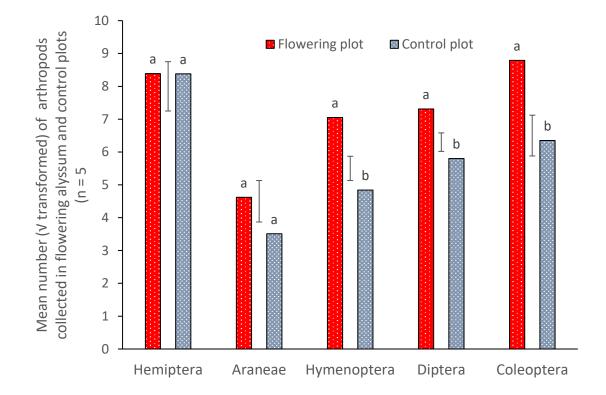
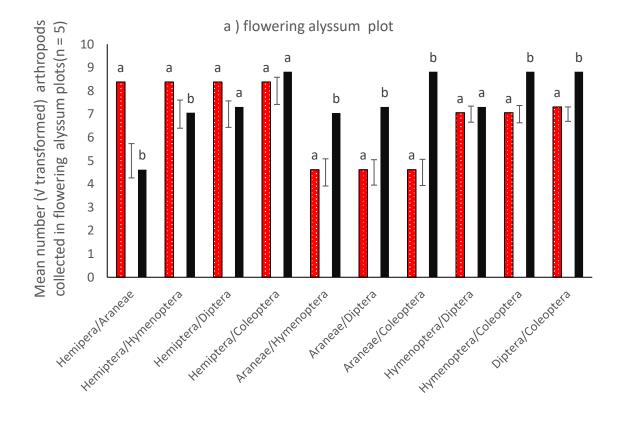


Figure 6.3 Mean number (V transformed) in each arthropod group averaged over six samplings in flowering alyssum (left-hand bar) and control plots (right-hand bar) (n = 5). The vertical bar is the least significant difference, LSD (5 %). The number of species (V transformed) in each group was compared using a paired sample *t*-test (p < 0.05) (n = 5). Group means with no letters in common are significantly different (p < 0.05).

In the flowering alyssum plots, Hemiptera were significantly higher than Araneae (t = 7.188; p = 0.001) and Hymenoptera (t = 2.858; p = 0.046), but not significantly different from Diptera (t = 2.600; P = 0.060) and Coleoptera (t = -1.091; p = 0.336) (Fig. 6.4 a). However, in the control plots, Hemiptera were significantly higher than Araneae (t = 8.669; p = 0.000), Hymenoptera (t = 6.545; p = 0.002), Diptera (t = 4.903; p = 0.008) and Coleoptera (t = 3.120; p = 0.035) (Fig. 6.4 b). In both the

alyssum and control plots, Araneae numbers were significantly lower than Hymenoptera (Flowering t = -4.271; p = 0.012; Control t = 3.918; p = 0.0172), Diptera (Fl: t = -5.199; p = 0.006; C: t = -6.297; p = 0.003) and Coleoptera (Fl: t = -7.075; p = 0.002; C: t = -3.784; p = 0.019) (Figs 6.4a and 6.4b).

In the flowering alyssum plots, Hymenoptera numbers were not significantly different from Diptera (t = -1.927; p = 0.126), but were significantly lower (t = -4.506; p = 0.010) in the control plots. However, both these insect orders (Hymenoptera and Diptera) were significantly lower than Coleoptera in both the alyssum (t = -9.032; p = 0.000) and control plots (t = -3.552; p = 0.023). Coleoptera were significantly higher than Diptera (t = -11.347; p = 0.000) in alyssum plots but not significantly different (t = -1.200; p = 0.296) in the control plots.



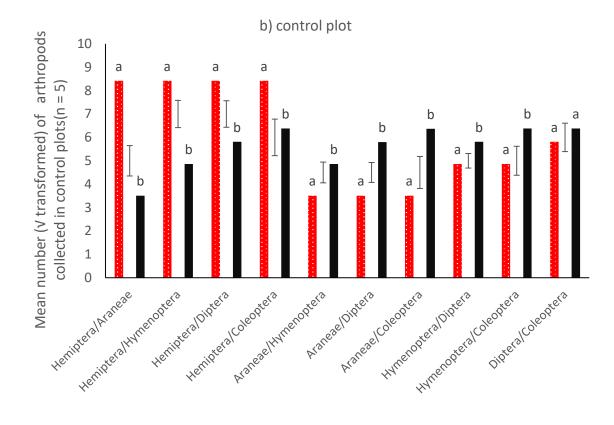


Figure 6.4 Mean number (\vee transformed) of arthropods in pairs for five arthropod groups averaged over six samples in: a) flowering alyssum and b) control plots (n = 5). For each group pair, the number of species (\vee transformed) was statistically compared between the two groups using a paired sample *t*-test (*p* < 0.05) (n = 5). The vertical bar is the least significant difference, LSD (5 %). Within each group pair, means with no letters in common are significantly different (unprotected LSD; *p* < 0.05).

6.4.2 Arthropods in flowering alyssum strips

In the flowering alyssum strips, the dominant insect species encountered in the yellow water traps was the flea beetle, *M. signata* (Coleoptera: Chrysomelidae) (Fig. 6.5), which was significantly more abundant than syrphid flies (t = 3.185; p = 0.033), ants (t = 3.796; p = 0.019), honey bees (t = 4.117; p = 0.014), *S. casigneta* (t = 4.622; p = 0.009), wasps (t = 11.271; p = 0.000), spiders (t = 5.084; p = 0.007) and *Epilachna vigintioctopunctata* F. (t = 9.355; p = 0.000).

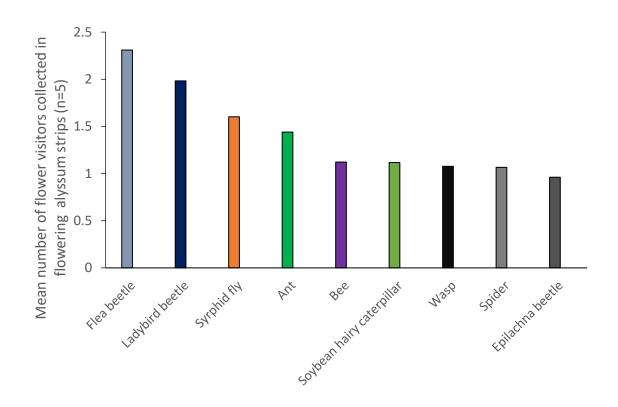


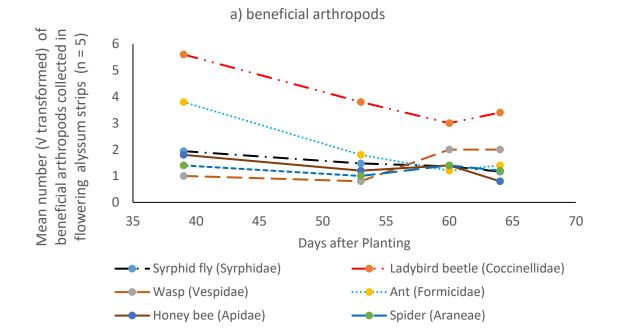
Figure 6.5 Mean number (\vee transformed) of beneficial and harmful arthropods averaged over four samples (from 05- 30 April) in alyssum strips (n = 5).

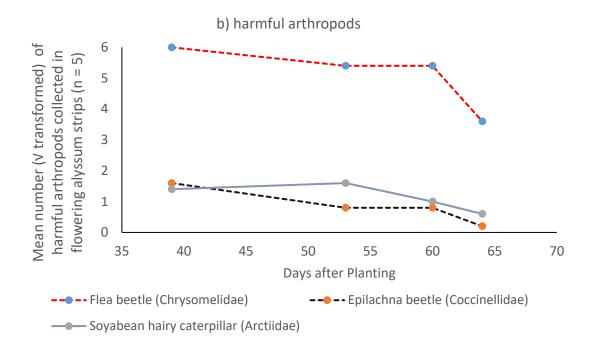
The second most dominant species in the flowering alyssum strips was *C. septempunctata* but its population was not significantly different from syrphids (t = 1.853; p = 0.137) and *M. signata* (t = 1.761; p = 0.153), but was significantly higher than ants (t = 3.432; p = 0.026), honey bees (t = 4.139; p = 0.014), *S. casigneta* (t = 3.511; p = 0.024), wasps (t = 10.223; p = 0.000), spiders (t = 2.883; p = 0.044) and *E. vigintioctopunctata* (t = 5.036; p = 0.007). The syrphid population was not significantly different from ants (t = 1.011; p = 0.369), but was significantly higher than honey bees (t = 4.947; p = 0.007), *S. casigneta* (t = 3.998; p = 0.016), wasps (t = 2.938; p = 0.042), spiders (t = 3.780; p = 0.019) and *E. vigintioctopunctata* (t = 3.484; p = 0.025).

