Alfalfa management affects infestations of Lygus rugulipennis (Heteroptera: Miridae) on strawberries in northwestern Italy

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Abstract (<400 words)

Lygus rugulipennis (Heteroptera: Miridae) is a highly polyphagous plant bug that causes severe damage on everbearing strawberries in NW Italy. In this area strawberry fields are frequently surrounded by alfalfa and other forage crops on which plant bugs usually live and reproduce until mowing or harvest. A 3-year research study was conducted to test the attractiveness of some herbs, including alfalfa, for ovipositing *L. rugulipennis* females, and to evaluate if appropriate management of neighbouring forage crops could affect bug infestation levels and damage to strawberries. In 2005, the attractiveness to ovipositing females of 4 plant species (strawberry, alfalfa, red clover and chamomile), each of which is abundant in NW Italy, was evaluated in laboratory trials. In 2006 and 2007, the seasonal abundance of plant bugs on strawberries and alfalfa was monitored in an experimental strawberry field with a strip of alfalfa along two sides. During field surveys, the influence of alfalfa management, including insecticide treatments in 2007, on plant bug numbers was assessed. In the laboratory, females of *L. rugulipennis* preferred, in decreasing order, chamomile, alfalfa, red clover and strawberry for oviposition. However, in the field, the majority of nymphs were collected on strawberries over most of the growing season, showing that *L. rugulipennis* is able to reproduce and develop on this plants. During field surveys, more species of plant bugs, and more individuals, were observed on alfalfa, which although attractive for mirid bugs did not prevent their migration to strawberries during periods of high population density. At such times, an insecticide treatment on alfalfa kept plant bug infestations on strawberries under the economic threshold.

Key words (3-6): European tarnished plant bug, plant attractiveness, alfalfa, field surveys, plant preference trials

Introduction

Plant bugs (Heteroptera: Miridae) include noxious pests of several crops worldwide. Severe damage due to their feeding activity is reported mainly on cotton in the US (Godfrey and Leigh, 1994; Greene et al., 1999; Stewart and Layton, 2000; Carrière et al., 2006), Africa (Stride, 1969) and Asia (Wu et al., 2002), but also on forage (Craig, 1963; Wipfli et al., 1990), vegetables (Varis, 1978; Boivin et al., 1991; Jacobson, 2002) and fruit crops (Pree, 1985; Michaud et al., 1989; Michaud and Stewart, 1990; Arnoldi et al., 1991; Rhainds et al., 2002; Hardman et al., 2004; Fleury et al., 2006). In Europe the most serious and widespread plant bug is *Lygus rugulipennis* Poppius, also known as the European tarnished plant bug (ETPB). This species is polyphagous, and it can live and reproduce on both herbaceous and arboreous, cultivated and wild plants. More than 400 host plants have been recorded, belonging to over fifty plant families, among which Brassicaceae, Asteraceae and Fabaceae are the most attractive (Holopainen and Varis, 1991).

In Italy, *L. rugulipennis* has affected both yield and quality of kenaf (Conti and Bin, 2001), lettuce (Accinelli et al., 2005), peach (Tavella et al., 1996) and strawberries (Bosio and Scarpelli, 1999). Beginning in the late 1990's, high rates of catfaced or buttoned fruits have been observed in strawberry fields in northwestern Italy. This is probably due to the reduction in use of broad-spectrum insecticides not compatible with IPM programmes largely adopted in the area (Bosio and Scarpelli, 1999). With their feeding on developing fruits, nymphs and adults of plant bugs disrupt the achenes and inhibit fruit development. Misshapen fruits bearing apical seediness are worthless for fresh market and often also for processing (Schaefers, 1966). Such symptoms are sometimes ignored because they are confused with malformation due to poor pollination or adverse weather conditions (Easterbrook, 2000).

In NW Italy *L. rugulipennis* usually migrates to strawberry fields late in the growing season, often as a consequence of cultural practices in the adjacent crops, such as mowing fodder or harvesting winter cereals. The removal, or the reduction, of herbaceous host plants in the agro-ecosystem induces the movement of ETPB adults to strawberry plants (Bosio and Scarpelli, 1999). Therefore, damage is more severe on everbearing varieties, which in NW Italy flower and fruit from June to October, as also observed in the UK (Easterbrook, 1996).

In strawberry fields, the control of plant bugs is generally achieved by applying insecticides (Cross and Easterbrook, 1998), according to damage thresholds developed in various countries (Schaefers, 1980; Jay et al., 2004). However, chemical control is difficult to apply successfully in northwestern Italy because, following the recent EEC regulations, very few selective and non-persistent products are now authorized on strawberries.

