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Open Science in Acarology

Climatic and cultivar effects on phytoseiid species establishment and seasonal abundance on citrus

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Original research

ABSTRACT

The structure of phytoseiid communities on citrus varies among orchards and seasons, with potential implications for biocontrol services. Such variation may be driven by meteorological conditions and cultivar-specific traits. We conducted an outdoor seedling experiment to determine the effects of different cultivars on the establishment of introduced predatory mites (Euseius stipulatus and E. scutalis) and colonization by naturally occurring phytoseiids (Amblyseius swirskii, Iphiseius degenerans, and Typhlodromus athiasae) across seasons. Euseius stipulatus persisted through the spring only where it was released, regardless of cultivar, and reduced colonization by *I. degenerans*. In early summer (hot and dry conditions), nearly all phytoseiid species essentially disappeared, and after that only the naturally occurring A. swirskii and T. athiasae re-established. In mid-summer, A. swirskii colonized cultivars differentially, especially where pollen was provisioned, and was negatively related with the colonization of T. athiasae, which is known as its intraguild prey. Additionally, we performed laboratory experiments that simulated daily temperature fluctuations typical to spring and mid-summer seasons, measuring key demographic parameters of A. swirskii and E. stipulatus on leaf disc arenas of two citrus cultivars. Simulated spring temperatures resulted in higher total reproductive output in E. stipulatus than in A. swirskii, whereas mid-summer temperatures resulted in the opposite pattern. Both results are consistent with the seasonal patterns observed in the outdoor experiment. Together with the phytoseiid dynamics observed previously in commercial orchards, these findings emphasize the role of meteorological conditions and cultivars in the structure and function of phytoseiid communities.

Keywords conservation biological control; predatory mites; ecosystem services; intraguild interactions

Introduction

Phytoseiids are mostly generalist predators that occur both in natural habitats and in crops (McMurtry *et al.*, 2013). They can therefore potentially provide ecosystem services in agricultural pest control (Gerson *et al.*, 2003). However, not all phytoseiid species are equally effective as biocontrol agents. In addition, negative and asymmetrical intraguild interactions such as intraguild predation (Rosenheim *et al.*, 1995) and priority effects (Montserrat *et al.*, 2008; van der Hammen *et al.*, 2010) can limit the capacity of phytoseiid communities to suppress pests such as phytophagous mites (although the frequency of itraguild predation

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is debated; Guzmán *et al.*, 2016). Furthermore, different environmental conditions, such as climate and host plant traits, may affect the relative dominance of different phytoseiid species in different habitats (McMurtry *et al.*, 2013; Tixier, 2018), or alter key interspecific interactions (Urbaneja-Bernat *et al.*, 2019) among them. Understanding the factors that influence mite community structure and dynamics across habitat types and seasons may help to manage ecosystem services and optimize biocontrol programs.

Overall forty six phytoseiid species have been collected from citrus in different Mediterranean countries (Vacante and Gerson, 2012). *Euseius stipulatus* Athias-Henriot was the most abundant species on citrus plants in most countries, except Israel, Egypt and Lebanon, where *Amblyseius swirskii* Athias-Henriot and *Typhlodromus athiasae* Porath and Swirski dominated (Rasmy *et al.*, 1972; McMurtry, 1977; Vacante and Gerson, 2012; Abdelgayed *et al.*, 2017). In Israel, phytoseiid species recorded in citrus orchards (Porath and Swirski, 1965) include *Iphiseius degenerans* Berlese and *A. swirskii* that are generally prevalent in orchards in the humid coastal region, *T. athiasae* that is more dominant in drier, inland regions, and *Euseius scutalis* Athias-Henriot, which is common on avocado (Maoz *et al.*, 2011) but rare on citrus (Porath and Swirski, 1965).

