

# Negative interaction between twospotted spider mites and aphids mediated by feeding damage and honeydew

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## Abstract

Among the herbivorous arthropods that feed on strawberry, the most important are the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, and several species of aphids. Mites and aphids belong to different guilds that coexist in the field and feed on the undersides of strawberry leaflets. However, the occurrence of large numbers of individuals of both species on the same leaflet is rarely recorded. We hypothesize that negative interactions between TSSM and aphids explain the intraplant distribution of these herbivores. We first examined the spatial coincidence of both herbivores in the field. Under experimental conditions, we then analyzed: (i) the rate of increase of TSSM and the aphid *Chaetosiphon fragaefolii* (Cockerell), growing individually and together; (ii) the effect of honeydew on TSSM preference; and (iii) the effect of previous strawberry leaflet damage by TSSM on *C. fragaefolii* preference. The proportion of TSSM that coincided with at least one aphid decreased as the percentage of leaflets with TSSM increased. The spatial coincidence index between aphids and TSSM increased together with the percentage of TSSM-infested leaflets. TSSM showed both a lower rate of increase when they shared the same leaflet with *C. fragaefolii* and lower fecundity on strawberry discs with honeydew. The rate of increase of *C. fragaefolii* did not change on co-occupied leaves, but the aphid species moved to the other side of leaflets shared with TSSM. Negative interactions resulting in a tendency for species to avoid each other, such as demonstrated herein, can affect distribution and performance of herbivorous arthropods.

**Keywords:** *Tetranychus urticae*, *Chaetosiphon fragaefolii*, negative interactions, strawberry, competition

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## Introduction

Competition may be either direct, when damage to a plant by one herbivore species deprives a second species of that

resource, or indirect, when it is plant-mediated (i.e. induced defences) (Ohgushi, 2005; van Veen *et al.*, 2006; Kaplan & Denno, 2007). Competition appears most likely when herbivores are closely related, sessile and feed on discrete resources (Denno *et al.*, 1995), but even herbivores feeding on distant portions of the plant may compete (e.g. foliar and root feeders: Bezemer *et al.*, 2003; Blossey & Hunt-Joshi, 2003). Plants are assemblages of heterogeneous tissue types and herbivores often specialize on certain plant parts.

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Accordingly, phloem feeders, leaf chewers, stem borers, seed predators, among others, exploit different resources but rarely consume the whole plant. Morphological or chemical changes in plants in response to damage caused by a guild of herbivores can increase or decrease the attractiveness of the plant to other guilds (Blossey & Hunt-Joshi, 2003; Jones & Russell, 2009).

Temporal or spatial segregation of consumers (Denno *et al.*, 1995) or fine-scale resource partitioning (Kagata & Ohgushi, 2001) are common mechanisms of coexistence in phytophagous insects, and several guilds of herbivores may coexist on the same resource by effectively avoiding each other (Daugherty, 2009).

Furthermore, local interactions can occur between herbivores belonging to different guilds that are feeding on the same structure of the plant (e.g. leaf). Physical and chemical alteration of leaf tissues caused by a guild may interfere with resource acquisition by another guild (Karban, 1986; van Zandt & Agrawal, 2004; Lynch *et al.*, 2006; Kaplan & Denno, 2007). Local interactions can also change the spatial distribution of different populations on a small scale (Faeth, 1986; Kagata & Ohgushi, 2001) and affect performance when space is co-occupied (Ohgushi, 2005, 2008).

The main herbivorous arthropods that feed on the leaves of strawberry (*Fragaria X ananassa* Duchesne) are the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), and several species of aphids, including *Aphis gossypii* Glover, *A. fabae* Scopoli, *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* Thomas and *Chaetosiphon fragaefolii* (Cockerell) (Cédola & Greco, 2010). TSSM are mesophyll feeders that penetrate epidermal cells and ingest cell contents (Kielkiewicz & van de Vrie, 1983; Kielkiewicz, 1985). Aphids are mobile feeders that suck mainly from the sap flow in phloem sieve elements. Aphids inject watery and solid saliva that remains in leaf tissues after feeding (Dixon, 1998; Powell *et al.*, 2005). The solid saliva deposit on the leaf surface called 'flange-salival' is continued within a tubular structure, the 'stylet-sheath', that represents the pathways of stylets into leaf tissues (Miles, 1972; Tjallingii, 1978; Peeters *et al.*, 2007). Aphids cause direct feeding damage because of their toxic saliva (van Emden *et al.*, 1969; Hill, 1983; Gharidi, 2002) and indirect damage from their sugary sticky excreta, the honeydew. Honeydew may decrease photosynthesis, induce senescence (Bardner & Fletcher, 1974) and favour the growth of saprophytic fungi that also may negatively affect photosynthesis (Vereijken, 1979).