Alternative control measures have been investigated, mainly in North America and the UK, to reduce the incidence of plant bugs on strawberries, i.e. reflective mulch between rows (Rhainds et al., 2001), vacuum devices to remove insects (Vincent and Chagnon, 2000; Rancourt et al., 2003; Swezey et al., 2007), and augmentation of the egg parasitoid *Anaphes* *iole* Girault (Hymenoptera: Mymaridae) (Norton and Welter, 1996; Udayagiri and Welter, 2000). However, these measures have often provided less effective control levels than with conventional insecticides. Also the use of “trap crops”, i.e. plants more attractive and suitable for the target pest than crop plants (Hokkanen, 1991), has been tested to avoid the migration of plant bugs to strawberries in UK with conflicting results (Easterbrook and Tooley, 1999).

In NW Italy, strawberries are usually grown in agricultural areas where livestock and cereal farming are widespread, often representing the main farm activities. Therefore, everbearing strawberry fields are frequently surrounded by alfalfa and other forage crops on which plant bugs live and reproduce until harvest. In recent years mirid damage to strawberries has been increasing but at the same time the market requires more organic or integrated production.

This research was aimed at: i) testing the attractiveness for ovipositing females of selected herbs, including alfalfa; ii) evaluating if appropriate management of the neighbouring forage crops could affect plant bug infestation levels and, as a consequence, damage to strawberries.

Materials and methods

Collection and mass rearing of *L. rugulipennis*

During spring and summer, 2005, *L. rugulipennis* adults were collected monthly using a sweep net in 3 sites: Moretta, Boves and Lagnasco (province of Cuneo, Piedmont, NW Italy), and transferred to our laboratories (Di.Va.P.R.A., University of Torino). Here, they were first identified using the key of Wagner and Weber (1964); then they were reared on French bean pods (*Phaseolus vulgaris* L., Fabaceae) and sprouted potatoes (*Solanum tuberosum* L., Solanaceae) inside 3L plastic boxes. The lid of these boxes (265mm×175mm) was cut in the middle and closed with net. Mass rearing was conducted in climatic chambers maintained at 25±1°C, 70±5% RH and a 16L:8D photoperiod.

Evaluation of plant attractiveness in the laboratory

In 2005, laboratory trials were carried out to evaluate the attractiveness to ovipositing females of 3 plant species [alfalfa, *Medicago sativa* L. (Fabaceae), var. Europe (10%) and Elena (90%) (Bottos Sementi, Italy); red clover, *Trifolium pratense* L. (Fabaceae), var. Viola (Bottos Sementi, Italy); chamomile, *Matricaria chamomilla* L. (Asteraceae) (Semeurop, Italy)] versus strawberry [*Fragaria x ananassa* Duchesne cv Diamante (Rosaceae)]. Alfalfa, red clover and chamomile were chosen because they are common in northwestern Italy and are well-known host plants of *L. rugulipennis* (Holopainen and Varis, 1991) and, more generally, of the genus *Lygus* (Esquivel and Mowery, 2007).

The trials were conducted using Plexiglass and net cages (300×300×300mm) placed in climatic chambers under the same conditions as the mass rearing. Inside each cage, 4 similarly-sized potted plants (1 strawberry, 1 alfalfa, 1 clover, 1 chamomile) were carefully arranged so that one plant was never in contact with another. Ten-day-old mass reared *L. rugulipennis* adults (12 females, 8 males) were introduced to the middle-bottom of each cage. Adults were kept for 7 days [i.e. embryonic development time assessed in previous experiments (unpublished data)], after which they were removed and counted. Plants were also removed from the cages, carefully surveyed and singly isolated in a Plexiglass cylinder (height 200mm, ∅ 120mm) placed on each pot. Each plant was then kept in climatic chambers under the conditions described above, and checked daily to assess the presence of newly hatched nymphs. To facilitate the inspection, a white paper disk (∅ 120mm) was placed at the base of the plant. The plants were checked until no nymphs were seen for 4 days. Overall, 12 replications of the experiment were made.

