Plant traits mediate effects of predators across pepper (*Capsicum annuum*) varieties

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Abstract. 1. The magnitude of plant intra-specific variation for indirect defence and the underlying plant traits influencing predators remain relatively unstudied, particularly in cultivated plants.

2. We tested whether differences in flower number, pollen production, and leaf trichome density among 17 pepper (*Capsicum annuum* Linnaeus) varieties influenced the abundance and predation intensity by the omnivorous mite *Amblyseius swirskii* Athias-Henriot.

3. A greenhouse experiment was conducted where pepper plants were infested with thrips (*Frankliniella cephalica* Crawford DL) and subsequently exposed to *A. swirskii*. We estimated thrips and mite density based on arthropod counts conducted over a 4-week period, and also performed flower and trichome counts, and estimated pollen production per anther.

4. Significant differences were found among varieties for all three traits, as well as mite and thrips density. After accounting for all traits in a multiple regression model, we found that flower and trichome number had significant positive effects on mite density (by providing food and shelter, respectively). Increased mite density was in turn associated with a decrease in thrips density, presumably as a result of mite predation. Moreover, we found that flower number (but not trichome density) increased the strength of thrips suppression and that such an effect was mediated by mite density.

5. These findings suggest that genetic variation for plant traits may indirectly influence herbivore suppression in peppers (although traits may vary in the strength or direction of their effects), and underscore the evolutionary potential and importance of selection not only for direct but also indirect resistance in crops.

Key words. Apparent competition, biological control, plant genetic variation, predation, tri-trophic interactions.

Introduction

There has been a long-standing interest for plant traits conferring resistance to herbivores in both wild and cultivated plant species (Harris & Frederiksen, 1984; Kennedy & Barbour, 1992; Marquis, 1992; Smith, 2005; Agrawal, 2007). Traditionally, ecological and agricultural research has focused on the

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evolution and selection (respectively) of plant traits that provide direct resistance by reducing herbivore fitness (Adkisson & Dyck, 1980; Eigenbrode & Trumble, 1994; Karban & Baldwin, 1997; Stamp, 2003; Agrawal, 2007). More recently, however, studies have addressed the role of plant traits that attract natural enemies of herbivores, and in turn lead to herbivore suppression (termed 'indirect defence'; Hare, 2002, 2011; Dicke *et al.*, 2003; Heil, 2008). Although variation in plant traits conferring indirect defence has been particularly well documented across species, evidence is also mounting for effects of a similar magnitude owing to trait variation within species (Hare, 1992, 2002; Poelman *et al.*, 2009; Stenberg *et al.*, 2011).

Accordingly, investigating plant intra-specific variation for such traits is important because it provides the basis for the evolution and selection for enhanced indirect defence (Bottrell *et al.*, 1998; Cortesero *et al.*, 2000; Hare, 2002; Kessler & Heil, 2011; Mooney & Singer, 2012).

Generalist predators, and in particular omnivorous predators, are well suited for biological control of herbivores because their populations may persist by feeding on alternative food when any single resource is scarce (Eubanks & Denno, 1999, 2000; Van Baalen *et al.*, 2001; Eubanks, 2005; Mooney *et al.*, 2010). More stable omnivore populations are in turn predicted to lead to stronger herbivore suppression and thus more effective indirect defence (reviewed by Symondson *et al.*, 2002; Eubanks & Styrsky, 2005). A prime example of this are omnivorous predatory mites which feed on pollen and nectar and are frequently used as pest control agents in cultivated systems (McMurtry & Croft, 1997; Solomon *et al.*, 2000; Symondson *et al.*, 2002). Previous work has shown positive effects of experimental pollen addition on the abundance of predatory mites (Messelink *et al.*, 2008; Nomikou *et al.*, 2010) as well as positive effects of leaf domatia, presumably because trichomes provide protection against other predators (reviewed by Walter, 1996; Agrawal, 2003; Loughner *et al.*, 2008; negative effects have been reported for other predator groups, see Styrsky *et al.*, 2006). To date, however, only a handful of studies have looked at the effects of plant intra-specific variation for traits influencing predatory mites (for examples with other predators see Hare, 2002). The few studies available have shown that differences in trichome density among crop genetic types (e.g. varieties and inbred lines) influence predatory mite abundance (Downing & Moilliet, 1967; Duso & Vettorazzo, 1999; Duso *et al.*, 2003; Loughner *et al.*, 2008), whereas evidence for the effects of plant genotype variation in other vegetative or reproductive traits is lacking (but see Stenberg *et al.*, 2011).

The generalist predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is commercially available and used worldwide to control thrips and whiteflies in cultivated plant species (Nomikou *et al.*, 2003). Recent studies have evaluated the role of prey species number (Messelink *et al.*, 2008, 2010), predator species number (Messelink *et al.*, 2011), crop diversity (Messelink *et al.*, 2009), and plant-based resources and traits (Nomikou *et al.*, 2010) on *A. swirskii* abundance and predation intensity. With regard to plant-based resources, past work has shown positive effects of experimental pollen addition on the establishment and herbivore suppression effects by this mite (e.g. Nomikou *et al.*, 2003), as well as positive correlations between leaf pubescence and mite abundance (Doğramacı *et al.*, 2011; Loughner *et al.*, 2011). Nonetheless, research has largely ignored plant intra-specific variation in these traits and the consequences of such variation for indirect defence mediated by this predatory mite.