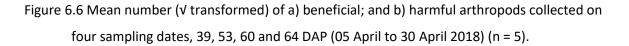
Ant numbers were not significantly different from honey bees (t = 2.173; p = 0.095), *S. casigneta* (t = 2.766; p = 0.050), wasps (t = 2.300; p = 0.082) and spiders (t = 1.391; p = 0.236) but significantly higher than the *E. vigintioctopunctata* (t = 3.361; p = 0.028). The number of honey bees was not significantly different from the *S. casigneta* (t = 0.037; p = 0.971), wasps (t = 0.208; p = 0.845), spiders (t = 0.250; p = 0.814) and *E. vigintioctopunctata* (t = 0.716; p = 0.513). *Spilarctia casigneta* numbers were not significantly different from wasps (t = 0.184; p = 0.862) and spiders (t = 2.074; p = 0.797). The lowest mean recorded in flowering alyssum was for *E. vigintioctopunctata*, which was not significantly different from *S. casigneta* (t = 1.038; p = 0.357), spiders (t = 0.484; p = 0.653) and

wasps (t = 0.745; p = 0.497). The numbers of wasps and spiders were not significantly different (t = 0.040; p = 0.969).

Among the beneficial arthropods, *C. septempunctata* was most abundant in alyssum strips in all sampling periods from 39 to 64 DAP (5 to 30 April 2018) (Fig. 6.6 a). Ants were the second most abundant group in alyssum strips. The number of wasps steadily increased throughout the sampling period. Similar numbers of spiders, bees and syrphids were collected throughout the sampling period. Alyssum hosted many harmful crop pests such as flea beetle (*M. signata*), *E. vigintioctopunctata* and *S. casigneta* (Fig. 6.6 b). *Monolepta signata* was the most abundant species in all sampling periods from 39 to 64 DAP (05 April to 30 April 2018).







6.4.3 Arthropod abundance (syrphids, *Coccinella septempunctata* and winged *Myzus persicae*) next to flowering alyssum strips and the control strips

The mean numbers of syrphids and seven-spotted ladybirds (*C. septempunctata*) were significantly higher in flowering alyssum plots than in the control plots (p = 0.024 and p = 0.036, respectively) (Fig. 6.7). However, the number of *M. persicae* was greater in the flowering alyssum plots than in the control plots, but not significantly so.

In general, the density of syrphids, *C. septempunctata* and winged *M. persicae* in radish plots decreased with increasing distance from the flowering alyssum strips (Figs 6.8, 6.9 and 6.10). However, this trend was less obvious in the control plots. Significantly greater numbers of syrphids were recorded close (0 m) to the alyssum strips compared with 2 m (t = 5.385; p = 0.005), 3 m (t = 8.340; p = 0.001), 5 m (t = 7.281; p = 0.001) and 7 m (t = 8.245; p = 0.001) (Fig. 6.8). Their numbers at 2 m from flowering alyssum strips were significantly higher than at other distances such as at 3 m (t = 2.677; p = 0.055), 5 m (t = 7.628; p = 0.001) and 7 m (t = 3.594; p = 0.022). The numbers were not significantly different between 3 m and 5 m (t = 2.147; p = 0.098), between 3 m and 7 m (t = 0.609; p = 0.574) and between 5 m and 7 m (t = -0.627; p = 0.565).

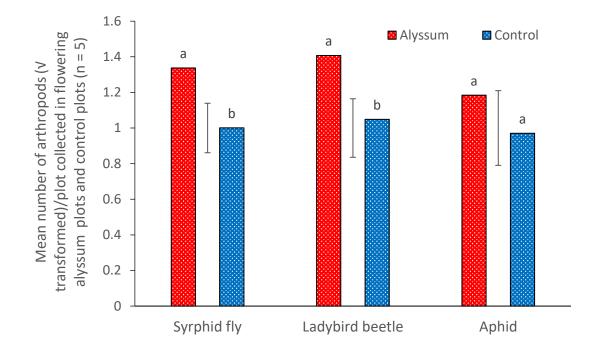


Figure 6.7 Mean numbers (V transformed) of adult syrphids, adult seven-spotted ladybirds (*Coccinella septempunctata*) and winged green peach aphid (*Myzus persicae*) collected in alyssum and control plots (n = 5). The first (left) bar in each pair is for alyssum and the second is the control. The vertical bar is the least significant difference, LSD (5 %). Means with no letter in common are significantly different at p < 0.05.

In the control plots, adult syrphids numbers did not differ significantly between flowering alyssum strips (0 m) and 2 m distance (t = 0.268; p = 0.801), between 0 m and 3 m (t = 0.898; p = 0.419), 0 m and 5 m (t = 1.063; p = 0.347), and 0 m and 7 m (t = 0.746; p = 0.496) (Fig. 6.8). Adult syrphid numbers in the control plots were not significantly different between 2 m and 3 m (t = 1.146; P = 0.315), 2 m and 5 m (t = 1.150; p = 0.313), and between 2 m and 7 m (t = 1.031; p = 0.360). Their populations were also not significantly different between 3 m from the flowering alyssum strips and 5 m (t = -0.690; p = 0.528), between 3 m and 7 m (t = 0.663; p = 0.543) and between 5 m and 7 m (t = 0.044; p = 0.966).

The adult syrphids population near flowering alyssum (0 m) was significantly higher (t = 4.475; p = 0.011) than the population at the same distance in the control plots (Fig. 6.8). The numbers were not significantly different for each of the 2 m, 3 m, 5 m and 7 m distances in both the flowering alyssum and control plots (Fig. 6.8).

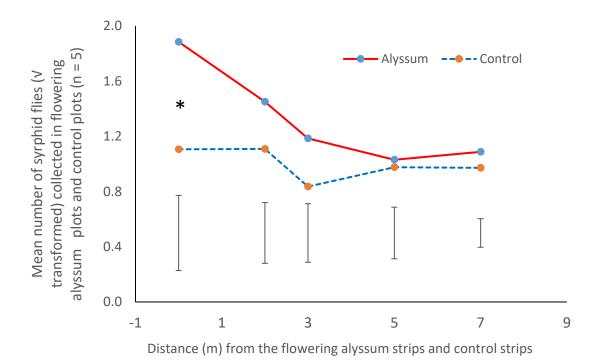
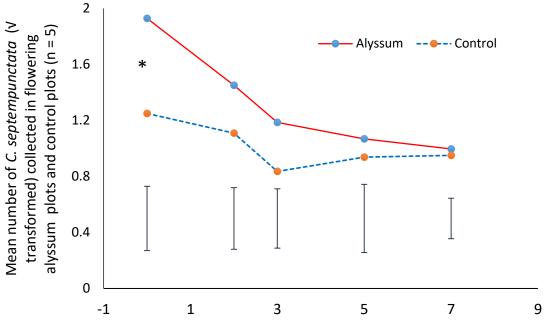


Figure 6.8 Mean number (V transformed) of adult syrphids averaged over five samples at 0 m, 2 m, 3 m, 5 m, and 7 m from flowering alyssum strips and control strips (n = 5). The vertical bar is the least significant difference, LSD (5 %); * represents a 5 % significant difference between alyssum and control plots.

The seven-spotted ladybird (*C. septempunctata*) density was significantly higher close to flowering alyssum strips and declined with distance from the strips (Fig. 6.9). Significantly higher numbers of *C. septempunctata* were recorded at 0 m from the flowering alyssum strips compared with 2 m (t = 3.418; p = 0.026), 3 m (t = 8.277; p = 0.001), 5 m (t = 5.926; p = 0.006), and 7 m (t = 7.953; p = 0.001). The numbers were also significantly higher at 2 m than at 3 m (t = 3.306; P = 0.029), 5 m (t = 11.24; p = 0.000) and 7 m (t = 3.202; p = 0.032) from the flowering alyssum strips. However, *C. septempunctata* adults were not significantly different between 3 m from flowering alyssum strips and 5 m (t = 1.471; p = 0.215), between 3 m and 7 m (t = 1.199; p = 0.296) and between 5 m and 7 m (t = 0.469; p = 0.663).

Coccinella septempunctata population in the control plots was not significantly different between 0 m from the control strips and 2 m (t = 1.604; p = 0.183), 3 m (t = 2.242; p = 0.088) and 7 m (t = 2.517; p = 0.065), but was significantly higher at 0 m compared with 5 m (t = 4.888; p = 0.008) from the control strips. The populations were not significantly different between 2 m and 3 m (t = 1.710; p = 0.162) or between 2 m and 7 m (t = 2.381; p = 0.075), but were significantly higher at 2 m compared with 5 m (t = 3.457; p = 0.025). The populations were not significantly different between 3 m and 5 m (t = -0.652; p = 0.549), 3 m and 7 m (t = -0.773; p = 0.482) and 5 m and 7 m (t = -

0.131; p = 0.901) distant from the control strips (Fig. 6.9). *Coccinella septempunctata* adult populations were significantly higher close to the flowering alyssum strips at 0 m than at the same distance from the control strips (t = 5.258; p = 0.006). The populations were not significantly different at 3 m, 5 m, and 7 m between the alyssum and control plots.



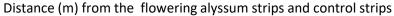


Figure 6.9 Mean number (V transformed) of *Coccinella septempunctata* averaged over five samples at distances of 0 m, 2 m, 3 m, 5 m and 7 m from alyssum and control strips (n = 5). The vertical bar is the least significant difference, LSD (5 %); * represents a 5 % significant difference between alyssum and control plots.