<u>Evaluation of the impact of alfalfa on bug infestation on strawberries in the field</u>

In 2006 and 2007, field trials were carried out in Boves, Piedmont, NW Italy (44°20’N 7°32’E, 580m a.s.l.) to survey the seasonal abundance of plant bugs on strawberries and on alfalfa, and to evaluate the influence of management of alfalfa on the bug infestation on strawberries. For the trials, a strawberry field of 432m^2 (80×5.4m), consisting of 5 rows of an everbearing variety (cv Albion), was planted in early April 2006. Throughout the growing season, from late May to September in both years, the field was covered with a black shading net, as usually performed on strawberries in this area. Along the two major sides, 2 strips of alfalfa (80×4m) were sown in late April 2006; in both years the alfalfa was irrigated monthly during spring and late summer, and weekly in July and August, and mowed at the beginning of flowering, namely 3 times in 2006 (4 July, 20 July, and 20 August) and 5 times in 2007 (25 May, 21 June, 8 July, 30 August, and 17 September). During mowing, alfalfa was cut alternately only on half of each strip, i.e. half near the strawberry field on one side and half far from the strawberry field on the other side (Fig. 1). This practice was to ensure that alfalfa was available to the plant bugs throughout the experiment.

In 2006, plant bugs were sampled every two weeks on strawberries and neighbouring alfalfa from 8 June to 11 October. On strawberries, sampling was carried out on 6 groups of 10 plants: the foliage of each plant was first visually inspected and then beaten on a white dish (∅ 330mm). On alfalfa, plant bugs were sampled using a sweep net, 3 series of 10 sweeps per each side. All nymphs and adults collected on strawberries and alfalfa were counted, and identified following Wagner and Weber (1964) and Rinne (1989).

In 2007, to evaluate if chemical treatments on alfalfa could prevent bug migration to strawberries, the field was divided into 2 plots (A_{str} and B_{str}), as well as the neighbouring alfalfa strips (A_{alf} and B_{alf}) (Fig. 1). On strawberries the same horticultural practices were adopted in the 2 plots; on alfalfa, in one plot per strip (A_{alf}, control) no treatments were applied, whereas in the other plot (B_{alf}) chemicals were used when plant bug populations increased and exceeded on average 2 individuals per sweep. Two treatments were made, one with λ-cyhalothrin (Karate xpress®, Syngenta Crop Protection, Switzerland) on 10 July, and one with etofenprox (Trebon star®, Sipcam, Italy) on 17 September. In 2007 plant bugs were sampled every two weeks from 16 May to 3 October. Sampling was carried out with the same methods adopted in 2006, on 3 groups of 10 plants per plot on strawberries, and with 4 series of 10 sweeps per each plot on neighbouring alfalfa.

Statistical analysis

In the laboratory trials, the numbers of nymphs detected on each plant species were compared using a non-parametric analysis (Kruskal-Wallis); values were separated by Mann-Whitney's test ($p<0.05$) when the Kruskal-Wallis analysis was significant (SPSS® 12.0 version). To compare plant bug populations in the plots (A_{str} and B_{str}; A_{alf} and B_{alf}) during field trials in 2007, sampling data on each date were firstly tested for homogeneity of variance (Levene test) and normality (Shapiro-Wilk test) (SPSS® 12.0 version). On strawberries, numbers of bugs collected on the 30 plants per plot were analyzed by a non-parametric analysis (Kruskal-Wallis) because variance homogeneity test and normality test failed. On alfalfa, numbers of bugs collected in 4 series of 10 sweeps per plot were compared with a one-way analysis of variance (ANOVA), with a 5% significance threshold (SPSS® 12.0 version).

Results

Evaluation of plant attractiveness in the laboratory

In the laboratory, for oviposition *L. rugulipennis* females preferred, in decreasing order: chamomile, alfalfa, red clover and strawberry (Fig. 2). During the trials, adult mortality ranged between 0-22%, and was on average slightly higher in males than in females (14% males and 9% females). Newly hatched nymphs were observed between the 6th and the 16th day after the isolation of single plants inside Plexiglass cylinders, with maximum densities between the 7th and the 11th day. Moreover, nymphs were not found on strawberries in 4 out of 12 replications, whereas they were always observed, although in different amounts, on the other plant species in all replications.

Evaluation of the impact of alfalfa on bug infestation on strawberries in the field

The total numbers of plant bugs collected on strawberries and alfalfa in 2006 and 2007 are reported in Tables 1 and 2, respectively. In both crops and years *L. rugulipennis* was the predominant plant bug species. Other plant bugs sampled only occasionally on strawberries but more frequently on alfalfa were *Lygus pratensis* L., and *Adelphocoris lineolatus* (Goeze). The latter species was rare on strawberries but very abundant on alfalfa especially in 2007. Another plant bug species, *Polymerus vulneratus* (Panzer), was common on alfalfa but never collected on strawberries. Generally, the majority of the field-collected plant bugs were nymphs. For example, in 2006, nymphs comprised 89% and 77% of the total plant bugs collected on strawberries and on alfalfa, respectively (Table 1). In 2007, nymphs were always more abundant than adults, even if with lower percentages, 77% and 59% of collected insects were nymphs on strawberries and alfalfa, respectively (Table 2). Within the genus *Lygus*, nymphs were not identified to the species level, but on the basis of adult collection they were considered to belong mostly to *L. rugulipennis* (Tables 1 and 2).