We conducted a greenhouse experiment to investigate if differences among 17 pepper (*Capsicum annuum* Linnaeus) varieties in flower number, pollen production, and leaf trichome density parallel variation in *A. swirskii* abundance as well as mediate the consumptive effects of this mite on thrips (*Frankliniella cephalica* Crawford DL). Specifically we asked: (i) Do pepper varieties differ in *A. swirskii* abundance and do flower number, pollen production, and leaf trichome density explain such differences? (ii) Do pepper varieties with greater *A. swirskii* abundance exhibit stronger thrips suppression, such that the measured traits mediate the strength of indirect defence in *C. annuum*? By assessing the effects of multiple plant traits, we also sought to determine if these traits had effects of a similar magnitude and in the same direction on *A. swirskii* (i.e. assessing if plant traits have antagonistic or synergistic effects on indirect defence), as well as the presence of trait genetic correlations that may influence selection for indirect defence.

Materials and methods

The present study was conducted at the Instituto Nacional de Investigaciones Agrícolas, Forestales y Pecuarias (INIFAP) in Mocochá, Yucatán (México) (21°6′40″N, 89°26′35″W) from September 2011 to January 2012. The third week of September 2011, we germinated seeds from 17 *C. annuum* varieties originating from different locations in central and southern Mexico (Table 1). One month later, we transplanted seedlings to 15-litre bags filled with a mix of sheep manure, composted agave (*Agave fourcroydes* Lem.) bagasse, and vertic luvisol soil (3 : 3 : 4). We then randomly selected 10 plants per variety for a total of 180 experimental plants, and placed them in a greenhouse that had been previously used for an experiment with *C. annuum* and was infested with thrips (*F. cephalica*); hence, the thrips population was already established and acclimated to a *Capsicum* diet. Within the greenhouse, we randomised positions of plants from each variety, and kept them there for 1 month to allow infestation by thrips.

On 15 November 2011, we moved experimental plants infested with *F. cephalica* thrips to an adjacent greenhouse free of thrips, where *A. swirskii* would be subsequently introduced. The entire surface of this greenhouse was occupied with plants, with a distance of 75 cm among each of six rows of plants, and 30 cm among plants within each row. We randomised plants positions throughout the greenhouse (completely randomised design) and divided the greenhouse into 10, 2×2.5-m imaginary quadrats which served as a template to determine the spatial distribution of *A. swirskii* inoculation (see ahead). On 1 December 2011, we placed a nylon mesh horizontally 1 m above the ground such that it came into contact with the apical leaves of all plants. The net was used to facilitate mite movement across plants. On 15 December 2011, we placed one bag with 250 *A. swirskii* (purchased from Koppert B. V., Berkel en Rodenrijs, The Netherlands) on the net at the centre of each quadrat, resulting in an approximate initial density of 13.88 mites per plant which is within the range used in biological control studies with *A. swirskii* (e.g. Messelink *et al.*, 2008; Calvo *et al.*, 2011). Bags and netting were removed 1 week later. Most plants from each variety [mean = 87.2 ± 4.9 (S.E.)%] were flowering at the time of *A. swirskii* inoculation, precluding initial biases in plant selection by mites owing to differences in flowering phenology influencing pollen availability. All varieties continued flowering until the end of the experiment in mid January 2012.

The mean daily temperature inside the greenhouse during the experiment was 24.76 ± 0.33 °C (SE), with mean minimum and maximum mean values of 17.08 ± 0.56 and 40.46 ± 0.52 °C,

Table 1. Studied pepper (*Capsicum annuum*) types (common names), including the inbred line used in each case and its origin (i.e. source site).

ID	Pepper type	Line	Origin
1	Dulce	IYCA-123	Yucatán (México)
2	X'cat	IYCA-276	Yucatán (México)
3	Chawa	IYCS-6.3	Yucatán (México)
4	Cayene	NuMex Mesilla	U.S.A.
5	Jalapeño	Don Pancho	Tamaulipas (México)
6	Peter	PI 593566	U.S.A.
7	Chilhuazcle	IYCA-284	Chiapas (México)
8	De agua	IYCA-287	Oaxaca (México)
9	Chilpaya	IYCA-281	Chiapas (México)
10	Ancho/Poblano	IYCA-283	Chiapas (México)
11	Serrano	Tampiqueño 74	Tamaulipas (México)
12	Criollo de Morelos 334	PI 636424	Morelos (México)
13	Filfil I Hind	PI 138565	Iran
14	California Wonder	PI 586661	U.S.A.
15	Jalapeño	IYCA-301	Chiapas (México)
16	Ya'ax ic	PI 138565	Yucatán (México)
17	Guaque	IYCA-302	Chiapas (México)

ID, code number used in study.

respectively. The mean relative humidity was $61.93 \pm 0.58\%$, with mean minimum and maximum averages of $33.98 \pm 0.31\%$ and $79.12 \pm 1.4\%$, respectively.