The winged green peach aphid (*M. persicae*) numbers in flowering alyssum and control plots declined with increasing distance from the alyssum and control strips but, in most cases, the decline rate between two distances was not significant (Fig. 6.10). The numbers were significantly higher only at 0 m compared with 7 m (t = 4.721; p = 0.009) from the flowering alyssum strips. However, the numbers collected at each distance from the alyssum strips were not significantly different from each other, i.e., between 0 m and 2 m (t = 1.454; p = 0.219), 0 m and 3 m (t = 1.945; p = 0.123), 0 m and 5 m (t = 2.094; p = 0.104), 2 m and 3 m (t = 1.956; p = 0.122), 2 m and 5 m (t = 2.434; p = 0.071), 2 m and 7 m (t = 2.893; p = 0.044), 3 m and 5 m (t = 1.660; p = 0.172), 3 m and 7 m (t = 1.175; p = 0.304), and 5 m and 7 m (t = 0.361; p = 0.736). However, winged *M. persicae* numbers in the control plots were significantly higher at 0 m than at 2 m (t = 3.133; p = 0.035), at 0 m than at 5 m (t = 4.486; p = 0.010), and at 2 m than at 5 m (t = 2.896; p = 0.044), but not significantly different between 0 m and 3 m (t = 1.534; p = 0.199), 0 m and 7 m (t = 1.948; p = 0.010), and at 2 m than at 5 m (t = 1.948; p = 0.034), but not

0.123), 2 m and 3 m (t = 0.911; p = 0.413), 2 m and 7 m (t = 0.904; p = 0.417), 3 m and 5 m (t = -0.093; p = 0.929), 3 m and 7 m (t = -0.729; p = 0.506) and 5 m and 7 m (t = -1.995; p = 0.116). *Myzus persicae* adult numbers at each distance (0 m, 2 m, 3 m, 5 m and 7 m) were not significantly different between alyssum and control plots (Fig. 6.10).

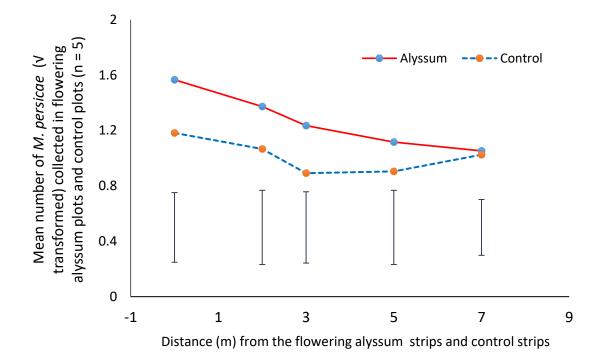


Figure 6.10 Mean number (√ transformed) of winged *Myzus persicae* averaged over five samples at 0 m, 2 m, 3 m, 5 m and 7 m from alyssum and control strips (n = 5). The vertical bar is the least significant difference, LSD (5 %); there was no significant difference at each distance between alyssum and control plots.

6.4.4 Visual observations

The abundance of syrphids larvae (t = 3.779; p = 0.019) and adults (t = 5.445; p = 0.005) was significantly higher in alyssum plots than in the control plots. *Coccinella septempunctata* larval (t =1.740; p = 0.156) and adult (t = 2.298, p = 0.083) densities were not significantly different between flowering alyssum and control plots. The green peach aphid (*M. persicae*) numbers in the flowering alyssum plots were significantly lower than in control plots (t = -7.095; p = 0.002) (Fig. 6.11).

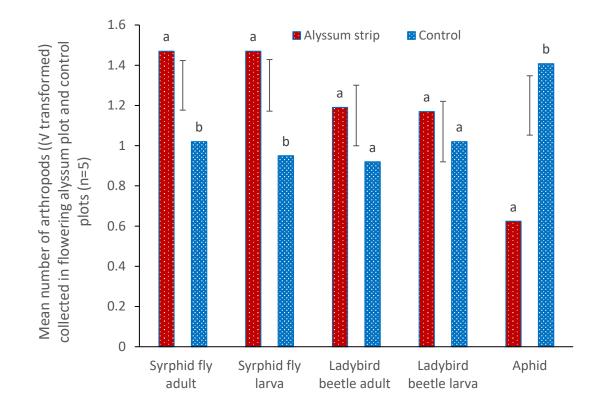


Figure 6.11 Mean number (V transformed) of syrphids (adults and larvae), seven-spotted ladybirds (*Coccinella septempunctata*) (adults and larvae) and winged green peach aphid (*Myzus persicae*) collected in flowering alyssum and control plots (n = 5). The first (left) bar in each pair is for the alyssum plots and the second is the control. The vertical bar is the least significant difference, LSD (5 %); means in each pair were statistically compared using a paired sample *t*-test (p < 0.05) (n = 5).

6.5 Discussion

This study evaluated the effect of alyssum flowers on the abundance of beneficial arthropods that likely play an important role in suppressing insect pests in radish fields. The frequently encountered beneficial predators and parasitoids in the study fields were syrphids, carabids, rove beetles, spiders, ants, *C. septempunctata*, and ichneumonids (Table 6.1). *Myzus persicae*, *S. casigneta*, *M. signata*, leafhoppers, plant hoppers, lygaeids etc. were the major insect pests collected in the plots. These pests are the economic pests of radishes in tropical and sub-tropical regions (Kunjwal & Srivastava, 2018). The study hypothesised that the provision of floral resources in a radish field would increase the abundance and 'fitness 'of predators and parasitoids, and improve the CBC of radish pests.

Alyssum has been commonly used in CBC (Ambrosino, Luna, Jepson, & Wratten, 2006; Aparicio, Gabarra, & Arnó, 2018; Colley & Luna, 2000; Gurr et al., 2000; Haseeb et al., 2018; Ribeiro &

Gontijo, 2017). Reasons for using this plant include: a perennial habit and spreads well in the Mediterranean (Austin & Dowton, 2000), an excellent source of pollen and nectar (Landis et al., 2000), a good competitor with weeds (Begum, Gurr, Wratten, Hedberg, & Nicol, 2006), a short corolla that makes nectar accessible for syrphid fly adults (Vattala, Wratten, Phillips, & Wäckers, 2006). Maintaining such flowering strips within-crop and at farm and landscape levels improves the availability of floral resources for predators and parasitoids (Colley & Luna, 2000; Landis et al., 2000), enhances the longevity, survival, searching efficiency and fertility of predators (Barbir et al., 2015; Brennan, 2016; Colley & Luna, 2000; Pineda & Marcos-García, 2008; Ribeiro & Gontijo, 2017) and reduces the herbivore populations and crop damage (Gurr et al., 2000). For example, flowering alyssum is attractive to predators such as the minute pirate bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), *C. septempunctata*, assassin bugs (Hemiptera: Reduviidae), damsel bugs (Hemiptera: Nabidae), syrphids and spiders (Araneae) (Haseeb et al., 2018).

6.5.1 Arthropods in flowering alyssum and control plots

Flowering alyssum strips bordering radish plots have increased the abundance of beneficial arthropods and herbivores compared with control plots (Table 6.1). Flowering alyssum significantly increased the abundance of Coleoptera, Diptera and Hymenoptera but had no significant effect on Araneae and Hemiptera compared with the control plots (Fig. 6.3). Beneficial predators such as C. septempunctata, syrphid larvae, carabids, rove beetles, spiders, ants, wasps, honey bees and parasitoids numbers were significantly increased in alyssum plots compared with the control plots. Similar results have been suggested by various researchers, e.g., the survival, fecundity, longevity and performance of aphidophagous syrphids and other beneficial arthropods were significantly increased by the provision of floral resources compared with non-flower plants (Ambrosino et al., 2006; Baggen & Gurr, 1998; Colley & Luna, 2000; Hickman & Wratten, 1996; Landis et al., 2000; Pineda & Marcos-García, 2008). Long et al., (1998), in an insect dispersal study, confirmed that beneficial arthropods, such as seven-spotted ladybirds, lacewings, syrphids and wasps, provided benefits in flowering farms compared with non-flower farms. The populations of crab spiders, chalcidoid wasps and predatory bugs were significantly higher in flowering mealy cup sage (Salvia farinacea Benth.) bordered fields than in non-flower fields (El-Nabawy, Tsuda, & Sakamaki, 2015). Increasing the diversity of natural enemies or interspecific differences may have a positive effect on biocontrol leading to resource partitioning, when natural enemies feed on different pests (Finke & Snyder, 2008). It may facilitate one natural enemy species' feeding by other species (Losey & Denno, 1998) or may have a negative effect by influencing the rate of intra-guild predation or interspecific interference (Prasad & Snyder, 2006) and inter-specific competition (Costamagna, Landis, & Brewer, 2008).

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Flowering plants in crop fields provide floral rewards to beneficial arthropods and increase their fitness (Irvin et al., 1999), resulting in lower herbivore populations and reduced crop damage (Gurr et al., 2000). Maintaining such non-crop floral habitats in- and off-farm potentially provide SNAP to the pests' natural enemies and to pollinators (Gurr et al., 2017), which improves the arthropod-mediated ecosystem services (AMES) (Isaacs et al., 2009). Floral nectar is a rich source of sugars, proteins, lipids and many other organic/inorganic substances used for growth and development by insect predators and parasitoids (Nicolson & Thornburg, 2007). Spiders use floral resources to supplement their food (Taylor & Pfannenstiel, 2008). However, the effectiveness of floral habitats can be influenced by a candidate plant that can alter the behaviour of natural enemies. For example, volatiles produced by molasses grass, *Melinis minutiflora* (Beauv.), repel female stem borers, *Chilo partellus* Swinhoe, and attracts female foraging *Cotesia sesamiae* (Cameron), which increases the parasitism rates of stem borer in maize fields (Khan et al., 1997). The spatial scale, structure, location and complexity of habitats also affect natural enemy efficiency (Landis & Menalled, 1998).