In 2006, plant bugs were first detected on alfalfa and on strawberries in early and late June, respectively (Fig. 3). On these sampling dates, only adults were collected, whereas in the following dates nymphs were collected in greater numbers as well on both alfalfa and strawberries. Plant bug populations showed similar trends on both crops with two peaks. The first peak occurred on alfalfa (52.7 individuals per 10 sweeps) on 13 July and later on strawberries (0.6 individuals per plant) on 26 July. The second peak occurred on both alfalfa (51.5 individuals per 10 sweeps) and strawberries (1.4 individuals per plant) on 5 September. Although alfalfa was cut only on half of each strip, population increase was observed on strawberries after mowing alfalfa on 4 July, 20 July, and 20 August.

In 2007, plant bugs were present in both plots on strawberries and alfalfa already during the first field survey in mid-May, before strawberry flowering (Fig. 4). From this date until mid-July the population trend on strawberries was similar in both plots A_{str} and B_{str} (near untreated and treated alfalfa, respectively), reaching infestation levels of 0.4 individuals per plant on 11 July, immediately after the treatment with λ-cyhalothrin on alfalfa in the plot B_{alf} on 10 July. Afterwards bug populations decreased on strawberries in both plots. However, higher population levels were observed in the plot A_{str} (near untreated alfalfa), where the population reached a second peak of 0.4 individuals per plant on 22 August, and thereafter declined gradually until the last sampling on 3 October. In the plot B_{str} (near treated alfalfa), the bug population was low until 22 August; then it increased until 3 October, reaching the highest density of 0.5 individuals per plant in the growing season.

The Kruskal-Wallis analysis highlighted significant differences between mirid densities in the two strawberry plots (Fig. 4a). On sampling dates following λ-cyhalothrin application in plot B_{alf} (treated) (25 July and 8 August), mirid densities were significantly lower in plot B_{str} (near treated alfalfa), when compared with plot A_{str} (near untreated alfalfa). However, after the etofenprox application to plot B_{alf} (treated), mirid densities were significantly greater in plot B_{str} (near treated alfalfa) (19 September and 3 October) (Fig. 4a).

On alfalfa, plant bugs reached an initial peak of 26.8 and 28.5 individuals per 10 sweeps in the plots A_{alf} (untreated) and B_{alf} (treated), respectively, on 27 June (Fig. 4b). Afterwards in the plot A_{alf} (untreated) the mirid population continued increasing until early September (48.5 individuals per 10 sweeps on 5 September), then it decreased dramatically until early October. By contrast, in the plot B_{alf} (treated) the bug population dropped following the treatment with λ-cyhalothrin and started rising gradually in August, reaching the maximum of the growing season of 76.3 individuals per 10 sweeps on 5 September. Then it decreased dramatically in both plots A_{alf} (untreated) e B_{alf} (treated), without being seemingly influenced by the treatment with etofenprox on 17 September. Significant differences between mirid densities in the two alfalfa plots were observed on 11 and 25 July, and 9 August, i.e. in the sampling dates following the treatment with λ-cyhalothrin on alfalfa in the plot B_{alf}. On these dates, mirid densities were significantly higher in the plot A_{alf} (untreated) (Fig. 4b).

Discussion

In laboratory trials, the most attractive plant species for *L. rugulipennis* oviposition was chamomile, belonging to the family Asteraceae, on which the highest numbers of nymphs were always found. Of the tested plants, strawberry was the least attractive plant species. However, in our region chamomile is an invasive weed, usually growing in wastelands and flowering throghout the summer, and hard to manage and control. It is not advisable to use this plant as a trap crop, although it resulted very attractive in the laboratory trials. Moreover, in previous field trials conducted in the UK, chamomile was not always able to prevent the migration of *L. rugulipennis* to strawberries (Easterbrook and Tooley 1999).

