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

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Abstract The New Zealand endemic wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae), is a pest of brassica seedlings. However, it has a wide host range comprising almost all cultivated brassicas, cereals and many other cultivated crops, as well as weeds. The brassica alyssum (*Lobularia maritima*) is a potential trap crop 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 ecosystem services that can contribute to mitigating diminished ecosystem functions in agriculture.

Keywords Alyssum, wheat bug, trap crop, choice test, ecosystem services

INTRODUCTION

The wheat bug, *Nysius huttoni* White 1878 (Hemiptera: Lygaeidae) is an endemic New Zealand insect (Eyles 1960b; He et al. 2003; Aukema et al. 2005) widely distributed in both main islands from sea level to 1830 metres above sea level (Myers 1926; Eyles 1960a; Eyles & Ashlock 1969). Although it is a seed feeder, it also feeds on plant host stems, petioles, leaves and fruit (He & Wang 1999). It is a primary pest of forage brassicas (Eyles 1965; He et al. 2003; Yang & Wang 2004) and many other cereal crops (Bejakovich et al. 1998) in New Zealand. The damage is most obvious in seedling brassicas and 80 – 90% damage has been reported on that crop in New Zealand (AgPest 2016; Speciality Seeds 2016). Insecticide use is the usual practice for *N. huttoni* management in New Zealand although using specialised trap crops is a potential alternative management option. A series of previous laboratory, field cage and open field experiments have studied on a range of potential trap plant species by this bug. Results showed that the popular garden plant alyssum, *Lobularia maritima* L. Desvaux (Brassicaceae), has a greater potential to trap this bug than did 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. 2018a). 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 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 wheat bug.

MATERIALS AND METHODS

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 wheat bug. 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, 16 L: 8 D h, and 60% (with a 10% range) humidity.

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 complete 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 (SE) cm for the vegetative stage and 13.3 ± 0.37 (SE) cm for the flowering cohort. In the choice tests, the mean height was 7.1 ± 0.29 (SE) cm for the vegetative stage and 13.0 cm ± 0.42 (SE) 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. *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, 69 h , 93 h , 108 h, 117 h, 132 h, 141 h, 156 h, 165 h, 180 h, 189 h, 204 h, 228 h, 237 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.

Data analysis

The mean number of wheat bugs 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. 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, UK). The survival rate (%) at 261 h at each stage, and first settlement time (mins.) 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.

RESULTS

First settlement time

Nysius huttoni settled significantly earlier on the flowering than on the vegetative stage in no-choice tests ($t = -2.9$; $df = 13$; $P=0.010$) and choice tests ($t = -5.6$; $df = 11$; $P<0.001$) (Fig. 1). In the latter tests, the bug took a mean at 14.07 minutes for first settlement on the vegetative stage and 9.92 minutes on the flowering stage of alyssum. In the choice tests, the bug took approximately 12.5 minutes for the first settlement on the vegetative stage and 5.25 minutes on the flowering stage (Fig. 1).

***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

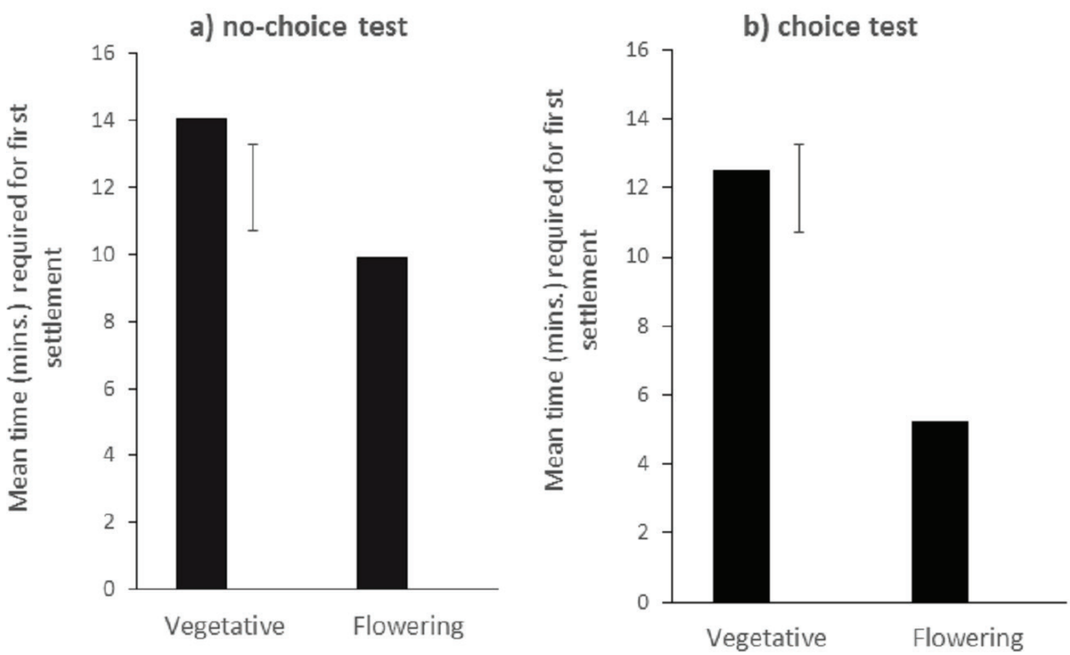

Figure 1 Mean time required for first settlement on the two growth stages of alyssum in; (a) no-choice (n = 14); and (b) choice experiments (n = 12). The vertical bars are least significant differences, (LSD) (5 %).

significantly higher at the flowering stage than on the vegetative one in the no-choice ($t = 3.39$; $df = 13$; $P=0.004$) and choice tests ($t = 12.4$; $df = 11$; $P<0.001$) (Fig. 2). 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, 132 h, and 261 h. By comparison, in the choice tests, the numbers collected on each stage differed significantly at each sampling time from 2 h to 261 h.