Adult TSSM females reach potential host plants either by random walking or by passive wind dispersal; hence, if by wind, the probability of finding and colonizing new resources depends in part on the host-plant range and the layout of the crop (Kennedy & Smitley, 1985). The dispersal of aphids occurs because winged adults are attracted to visual and chemical clues (Powell *et al.*, 2005). On a smaller scale, both TSSM and aphids may increase emigration rates from unsuitable leaflets by changing the patterns of trivial movements (Kareiva, 1986; Andow, 1991). When mites have arrived at a leaflet, they can move to other leaflets or to the other surface of the same leaflet if there is injury to the leaf tissue (e.g. injury caused by the feeding of aphids). In turn, aphids may express similar behaviour in response to tissue damage caused by mites.

Although both herbivorous guilds can coexist on the same plant and attain high abundances (Cédola & Greco, 2010; Greco *et al.*, 2011), a large number of both species on the

same leaflet is uncommon (N.M. Greco, personal observation). Our hypothesis is that there exist negative interactions between TSSM and aphids in strawberries. Leaf tissue injury caused by TSSM feeding could negatively affect aphid feeding, and aphid honeydew may interfere with TSSM feeding and oviposition. If negative interactions influence the spatial distribution and pattern of co-occurrence of TSSM and aphids, we expect to find: (i) a relationship between the spatial co-occurrence at leaflet level and the density and dispersion of each species; (ii) low numbers of individuals of both species coinciding on the same leaflet; (iii) lower population growth rates of both herbivores when together on leaflets; (iv) preference of TSSM for leaflets without honeydew; (v) a higher number of eggs on leaflets without honeydew; (vi) preference of aphids for leaflets without TSSM feeding damage; and (vii) higher numbers of offspring on leaflets with TSSM feeding damage.

The purpose of this study was to determine if there is negative interaction between TSSM and aphids, and whether the interaction is mediated by feeding damage and the presence of honeydew.

## Materials and methods

### *Spatial coincidence*

The populations of TSSM and aphids, species mentioned above, were sampled once or twice per month in greenhouses (GH) and from open-field (crop with plastic covered tunnels, CCT) commercial strawberry crops (Aromas) located in La Plata, Province of Buenos Aires, Argentina (38° 52'S, 57°59'W). Irrigation and soil management were standard for the region. Granulated fertilizer composed of total nitrogen (N) 15.0%, (ammoniacal nitrogen 8.89%, nitrate nitrogen 6.11%), assimilable phosphorus (P<sub>2</sub>O<sub>5</sub>) 15.0% and water soluble potassium (K<sub>2</sub>O) 15.0% was applied to the soil 20 days before planting. Methyl bromide (Bromopic®, 70% methyl bromide + 30% chloropicrin, Brometan SRL, Burzaco, Argentina, on 12 fields, and Vendraal Fumigante 51®, metam sodium 51%, Síntesis Química SAIC, Buenos Aires, Argentina, on 17 fields) was used to fumigate the soil. The beds were covered with black polyethylene mulch Coverfilm® and irrigated by drip. In all fields, fungicide Benosem 50 PM® (benomyl 50%) was used weekly and acaricide New Mectin® (abamectin 1.8%, Agrimarketing S. A., Buenos Aires, Argentina) was applied when *T. urticae* reached the economic threshold level (50 active mites per leaflet: Wyman *et al.*, 1979). Insecticides were not applied because densities of other pests, such as thrips, whiteflies and aphids were low. Samplings were performed on 29 strawberry fields. Each field was approx. 1200 m<sup>2</sup> (20 beds of 0.70m width and 50m length, and 0.5m between beds). Systematic sampling (Greco *et al.*, 2004) was conducted from May 2006 to January 2007 (17 fields: 6 GH and 11 CCT) and from September 2008 to January 2009 (12 CCT fields). An expanded leaflet was randomly taken from a plant at intervals of 10m until the end of each row. The number of leaflets per sample varied from 90 to 115. Each sample was put in a plastic bag and the number of TSSM (active mites: larvae, nymphs and adults) and aphids (nymphs and adult females, without discriminating among species) per leaflet, on the abaxial surface, was counted in the laboratory using a binocular microscope (NIKON SMZ 645, 20×).