A key cosmopolitan pest of citrus is the citrus rust mite (CRM) *Phyllocoptruta oleivora* Ashmead. A field study conducted by Smith and Papacek (1991) in Australia demonstrated that better control of *P. oleivora* and of the brown citrus rust mite *Tegolophus australis* Keifer can be achieved with *Euseius victoriensis*, using a windborne pollen-releasing Rhodes grass *Chloris gayana* Kunth cover crop. With the aim of establishing new biocontrol agents for CRM control, five phytoseiid species, among them *E. stipulatus* were imported and released during 1994-1996 in all citrus growing regions of Israel (Argov *et al.*, 2002). Studies conducted in the laboratory with citrus seedlings provisioned with pollen as supplemental food, indicated that the indigenous *E. scutalis* could be an effective CRM biocontrol agent. In contrast, *A. swirskii*, an intraguild predator of *E. scutalis*, had no effect on CRM control (Maoz *et al.*, 2016). Thus, the success of biological control may depend critically on the phytoseiid community structure and seasonal dynamics, particularly of these two functionally distinct genera.

Warburg et al. (2019) followed the dynamics and abundance relationships between released *E. stipulatus* and other naturally occurring phytoseiids at varying distances from Rhodes grass cover crop strips in two orchards in Israel's coastal plain; a Shamouti *Citrus sinensis* (L.) Osbeck orchard, and a pomelo *Citrus grandis* (L.) Osbeck orchard. In the pomelo orchard, *E. stipulatus* did not establish, and *A. swirskii* was found to be the dominant species from May through October. In contrast, in the Shamouti orchard, *E. stipulatus* was the dominant species in April and May. During June and July, the densities of all phytoseiids were extremely low, but *E. stipulatus* re-established from August through October, co-existing with *A. swirskii* at similar densities. In both orchards, pollen provisioning next to the Rhodes grass strips resulted in substantially higher phytoseiid population densities. However, negative relationships between *E. stipulatus* and *A. swirskii* (possibly due to intraguild predation) were observed only in the Pomelo orchard. In the Shamouti orchard, no such relationship was detected, *E. stipulatus* densities were high, and a significant effect on CRM control was observed.

We hypothesized that seasonal phytoseiid community structure in orchards is affected by cultivar. We also hypothesized that temperature and cultivar affect the relative performance of different mite species, specifically *E. stipulatus* and *A. swirskii*. To test these hypotheses, we evaluated phytoseiid establishment on potted seedlings of several cultivars, from spring through mid-summer, with and without pollen provisioning. To determine the potential role of fine-scale cultivar effects that might be mediated by leaf morphological or chemical traits, we also conducted controlled laboratory experiments on leaf discs of the two previously studied cultivars, pomelo and Shamouti (Warburg *et al.*, 2019). These experiments evaluated the survival and reproduction of *A. swirskii* and *E. stipulatus* on each cultivar, under daily-fluctuating temperature regimes that simulated the spring and mid-summer seasons in the coastal plain of Israel.

Materials and methods

General methods

Experiments were performed at the Newe-Ya'ar Research Center ($32^{\circ}70'$ N; $35^{\circ}18'$ E), located in the Jezreel Valley. A pollen mix from Mount Tabor oak *Quercus ithaburensis*, cattail *Typha domingensis* and maize *Zea mays* (in even parts) was used as a source of supplementary food in all experiments. Oak pollen was collected from trees in Newe-Ya'ar in March, cattail pollen from plants growing on drainage ditch banks near Newe-Ya'ar in May, and maize pollen was collected from commercial maize fields in June, before any insecticide sprays were applied. The flowers bearing pollen grains were dried for a week on paper. Dried flowers and loose pollen grains were sieved (180 µm sieve) and stored at -20 °C. Mites that were used in both experiments (*E scutalis* and *E. stipulatus* in the seedling experiment, and *A. swirskii* and *E. stipulatus* in the lead disc experiment) were received from Bio-Bee, Sdeh Eliyahu, Israel (http://www.biobee.com).

Outdoor seedling experiment

Eighteen seedlings (1 year old trees) of the following six rootstock / rootstock-scion combinations (hereafter referred to simply as "cultivars"), were purchased from the Messilot nursery (https://www.messilot.net/greenhouse): Sour orange (SO), Or grafted on SO (Or-SO), Pomelo grafted on SO (Pomelo), Shamouti grafted on SO (Shamouti), Volka, Or grafted on Volka (Or-Volka) (for full common and Latin names of citrus rootstock and scions see Table 1). Seedlings were approximately 1.5 m high when transplanted to ten liter pails and arranged randomly on a levelled gravel surface at least one meter apart. The seedlings were irrigated and fertilized following standard practice.