Sampling of predatory mites and thrips

We conducted weekly surveys of *A. swirskii* and *F. cephalica* abundance from mid-December 2011 to mid-January 2012. For each survey, we randomly selected five leaves per plant, measured leaf blade lengths and widths and inspected both leaf surfaces for mites and thrips. Leaf lengths and widths were converted to leaf area based on a regression between leaf area and leaf length × width (R^2 values ranged from 0.96 to 0.99 across all pepper varieties). Leaf areas used in these regressions were estimated with a Li-Cor area meter (Li-3000A; LI-COR, Lincoln, Nebraska) for a sample of 20 leaves per variety from a group of non-experimental plants grown under the same conditions. We used thrips and mite densities (arthropods/cm^2 of leaf area sampled) as response variables for statistical analyses.

Plant traits

Flower number and pollen production. During each arthropod survey, we counted all open flowers per experimental plant. To quantify pollen production per anther, we collected both closed and open anthers from flowers of plants not used in the experiment ($n = 3$ per variety), but grown under the same conditions in another greenhouse. We did not collect anthers from experimental plants to avoid influencing mite abundance owing to unintended effects of flower manipulation (e.g. changes in pollen availability, reallocation of resources, etc.). For each plant we collected three groups of 15 closed anthers, placing each group in an individual 2-ml plastic vial; a group of anthers consisted of a pool of anthers from different flowers of a given

plant. Vials were previously dried and weighed. Each group of anthers, together with the vial in which they were contained, were dried at $70\,°C$ for 18 h and weighed with an analytical scale accurate to the nearest 10^{-10} g. Based on this, the mass (i.e. weight) of a closed anther was calculated as: [(vial weight + weight of 15 closed anthers) − vial weight]/15. The same procedure was followed for each group of anthers, resulting in three estimates of closed anther weight per plant. Open anthers were sampled in the same way, but carefully washed twice with 1.5 ml of bidistilled water and then twice again with 1.5 ml of pure ethanol to remove all the pollen grains. After washing, anthers were dried at $70\,°C$ for 18 h. The mass of each open anther was calculated as: [(vial weight + weight of 15 open anthers) − vial weight]/15, resulting in three estimates per plant. For each plant, we then averaged the weight of the three groups of open anthers and estimated the amount of pollen produced (mg) per anther as: weight of a closed anther (i.e. with pollen) − mean weight of an open anther (without pollen). This procedure was repeated three times for each plant (i.e. for each estimate of closed anther weight, in all three cases using the same mean value of open anther weight), and the three estimates of pollen weight per anther were then averaged per plant for statistical analyses.

Leaf trichomes. At the end of the experiment, we collected two fully-expanded leaves from three to five experimental plants per variety. Glandular trichomes were not quantified as they were only observed on the abaxial surface of two pepper varieties, in both cases at very low densities. Non-glandular trichomes, however, were present in 15 out of the 17 studied varieties and were especially abundant on leaf veins of the abaxial leaf surface (previous studies have shown positive effects of this trichome type on predatory mites; Walter, 1996; Loughner *et al.*, 2008). Therefore, we only measured the abundance of non-glandular trichomes, hereafter referred to simply as 'trichomes'. Using high-resolution digital images, we counted all trichomes present along a 1-cm^2 segment of the midvein immediately adjacent or proximal to the petiole, because trichomes become gradually less abundant when progressing from the base to the tip of the leaf blade. The sampled segment of the midvein was representative of the leaf blade region where trichomes are most abundant and where mites more frequently aggregated (L. Abdala-Roberts, pers. obs.). We expressed trichome density as the number of trichomes found in the 1-cm^2 sampled portion of the midvein, and used the average of both leaves per plant for statistical analyses.

Statistical analyses

Trait differences among pepper varieties. We performed generalised linear models using PROC GENMOD in SAS ver. 9.1 (SAS Institute, 2002) to determine if there were differences among pepper varieties in the traits measured. Pepper variety was treated as a fixed effect given that varieties were selected *a priori* based on their phenotypic characteristics, as well as because we were interested in assessing differences among specific varieties to inform future breeding and biocontrol research. For flower

number, we used the full data set, i.e. all experimental plants used in arthropod surveys ($n = 180$), whereas trichome data originated from a subset of three to five experimental plants per variety ($n = 67$), and pollen production per anther came from a set of non-experimental plants ($n = 54$). The flower count model was based upon a Poisson distribution with a log link function (there was no evidence of overdispersion for these data), whereas the other two models with continuous data were based upon a normal distribution (identity function). Trichome data (mean value per plant) were log-transformed to achieve normality while pollen production per anther data were normally distributed without transformation (normality was verified with Shapiro–Wilk tests). For all traits we provide raw means and standard errors as descriptive statistics.

Variation in mite and thrips abundance among pepper varieties. To determine if pepper varieties exhibited differences in mite and thrips density, we performed generalised linear models using PROC GENMOD testing for an effect of pepper variety (treated as a fixed effect, see above) on the density of each arthropod species. For both models, we used the mean density of each species across all surveys as a response variable (to avoid overestimates based on cumulative data). For the *A. swirskii* model, we used the gamma distribution (with a log link function) which handles continuous data with different types of non-normal distributions (Zuur *et al.*, 2009). For the thrips density model, we used a normal distribution (normality verified with a Shapiro–Wilk test). In both cases, we report raw means and standard errors as descriptive statistics.