When both populations were in the same field at the same time, the spatial coincidence at leaflet level was estimated using the indices proposed by Griffiths (1969):

$$I_{CTSSM} = \frac{\sum_{i=1}^n TSSMA_i}{\sum_{i=1}^n TSSM_i}$$

where  $I_{CTSSM}$  is the spatial co-occurrence of TSSM and aphids,  $TSSMA_i$  is the number of TSSM that occurred with at least one aphid on the  $i$ th leaflet, and  $TSSM_i$  is the number of TSSM on the  $i$ th leaflet;  $n$  is the number of leaflets in the sample, and

$$I_{CA} = \frac{\sum_{i=1}^n ATSSM_i}{\sum_{i=1}^n A_i}$$

where  $I_{CA}$  is the spatial co-occurrence between aphids (A) and TSSM,  $ATSSM_i$  is the number of aphids that occurred with at least one TSSM on the  $i$ th leaflet, and  $A_i$  is the number of aphids on the  $i$ th leaflet.

Generalized linear/nonlinear models (Lindsey, 1997) were used to examine the relationship between spatial coincidence indices and TSSM density, aphid density, the percentages of TSSM and aphid-infested leaflets. Time periods (2006–2007 and 2008–2009), the time of sampling (month) and type of crop cover (plastic covered greenhouses or plastic low tunnels) were added as categorical variables to incorporate the structure of sampling in analyses of coincidence. The statistical significance of each variable was tested in turn in the model by a forward step-wise procedure, and those that contributed to the most significant change in deviance from the null model were retained. The change in the deviance was tested using the log-likelihood ratio test considering a Chi-square distribution with a significance level of 0.05. The Wald statistic was used to test the significance of the regression coefficient (Lindsey, 1997; Fox, 2008). Preliminary analyses were performed in order to facilitate pooling of data. Effects of time period, time of sampling and type of crop cover on coincidence indices were analysed by ANOVA. Before analysis, data were checked for normality (normal probability plots) and homogeneity of variance (Levene test). Kruskal-Wallis test was used when the assumptions for an analysis of variance were not met (Zar, 1996).

#### *Growth rate of T. urticae and C. fragaefolii (singly and in combination)*

Colonies of TSSM and aphids were reared on strawberry leaves in the laboratory under controlled conditions. All experiments were conducted at  $24 \pm 1^\circ\text{C}$ , 60–70% RH and 14:10 light:dark. The experimental unit was a new and expanded strawberry leaflet. The petiole of each leaflet was placed in a water-filled tube that was stored in a plastic container covered with plastic film to prevent the escape of individuals. To carry out the experiments, we selected *C. fragaefolii* because this was the most abundant aphid species during the survey period (Cédola & Greco, 2010). The following abundances of TSSM and aphids were assayed: 20:0–10:10–0:20. Two-day-old adult females of TSSM and *C. fragaefolii* were put on the underside of the leaflets with a fine brush. Each treatment was replicated 20 times and the experimental time was seven days. The intrinsic rate,  $r$ , of growth of each species was calculated as:

$$r = \ln(N_t/N_0)/t$$

where  $N_0$ =number of individuals at the beginning of the experiment;  $N_t$ =number of individuals at the end of the experiment and  $t$ =seven days. In addition, we recorded the number of individuals of each species per side of leaflet (adaxial or abaxial) once at the end of the seven-day trial in all treatments. Data were analysed by ANOVA, and the Kruskal-Wallis test was used when variances were heteroscedastic (Levene test).

#### *Preference tests*

The experimental unit was a Petri dish (10 cm in diameter) with strawberry leaf discs (1.8 cm in diameter) placed on moistened filter paper. Choice and no-choice experiments were performed with 15 replicates for all conditions. In choice experiments, five TSSM females (48–72-h-old taken from pure cohort) were placed between two discs: one with honeydew on the abaxial side (selected from a strawberry leaf previously infested with approximately 15 females of *C. fragaefolii* for four days, and then removed) and the other from a similarly-aged leaf without honeydew. The disc acceptance was measured by counting females that settled on each test disc after 48 h and the number of eggs per female. Similarly, five adult aphids (48-h-old taken from pure cohort) were placed between two discs: one selected from a strawberry leaf infested with approximately 25 active TSSM for four days and then removed, and the other without TSSM damage. The disc acceptance was measured by counting aphids that settled on each test disc after 48 h and the number of offspring per female. The proportion of TSSM and *C. fragaefolii*, and the number of eggs or offspring per female on each kind of disc, were analysed by the Kruskal-Wallis test.