Seedlings were randomly assigned to three predator and pollen treatments: *E. scutalis* release + pollen augmentation, *E. stipulatus* release + pollen augmentation, and control (no predators or pollen added), with 6 replicates per treatment. Rearing of *E. scutalis* and *E. stipulatus* was done on paper arenas (Argov *et al.*, 2002) with maize pollen provided by Bio-Bee (http://www.biobee.com). Predator release was performed once, on March 17, 2016, by fastening a 1 ml pipette tip containing 200 motile stages (or empty tips in the control) to each seedling. To facilitate the establishment of released predators, a pollen mix (described in the general methods) was added with a fine forceps on seedling leaves, starting at the time of predator release, and subsequently three times per week throughout the experiment. Seedlings were sampled for phytoseiid abundances in April, in June, and in August, by beating the top, middle and bottom of the seedling (three times each) with a section of flexible plastic irrigation pipe, being careful not to damage the seedling. Mites were collected from the beating tray with aspirators prepared from 200 μ l micropipette tips (Argov *et al.*, 2002). Collected mites were cleared, mounted in Hoyer medium and identified to the species level (using the description provided in Papadoulis *et al.*, 2009). The spring sampling, in April, was indicative of cultivar

 Table 1
 Common and Latin names of citrus rootstock and scion, combinations of rootstock-scion, and name of rootstock-scion combination used in text.

Common name	Rootstock scientific name	Scion scinetific name	Name in text
Sour orange (bitter orange)	Citrus aurantium		SO
Mandarin cultivar 'Or' CV	C. aurantium	C. reticulata	Or-SO
Pomelo	C. aurantium	C. grandis	Pomelo
Shamouti orange (Jaffa orange)	C. aurantium	C. sinensis	Shamouti
Volkamer lemon (Volka)	C. volkameriana		Volka
Mandarin cultivar 'Or' CV	C. volkameriana	C. reticulata	Or-Volka

compatibility with the establishment of the released predatory species, and with the colonization of other phytoseiid species from the surroundings natural environment, with and without pollen provisioning. The early summer sampling, in June, allowed to evaluate the persistence of the established phytoseiid species on the different cultivars during a season characterized by extreme events of high temperature and low humidity that resulted in phytoseiid population collapses in previous field studies (Warburg *et al.*, 2019). Finally, the mid-summer sampling, in August, several weeks after humidity levels typically increase and stabilize, tested the compatibility of cultivars to re-establishment by phytoseiid species from the surrounding natural environment, with and without pollen provisioning.

Leaf disc experiment

To evaluate potential cultivar leaf effects on the survival and reproductive success of A. swirskii and E. stipulatus under different climatic conditions, we conducted an experiment in climate-controlled growth chambers (https://www.conviron.com/products/ a1000-reach-in-plant-growth-chamber). The chambers were programed to follow two regimes based on typical daily temperature fluctuations in the spring (April) and mid-summer (July-August) in the coastal plain of Israel (Table 2). Rearing of E. stipulatus and A. swirskii was done at 24 ± 5 °C at Newe Ya'ar, using the pollen mix described above. Prior to each experiment, polyester fibers as substrate for egg laying were placed on rearing arenas of E. stipulatus and A. swirskii for 24 hr. Fibers with eggs were then placed on similar rearing units in each growth chamber and allowed to develop, mate and oviposit, thus rearing a full generation under the experimental temperature regimes. Second-generation eggs of each species at standardized ages (0-1 day old) were then collected and placed in groups of ten on leaf discs (20 mm in diameter) of either pomelo or Shamouti. Each leaf disc was floated on polyacrylamide gel in a 120 ml container, and replicated 6–10 times for each species by cultivar combination, under each temperature regime. Pollen mix was applied on each leaf disc twice per week with a fine forceps. On the day following the onset of oviposition in each temperature regime (days 6 and 13 in the summer and spring regimes, respectively), we recorded the number of females in each of the containers. Eggs oviposited during the experiment were counted and removed daily until the experiment was terminated (on days 10 and 21, in the summer and spring regimes, respectively). We calculated the mean daily reproductive output per female for each container as the total number of eggs oviposited in the container divided by the number of females observed at the onset of oviposition and by the number of reproductive days (5 days in the summer regime, and 9 days in the spring regime). This measure of mite fitness, or net contribution to population growth, incorporates variation in multiple underlying factors that were not recorded in the experiment, such as in female fecundity and survival, sex ratio, and mating success. In addition, mean survival per container was calculated for all individuals (regardless of sex) through the entire duration of the experiment.