Survival rate

The survival rate of *N. huttoni* did not differ significantly between the two alyssum stages. Only no-choice tests were carried out for this parameter ($t = 1.121$; $df = 13$; $P=0.282$).

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. The parameters were: time to first bug settlement, mean number settled over time, and (in no-choice tests only) survival rate. 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 et al. (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

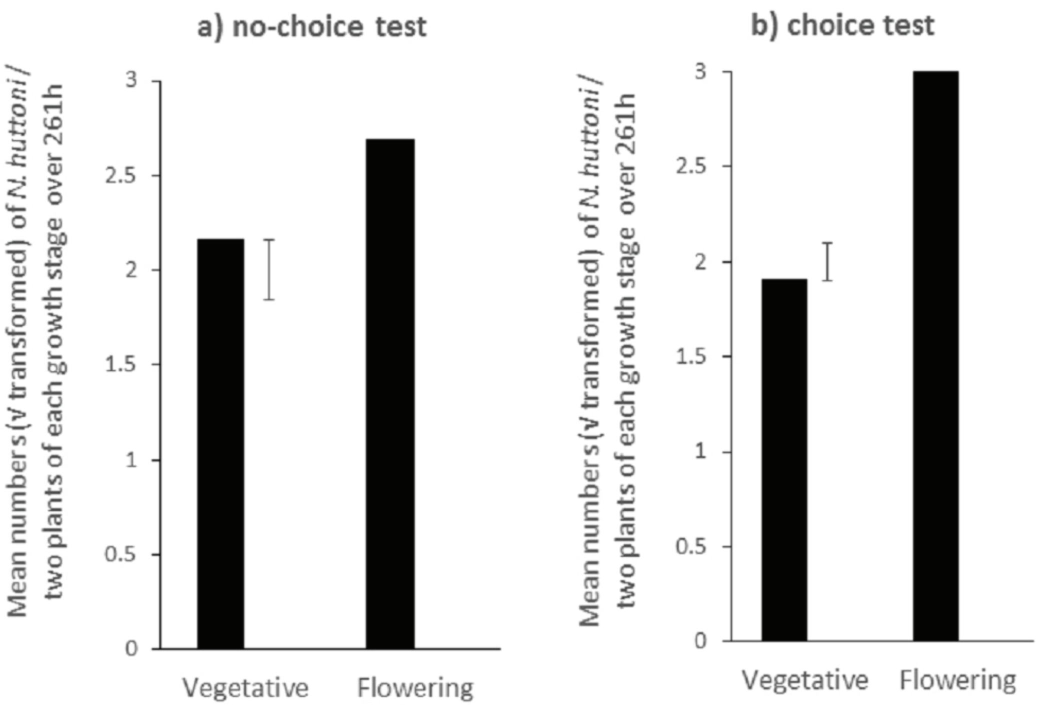

Figure 2 (a) a no-choice; and (b) a choice tests in the laboratory. Mean numbers (√ transformed) of *Nysius huttoni* adults on two alyssum growth stages over 261 h in no-choice (n=14) and choice tests (n=12). The vertical bars are the least significant differences, LSD (5%).

Vigna radiata (L.) Wilczek (Leguminosae), *Gossypium hirsutum* L. (Malvaceae), *Helianthus annuus* L. (Compositae) and *Chrysanthemum coronarium* L. (Compositae) plants over the vegetative stages of these plants (Pan et al. 2013). In cotton (*Gossypium hirsutum* L.) crops, *Lygus hesperus* (Knight) was more attracted to the flowering stage of the trap-crop plant species, alfalfa (*Medicago sativa* L.) and Russian thistle (*Salsola iberica* L.), than it was to flowering stages of sunflower (*Helianthus annuus* L.) and pigweed (*Amaranthus palmeri* L.), (Barman et al. 2010). In another study, flowering sunflower and the 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 et al. 2016). Flowering sunflower (*Helianthus annuus* L.) or flowering lucerne (*Medicago sativa* L.) 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 southern 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 et al. 2017). It was also trapped by the fruits of beans (*Phaseolus vulgaris* L.) around sweetcorn (*Zea mays* L.) 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 et al. 2010).

Physical, nutritional and chemical cues are responsible for the attraction of herbivores to host plants (Hokkanen 1991; Shelton & Badenes-Perez 2006; Bernays & Chapman 2007; Lucas-Barbosa et al. 2011). In general, the flowering stage of a plant releases more volatile chemicals (Ceballos et al. 2015) and provides nutritional rewards to many generalist herbivores than other stages (Wäckers et al. 2007). However, the type of volatiles and their concentrations can vary between growth stages (Silva et al. 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 et al. 2005). However, this idea needs to be verified by, for example, beginning with Y-tube olfactometer tests in the laboratory.

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 wheat bug 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 ecosystem services (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 pests more than natural enemies) (Baggen & Gurr 1998; Gurr et al. 2017). For example, the soybean hairy caterpillar, *Spilaractica casigneta* L. (Lepidoptera: Erebidae), a pest of soybean and other brassica crops damaged the alyssum flowers that were planted to improve the conservation biological control of radish pests in a 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 crop (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. 2018b) and potential trap crop 'alyssum' (Tiwari et al. 2018a) and their preferred growth stages 'flowering stage' as a 'pull' factor to attract the wheat bugs from the main crop that can also support conservation biological control and enhancement of natural enemies.

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