We also performed for TSSM and *C. fragaefolii* a no-choice test (as control) with each kind of disc (+honeydew, –honeydew, +TSSM damage, –TSSM damage, respectively). The frequency of individuals on each kind of disc and the position on different places of the experimental unit (on abaxial surface or the adaxial surface or on any other place in the Petri dish) were analysed by a  $2$  (kind of disc)  $\times$   $3$  (different places of the experimental unit) contingency table. Chi-square statistic was used to determine the significance of departure from expected in the contingency table (Zar, 1996). The number of eggs and offspring per female of TSSM and *C. fragaefolii*, respectively, were recorded and analysed by one-way ANOVA.

## **Results**

### *Spatial coincidence*

Populations of TSSM and aphids were recorded together in 82.75% (23/29) of sampled fields. Considering all samplings performed during 2006–2007 ( $N=338$ ) and 2008–2009 ( $N=50$ ), field coincidence was 29% (98/338) and 98% (49/50), respectively. Coincidence at the leaflet level occurred in 26.53% (26/98) and 87.75% (43/49) of cases in 2006–2007 and 2008–2009, respectively. Preliminary analyses of data indicated that  $I_{CTSSM}$  was similar between sampling periods (Kruskal-Wallis test:  $H_{(1,N=70)}=0.28$ ,  $P=0.597$ ), between times of sampling in both sampling periods (2006–2007: Kruskal-Wallis test:  $H_{(5,N=26)}=7.94$ ,  $P=0.160$ ) (2008–2009: Levene test:  $F=1.26$ ,  $df=3$ ,  $40$ ,  $P=0.301$ ; ANOVA:  $F=1.42$ ,  $df=3$ ,  $40$ ,  $P=0.250$ ) and between types of crop cover (Kruskal-Wallis test:  $H_{(1,N=70)}=0.58$ ,  $P=0.750$ ).  $I_{CA}$  was also

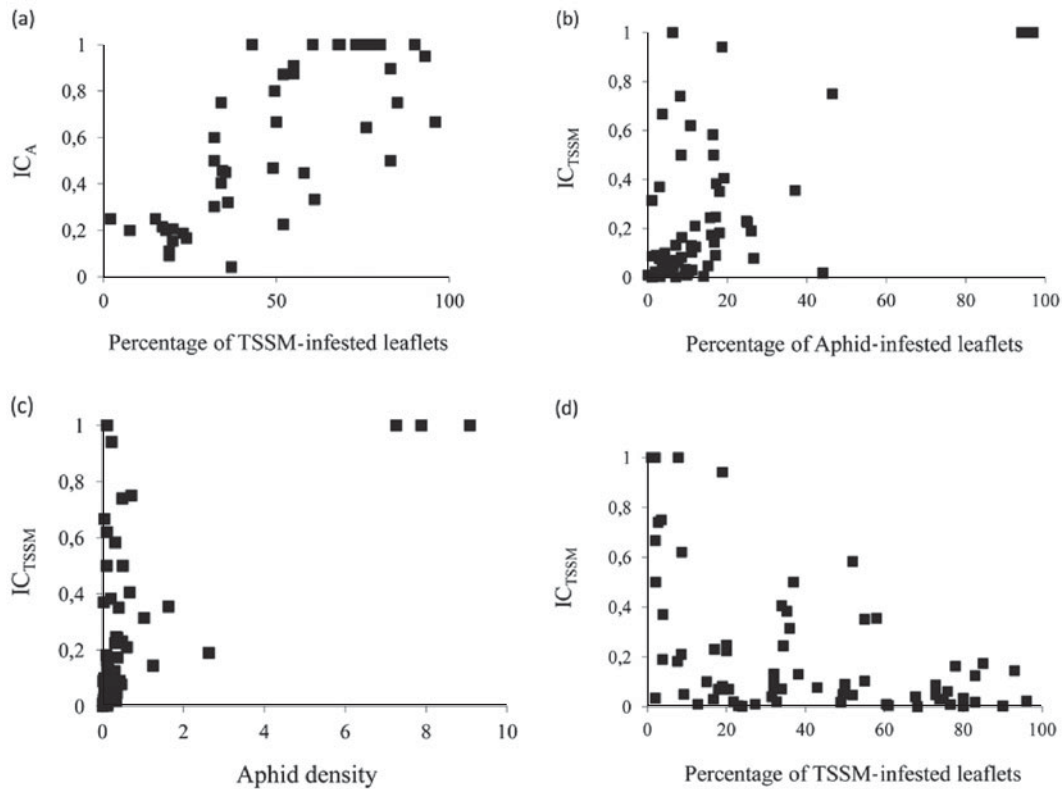


Fig. 1. Spatial coincidence indices:  $IC_{TSSM}$  (the spatial coincidence between TSSM and aphids) and  $IC_A$  (the spatial coincidence between aphids and TSSM). (a) Relationship between  $IC_{TSSM}$  and percentage of TSSM-infested leaflets; (b) relationship between  $IC_{TSSM}$  and percentage of aphid-infested leaflets; (c) relationship between  $IC_{TSSM}$  and aphids density; (d) relationship between  $IC_A$  and percentage of TSSM-infested leaflets.