Statistical analyses

Statistical analyses were done in R 3.5.1 statistical computing software (R Core Team, 2018). To analyze phytoseiid abundances in April in the seedlings experiment, we used general linear models (LM) for *E. stipulatus* and *I. degenerans*, and generalized linear models (GLM) with negative binomial error distributions and a log link function for *A. swirskii* and *T. athiasae*, with predator release treatment and cultivar as predictors. In August, we used LM for *A. swirskii* abundances, and GLM (negative binomial, log link) for *T. athiasae* abundances. In the August analyses, we considered our treatments to contrast the presence vs. absence of pollen augmentation, because the released *E. stipulatus* and *E. scutalis* populations had disappeared in June without recovering. In modeling *T. athiasae* abundance in August, we included the abundances of its intraguild predator, *A. swirskii* (Maoz *et al.*, 2016), as a predictor, and removed the pollen \times cultivar interaction term to maintain statistical power. In cases of

Table 2 Daily temperature regimes used in climate chambers experiments based on typical seasonal temperatures in the coastal plain of Israel, as measured in April (Regime 1), and in July-August 2014 (Regime 2), at meteorological stations near Pardessiya (32°18'N, 34°55'E) and Akko (32°56'N, 35°06'E).

Regime 1 (spring)		Regime 2 (summer)		
Time of day	Temperature (°C)	Time of day	Temperature (°C)	
00:00 - 06:00	11	00:00 - 06:00	24	
06:00 - 10:00	19	06:00 - 09:00	26	
10:00 - 15:00	25	09:00 - 11:00	28	
15:00 - 18:00	20	11:00 - 18:00	30	
18:00 - 24:00	14	18:00 - 20:00	28	
		20:00 - 23:00	26	
		23:00 - 24:00	24	
Mean	17.1	Mean	26.9	
Range	11 - 25	Range	24 - 30	
Amplitude	14	Amplitude	6	

heteroscedasticity of model residuals, we used the HC3 heteroscedastic-consistent covariance matrix method (Zeileis, 2004) in ANOVAs and post-hoc comparisons.

In the leaf discs experiment, female reproduction was modeled as a general linear model of species and cultivar under each temperature regime. Mite survival throughout the experiment was modeled as a GLM of species and cultivar under each temperature regime, with binomially-distributed errors and logit link function.

Results

Outdoor seedling experiment

The results of the statistical analyses used for this experiment are presented in Table 3.

In April — five weeks post release, *E. stipulatus* was recovered in substantial abundances (mean \pm SE: 15.0 \pm 2.1 individuals per sample) on seedlings on which it was released, whereas very few mites of this species were found on the other seedlings (mean \pm SE: 1.85 \pm 0.34; p < 0.0001). The cultivars had no significant effect (p = 0.671), and no significant interaction between cultivar and predator release was observed (p = 0.821) (Figure 1A). In contrast, despite its recent release, abundances of *E. scutalis* were very low on all seedlings (mean: 0.37, range: 0–6 individuals per sample), and it was found almost exclusively on seedlings on which it was released.