In the laboratory, alfalfa and red clover were very attractive to ovipositing females of *L. rugulipennis*. Since alfalfa is a common forage crop in the surveyed area, in the field trials this plant species was chosen and grown around the strawberry plots to assess its role in influencing bug infestations on strawberries. In field trials in Sweden red clover was used successfully to control an infestation of *L. rugulipennis* on lettuce [*Lactuca sativa* L. (Asteraceae)] (Rämert et al., 2001).

During the 2-year field surveys, the majority of plant bugs were collected from alfalfa in both total numbers of individuals and total numbers of species; in fact, *A. lineolatus* and *P. vulneratus* were captured almost exclusively on alfalfa. By contrast, *L. rugulipennis* was found both on alfalfa and strawberries; actually, on the latter crop it can be considered one of the most noxious pests, as observed also in other European countries (Easterbrook, 1996; Cross and Easterbrook, 1998). Except at the beginning and end of each growing season, most of the plant bugs captured on both alfalfa and strawberries were nymphs. *L. rugulipennis* overwinters as adults (males and females), and accomplishes 3-4 generations a year in northwestern Italy (Rancati et al., 1996). Inter-crop movement is effected by adults in the search of suitable host plants; so adults prevailed early and late in the season, in conjunction with the initial colonization of the crop and before the final migration towards overwintering refuges, respectively. The presence of large numbers of nymphs on strawberries indicates that *L. rugulipennis* can reproduce and develop on this plant despite it being the least attractive species for oviposition in our laboratory tests.

In 2006, alfalfa proved to be attractive to mirids, but was unable to prevent their migration to strawberries when populations reached high levels, as observed by Easterbrook and Tooley (1999) in strawberries and Accinelli et al. (2005) in lettuce. Starting from mid-July, plant bug density on strawberries was always higher than the economic threshold proposed by Jay et al. (2004) of 1 *L. rugulipennis* individual per 40 plants. In 2007, bug populations on strawberries generally exceeded this threshold during the growing season, even one day after the first chemical treatment on alfalfa. However, the obvious population increase on strawberries on 11 July could be due to a temporary movement from alfalfa to neighbouring crops of plant bugs disturbed by the insecticide intervention. Afterwards, plant bugs returned to alfalfa, and in the plot treated by the pesticide they were controlled. In the following dates in the plot near alfalfa treated with λ-cyhalothrin mirid populations declined next to the economic threshold (1.33 individuals of *L. rugulipennis* per 40 plants on 25 July and 9 August), whereas in the plot near untreated alfalfa the population level remained higher than the economic threshold (8 individuals of *L. rugulipennis* per 40 plants). On these two dates populations were significantly different in the two plots near treated and untreated alfalfa.

The application of λ-cyhalothrin was able to reduce pest density in alfalfa and, as a consequence, prevent their movement into strawberries for approximately one month. Only in late August, bug populations did return to damaging levels, thereby requiring another insecticide intervention. But the treatment with etofenprox in mid-September did not seem to positively influence the infestation level on strawberries since significantly higher numbers of plant bugs were sampled in the plot near treated alfalfa in the two dates following the intervention. The efficacy of etofenprox against *L. rugulipennis* was already assessed in both laboratory and semi-field trials in peach orchards of NW Italy (Pansa et al., 2008). Therefore, this treatment in mid-September could have advanced the natural dispersal towards overwintering refuges occurring at the end of the season, and attested by the decline until disappearance of bug populations on alfalfa in both plots. In particular, after the treatment with etofenprox, plant bugs moved from the treated alfalfa to the strawberries near treated alfalfa and caused high infestation levels before migrating to winter refuges.

In conclusion, forage crops, such as alfalfa, can act as either a sink or a source of plant bugs. The appropriate management of fields adjacent to strawberries is crucial to prevent the insect's inter-crop movement in fragmented landscapes. Time and methods of alfalfa mowing should be investigated in relation to life cycle and dispersal ability of plant bugs; in particular, the dispersal ability of nymphs should be studied, which so far remains unknown. During this research project, greater numbers of plant bugs, mainly nymphs, were collected on strawberries after mowing even if alfalfa was cut alternately (only half of each strip at a time). Therefore, when bug populations increased on alfalfa, a localized insecticide application can be useful to reduce their migration to strawberries. However, further investigations, being to be carried out on a larger scale including more replications, should be focused to assess the minimum surface to be treated to protect yield quality and safeguard environmental health.

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Figure captions

Figure 1 – Experimental strawberry field, with 2 strips of alfalfa along the sides, surveyed in 2006-2007.