similar between sampling periods (Levene test:  $F=0.71$ ,  $df=1, 68$ ,  $P=0.402$ ; ANOVA:  $F=3.51$ ,  $df=1, 68$ ,  $P=0.065$ , log transformation of variable), times of sampling in both periods (2006–2007: Kruskal-Wallis test:  $H_{(5, N=26)}=6.53$ ,  $P=0.258$ ) (2008–2009: Levene test:  $F=1.87$ ,  $df=3, 40$ ,  $P=0.150$ ; ANOVA:  $F=0.65$ ,  $df=3, 40$ ,  $P=0.589$ ) and between types of crop cover (Kruskal-Wallis test:  $H_{(1, N=70)}=4.48$ ,  $P=0.108$ ).

The TSSM spatial coincidence index was affected by the percentage of TSSM-infested leaflets, the percentage of aphid-infested leaflets and aphid density (fig. 1a–c). The model that included the most significant variable, the percentage of TSSM-infested leaflets, explained 65.09% (deviance change was 13.76 out of 21.14) of the variation in the TSSM spatial coincidence index (table 1). The proportion of TSSM that coincided with at least one aphid decreased as the percentage of leaflets with TSSM increased.

The spatial coincidence index between aphids and TSSM increased together with the percentage of TSSM-infested leaflets. Complete coincidence ( $IC_A=1$ ) was registered from 40% of TSSM-infested leaflets (fig. 1d). This was the only significant variable (table 2) when it was added to the normal-log model, demonstrating that this relationship is linear and not curvilinear ( $r^2=0.71$ ,  $F_{1,68}=167.13$ ,  $P<0.001$ ). The model explained 64% (total deviance change was 5.30 out of 8.28) of the variation in aphid spatial coincidence index (table 2). Sample period, time of sampling and type of crop cover did not affect the coincidence indices.

When there was spatial coincidence between aphids and TSSM at the leaflet level, each population reached its highest density at the lowest density level of the other (fig. 2a). In most cases, the TSSM coincided with very few aphids on the same leaflet and vice versa (fig. 2b).

#### *The intrinsic rate of increase of TSSM and C. fragaefolii (singly and in combination)*

The mean rate of increase of TSSM in pure culture ( $r=0.39 \pm 0.028$  measured over seven days) ( $\pm SE$ ,  $N=20$ ) was marginally but significantly diminished, 3%, in the presence of *C. fragaefolii* ( $r=0.378 \pm 0.01$ ,  $N=20$ ) (Levene test:  $F=0.014$ ,  $df=1, 38$ ,  $P=0.906$ ; ANOVA:  $F=8.08$ ,  $df=1, 38$ ,  $P=0.0071$ ). In contrast, the mean intrinsic rate of growth of *C. fragaefolii* in pure culture ( $r=0.19 \pm 0.009$ ,  $N=20$ ) was not reduced in the presence of TSSM ( $r=0.20 \pm 0.004$ ,  $N=20$ ) (Kruskal-Wallis:  $H_{(1, N=49)}=0.11$ ,  $P=0.74$ ).

The number of individuals settled on the abaxial side of the leaflet was higher than on the adaxial side in pure cultures of *T. urticae* (Kruskal-Wallis:  $H_{(1, N=40)}=30.41$ ,  $P<0.05$ ) and *C. fragaefolii* (Kruskal-Wallis:  $H_{(1, N=40)}=29.60$ ,  $P<0.05$ ). The number of aphids on the adaxial side of leaflets in mixed culture was significant higher than the number of aphids on the adaxial side in pure culture (Levene test:  $F=0.81$ ,  $df=1, 37$ ,  $P=0.37$ ; ANOVA  $F=11.78$ ,  $df=1, 37$ ,  $P=0.001$ ). The number the TSSM on the adaxial side was similar in pure and mixed

Table 1. Summary of the forward step-wise procedure used to build a multiple regression model for the spatial coincidence ( $I_{c_{TSSM}}$ ) between two-spotted spider mites (TSSM) and aphids (A).