Genetic correlations among pepper traits and effects on mite abundance. Using pepper variety means, we first tested for relationships among plant traits by performing pairwise simple regressions between trichome density, pollen production per anther, and flower number. Second, to test for the independent effects of plant traits on mite abundance we performed a multiple regression using variety means where all three traits were predictors of *A. swirskii* density. For both the simple regressions among traits and the multiple regression, quadratic terms were dropped when not significant. Whenever a predictor variable was significant in the multiple regression, we presented a bivariate plot depicting the predicted relationship between that trait and mite density based upon a simple regression. We previously removed thrips density as a predictor of mite density in the multiple regression because this effect was non-significant (partial $r^2 = 0.06$; $P = 0.19$), indicating that pepper trait effects on mites were not mediated by thrips density. Two observations might explain this previous finding and support the argument that pepper effects on mites were not mediated by thrips: First, thrips were present at low abundances and thus bottom-up effects of thrips abundance on mite abundance were expected to be negligible (Mooney & Singer, 2012). And second, *A. swirskii* is omnivorous and thus expected to track thrips abundance weakly owing to the presence of more predictable and abundant plant resources such as pollen (Eubanks & Denno, 1999; Eubanks, 2005). Accordingly, we predicted that mite density

would be influenced mostly by plant traits (pollen availability, trichomes) and that mites would suppress thrips resulting in a negative relationship between mite and thrips density (see *mite predation effects* in next section), analogous to an apparent competition effect of pollen or other flower resources (e.g. nectar) on thrips (Nomikou *et al.*, 2010).

Previous simple regressions using the mean value of thrips density across surveys showed that variation in thrips density among pepper varieties was not influenced by any of the measured traits [flower number (quadratic model): $R^2 = 0.21$, $P = 0.18$; trichome density (quadratic model): $R^2 = 0.26$, $P = 0.12$; pollen production: $R^2 = 0.03$, $P = 0.54$]. Likewise, effects of pepper traits on initial thrips density (2-week period subsequent to setting up the experiment, but prior to mite introduction) were not significant either (initial flower number: $R^2 = 0.08$, $P = 0.26$; pollen production: $R^2 = 0.002$, $P = 0.96$; trichome density: $R^2 = 0.04$, $P = 0.40$), suggesting that direct effects of plant traits on thrips were weak. It is possible that low levels of thrips colonisation observed in our study limited the detection of direct effects of plant traits on this herbivore. In contrast, previous work has shown that plant-based resources such as pollen are important determinants of thrips performance (Hulshof *et al.*, 2003; Skirvin *et al.*, 2006). All regression analyses were performed in PROC REG in SAS and normality was verified with Shapiro–Wilk tests.

Differences among pepper varieties in mite predation effects. First, we performed a regression using pepper variety means where *A. swirskii* density was a predictor of thrips density. Second, we tested if increasing mite density conferred stronger thrips suppression by regressing pepper variety means for the strength of thrips suppression onto variety means for *A. swirskii* density. Thrips density declined substantially after the third week of sampling, which was assumed to be at least partly as a result of mite predation. Therefore, we quantified thrips suppression prior to the third week as the per cent change (i.e. decrease) in thrips density from the first (22 December 2011) to the second (29 December 2011) survey as follows: [(thrips density survey 2 − thrips density survey 1)/thrips density survey 1] × 100. This estimate of suppression was based upon a subset of experimental plants ($n = 70$; 2–9 plants per variety) for which we recorded at least one thrips count during the first survey. In addition, we used *A. swirskii* density from the second survey in this regression as it was a more precise measure of mite abundance (as opposed to mite density averaged across surveys) during the time period for which thrips suppression was estimated. Finally, we tested for the effects of plant traits on thrips suppression (estimated as previously described) using s multiple regression based upon variety means. For this model, we only included traits that had a significant effect in the mite density multiple regression. One pepper genotype was excluded from all regressions involving thrips suppression, as thrips were not recorded on any of its plants during the first week of sampling. When significant, quadratic terms were retained in the regression models. All regression analyses were performed in PROC REG in SAS, and normality was verified by Shapiro–Wilk tests.

Results

Plant trait and arthropod variation among pepper varieties

We found strong differences among pepper varieties for all three measured traits. Specifically, we observed a significant effect of pepper variety on flower number ($F_{16,150} = 18.23$, $P < 0.0001$), with up to 11-fold variation among varieties (from 8.42 ± 0.96 to 88.48 ± 3.13 flowers) (Figure S1a). Likewise, we found significant differences among varieties for the amount of pollen produced per anther ($F_{16,34} = 11.60$, $P < 0.0001$) and leaf trichome density ($F_{16,46} = 36.12$, $P < 0.0001$). Pollen production varied up to 5.5-fold among pepper varieties (0.13 ± 0.01 to 0.71 ± 0.08 mg per anther) (Figure S1b), whereas trichome density ranged from 0 up to 102.81 ± 30.25 trichomes/cm^2 (Figure S1c).