In contrast, crop pests such as *M. signata*, weevils, fruit flies and bugs (mirids, leafhoppers, planthoppers, aphids, and lygaeids) did not differ significantly in abundance between flowering alyssum and control plots (Table 6.1). However, a similar study by Lee and Heimpel (2005) reported that buckwheat (Fagopyrum esculentum Moench) in cabbage plots had no effect on cabbage pests such as cabbage looper (Trichoplusia ni Hübner), white butterfly (Pieris rapae L.) and diamondback moth (Plutella xylostella L.). Herbivore populations were higher in flowering plots than in the control plots (Lee & Heimpel, 2005) (Table 6.1). Flowering plants increase herbivore populations when they feed on floral nectar and pollen (Begum et al., 2006; Pinheiro, Torres, Raimundo, & Santos, 2015; Winkler, Wäckers, Termorshuizen, & van Lenteren, 2010; Zhao, Ayers, Grafius, & Stehr, 1992) and such pests potentially demonstrate antagonistic or synergistic effects to their natural enemies in the presence of floral resources (Jonsson, Wratten, Robinson, & Sam, 2008; Robinson et al., 2008). For example, the longevity of Tasmanian lacewing, Micromus tasmaniae Walker (Neuroptera: Hemerobiidae), was higher in buckwheat (F. esculentum: Polygonaceae) in the absence of the pea aphid, Acyrthosiphon pisum Harris (Homoptera: Aphididae). Micromus tasmaniae's fecundity was unaffected by buckwheat in an abundance of prey (A. pisum). When aphids were less abundant, lacewings fed on buckwheat flowers which positively influenced their pre-oviposition and oviposition rate. Hence, the risk of flowering plants, pests and natural enemies potential interactions need to be evaluated or considered in ecological engineering (Zhu et al., 2015).

6.5.2 Arthropods in flowering alyssum strips

In this study, nine main groups of insects were encountered in the alyssum strips. Some were beneficial insects and some were crop insect pests (Fig. 6.5). Spiders, syrphids, wasps, ants and C. *septempunctata* were the dominant insect predators collected from the floral strips. *Coccinella septempunctata* and syrphids were the most frequently encountered beneficial arthropods in the alyssum strips followed by ants, honey bees, wasps and spiders. This result corroborates the findings of Haseb et al., (2018), and Colley and Luna (2000). *Coccinella septempunctata* and syrphids are predators of soft-bodied insects such as aphids and thrips (Tenhumberg & Poehling, 1995) and potentially control aphids in brassica fields (Fig. 6.11). However, their predation rate might decrease when they encounter sufficient pollen and nectar in floral habitats (Prasad & Snyder, 2006).

Monolepta signata, S. casigneta and epilachna beetles are generalist crop pests frequently encountered in alyssum strips. In many agricultural systems, mixed cropping and habitat diversity have elevated pest numbers (Bianchi, Booij, & Tscharntke, 2006; Winkler et al., 2010; Zhao et al., 1992). These generalist pests were supposed to migrate from other adjoining crops/non-crop hosts and potentially pose a threat to a variety of crop species near the research fields. The abundance of *M. signata* in the alyssum strips was significantly higher than that of beneficial arthropods (Figs 6.5 and 6.6 b). The second most encountered pest was S. casigneta (Figs 6.5 and 6.6b). Both species were major pests of crucifers and significantly damage crops such as cabbage, cauliflower, broadleaf mustard and radishes (Kunjwal & Srivastava, 2018; Neupane, 2011). The relatively higher populations of *M. signata* and *S. casigneta* in alyssum strips, potentially damaging the alyssum flowers, could lead to the death of alyssum plants and limit the provision of multiple ES (Fig. 6.12) (Gurr et al., 2017). Outbreaks of such pests in alyssum strips could damage adjoining radish crops and needs immediate pest management action before such outbreaks occur. In large commercial fields, careful consideration should be given during pesticide use on flower strips to protect beneficial flower visitors such as syrphids, honey bees, spiders and seven-spotted ladybirds. Dead alyssum plants or other refuges can provide an overwintering shelter for the pest and it will likely damage next season's crop (Hokkanen, 1991). Epilachna vigintioctopunctata was another common pest in the research fields. It is a serious pest particularly of bitter gourd, cucumbers, crucifers and legumes (Nair, Thangjam, Bhattacharjee, & Debnath, 2017) and was presumed to have migrated from nearby cucurbit fields. However, such insects and potential biotic competitors need to be evaluated before adopting a CBC approach (Hogg et al., 2011).



Figure 6.12 Soybean hairy caterpillar, *Spilarctia casigneta*, feeding on alyssum flowers potentially limits ecosystem services in radish fields.

6.5.3 Arthropod abundance (syrphids, *Coccinella septempunctata* and winged *Myzus persicae*) next to flowering alyssum strips and the control strips

In yellow pan traps, a significantly higher number of adult syrphids and seven-spotted ladybird adults were recorded in alyssum plots than in the control plots (Fig. 6.7). However, from visual observations, seven-spotted ladybird populations (adults and larvae) were not significantly different between the flowering alyssum and control plots. However, the syrphids populations (adults and larvae) were significantly affected by flowering alyssum plots compared with the control plots (Fig. 6.11).

Two contrasting results for *M. persicae* were obtained from the two sampling methods used in this study. In the yellow water traps, winged *M. persicae* counts were higher in the alyssum plots than in the control plots, but not significantly so (Fig. 6.7). In contrast, visually, *M. persicae* numbers (winged and wingless) were significantly lower in alyssum flower plots than in the control plots (Fig. 6.11). In first case, the food resources close to the flowering alyssum strips potentially draw more winged *M. persicae* than the control strips (Begum et al., 2006). However, these results can be influenced by prevailing weather, sampling methodology, type of insect, monitoring tools and the collector's efficiency (Dent, 2000; Flint & Resources, 2012; Pedigo, 1989). The higher number of aphids near the flowering plants can damage alyssum flowers and likely limit multiple ES (Baggen & Gurr, 1998; Begum et al., 2006; Gurr et al., 2017). The low number of aphids in the alyssum plots

could be an effect of flowering resources on the fitness of natural enemies and the promotion of CBC that potentially reduces the pest population (Gurr et al., 2017; Landis et al., 2000).

In this study, higher numbers of syrphids, seven-spotted ladybirds and aphids were found near the alyssum which indicates that those arthropods concentrate in rich floral resources and use them for SNAP and leads them to aggregate (Hickman, Lövei, & Wratten, 1995). The numbers of syrphids and seven-spotted ladybirds decreased with increasing distance from the floral resources (Wratten et al., 2003b). These species collected close to the alyssum strips (0 m) were in significantly higher numbers than those collected at the same distance from the control strips but there were no significant differences at other distances (2, 3, 5 and 7 m) between flowering and control plots (Figs 6.8, 6.9 and 6.10). The gradual decline of aphid numbers from the flowering alyssum strips indicated that the manipulation of floral strips did not possess strong positive effects on the reduction of aphid populations (Long et al., 1998). However, in the control plots, aphid numbers slightly increased from 5 to 7 m, which supports the result of Baggen and Gurr's (1998) result and indicates there were not enough predators to prey on the pest. Such a trend in syrphids adults and larvae, and aphids have been reported in New Zealand in a study on the effect of a strip of coriander (*C. sativum*) on syrphid populations and cabbage pests (Morris & Li, 2000). These researchers suggested that such a population variation could be because of variation in soil fertility and moisture rather than an effect of the floral strips.

6.6 Conclusions

In summary, the results suggest that increased floral resources in a habitat management strategy significantly increase the abundance of beneficial arthropods that can potentially reduce pest populations in radishes. This suggests that polyculture practices and non-crop habitats in- and off-farms can reduce pest pressure and potentially reduce pesticide use (Gurr et al., 2017; Landis et al., 2000; Zehnder et al., 2007). The plant species should be selective for natural enemies and pests, which is the major challenge in designing a habitat management protocol. The selection of potential plant species in CBC has to follow the ARMED (access, rank, manipulate, evaluate and develop) selection strategy before deployment in fields (Morgan Shields, Bio-Protection Research Centre, pers. comm.). The evaluated plants should be less attractive to the pest (Baggen, Gurr, & Meats, 1999). If the insect pest's preference is significantly higher for the flowering plant and causes significant damage to it, such flowering plants could be a potential pest food source (Begum et al., 2006; Winkler et al., 2010) and potentially cause ecosystem DS (Gurr et al., 2017). Hence, habitat manipulation modelling has been suggested to reduce such potential ecosystem DS (Kean, Wratten, Tylianakis, & Barlow, 2003). Further, ecosystem service providers (SP) and associated service providing units (SPU) need to be clearly identified, followed by the development of a

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service providing protocol (SPP) for growers' adoption (Gurr et al., 2017). The development of a habitat management protocol for the candidate plant is a suitable pathway for small farmers who cannot afford pesticides. Arthropod-mediated ecosystem services (AMES) in local conditions can be promoted using local, native perennial plants, which enhances the adoption of multiple conservation goals in developed and developing countries (Isaacs et al., 2009).