| Model  | Deviance | Change in deviance | df | <i>P</i> | Wald  | <i>P</i> |
|--|----------|--------------------|----|----------|-------|----------|
| No terms added (null model)                      | 21.136   |                    | 69 |          |       |          |
| Int + A density                                  | 15.932   | 5.204              | 69 | 0.022    | 7.469 | 0.006    |
| Int + TSSM density                               | 16.771   | 4.365              | 69 | 0.037    | 2.507 | 0.113    |
| Int + % of A- infested leaflets                  | 14.237   | 6.899              | 68 | 0.008    | 9.476 | 0.002    |
| Int + % of TSSM-infested leaflets                | 13.758   | 7.378              | 68 | 0.006    | 5.614 | 0.018    |
| Int + sampling time                              | 19.815   | 1.321              | 68 | 0.250    |       |          |
| Int + sampling time                              | 14.687   | 6.456              | 68 | 0.011    | 5.960 | 0.428    |
| Int + type of crop cover                         | 19.793   | 1.343              | 68 | 0.246    |       |          |
| Int + % of TSSM-infested leaflets + TSSM density | 13.669   | 0.089              | 67 | 0.956    |       |          |
| Int + % of A- infested leaflets + TSSM density   | 12.242   | 1.995              | 67 | 0.369    |       |          |
| Int + % of A- infested leaflets + A density      | 13.717   | 0.519              | 67 | 0.771    |       |          |

The model assumes a poisson distribution of errors and uses the log-link function. The change in deviance after inclusion of a term in the model was tested through a log-likelihood ratio test ( $P < 0.05$ ).

Table 2. Summary of the forward stepwise procedure used to build a multiple regression model for the spatial coincidence ( $I_{c_A}$ ) between aphids (A) and two-spotted spider mites (TSSM).

| Model                             | Deviance | Change in deviance | df | <i>P</i> | Wald   | <i>P</i> |
|-----------------------------------|----------|--------------------|----|----------|--------|----------|
| Intercept                         | 8.280    |                    | 69 |          |        |          |
| Int + A density                   | 7.418    | 0.862              | 68 | 0.353    | 0.181  | 0.670    |
| Int + TSSM density                | 6.461    | 1.820              | 68 | 0.177    | 0.569  | 0.450    |
| Int + % of A infested leaflets    | 6.680    | 0.738              | 68 | 0.390    | 0.862  | 0.353    |
| Int + % of TSSM-infested leaflets | 2.981    | 5.299              | 68 | 0.021    | 60.912 | 0.000    |
| Int + sampling period             | 7.787    | 0.493              | 68 | 0.482    |        |          |
| Int + sampling time               | 6.710    | 0.571              | 68 | 0.451    |        |          |
| Int + type of crop cover          | 7.764    | 0.516              | 68 | 0.472    |        |          |

The model assumes a normal distribution of errors and uses the log-link function.

The change in deviance after inclusion of a term in the model was tested through a log-likelihood ratio test ( $P < 0.05$ ).

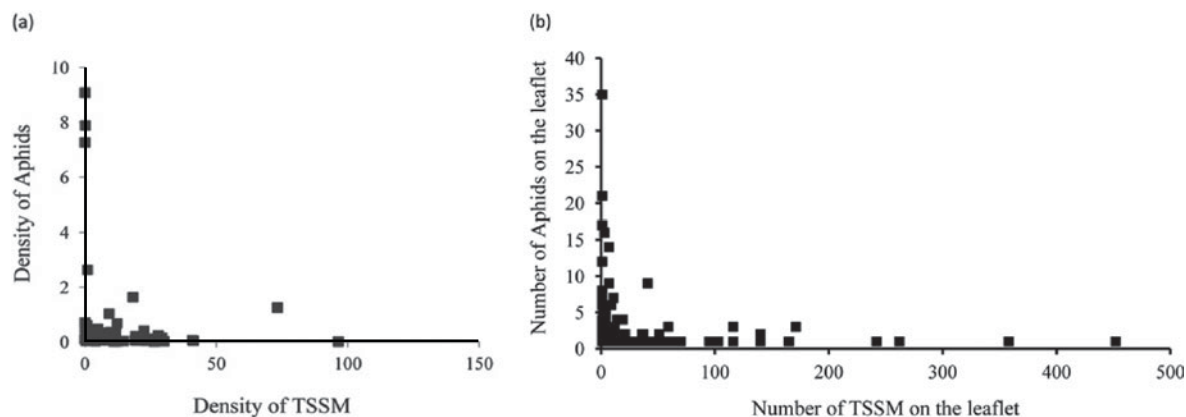


Fig. 2. (a) Relationship between aphid density (number of aphids/leaflet) and TSSM density (number of active *Tetranychus urticae*/leaflet) and (b) relationship between the number of aphids and the number of active *T. urticae* on the same leaflet.

cultures (Levene test:  $F = 3.97$ ,  $df = 1, 38$ ,  $P = 0.053$ ; ANOVA  $F = 0.17$ ,  $df = 1, 38$ ,  $P = 0.678$ ).