Colonizing *I. degenerans* established in high abundances on seedlings without release (mean \pm SE: 11.03 \pm 2.77 individuals per sample), and on *E. scutalis*-augmented seedlings (mean \pm SE: 11.97 \pm 3.47 individuals per sample). It generally had lower abundances on seedlings where *E. stipulatus* was released (mean \pm SE: 2.06 \pm 0.55 individuals per sample), despite the provisioning of pollen (p = 0.010; Tukey's comparisons between *E. stipulatus* augmentation and no augmentation treatments t = 3.152; p = 0.006; between *E. stipulatus* augmentation and *E. scutalis* augmentation treatments t = 3.337; p = 0.003) (Figure 1B). Whereas the main effect of cultivar on *I. degenerans* abundance was non-significant (p = 0.362), the negative effect of *E. stipulatus* on the establishment of *I. degenerans* appeared mostly on Shamouti-SO, Volka and Or-Volka and was not consistent across all cultivars (p = 0.054).

Relatively few colonizing A. swirskii were recovered on all seedlings (mean: 1.21, range: 0

Table 3 Statistical analyses of phytoseiid abundances in the seedling experiment. In GLMs, the significance of model terms was tested using likelihood-ratios (LR).

RV / Predictors			
April, E. stipulatus abundance	df	F	Р
Cultivar	5, 88	0.638	0.671
Predator augmentation	2, 88	45.629	< 0.0001
Cultivar \times Predator augmentation	10, 88	0.586	0.821
April, I. degenerans abundance	df	F	Р
Cultivar	5,88	1.109	0.362
Predator augmentation	2, 88	4.806	0.010
Cultivar \times Predator augmentation	10, 88	1.914	0.054
April, A. swirskii abundance	df	LR	Р
Cultivar	5, 89	20.141	0.001
Predator augmentation	2, 89	3.924	0.141
Cultivar \times Predator augmentation	10, 89	13.860	0.179
April, T. athiasae abundance	df	LR	Р
Cultivar	5, 89	1.871	0.867
Predator augmentation	2, 89	3.189	0.203
Cultivar \times Predator augmentation	10, 89	6.221	0.796
August, A. swirskii abundance	df	F	Р
Cultivar	5, 95	3.549	0.006
Pollen augmentation	1, 95	8.958	0.004
Cultivar \times Pollen augmentation	5, 95	0.767	0.576
August, T. athiasae abundance	df	LR	Р
Cultivar	5, 99	13.203	0.031
Pollen augmentation	1, 99	2.376	0.123
A. swirskii	1, 99	15.219	< 0.0001

-11 individuals per sample), without significant effects of predator augmentation (p = 0.141), but with a significant cultivar effect (p = 0.001), with lower densities on Or-Volka and on Shamouti than on the other cultivars (Figure 1C).

Abundances of *T. athiasae* were also low on all seedlings (mean: 1.71, range: 0 - 9 individuals per sample), and not affected by phytoseiid augmentation or by cultivar (Table 3).

In June — only two specimens of *E. stipulatus*, two specimens of *E. scutalis*, and no *I. degenerans* were recovered from all the seedlings. Abundances of *A. swirskii* (mean: 0.43, range: 0-7 individuals per sample) and of *T. athiasae* (mean: 1.73, range: 0-6 individuals per sample) were reduced compared to April, or remained similarly low, without any cultivar or augmentation treatment effects.

In August — the abundances of *E. stipulatus* and *E. scutalis* remained negligible (mean: 0.17 and 0.27, range: 0-5 and 0-10 individuals per sample, respectively). Similar to June, no *I. degenerans* mites were detected in August. In contrast, abundances of *A. swirskii* were very high, especially on the seedlings that were provisioned with pollen (compared to unprovisioned seedlings, p = 0.004) (Figure 2A). There was also a significant cultivar effect (p=0.006), with weakest *A. swirskii* establishment on pomelo and Shamouti, and strongest establishment on

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Figure 1 Phytoseiid species abundances (number of individuals per beating sample) on different cultivars in April, 5 weeks post release, on seedlings where *Euseius stipulatus* was released, with pollen provisioning (white bars), on seedlings where *Euseius scutalis* was released, with pollen provisioning (gray bars), and on seedlings where no predator was released, without pollen provisioning (black bars). A – *Euseius stipulatus* abundances. B – *Iphiseius degenerans* abundances. C – *Amblyseius swirskii* abundances. Error bars are ± 1 SE.

both rootstocks (Sour and Volka) and on Or-Volka.