Similarly, we also found a strong effect of pepper variety on mite density ($F_{16,150} = 13.90$, $P < 0.0001$), with up to 12.5-fold differences among varieties (range from 0.016 ± 0.002 to 0.20 ± 0.035 mites/cm^2) (Figure S1d). Likewise, although thrips were low in abundance throughout the experiment, we also observed a significant effect of pepper variety on thrips density ($F_{16,150} = 2.00$, $P = 0.01$) with up to four-fold differences among varieties (0.0008 ± 0.0004 to 0.0032 ± 0.0005 thrips/cm^2).

Effects of pepper traits on mite abundance

Genetic correlations among plant traits. Across pepper varieties, we found a negative relationship between flower number and pollen production per anther (pollen weight per anther $= -0.003 \times$ flower number $+ 0.501$; R$^2 = 0.33$, $P = 0.02$) (Figure S2a), suggesting a reproductive trade-off in *C. annuum*. In addition, we found a significant negative relationship between trichome density and pollen production per anther (pollen weight per anther $= -0.002 \times$ trichome density $+ 0.456$; R$^2 = 0.27$, $P = 0.03$) (Figure S2b), but no relationship between trichome density and flower number ($R^2 = 0.16$, $P = 0.11$) (Figure S2c).

Effects of plant traits on mite abundance. Multiple regression showed that accounting for all three traits explained over 70% of the variation among pepper varieties in mite density ($R^2 = 0.71$, $P = 0.0004$). Trichome density and flower number were significant predictors of mite density after accounting for trait correlations (Table 2 and Fig. 1a,b), whereas pollen production per anther did not have an independent effect on mite density (Table 2). The regression model included trichome density^2 because this variable predicted mite density non-linearly (Table 2) with mite density being greater at intermediate trichome numbers (Fig. 1a).

Variation among pepper varieties in mite predation effects

We found a negative relationship between mite density and thrips density (thrips density $= -0.009 \times$ mite density $- 0.002$;
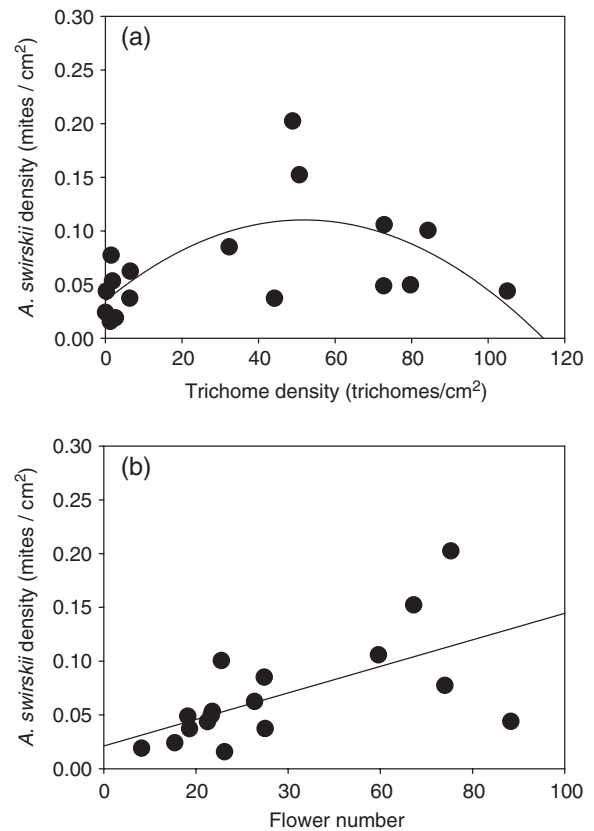

Fig. 1. Relationships between pepper (*Capsicum annuum*) leaf trichome density and density of the predatory mite *Amblyseius swirskii* (a), and between flower number and *A. swirskii* density (b). Dots are pepper variety means. Each panel shows predicted relationships from quadratic (a) and linear (b) simple regression models. Mite densities were estimated as the number of mites per cm^2 of leaf area sampled.

$R^2 = 0.29$, $P = 0.03$), suggesting that mite predation reduced thrips abundance across pepper varieties (Fig. 2a). Accordingly, we found a significant relationship between mite density and thrips suppression (linear regression: thrips suppression $= -519.92 \times$ mite density $- 50.55$; R$^2 = 0.28$, $P = 0.03$) where the magnitude of thrips suppression increased with increasing mite density (Fig. 2b). To test for an indirect association between pepper traits and thrips suppression, we performed a multiple regression where flower number (for survey 1, immediately prior to suppression period) and trichome density were predictors of suppression. We did not include pollen production per anther as a predictor in the model because this trait did not influence mite density. Results showed a negative relationship between flower number and thrips suppression (Table 2), where the strength of thrips suppression increased with flower number (Fig. 2c). Importantly, the effect of flower number on thrips suppression became non-significant once mite density (in survey 2) was accounted for in the model (flower number partial $r^2 = 0.23$, $P = 0.10$), suggesting that the effects of flower number on thrips suppression were mediated by mite abundance. In contrast, trichome density had no effect on thrips suppression (Table 2); a quadratic term was included in this multiple regression model

Table 2. Results from separate multiple regression models evaluating the effects of pepper (*Capsicum annuum*) intra-specific (varietal) differences in flower number, leaf trichome density, and pollen production per anther (mg) on the density of the predatory mite *Amblyseius swirskii* (mites/cm^2 of leaf surface) (A) and mite predation effects measured as thrips suppression (B). The quadratic term for trichome density was included because this trait had a non-linear influence on mite density.