#### Preference assays

TSSM showed preference for strawberry discs without honeydew (Kruskal-Wallis:  $H_{(1,N=30)} = 22.55$ ,  $P < 0.05$ ) (fig. 3). The number of eggs per TSSM female was higher on discs without honeydew (Kruskal-Wallis:  $H_{(1,N=30)} = 17.36$ ,

$P < 0.05$ ). In some replicates, when discs had honeydew, leaflet hairs were chosen by females to lay the eggs (fig. 4). Strawberry discs without TSSM damage were preferred by aphids (Kruskal-Wallis:  $H_{(1,N=30)} = 22.41$ ,  $P < 0.05$ ) (fig. 4). No aphid offspring were observed on either kind of disc.

In no-choice experiments, the location of TSSM females on the abaxial disc surface, the adaxial disc surface or any other place in the Petri dish was not independent of the kind of disc, both with honeydew and without honeydew ( $\chi^2 = 8.23$ ,  $df = 2$ ,

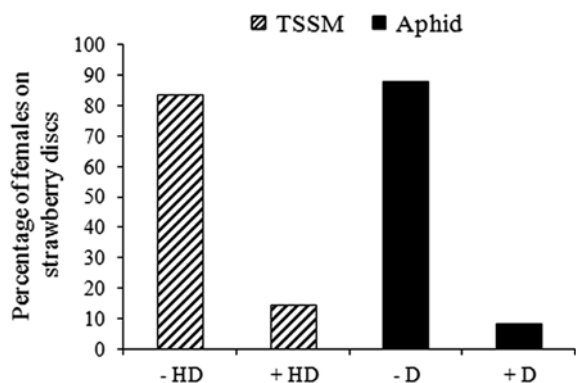


Fig. 3. Results of no-choice test showing percentage of TSSM, *Tetranychus urticae*, and aphid, *Chaetosiphon fragaefolii*, females on strawberry discs with honeydew and mites damage, respectively. (–HD, discs without honeydew; +HD, disc with honeydew; –D, discs without *Tetranychus urticae* damage; +D, discs with *Tetranychus urticae* damage).

$P=0.016$ ). Subdividing a contingency table (Zar, 1996) and ignoring data of individuals on any other place in the Petri dish, the results indicated that the position of individuals was independent of the kind of disc ( $X^2=0.292$ ,  $df=1$ ,  $P=0.588$ ). This evidenced that there were more individuals on any other place in the Petri dish when discs had honeydew and suggests that, when this occurred, TSSM started wandering in search of another food source. The mean number of TSSM eggs per female on discs without honeydew was higher ( $11.69 \pm 0.69$ ) than that on discs with honeydew ( $2.96 \pm 0.49$ ) (Levene test:  $F=3.49$   $df=1$ ,  $28$   $P=0.07$ ; ANOVA:  $F=102.58$ ,  $df=1$ ,  $28$ ,  $P<0.05$ ). The location of aphids on the abaxial disc surface, the adaxial disc surface or any other place in the Petri dish was dependent on kind of disc, with honeydew and without honeydew ( $X^2=71.29$ ,  $df=2$ ,  $P<0.001$ ). Subdividing the contingency table and ignoring data of individuals on any other place in the Petri dish, the results indicated that the position of individuals was also dependent on kind of disc ( $X^2=61.31$ ,  $df=1$ ,  $P<0.001$ ). In this case, the results show that more individuals were located on any other place in the Petri dish and on the adaxial surface when discs had TSSM damage. No aphid offspring were observed on either kind of disc.

## Discussion

Ecological theory assumes that competition occurs mainly between members of the same guild (Pianka, 1983); however, studies have demonstrated that herbivores in different guilds can exert strong effects on each other (Kaplan & Denno, 2007; Kaplan *et al.*, 2009). Indeed, temporally and spatially separated competitive effects mediated by the host plant appear to be common among phytophagous insects (Faeth, 1986; Masters & Brown, 1997). Although TSSM and aphids feed on different parts of the leaf and exploit different resources (epidermal cell content and phloem sap, respectively) the results derived from this study suggest a negative interaction between them.