Chapter 7

Discussion and Conclusions

The world's human population is estimated to reach 11 billion by the end of this century, with the highest population growth rate in tropical nations (Laurance et al., 2014). By the middle of this century, food production needs to increase by 70 - 110 % to meet global food demand (Tilman et al., 2001). Fulfilling the global food demand from the limited land area is the most challenging task for agriculture. Before the 1960s, land area expansion and modernization of agricultural practices were key options in food production (Laurance et al., 2014). The agricultural area can be expanded to a certain degree from the fixed land area by converting current forest, woodlands or other uncultivated fallow lands. Currently, approximately 53 % of the total earth's land area is used for agricultural purposes (Hooke, Martín-Duque, & Pedraza, 2012) and a further 10 % is expected by 2030 in the developing countries (Haines-Young, 2009). Modernization of agricultural practices is the second option to increase food production to meet global food demand. Current practices consume large amounts of chemical fertilizers, fuel, electricity, synthetic chemical pesticides, high yielding hybrid varieties and also cause intensive soil disturbance (Tilman, Cassman, Matson, Naylor, & Polasky, 2002) that leads to negative environmental impacts on soil, water, air and biodiversity (Firbank, Petit, Smart, Blain, & Fuller, 2007). These anthropogenic activities have been linked to the natural habitat destruction and land fragmentation (Fahrig et al., 2011; Kovács-Hostyánszki et al., 2017). Fewer crop and non-crop habitats in heterogeneous fragmented landscapes exacerbate biodiversity loss and a loss of ES on which current agriculture depends (Landis, 2017). These practices have also been associated with environmental challenges such as biodiversity loss and negative impacts on beneficial arthropods such as predators, parasitoids and pollinators (Senapathi et al., 2015; Tscharntke et al., 2012), which exacerbates ecological functions and ES (Costanza et al., 2017; Costanza et al., 1997).

After the publication of *'Silent Spring'* by Rachel Carson in 1962 (Carson, 1962), negative impressions of agricultural intensification have seen attempted to replace it by ecological or sustainable intensification, organic farming and other agro-ecological pest management practices (Godfray & Garnett, 2014; Pretty & Bharucha, 2015). These important practices include intercropping (Cook, Khan, & Pickett, 2007), trap cropping (Shelton & Badenes-Perez, 2006), cover cropping (Hokkanen & Menzler-Hokkanen, 2018), improving habitat quality using floral strips to support CBC (Baude et al., 2016; Fiedler et al., 2008; Goulson et al., 2008; Gurr et al., 2000). These practices are also associated with improved ES such as pest control, pollination and improved soil fertility that are needed for sustainable crop production (Fiedler et al., 2008; Kovács-Hostyánszki et al., 2017; Landis et al., 2000; Power, 2010).

Insect pests are major crop-limiting factors in agricultural production (Oerke, 2006). Integrated pest management, a form of ecological intensification with trap cropping, use of less susceptible varieties and intercropping, can be used to develop a pest management protocol (Ehler, 2006; Flint & Van den Bosch, 2012). Such habitat management protocol can be developed to keep the pests away from the main fields (Shelton & Badenes-Perez, 2006), and also improve CBC (Gurr et al., 2017; Landis et al., 2000) that ultimately reduces the pesticide load in agricultural fields (Kovács-Hostyánszki et al., 2017). This study aimed to reduce the damage on brassica seedlings by Nysius huttoni White using agro-ecological pest management approaches such as trap cropping and less susceptible kale cultivars (Chapters 2 to 5) and improving CBC in radish fields (Chapter 6). Nysius huttoni damages brassicas during the seedling stage and reduces seedling populations. Hence this pest is sometimes called a crop establishment pest (PGG, 2009). This study is the first environmentally-sound investigation of N. huttoni management in New Zealand. The protocol developed in this study is not only useful for *N*. huttoni management but is also important for the management of other pests in forage brassicas and other crops. The trap crop (alyssum) with the most potential for N. huttoni could be deployed in forage brassicas to attract, 'pull', bugs from the main crop and reduce pest pressure in fields (Tiwari et al., 2018). It has also been suggested that flowering alyssum strips should be maintained at field edges at the kale seedling stage. Less susceptible kale cultivars, such as Corka and Regal, can be used to deter, 'push', N. huttoni from brassica fields. This is a 'push-pull' strategy (Khan et al., 2001). The most suitable trap crop for N. huttoni was selected after a series of laboratory, field-cage and open-field experiments using the ARMED (access, rank, manipulate, evaluate and develop) procedure. A series of choice, no-choice and paired-choice tests was established at the Bio-Protection Research Centre (BPRC), Lincoln University, to choose suitable host plants for *N. huttoni* from a range of potential trap plant species (Chapter 2). A laboratory test of the susceptibility of kale cultivars to *N. huttoni* was performed on a range of kale cultivars (Chapter 3). The preferred growth stage of the potential trap plant, alyssum, was evaluated in laboratory choice and no-choice tests (Chapter 4). The most promising potential trap crops for the *N. huttoni*, such as alyssum and wheat, based on a laboratory study, were evaluated in field cages and open-field experiments (Chapter 5). These potential trap crops were compared with the most susceptible kale cultivar, i.e., Kestrel, in both field-cage and openfield experiments (Chapter 5). The experimental work in an open field evaluated the damage potential of *N. huttoni* on brassica seedlings and the suitable growth stages of potential trap plants. This information is important in developing a sustainable bug-management protocol for brassicas. Last, but not least, alyssum (L. maritima) which is the potential trap crop of the N. huttoni, also can

be used to improve CBC of other brassica pests (Chapter 6). Because of financial and timing constraints, many unanswered questions arising in each set of experimental work could not be addressed, but do provide suggestions for future study (see section 7.4).

7.1 Study approach and outcomes

The overall aim of this study was to develop an integrated management protocol for *N. huttoni* and so reduce the pesticide load in forage brassicas. The protocol comprises the deployment of the most promising trap crops in kale fields that are less susceptible to *N. huttoni*.

7.1.1 Host plant selection by *Nysius huttoni* on a range of potential trap plant species

The first experimental chapter (Chapter 2) described three major approaches: choice, no-choice and paired-choice tests. First, wheat bug's (N. huttoni) identity was confirmed by hemipteran taxonomist, Dr Marie-Claude Lariviere, and a laboratory culture was maintained at the BPRC for the regular supply of bugs for the bioassays. The choice and no-choice tests were performed in a controlled-temperature (CT) room to evaluate the bug's preference for seedlings of Lobularia maritima L. Desv. (alyssum), Triticum aestivum L. (wheat), Phacelia tanacetifolia Bentham (phacelia), Fagopyrum esculentum Moench (buckwheat), Coriandrum sativum L. (coriander), Trifolum repens L. (clover) and Medicago sativa L. (lucerne). This study suggested that alyssum and wheat could be the most useful hosts of *N*. huttoni and could be used as a potential trap crop combination in brassica fields. The preferences of *N. huttoni* could be affected by physical and chemical cues of the host plants (Badenes-Perez et al., 2004; Coffey, Simmons, Shepard, & Levi, 2016; Du et al., 1998). In this study, a Y-tube olfactometer test was not used to evaluate the bug's 'preferences' on the basis of plant volatiles. The highly preferred plant species, such as alyssum and wheat, could be deployed to attract or 'pull' bugs into the trap crop in brassica fields, and less preferred plants (feeding deterrence), such as clover, coriander, lucerne and buckwheat, could be used to deter/repel or 'push' the bugs away from brassica fields. These findings suggest that the 'push-pull' strategy to repel or deter (repellent crops) the bugs from the main fields and/or attract or trap (trap crops) the bugs could be a possible strategy to suppress N. huttoni populations in brassica fields (Khan et al., 2001; Pickett, Woodcock, Midega, & Khan, 2014). Flowering alyssum in brassica fields can improve CBC (Barbosa, 1998; Gurr et al., 1998; Gurr et al., 2000) and potentially deliver multiple ES (Gurr et al., 2017). Significant weight loss and height reduction in alyssum and wheat plants could produce negative consequences such as death of the trap plants when these plants are deployed as a trap crop in brassica fields. In some cases, flowering trap crops harbour many pests that damage alyssum flowers. These consequences are ecosystem dis-services (Baggen & Gurr, 1998; Zhang et al., 2007).