Figure 2 – Attractiveness for *Lygus rugulipennis* ovipositing females, expressed as mean number of nymphs emerged on alfalfa, chamomile, red clover, and strawberry in laboratory trials. Values followed by different letters are significantly different (test Mann-Whitney, $p<0.05$).

Figure 3 – Mean numbers (± SE) of plant bugs per plant on strawberries (a) and of plant bugs per 10 sweeps on alfalfa (b) in 2006.

Figure 4 – a. Mean numbers (± SE) of plant bugs per plant sampled in the 2 plots A_{str} and B_{str} of strawberries in 2007. In the dates with * values are significantly different between plots (Kruskal-Wallis analysis, $p<0.05$). b. Mean numbers (± SE) of plant bugs per 10 sweeps in the 2 plots A_{alf} (untreated) and B_{alf} (treated) of alfalfa in 2007. The arrows indicate the insecticide treatments in the plot B_{alf}. In the dates with * values are significantly different between plots (ANOVA, $p<0.05$).

Table 1 – Total numbers of plant bugs collected on strawberries and alfalfa during field surveys carried out every two weeks from 8 June to 11 October 2006.

Plant bugs	strawberry	alfalfa
Lygus rugulipennis, adults	20	164
Lygus pratensis, adults	1	6
Lygus spp., nymphs	202	798
Adelphocoris lineolatus, adults	5	131
Adelphocoris lineolatus, nymphs	16	104
Polymerus vulneratus, adults	0	64
Polymerus vulneratus, nymphs	0	300
total bugs	244	1567

Table 2 – Total numbers of plant bugs collected on strawberries and alfalfa during field surveys carried out every two weeks from 16 May to 3 October 2007.

Plant bugs	strawberry near		alfalfa	
	treated alfalfa	control alfalfa	treated plot	control plot
Lygus rugulipennis, adults	10	10	114	179
Lygus pratensis, adults	0	0	3	4
Lygus spp., nymphs	34	33	267	349
Adelphocoris lineolatus, adults	0	2	141	214
Adelphocoris lineolatus, nymphs	4	6	98	181
Polymerus vulneratus, adults	0	0	40	44
Polymerus vulneratus, nymphs	0	0	91	84
total bugs	48	51	754	1055

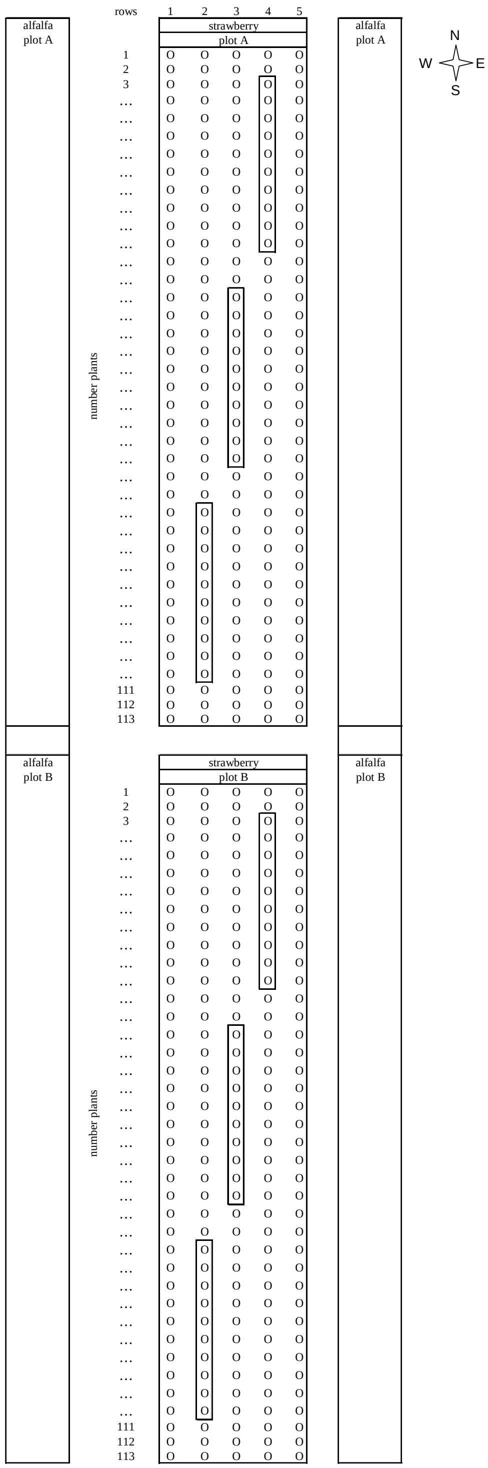


Figure 1 – Experimental strawberry field, with 2 strips of alfalfa along the sides, surveyed in 2006-2007.