Model	Predictor	β ($\pm$ S.E.)	P-value	Partial r^2
(A) Mite density	Flower number	0.0013 ± 0.0003	0.007	0.22
	Trichome density	0.0028 ± 0.0007	0.005	0.25
	Trichomes^2	$-2 \times 10^{-5} \pm 8 \times 10^{-6}$	0.006	0.23
	Pollen production	0.021 ± 0.075	0.803	0.001
(B) Thrips suppression	Flower number	-0.025 ± 0.0103	0.028	0.34
	Trichome density	0.0007 ± 0.0067	0.915	0.0009
	Trichomes^2	0.00001 ± 0.00007	0.998	0.00005

because the relationship between mite and trichome density was non-linear (see Fig. 1a).

To verify that mite density and flower number effects on thrips suppression were not influenced by correlations between the X-axis variable and variables used to construct the Y-axis variable (Brett, 2004), we performed regressions between mite density and flower number with thrips density in survey one and two. Results yielded non-significant relationships in all cases ($R^2 < 0.25$, $P > 0.05$), suggesting that the effects of mites and flowers on thrips suppression were biologically significant and not influenced by a statistical artefact.

Discussion

We found marked differences in *A. swirskii* density among *C. annuum* varieties and these differences were largely explained by variation in flower number (which provides resources, i.e. pollen) and leaf trichome density (which provides shelter). Both traits had positive effects on the abundance of this predatory mite (although trichomes exhibited a non-linear effect), and flower number (but not trichome density) predicted the magnitude of thrips (*F. chephalica*) suppression across varieties. This latter effect was mediated by flower number affecting mite density, where varieties with greater flower number had greater mite density and thus exhibited greater thrips suppression. Although trichome density did not predict thrips suppression, we argue (below) that this trait may also contribute to indirect defence in peppers. Finally, flower number and trichome densities were uncorrelated, suggesting that genetic correlations among traits will not constrain selection for increased trichome density and flower number in *C. annuum*. Overall, these findings indicate that plant traits mediate predator top-down control of herbivores in peppers and that the presence of intra-specific variation for such traits provides an opportunity for artificial and natural selection to maximise indirect defence in this crop species.

Effects of pepper traits on predatory mite abundance and herbivore supression

Although previous work has shown positive effects of experimental pollen addition (Messelink *et al.*, 2008; Nomikou *et al.*, 2010) and trichome density (reviewed by Walters, 1996;

Agrawal, 2003) on predatory mites, our study additionally shows effects of plant genetically-based variation for pollen availability (via flower number) and leaf trichome density on this important group of predators. We found that differences among *C. annuum* varieties for flower and leaf trichome density were positively related with *A. swirskii* density, and that these two traits independently explained 22–25% and in combination up to 70% of the observed variation in mite density among peppers. It is important to mention that effects of flower number may have been mediated by other unmeasured floral resources (e.g. nectar, presence of thrips larvae in flowers) and not only pollen availability (van Rijn & Tanigoshi, 1999; Faraji *et al.*, 2002).

Although weak, the effect of trichome density on mite density was non-linear such that *A. swirskii* density tended to be greatest for varieties with an intermediate trichome density. Whereas previous work has reported that trichomes provide shelter to mites against other predators (Agrawal, 2003; Loughner *et al.*, 2008), our finding suggests that high trichome densities may interfere with *A. swirskii* foraging as observed for omnivorous mites (Krips *et al.*, 1999) and other predators and parasitoids (see studies in Hare, 2002; Gassman & Hare, 2005), showing that this trait may incur an ecological cost by reducing indirect defence. There are only a few examples of studies looking at genetic variation for trichome density in cultivated species and its effects on predatory mite abundance (Duso & Vettorazzo, 1999; Duso *et al.*, 2003; Loughner *et al.*, 2008, 2011; Doğramaci *et al.*, 2011). Future work is thus needed to improve our understanding of the functional relationship between trichomes and predatory mite abundance and its variation among plant genetic types.