The coincidence between TSSM and aphids populations in strawberry crop was, in general, high. During one of the study periods, the coincidence was low, probably because it covered the winter when these herbivores are absent or at very low density (Greco *et al.*, 1999; Cédola & Greco, 2010). In field and

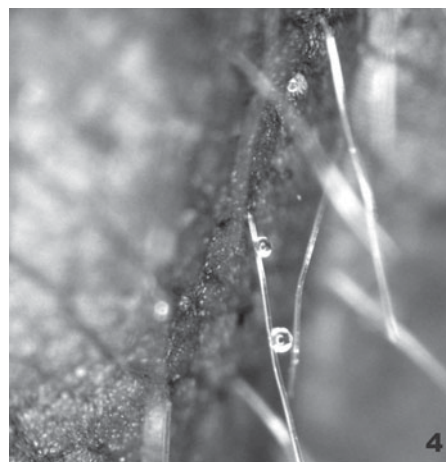


Fig. 4. Eggs of *Tetranychus urticae* on a hair from the abaxial surface of a strawberry leaflet with honeydew.

greenhouse surveys, the main factor that affected the coincidence indices at the leaflet level was the percentage of TSSM-infested leaflets. The cause of this could be that TSSM would have a greater capacity for population growth and greater ability to move than aphids, so they would occupy new leaflets before aphids do. The proportion of TSSM that coincided with at least one aphid decreased as the percentage of TSSM-infested leaflets increased. The cause for this could be that when TSSM population is higher, the proportion of individuals that coincide with at least one aphid is lower. On the other hand, the proportion of aphids that coincided with at least one TSSM increased as the percentage of TSSM-infested leaflets also increased. Bearing in mind that TSSM density was always higher than aphid density, the probability of an aphid coinciding with at least one TSSM is expected to be higher as TSSM density, and consequently the percentage of mite-infested leaflets, increase. The sampling time did not affect the coincidence at leaflet level; therefore, we found no evidence for seasonal effects independent of density.

Sharing the same leaflet caused disturbances for both species. TSSM showed a lower increase rate when they shared the same leaflet with *C. fragaefolii*, as well as lower fecundity on strawberry discs with honeydew. Some compound in honeydew or the saliva of aphids may be toxic (Miles, 1999) and can cause changes in leaf quality (Dixon & Wratten, 1971; Cammell, 1981). An initial piercing before ovipositing allows TSSM to assess host-plant quality; nevertheless, females might settle on an unfavourable resource and have low fecundity (Yano *et al.*, 1998).

There are antecedents of negative interactions between phloem feeders and other herbivores. Soroker *et al.* (2010) found that whiteflies negatively affect the development of broad mite populations on tomato plants, and some negative effects of broad mites on whiteflies were also observed. Arouni *et al.* (2008) observed negative interactions between citric aphids, *Aphis spiraeicola* Patch, and leafminers, *Phyllocnistis citrella* Stainton, and a short coexistent period in which leafminer survival was lower on leaves with aphid infestations.

The rate of growth of *C. fragaefolii* did not change, but individuals moved to the other side of the leaflets when they

were with TSSM. Furthermore, the damage on strawberry leaflets produced after *T. urticae* feeding could interfere with the normal stylet pathway of *C. fragaefolii* in plant tissues. No offspring were produced on leaf discs with TSSM damage; however, the same behaviour was observed in discs without damage, suggesting that the experimental conditions could affect this variable. Many plant species emit volatiles in response to herbivory. Secondary metabolites or allelochemicals (e.g. alkaloids, phenolic compound, glucosinolates) are mediators in the response to herbivores attack (Karban & Baldwin, 1997). *Tetranychus urticae* is a generalist that can feed on several hundred host plant species, and its feeding induces emission of volatiles by certain plants including strawberry (Karban & Baldwin, 1997; van den Boom *et al.*, 2004). The landing of flying aphids on a host plant involves a sequence of visual cues to give specific information, but the landing response may be modified by plant volatiles (Powell *et al.*, 2005). In this study, there were no measurements of volatile compounds, but we suspect that phytochemical cues from *T. urticae* feeding may determine aphid behaviour in the field and in laboratory experiments.

Phenolic compounds are known to play an important role in mite-strawberry relationships (Luczynski *et al.*, 1990). Kielkiewicz & van de Vrie (1983) suggested that displacement of phenolics to the places of mite penetration and/or their synthesis 'de novo' in these places might be one of the factors which reduce mite injury to leaves. This mechanism could affect aphids such as Zucker (1982) observed.

Several studies involving temporally or spatially separated species in different feeding guilds (summarized in Kaplan & Denno, 2007) suggest that traditional resource-based competition theory may underestimate the frequency and intensity of within trophic level interactions. Negative interactions such as demonstrated herein can affect distribution of herbivorous arthropods (Faeth, 1986). Studies of negative interactions can offer important insights into how interactions structure ecological communities.

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