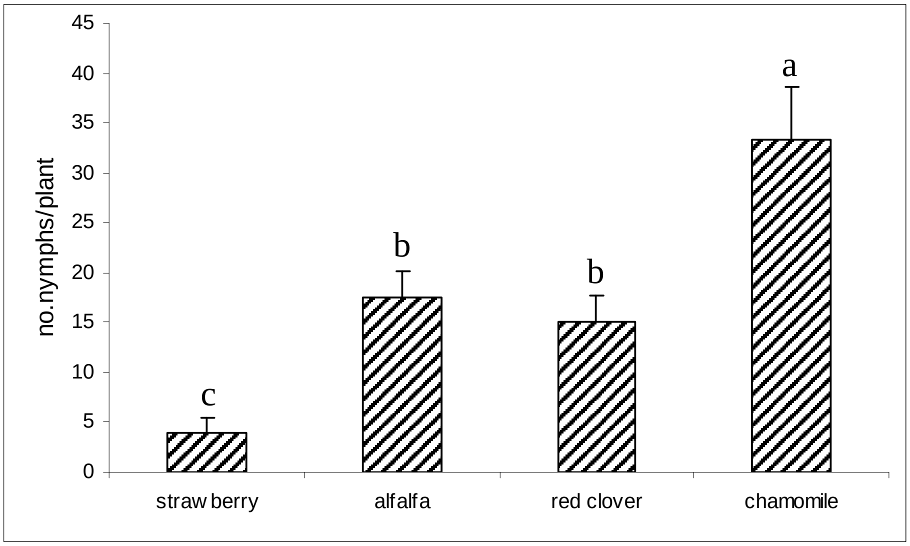
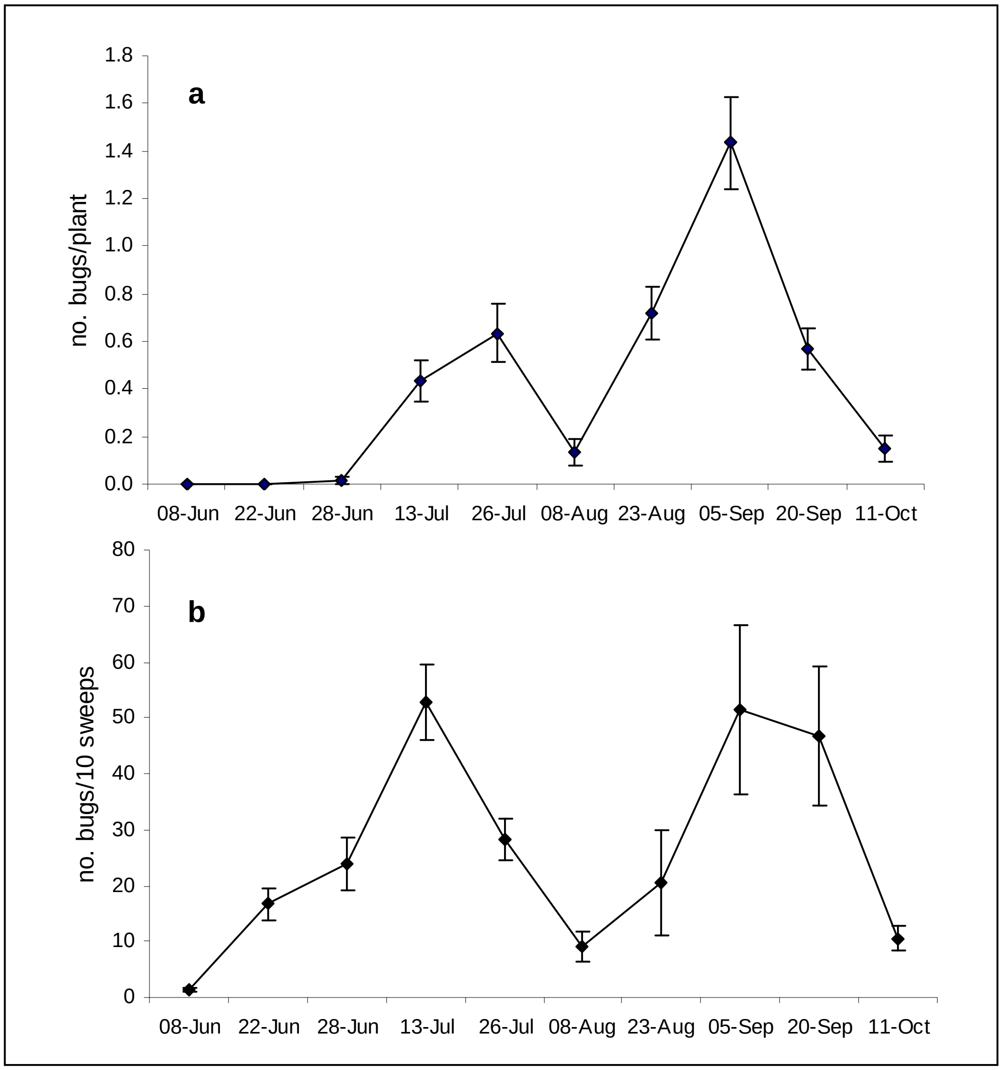