In agreement with our results, two previous studies, one with *A. swirskii* (Nomikou *et al.*, 2010) and another with the big-eyed bug *Geocoris punctipes* Fallén (Eubanks & Denno, 2000), reported positive effects of plant reproductive tissue availability (pollen and fruit pods, respectively) on these omnivorous predators. Both studies also reported positive effects of these traits on herbivore suppression. However, previous research has also shown that the availability of plant-based resources (reproductive or vegetative) may weaken herbivore suppression by omnivorous predators because the latter prefer to feed on plant tissues which are frequently more abundant and nutrient rich (see studies in Stenberg *et al.*, 2011). These contradictory findings have been attributed, at least partly, to the duration of the study as well as differences in predator traits (e.g. generation time, food
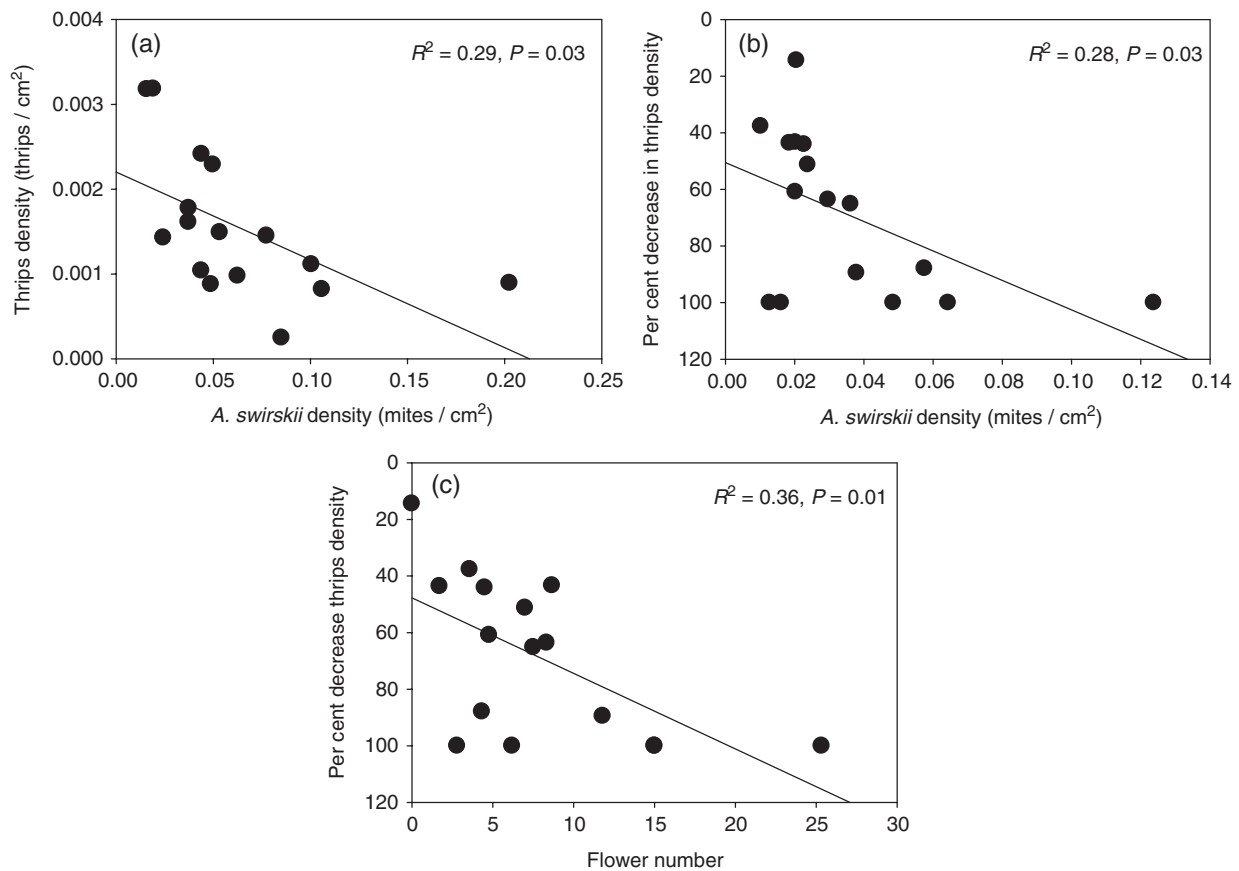
Fig. 2. Relationships between density of the predatory mite *Amblyseius swirskii* and density of thrips (*Frankliniella cephalica*) on pepper (*Capsicum annuum*) varieties (a), between *A. swirskii* density (during survey 2) and thrips suppression (percent change in thrips density from survey 1 to survey 2) (b), and between flower number (in survey 1) and thrips suppression (c). Dots are pepper variety means. Arthropod densities were estimated as the number of individuals per cm² of leaf area sampled. Each panel shows predicted relationships and statistics from simple linear regression models.

preferences, and prey switching) influencing their functional responses (Eubanks & Styrsky, 2005; Messelink *et al.*, 2009). For example, in short-term experiments the presence of one prey may result in short-term satiation by the predator and weakening of predation on a second prey (i.e. apparent mutualism; Abrams & Matsuda, 1996; see Koss & Snyder, 2005; van Maanen *et al.*, 2012). However, over longer time periods, increased predator abundance resulting from feeding on plant resources may lead to stronger herbivore suppression (i.e. apparent competition effect; Holt, 1977; van Rijn *et al.*, 2002; Eubanks & Styrsky, 2005; Messelink *et al.*, 2008). Collectively, these studies show that understanding the conditions under which alternative prey (or plant resources) enhance or dampen predation on target herbivores and the time scale over which such dynamics take place is fundamental in order to predict and enhance indirect defence (Eubanks & Denno, 2000; Eubanks, 2005; Messelink *et al.*, 2009).