The abundance of *T. athiasae* also increased substantially from June to August, but still remained low compared to *A. swirskii* abundance. There was a significant negative relationship between *T. athiasae* abundances and those of *A. swirskii* (p<0.0001), its intraguild predator (Maoz *et al.*, 2016). In addition, cultivar has a significant effect (p = 0.031), with the greatest difference occurring between Pomelo and Shamouti (Figure 2B). Pollen provisioning did not have a significant impact on *T. athiasae* abundance (p = 0.123).

Throughout the experiment, no citrus arthropod pests were observed, probably due to the absence of nearby orchards.



Figure 2 Abundances (number of individuals per beating sample) of phytoseiid mite species on seedlings in August. A – mean *Amblyseius swirskii* abundance with and without pollen provisioning. B – The relationship between *Typhlodromus athiasae* and *A. swirskii* abundances on different cultivars. The order of cultivars appearing in the legend corresponds to the magnitudes of their fitted intercepts (Pomello > Volka > ... > Shamouti). Error bars are ± 1 SE

Leaf disc experiment

Female reproductive output, combining female survival and fecundity, was much lower in the temperature regime simulating spring conditions (Figure 3A) than in the summer regime (Figure 3B), for both phytoseiid species and cultivars, despite the shorter experimental period of the latter regime. Under the spring regime, *E. stipulatus* reproductive output outperformed that of *A. swirskii* three-fold on Pomelo and six-fold on Shamouti (LM: species effect $F_{1,31} = 19.436$; p = 0.0001), without significant difference between cultivars (cultivar effect $F_{1,31} = 0.007$; p = 0.932). In contrast, under the summer regime, *A. swirskii* significantly outperformed *E. stipulatus* on both cultivars (LM: species effect $F_{1,29} = 10.460$; p = 0.003), without a significant difference between cultivars (cultivar effect $F_{1,29} = 0.249$; p=0.621).

Survival of *A. swirskii* mites (of both sexes) under the spring regime was greater than that of *E. stipulatus* on both cultivars (Figure 3C; GLM: species effect $\chi^2 = 16.304$; p<0.0001), and both species showed greater survival on Pomelo than on Shamouti (cultivar effect $\chi^2 = 18.456$; p < 0.0001). Under the summer regime, *A. swirskii* survival was greater than that of *E. stipulatus* on Pomelo, but lower than that of *E. stipulatus* on Shamouti (Figure 3D; GLM: species×cultivar interaction $\chi^2 = 8.853$; p = 0.003).



Figure 3 Mean daily reproductive output per female (panels A and B) and survival rate (of both sexes, panels C and D), of *A. swirskii* and *E. stipulatus* on Pomelo and Shamouti leaf discs in climate-controlled chambers. Panels A and C – Temperature regime 1 (simulating spring temperatures). Panels B and D – Temperature regime 2 (simulating summer temperatures). See Table 2 for the daily temperature schedule of each regime. Note the different scales of reproductive output between the two temperature regimes. Error bars are ± 1 SE.

Discussion

This study combined an outdoor seedlings experiment and a laboratory leaf disc experiment, both focusing on the roles of citrus cultivars and climatic conditions on the seasonal performance and abundances of introduced and naturally occurring phytoseiid species. The continuous pollen augmentation to some of the seedlings resembled orchards where pollen is either augmented manually (Biobest Belgium, 2013), or via wind-pollinated cover crops (Warburg *et al.*, 2019), to support phytoseiid populations.