Figure 2 – Attractiveness for *Lygus rugulipennis* ovipositing females, expressed as mean number of nymphs emerged on alfalfa, chamomile, red clover, and strawberry in laboratory trials. Values followed by different letters are significantly different (test Mann-Whitney, $p<0.05$).



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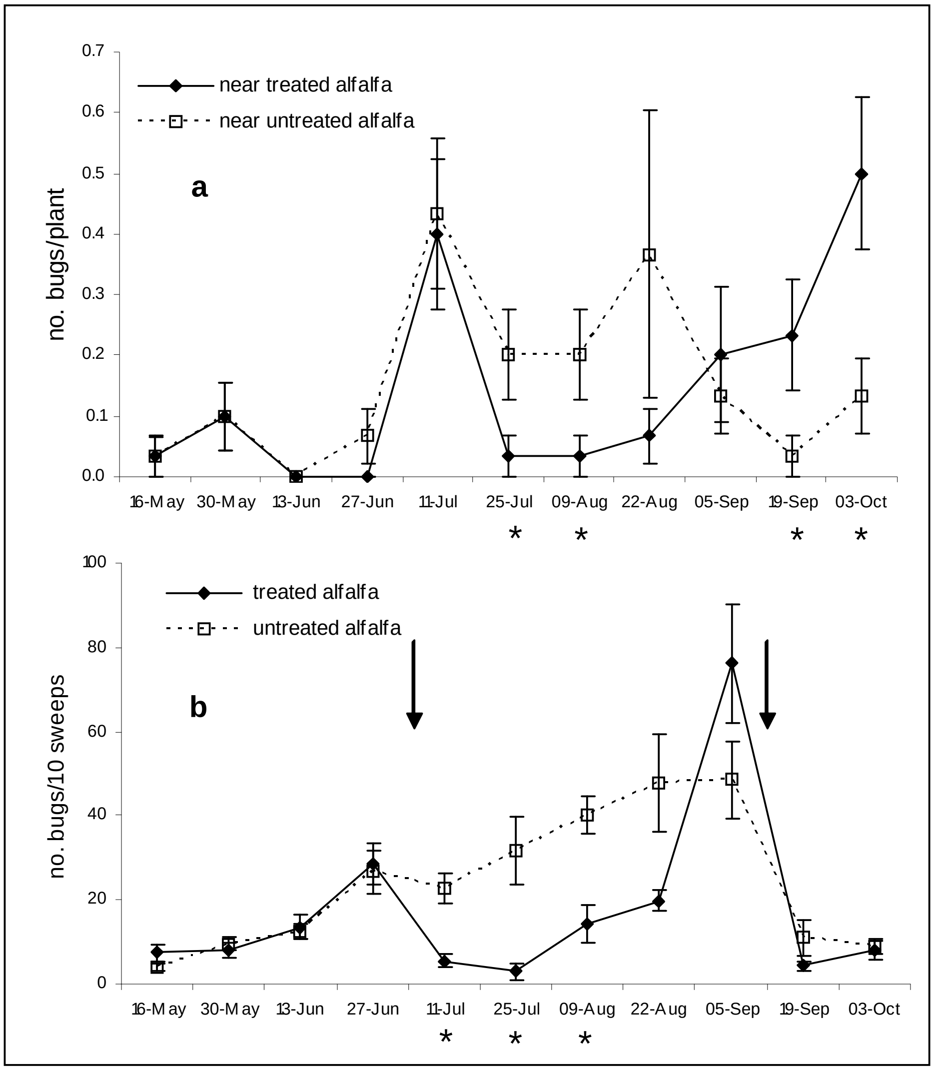

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