In contrast to flower number, and in spite of influencing mite density, trichome density did not predict thrips suppression. It is possible that using flower number during the first survey represented a more precise measure of pollen availability during the time period used to estimate thrips suppression (as opposed

to flower number across all surveys). By contrast, not having an analogous measure for trichomes (i.e. single estimate at the end of experiment) could have limited the detection of an effect of this latter on suppression. Nonetheless, because trichome density influenced *A. swirskii* density and increased mite density strengthened thrips suppression, we argue that this trait may be important in mediating indirect defense in *C. annuum* (Agrawal & Karban, 1997; Agrawal, 2003). This could be particularly true under field settings where mites use domatia and trichomes more frequently owing to adverse climatic conditions or the presence of predators (Faraji *et al.*, 2002). Finally, trichomes and flowers serve different functions for predators (refuge and food, respectively) and may thus have complementary or synergistic effects (Rasmann & Agrawal, 2009) that are stronger than the individual effects of each trait. Such interactive effects of traits are usually ignored and deserve further attention in the context of indirect defence (Heil, 2008).

Ecological costs and genetic correlations among plant traits

Plant traits that confer direct and/or indirect defence against herbivores may incur ecological trade-offs (Styrsky *et al.*, 2006),

particularly in agricultural species where genotypes exhibit strong phenotypic divergence. Such trade-offs may arise when plant traits have opposing effects on different species of herbivores or predators (Hare, 1992, 2002; Gassman & Hare, 2005). For example, although trichome density did not directly influence thrips in our study, previous work has shown that this trait can have positive effects on other herbivores such as the whitefly *Bemisia tabaci* (Gennadius) (Firdaus *et al.*, 2011). Furthermore, in spite of having a positive effect on predatory mite abundance, trichomes may also have negative effects on other predators or parasitoids (Hare, 1992; but see Styrsky *et al.*, 2006). Accordingly, previous work has suggested that breeding for intermediate levels of pubescence represents the best option under a multi-species or multi-trophic setting (Hare, 1992), and this may be the case for *C. annuum* where high trichome densities had a negative effect on *A. swirskii* abundance (and may potentially benefit some herbivorous pests). In addition, direct effects of flower resources (e.g. pollen, nectar; Hulshof *et al.*, 2003; Skirvin *et al.*, 2006) on thrips cannot be ruled out because we did not use a control group of plants that were not flowering and did not record thrips abundance in flowers. Accordingly, effects of flower traits on thrips could act against the effects of flowers on predatory mites and must be properly evaluated.

In selecting for plant herbivore resistance, genetic correlations among target traits are also important to consider because they can determine the outcome of breeding efforts (Simms & Rausher, 1992). Here, we found a negative relationship between *C. annuum* pollen production per anther and flower number across pepper varieties, suggesting a reproductive trade-off. However, pollen production did not influence mite density, and the effect of flower number was significant after accounting for pollen production in the multiple regression. This suggests that mite abundance responds mostly to pollen availability via increased flower number, and that this trade-off represents a weak constraint in selecting for greater pollen production. Similarly, although trichome density was also negatively related to pollen production per anther, the latter trait did not influence mite density and this precluded constraints in selecting for indirect defense via increased trichome density. Additionally, flower and trichome density were uncorrelated across pepper varieties. Overall, these findings suggest that based on the measured traits, genetic correlations are not important in selecting for indirect defence by *A. swirskii* in peppers. Reproductive trade-offs (Marcelis & Baan Hofman-Eijer, 1997) as well as unmeasured genetic correlations with other plant traits (Strauss *et al.*, 2002; Kaplan *et al.*, 2009) remain to be evaluated in breeding for increased flower and trichome number.

Conclusions

Over the last two decades, it has become increasingly clear that breeding for plant traits that enhance indirect defence is a key component of herbivore biological control (Bottrell *et al.*, 1998; Hare, 2002; Poelman *et al.*, 2009). Accordingly, this study documents variation among plant genotypes for traits that mediate the strength of herbivore suppression by predators, thus informing future work on indirect defence in *C. annuum*

as well as other cultivated species that use predatory mites as herbivore control agents. Our findings also point out the need to examine the functional relationships between plant traits, herbivore, and predator abundance, as well as test for trait genetic correlations to understand the evolution and outcomes of selection for indirect defence. Overall, our study is one of few (in both wild and cultivated plant species) that have identified key plant traits influencing predatory mites and documented the magnitude of plant intra-specific variation for these traits. Assessing such variation is fundamental in considering the evolution of indirect defence as well as artificial selection to maximise predator top-down control of herbivores.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12107

Fig. S1. Pepper (*Capsicum annuum*) intra-specific (varietal) differences ($\pm$ SE) in flower number (a), pollen production per anther (b), leaf trichome density (c), and density of the predatory mite *Amblyseius swirskii* (d).

Fig. S2. Relationships between pepper (*Capsicum annuum*) flower number and pollen production (weight, g) per anther (a), mean leaf trichome density and pollen production per anther (b), and leaf trichome density and flower number (c). Dots are pepper variety means. R^2 and *P*-values from simple regression models are shown in each case.

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