7.1.2 Susceptibility of kale cultivars to Nysius huttoni

Chapter 3 focused on evaluating relatively susceptible kale cultivars on the basis of N. huttoni preference. A series of choice and no-choice tests were performed in a replicated design in a CT room at the Bio-Protection Research Centre, Lincoln University. The experimental approaches included host-preference work on kale seedlings under the choice and no-choice tests as suggested by Ulmer et al. (2001). The results confirmed that *N. huttoni* favoured one group of kale cultivars (Kestrel, Coleor, Gruner and Sovereign) over another (Corka and Regal). Ironically, Kestrel and Coleor, the most popular kale cultivars in New Zealand (PGG, 2009), are also the most susceptible to N. huttoni. Regal was the least preferred cultivar based on damage by the bug, followed by Corka and Sovereign. This information is important in designing a pest management protocol using a 'push-pull' strategy where the least susceptible kale cultivars are used as a 'push' component and highly susceptible kale cultivars as a 'pull' component in the 'push-pull' pest management approach (Khan et al., 2001). The 'push' and 'pull' characteristics of the cultivars can be further confirmed by chemical analysis using Y-tube olfactometer tests (Koschier, De Kogel, & Visser, 2000; Koschier, Nielsen, Spangl, Davidson, & Teulon, 2017). Future research could focus on improving the 'push' or 'pull' nature of cultivars by biotechnology or standard breeding (Ashkani et al., 2015; Moose & Mumm, 2008; Sharma, Crouch, Sharma, Seetharama, & Hash, 2002; Zhou et al., 2015).

It has also been suggested that the less susceptible cultivars, such as Regal and Corka (Tiwari et al., 2019), can be integrated into other habitat manipulation approaches such as trap cropping or intercropping to keep *N. huttoni* away from the main crop and reduce the pesticide load in brassicas (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Less susceptible cultivars can be incorporated into other pest management options such as microbial and biological control, 'soft' chemicals and other IPM tools for better results (Dent, 2000).

However, the selection of cultivars by farmers is not only influenced by insect resistance characteristics but, equally important, other plant attributes such as environmental adaptability, productivity and yield stability (Asrat et al., 2010), crop quality (Bruch & Meng, 1998), pest, disease, lodging resistance (Burman et al., 2018), and tolerance to abiotic stress (De Micco, Buonomo, Paradiso, De Pascale, & Aronne, 2012). Field susceptibility of kale cultivars to *N. huttoni* also depends on habitat (Eyles, 1965), e.g., hot dry weather conditions and plant spacing (Gurr, 1957), and vigorous growth of grass or weeds around the field edges (Eyles, 1965). The outcomes of Chapter 3 and other information can help farmers to develop a pest management protocol and potentially decrease their reliance on pesticides.

7.1.3 Preferences of *Nysius huttoni* for particular growth stages of the potential trap plant, alyssum (*Lobularia maritima*)

Plant growth stages have different preference levels for insect pests (Hokkanen, 1991). Chapter 4 focused on evaluating the preference level of *N. huttoni* for alyssum in laboratory choice and no-choice tests. The results showed that flowering alyssum was more favoured by *N. huttoni* than the vegetative or seedling stages. This result suggests maintaining flowering alyssum strips at the brassica seedling stage in the main fields to trap maximum numbers of *N. huttoni*. Flowering volatiles or other chemicals could be regulating the host plant preference of the insects (Ceballos et al., 2015; Yang et al., 2017). Hence, it is suggested that the flowering volatiles present in flowering alyssum be evaluated. In future, such volatiles could be extracted or artificially produced to be sprayed on flowering plants to increase trap crop efficiency (Bruce et al., 2005). Flowering plants in agro-ecosystems also improve habitat quality and promote CBC (Begum et al., 2006; Berndt & Wratten, 2005; Landis et al., 2000) and improve multiple ES (Fiedler et al., 2008; Olson & Wäckers, 2007).

7.1.4 Evaluation of potential trap plant species for *Nysius huttoni* in forage brassicas

Chapter 5 evaluated potential trap plant species for *N. huttoni* in field-cage and open-field experiments. In field cages, the favoured trap plant species, such as alyssum and wheat, and least preferred plant species, such as clover and coriander, were evaluated; all species were compared with kale. These plant species were selected based on host plant selection by *N. huttoni* in a series of laboratory studies (Tiwari et al., 2018). In open-field experiments, only two plant species, alyssum and wheat, were used; the other species were discarded based on their poor performance. These studies confirmed that alyssum and wheat are the most likely trap crops for *N. huttoni*. A mixture of alyssum and wheat trap crops in brassica fields trapped a greater number of *N. huttoni* than wheat alone. This suggests that *N. huttoni* can be trapped either by using a single trap crop (alyssum) or a multiple trap crop (a mixture of alyssum and wheat). However, multiple trap cropping may not be practical for farmers because it may increase production costs and labour intensity (Shelton & Badenes-Perez, 2006).

Adult and nymph numbers of *N. huttoni* were higher at brassica field edges than in the centre. This finding suggests focussing a pest management strategy at the field edge, which can reduce pesticide load in the main field. However, this study was not focussed on *N. huttoni* distribution in brassica fields, the bugs' initial arrival direction, and appropriate deployment of the trap crop in brassica fields.

The population dynamics of *N. huttoni* vary through the cropping season and growth stages. The reproductive stages of alyssum and wheat were more favoured by *N. huttoni* than the vegetative stage. Flowering, fruiting and senescent stages of alyssum and the seed ripening and senescent stages of wheat are suitable crop growth stages for *N. huttoni*. This information is important for the deployment of trap plants and main crops in brassica fields (Shelton & Badenes-Perez, 2006). *Nysius huttoni* is a seedling pest of brassicas (PGG, 2009) and its damage is more obvious during that stage (Eyles, 1965). Hence farmers should be alerted to maintaining alyssum or wheat trap plants at the seedling stage of brassica crops. This would help farmers to reduce pest density and pesticide pressure in the main field. Furthermore, flowering alyssum in brassica fields can provide SNAP for predators and parasitoids and potentially improve CBC of *N. huttoni* and other pests (Gurr et al., 2007). The worst case is that flowering plants could provide habitat for crop pests (Baggen & Gurr, 1998), which limits ES (Gurr et al., 2017) and finally leads to deterioration of the habitat (Baggen & Gurr, 1998; Zhang et al., 2007).

7.1.5 Alyssum flowers promote arthropod diversity and biological control of radish pests

From Chapters 2 - 5, it is recommended that alyssum be used as a trap crop for *N. huttoni* in forage brassicas. This plant is one of the most used plant species in CBC of insect pests (Badenes-Pérez, 2018; Meyhöfer & Poehling, 2006; Wratten et al., 2013). Chapter 6 evaluated the influence of floral resources on arthropod diversity and so improve CBC of aphids and other pests in radishes. Added flowering resources in a farming system can deliver multiple ES such as biological control as well as improved soil fertility and can improve habitat quality (Gurr et al., 2017; Robinson et al., 2008). A replicated design was established in radish fields with flowering alyssum strips as a treatment plot that was compared with the control (non-alyssum flower) plot.

Flowering alyssum plus radish plots increased the abundance of natural enemies such as carabids, rove beetles, seven-spotted ladybirds, syrphids, wolf spiders, ants and ichneumonids, compared with the control plots. Visual observation of five radish plants in the treatment plots suggested that flowering plots increased the abundance of syrphids and seven-spotted ladybirds and improved biological control of *M. persicae*. The aggregation of syrphids and seven-spotted ladybirds was significantly higher in flowering strips and the numbers declined with distance from the flower strips. These results demonstrate that alyssum flowers in radish fields increase the abundance of generalist and specialist predators, which strongly suppresses aphids and other pests (Berndt & Wratten, 2005; Fiedler et al., 2008; Haseeb et al., 2018; Landis et al., 2000; Ribeiro & Gontijo, 2017). For this reason, it is expected that farmers would use this technology as a part of an IPM

approach to manage aphids and other pests in radishes in Nepal. In some instances, pests can also benefit from flowering plants and can kill alyssum and limit ES (Baggen & Gurr, 1998; Begum et al., 2006; Gurr et al., 2017).

7.2 Implications for Nysius huttoni management

Habitat manipulation using the cultural aspects of pest management such as trap cropping and intercropping has been recognised as an important strategy in pest management of agricultural fields (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). This study investigated alternative techniques of pest management that are more sustainable, environmentally friendly and economically viable (Pamminger et al., 2018; Rusch, Valantin-Morison, Sarthou, & Roger-Estrade, 2010). Insect pest management in forage brassicas in New Zealand mostly relies on chemical pesticides (Manktelow et al., 2005). These practices are not sustainable and could create many negative consequences for human health, biodiversity and the environment (Cimino, Boyles, Thayer, & Perry, 2016; Matthews, 2015; Pamminger et al., 2018). Hence, there is an increasing interest in trap cropping (Hokkanen, 1991; Shelton & Badenes-Perez, 2006) and multiple ES in farming systems for sustainable agriculture (Gillespie, Gurr, & Wratten, 2016; Gurr et al., 2017).

It has been suggested that alyssum can be used as a trap crop for *N. huttoni*. Alyssum and wheat plants as a multiple trap crop has been suggested as a second option for trap cropping (Tiwari et al., 2018). These trap crops can be maintained in small area of a brassica field (10 % of total crop area) (Badenes-Perez et al., 2005) to attract or divert the pest away from the main crop to where pest can be managed by cultural, mechanical, biological or 'soft' chemicals (Shelton & Badenes-Perez, 2006; Shelton & Nault, 2004). Flowering or fruiting alyssum and seed ripening and senescent wheat are the most suitable stages to attract N. huttoni. It has also been suggested that the trap crop be deployed at the edges of fields to intercept the bugs and restrict their movement into the main crop, thereby protect the brassica seedlings from bug damage (Boucher et al., 2003). Nysius huttoni normally damages brassica crops at the seedling stage rather than other plant growth stages (Eyles, 1965; He & Wang, 2000). Protecting the seedling stage in brassicas could be a challenging task for farmers. Hence, the timing of planting trap crops and the main crop should be harmonized so that, at the brassica seedling in the main field, alyssum strips should be flowering in the field edges to protect the brassica seedlings from bug damage. Weed vegetation near brassica fields is the main source of bugs arriving in the main crop. Hence, all primary weedy vegetation should be removed to reduce bug outbreaks in the main crop. The implications of these results can be exploited to develop IPM protocols and pesticide reduction in brassica fields.