Phytoseiid communities on citrus during spring

The outdoor seedling experiment showed that augmented E. stipulatus population established and persisted through spring, regardless of cultivar (Figure 1A). However, the lack of E. stipulatus dispersal from where they were released to other seedlings seems to indicate limited dispersal of the augmented mites. Provisioning of pollen did not seem to be a limiting factor because of the large amounts of pollen shed from the nearby oak trees during late March and early April. Indeed, *I. degenerans*, a naturally-occurring species classified as a pollen feeding generalist (McMurtry et al., 2013), was found in high abundance on seedlings without pollen supplementation. Euseius stipulatus appeared to locally reduce I. degenerans colonization on some of the cultivars, likely due to asymmetrical intraguild interactions or priority effects between these species (Montserrat et al., 2008; van der Hammen et al., 2010). This appeared to be more prominent on some citrus cultivars than on others (namely, on Shamouti, Volka, and Or-Volka, but not on Sour orange, Or-SO, and Pomelo). This intraguild interaction coupled with the cultivar effect might be higher in commercial orchards where cultivar monocultures preclude spatial heterogeneity and small-scale source-sink dynamics among *I. degenerans* populations. Indeed, *I. degenerans* occurred at very low abundances in the Shamouti orchard studied by Warburg et al. (2019), and was far less prevalent than E. stipulatus in the spring. These results differ from those of a recent laboratory study (Calabuig et al., 2018), where gravid females of *I. degenerans*, with and without pollen, consumed substantially more heterospecific larvae than gravid females of E. stipulatus. Maoz et al. (2016) also reported very strong predation by I. degenerans on heterospecific (both E. scutalis and A. swirskii) larvae in leaf disc arena experiments. Thus, it seems that in outdoor and orchard conditions, or as a result of priority effects, the laboratory-observed direction of intraguild predation is either reversed or outweighed by a counteracting factor, leading to the displacement of *I. degenerans* by *E.* stipulatus.

Colonization by *A. swirskii* in the spring sampling (April) occurred in extremely low densities (Figure 1C), mirroring the springtime (March-May) low abundances of this predator in the large scale field experiments in both Pomelo and Shamouti orchards conducted by Warburg et al (2019). Under the temperature regime simulating spring conditions, the reproductive output of *E. stipulatus* females was much greater than that of *A. swirskii* females (Figure 3A), despite greater survival of *A. swirskii* (Figure 3C). Other laboratory studies conducted at a constant temperature of 18 °C reported similar differences between these two species both in productivity and in fecundity (Ferragut *et al.*, 1987; Lee and Gillespie, 2011). However, survival in the leaf disc experiment reported herein may have been high mainly among the males, whose abundance contributes little to female reproductive success and to population dynamics.

Collapse of phytoseiid communities during early summer

The collapse of populations of all phytoseiid species in June also mirrors the dynamics observed by Warburg *et al.* (2019) in the commercial orchards. The population collapse in the current seedlings experiment likely occurred due to low humidity, in combination with high temperatures; the driest conditions were recorded in May, with a total of forty hours below 30% relative humidity during the fortnight preceding the June sampling, whereas relative humidity

never dropped below 30% during the fortnight preceding the August sampling. Reports in the literature show that *E. stipulatus* is sensitive to both temperature and relative humidity (Ragusa, 1986; Ferragut *et al.*, 1987; Sahraoui *et al.*, 2014); In Sicily, *E. stipulatus* populations collapsed after four hours of temperatures above 40 °C, coupled with strong dry winds (Ragusa, 1986). Similar reductions in *E. stipulatus* populations occurred in Tunisia during July-August, when temperatures exceed 30–35 °C (Sahraoui *et al.*, 2014), and in Spanish citrus orchards, after several days with temperature exceeding 34–38 °C and 30–40% RH (Ferragut *et al.*, 1987). Thus, in the absence of viable populations of *E. stipulatus* in the surrounding habitat that can serve as sources of orchard re-colonization, early summer weather in Israel can be an obstacle for effective CRM biocontrol with this phytoseiid species.

Phytoseiid communities on citrus during mid-summer

By August, none of the species that were abundant during spring had rebounded, and they have been succeeded by naturally occurring populations of *A. swirskii* and *T. athiasae* (Figure 2). The nearly complete absence of *E. stipulatus* differs from the densities observed in the commercial (primarily Shamouti) orchards (Warburg *et al.*, 2019), and may result from three, non-mutually exclusive factors: (i) reduced availability of refugia from the extreme climatic conditions in early summer on the small seedlings, compared to fully grown trees, (ii) suppression by the dominant intraguild predator, *A. swirskii*, as was also indicated by the patterns observed in the commercial Pomelo orchard (but not in the Shamouti orchard) (Warburg *et al.*, 2019), and (iii) the higher reproductive output of *A. swirskii* under summer temperatures (Figure 3B).