However, trap cropping is not popular with farmers. The suggested reasons are: it is a knowledgeintensive practice (Shelton & Badenes-Perez, 2006); can get lower economic returns than from conventional methods; it is species-specific; and it needs extra investment to manage trap crops and main crops (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Communicating these practices to farmers for wider adoption is not the responsibility of the scientific community. It has been suggested that researchers and scientists should focus their research on being farmercentred and developing the right pathway for implementation in a farmers field (Warner, 2007).

This benign technology can be integrated with other agro-ecological approaches such as using less susceptible cultivars (Fathipour & Sedaratian, 2013). Kale cultivars, such as Corka and Regal, are less susceptible to the *N. huttoni* (Tiwari et al., 2019). However, the cell chemicals' or volatiles' relationships of these cultivars to *N. huttoni* could not be addressed in this study. Van Emden (1991) suggested that the use of less susceptible cultivars can reduce pesticide use three-fold without increasing the pest populations. The repellent efficiency of kale cultivars enhanced by other novel pest management strategies such as the genetic modification or the development of cultivars that produce chemicals to repel *N. huttoni* and other pests, is possible.

The integration of trap cropping technology using alyssum as the trap crop, preferably flowering, along with sowing less susceptible kale cultivars such as Corka and Regal in main fields has been suggested to protect brassica seedlings from bug damage (Chapters 2, 3 and 5). These two IPM technologies can be integrated as a 'push-pull' strategy (Khan et al., 2001). Flowering alyssum strips at the edge of kale fields can attract or 'pull' *N. huttoni* from the surrounding vegetation including the main field and keep the bugs away from the main crop (Chapters 2 and 5). 'Pushing' out *N. huttoni* from the kale fields can be facilitated by using less susceptible kale cultivars (Chapter 3). Genetic manipulation of alyssum as well as the use of pheromone lures in trap crops, can increase the efficiency of the bug's preference for alyssum strips. Similarly, the repellent or deterrent capacity of kale cultivars can be increased by applying organic bio-pesticides or other 'soft' chemicals such as neem-based pesticides (Campos et al., 2018; Chaudhary et al., 2017).

These important strategies for controlling *N. huttoni* can lead to pesticide reduction in brassicas. Significantly higher numbers of nymph and adult *N. huttoni* at the edges could be managed by localised spray of pesticide that can reduce pesticide costs for the entire brassica fields (Boucher et al., 2003; Morrill, Weaver, & Johnson, 2001). Such a strategy has been suggested to control western flower thrips, *Frankliniella occidentalis* Pergande, which was concentrated in the field margins of tomato fields (Navas, Funderburk, Beshear, Olson, & Mack, 1991). This localized approach has also been suggested for other pests such as cabbage seedpod weevil (*Ceutorhynchus* *obstrictus* Marsham) (Coleoptera: Curculionidae) in perimeter trap crop of *B. rapa* in *B. napus* fields (Cárcamo, Dunn, Dosdall, & Olfert, 2007).

Flowering alyssum strips in kale or other brassica fields can also offer SNAP to predators and parasitoids, which ultimately improves CBC (Gurr et al., 2000; Gurr et al., 2017; Landis et al., 2000) and suppresses pest populations (Chapters 4 and 6) (Fiedler et al., 2008; Tscharntke et al., 2005). However, in certain conditions, flowering alyssum can also attract herbivores that might damage the alyssum and limit the ES (Begum et al., 2006). Hence, both the positive and negative sides of added floral habitats in a farming system need to be evaluated before recommending their deployment on a large scale. Other parameters of the candidate insectary plants such as area, crop stage, nectar and pollen content, agronomic characteristics, could influence the efficiency of the biological control of pests (Gurr et al., 2000). Hence, ARMED (access, ranking, manipulate, evaluate and develop) principles should be followed during the selection of candidate plants. Concerted efforts by farmers at the community level have been suggested for wider adoption of this technology. Farmers' efforts in sustainable pest management practices should be supported by the community, local government and central government policy.

7.3 Conclusions

The overall findings of this study contribute to reducing prophylactic pesticide use in forage brassicas and promote agro-ecological pest management approaches. In this study, habitat manipulation in brassica fields using a trap crop and less susceptible cultivars has been considered as an integrated management strategy.

The laboratory bioassay, no-choice field cages and open-field experiments on a range of potential trap plant species for *N. huttoni* suggested that alyssum (*L. maritima*) and wheat (*T. aestivum*) are the suitable trap crops (Tiwari et al., 2018). Deployment of these trap crops in a trap cropping (alyssum only) or multiple trap cropping (alyssum plus wheat) can intercept/divert *N. huttoni* and prevent their movement from the trap crops to the main crop (Hokkanen, 1991). In this study, a single trap crop using alyssum was more efficient than a multiple trap cropping with alyssum plus wheat. This study also showed that the flowering and fruiting stages of alyssum are the stages more favoured by *N. huttoni* than the vegetative stage. Habitat manipulation using the appropriate growth stages of alyssum at the seedling stage of kale in main fields can produce risk-free brassica seedlings from bug damage.

It is also strongly recommended that management strategies for this endemic lygaeid bug in brassicas should focus at field edges because higher bug aggregation takes place at the edge and so limit further dispersal to the main field (Shelton & Badenes-Perez, 2006). Integrated management strategies such as cultural and biological control and the use of more benign 'soft' chemicals are recommended to suppress this pest at the edges and keep them below the economic injury level in the main field. The pest management practices in the smaller area would reduce the pesticide costs compared with spraying the entire field. In this study, flowering alyssum strips harboured a significant number of generalist predators such as spiders, which are potential natural enemies of *N. huttoni*. However, this study did not focus on the CBC aspect of *N. huttoni*. A completely different study is suggested to address the role of CBC in *N. huttoni* management.

A series of laboratory choice and no-choice experiments on a range of kale cultivars with *N. huttoni* suggested that the kale cultivars Corka and Regal are relatively less susceptible cultivars to the *N. huttoni* damage. This fact can be integrated into other pest management approaches such as trap cropping, biological control and using selective chemicals for sustainable results. Highly susceptible kale cultivars such as Kestrel, Gruner, Sovereign, and Coleor, should be avoided by farmers (Tiwari et al., 2019). Maintaining flowering or fruiting alyssum strips at kale (cv. Corka or Regal) field edges can reduce bug damage on kale seedlings. The mechanism is called a 'push-pull' strategy (Khan et al., 2001). In this agro-ecological pest management strategy, kale cultivars such as Corka and Regal, can be used in main fields as a 'push' (repel) factor and alyssum crops at the edges can be used as a 'pull' (trap) factor. Further investigation of the changed behaviour of the *N. huttoni* using an alyssum trap crop and less susceptible kale cultivars such as Corka and Regal has been suggested.

In all the work in this thesis, potentially all or some of the components of SNAP are implicated. However, the effects of this acronym are likely to vary depending on how the interactions of SNAP differ whether the pest is being explored or its natural enemies too. Those complex interations are summarised in Tables 7.1 and 7.2 below.

Components	Resoruces to Nysius huttoni	Resources to the pest's NEs
Shelter (S)	\checkmark	\checkmark
Nectar (N)	х	\checkmark
Alternative food (A)	\checkmark	\checkmark
Pollen (P)	х	\checkmark

Table 7.1 Alyssum (Lobularia maritima) in brassica (kale) fields can provides the following resources

to Nysius huttoni and its natural enemies

ComponentsResoruces to radish pestResources to the pest NEsShelter (S)√√Nectar (N)√√Alternative food (A)√√Pollen (P)X√

Table 7.2 Alyssum (*Lobularia maritima*) in brassica (radish) fields can provides the following resources to radish pests and their natural enemies

Alyssum, which has been evaluated as a potential trap plant for the *N. huttoni* in forage brassicas in New Zealand, was also assessed for improving the CBC of radish pests, including *M. persicae*, in Nepal. The study results show that flowering alyssum in radish fields increases the abundance of generalist and specialist predators such as syrphids, carabids, spiders, and seven-spotted ladybirds, and reduces the abundance of pests such as leafhoppers, planthoppers, and aphids. The study also suggests potential ecosystem DS from the added floral habitat in an agro-ecosystem have to be considered in CBC control work (Begum et al., 2006; Gurr et al., 2017). Ecosystem dis-services are insect pests benefitting from floral resources more than natural enemies.