A greater abundance of *A. swirskii* was found in August on seedlings where pollen was augmented than those that were not augmented, and the abundances of this species were significantly different among cultivars. A negative relationship was evident between *T. athiasae* and *A. swirskii* abundances (Figure 2B), as observed also in the commercial Pomelo orchard (Warburg *et al.*, 2019), likely due to intraguild predation (Maoz *et al.*, 2016).

The success of Euseius spp. introductions

After its introduction in 1994 (Argov *et al.*, 2002), *E. stipulatus* was recovered for the first time in Northern Israel adjacent to its release site in 2011 (Maoz *et al.*, 2016), and then in the coastal region approximately 10 km from their closest release sites in 2013 and 2014 (Warburg, unpublished). As far as we know, Warburg et al. (2019) was the first to report coexistence of *A. swirskii* and *E. stipulatus* on citrus in the field in Israel. In Egypt and Saudi Arabia (Elmoghazy, 2016; Abdelgayed *et al.*, 2017) *A. swirskii* is abundant and *E. stipulatus* has not been reported. In the other citrus growing countries in the Mediterranean, *E. stipulatus* is the prevalent species (Abad-Moyano *et al.*, 2009; Faraji *et al.*, 2011; Tsagkarakis *et al.*, 2011; Vacante and Gerson, 2012; Barbar, 2013; Satar *et al.*, 2013) without record of *A. swirskii*. We believe that it is important to continue to follow the regional establishment of this important introduced phytoseiid species.

Interestingly, in the current seedlings experiment, *E. scutalis* found in abundance on citrus in other Mediterranean countries (Bounfour and McMurtry, 1987; Barbar, 2013; Barbar, 2014), failed to establish on any of the cultivars, despite releases in large numbers, pollen provisioning, and the lack of dominant intraguild predators in the spring, at the time of augmentation. A similar observation was made in our previous field experiment in Shamouti (Warburg *et al.*, 2019), where, remarkably, high abundances of *E. scutalis* were found on adjacent avocado trees. Thus, while *E. scutalis* proved to be easily enhanced by Rhodes grass pollen and efficient in controlling spider mites on avocado (Maoz *et al.*, 2011), it repeatedly proves to be unsuccessful on citrus in Israel.

Conclusions, prospects, and implications

Successful establishment of introduced phytoseiid species for biocontrol may critically depend on taking into account seasonal and climatic conditions in the orchard. Specifically, *E. stipulatus* is sensitive to hot and dry conditions that typically occur in early summer, in Israel, resulting in severe declines or extinction of augmented populations. This can potentially be mitigated proactively, by providing humid refugia such as cover crop strips or perennial plants in orchard margins. Introductory mite releases can be considered earlier in the spring, when *E. stipulatus* performs well, to allow the establishment of more robust populations prior to the early-summer period of adverse conditions.

The performance of *E. stipulatus* did not appear to be directly affected by citrus cultivars. However, our results suggest that the naturally-occurring phytoseiids may be more sensitive to cultivar effects, potentially also affecting *E. stipulatus* indirectly. Specifically, *A. swirskii* performance differed between cultivars in our seedling experiment, although not on the leaf discs, suggesting the relevant scale of cultivar-specific factors may be the tree level. In the current experiment, *E. stipulatus* did not co-occur with *A. swirskii*, and the laboratory experiment was not designed to test for interspecific interactions among the phytoseiids. However, a key observation in Warburg *et al.* (2019) was the indication of negative intraguild interactions between *A. swirskii* and *E. stipulatus* only in the Pomelo orchard, but not in the Shamouti orchard. Moreover, in the current outdoor experiment, cultivar-dependent negative interactions appeared to occur between *E. stipulatus* and *I. degenerans*, and between *A. swirskii* and *T. athiasae*. These may have implications for the control of different pest species that these phytoseiids can suppress. Further research should explicitly examine cultivar and temperature effects on the behavioral responses of phytoseiid species pairs, investigating, for instance, the expression of intraguild predation and oviposition habitat selection.

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