The outcomes of this study are valuable to New Zealand dairy farmers to produce pesticide-free forage brassicas and likely reduce pesticide residues in milk which is demanded by consumers. In general, pesticide-free products are more valuable than conventional products in promoting New Zealand dairy business nationally and globally. A pesticide-free environment in pasture can also improve many other ES such as groundwater purification, a suitable environment for aquatic species, pollinator conservation, and improved human health and agro-biodiversity (Gurr et al., 2017). Conservation biological control of radish pests by maintaining flowering alyssum strips can minimize pest pressure in radishes. In future, flowering strips can be integrated to other pest management approaches to suppress the pest populations by CBC (Gurr et al., 2017; Jonsson, Wratten, Landis, et al., 2008). Overall, this study suggests that habitat manipulation using alyssum trap cropping in brassica fields and integrating them to other compatible pest management approaches, such as using less susceptible kale cultivars (Cork and Regal), can protect brassica seedlings from *N. huttoni* damage and reduce pesticide costs. Alyssum floral strips in radish fields also improve the CBC of aphids and other pests. All these techniques are components of 'ecological' or 'sustainable intensification' practices, an alternative to 'agricultural intensification'.

7.4 Future work

All approaches to IPM have not been considered in this study because of limited funds and time. In a laboratory bioassay for the selection of potential trap plants (Chapter 2), only a few potential trap plants were tested and evaluated because of the constraints of a limited budget and time. Future work to evaluate potential trap crops should focus on a wide range of cultivated plant species and include many annual weeds.

In the laboratory study (Chapter 2), host plant selection by *N. huttoni* was measured visually. The visual sampling parameters were: first settling time, settling number over time, time taken to observe the first feeding damage, survival rate. Herbivore preference is influenced by visual, tactile or chemical/olfactory cues of the host plants (Eigenbrode, Birch, Lindzey, Meadow, & Snyder, 2016; Schoonhoven, Van Loon, van Loon, & Dicke, 2005). The results based on these visual observations should be proved by evaluating the chemical cues of the host plants (Bernays & Chapman, 2007; Blaauw, Morrison III, Mathews, Leskey, & Nielsen, 2017; Finch & Collier, 2012; Renwick, 1989, 2018) since herbivores locate or choose a host plant using chemical cues (Dicke, 2000; Finch & Collier, 2012; Johnson & Gregory, 2006; Renwick, 1989). A Y-tube olfactometer test is commonly used to identify the attractiveness of a plant to insects (Ballhorn & Kautz, 2013; Bruce et al., 2005). Hence the host selection behaviour of *N. huttoni* using an olfactometer for host plant selection could reinforce in this study.

The potential trap plants for *N. huttoni* based on the laboratory findings were further tested in field cages and non-cage field experiments (Chapter 5). From both test conditions, future work is suggested to test a wider range of potential trap plants including cultivated crops as well as weeds to select the most favoured host plants from a larger group of host plant species. Greenhouse cage experiments are suggested before conducting field-cage experiments to reduce the mortality of *N. huttoni* in field cages. The higher mortality rate of laboratory cultured bugs in field cages could be the biotic and abiotic factors in a changing environment.

In field cages, only a little information, such as bugs settling over time and survival rate were recorded because of rainfall during the study periods. However, other parameters of host plant selection by the bug such as population growth rate over time, mortality rate, sex ratio, and other morphological characteristics are suggested for future studies to get more reliable data on host plant selection by *N. huttoni*. The open-field experiments were conducted in small plots; it is suggested that they should be conducted, in the future, on a large commercial scale. The results from the small plots could not generate enough data for future recommendations to farmers. The minimum distance between two research plots can have a mixed effect both ways. A large gap

between plots has been suggested to minimize the dispersal of *N. huttoni* from one trap plot to the other. Future research on the ratio of the main crop to trap crop is suggested (Badenes-Perez et al., 2005; Hokkanen, 1991), as well as the temporal and spatial arrangement , the planting time for both the trap and main crop, the cultivars to use, the stage of the trap and main crop, the chemical and physical characteristics of the trap and main crop, the insect stage (Renwick, 1989), the ecology and behaviour of the insect pest and its natural enemies (Shelton & Badenes-Perez, 2006), and insect and trap crop characteristics (Shelton & Badenes-Perez, 2006), have been suggested for consideration before recommending this technology to farmers. The biology and behaviour of insect species are influenced by both the genetics of the species and the environment where they are currently present (Papaj & Prokopy, 1989). The genetics of a bug in a particular location can vary from the same species in another location. A pest management strategy using habitat management targeted for one species in a particular location might not fit the same species in other geographical regions and agro-ecosystems. Hence, a future genetic study of *N. huttoni* and its host plant preference behaviour has been suggested for various locations and in many seasons for wider applicability and wider adoption of this technology.

Some plant species simultaneously attract both herbivores and their natural enemies (Cook et al., 2007). However, some other plants can attract multiple natural enemies that would strengthen pest control activity in CBC. Semiochemicals or various volatiles and herbivore-induced plant volatiles (HIPVs) present in plant(s) could attract many beneficial arthropods, which can be useful in CBC of insect pests (Collier & Van Steenwyk, 2004; Cook et al., 2007; Landis et al., 2000; Symondson, Sunderland, & Greenstone, 2002). Future research could focus on the various semiochemicals and volatiles present in potential trap plants and their uses in IPM. Exogenous application of such compounds such as methyl salicylate and sugar-rich food sources, potentially increases predation and parasitism rates of natural enemies so improving pest control (Birkett et al., 2000; James & Price, 2004). Some flowering trap plants potentially provide all or each component of SNAP for pest natural enemies and increase their fitness in agro-ecosystems (Gurr et al., 2017). Hence, in future, a pest management strategy by habitat manipulation such as trap cropping is suggested and should be considered in a holistic-system approach to IPM with the 'push-pull' strategy, CBC, and the use of deterrents or attractants to stimulate herbivores, predators and parasitoids.

Future research on the suitability of kale cultivars to *N. huttoni* (Chapter 3) has been suggested for a wider range of kale cultivars, evaluating their preference on the basis of chemical, tactile or physical cues of the host plant, conducting research at more sites and seasons, large scale verification, and the use of latest biotechnological and breeding techniques to increase the

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resistance level of cultivars. Similarly, in the alyssum growth stage preference study (Chapter 4), only two growth stages of alyssum (vegetative and flowering) were considered for study following visual observations to evaluate the preference by *N. huttoni*. Plant growth stages significantly affect insects' host selection behaviour (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Hence, future study is suggested to consider other alyssum growth stages such as pod formation, seed maturity and senescence. Other variables such as bug population growth over time, nymph development, fecundity, the mortality rate of nymphs and adults, the sex ratio, lifespan are suggested for future study. The attraction of *N. huttoni* to the various growth stages of alyssum could also be verified using chemical cues released by the host plants. Experiments repeated at least three times, in more seasons, on a large field scale, and at more sites have been suggested to get more reliable information for assessing the preferred growth stages of alyssum. This information could be useful in developing an IPM protocol for *N. huttoni*.

Alyssum can improve CBC of many pests (Berndt & Wratten, 2005; Irvin et al., 2006). Alyssum flowering strips were maintained in a radish field to control a broad range of radish pests by improving CBC (Chapter 6). However, the study was conducted in a small plot and the alyssum flowering strips are less likely to influence pest numbers in radish field. Hence, in future, the following issues such as the area of the main crop and flower strips, the deployment time of both, the cultivars used, and research at more sites and in more seasons, multiple flower effects, spatial effects, the effect on landscape level etc., need to be considered for good results.

Finally, the results from all experimental chapters can be combined to develop a 'push-pull strategy' to manipulate the behaviour of pest and beneficial insects (Pyke, Rice, Sabine, & Zalucki, 1987), which is certainly useful in developing a future integrated pest management strategy in brassica fields (Aldrich et al., 2003; Cook et al., 2007; Cox, 2004). The 'push' factor could be less susceptible kale cultivars, deployment of such kale cultivars in kale fields can repel or deter *N. huttoni* or make the field unattractive or unsuitable for landing or feeding (Khan & Pickett, 2004), which could lead to preventing the brassica crop from bug damage. The efficiency of a less susceptible kale cultivar 'push' factor can be enhanced by exogenous application of repellents (Gerard, Perry, Ruf, & Foster, 1993; Griffiths et al., 1991) or other biotechnological approaches (Eigenbrode, Stoner, Shelton, & Kain, 1991). The 'pull' factor could be the alyssum plants that can lure or attract *N. huttoni* and keep them away from the main crop (Cook et al., 2007; Khan & Pickett, 2004). Certain crop stages are relatively more preferred by insects than other growth stages (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). The efficiency of the trap crop 'pull' factor can be enhanced by maintaining the flowering or pod stage of alyssum strips in brassica fields or by using attractants at peak pest population time. Flowering alyssum in kale or other

brassica fields can improve CBC in and off-farms by providing shelter, nectar, alternative food or pollen (SNAP) to insect predators and parasitoids and so increase pest control activity (Gurr et al., 2017; Wratten et al., 2003a). *Nysius huttoni* normally damages 4 - 6 - week old brassica seedlings (AgPest, 2016). Hence, kale seedling protection using a trap crop could be a future challenging issue. Future research could focus on the time of deployment of alyssum in kale fields so that 4-6week old kale seedlings are protected from bug damage. In summary, the use of potential trap crops and their preferred growth stages, suitable cultivars for both main and trap crop, and their appropriate deployment time considering the importance of CBC and 'push-pull' strategies would be a holistic agro-ecological *N. huttoni* management strategy in brassica fields.

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