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Pollen supply promotes, but high temperatures demote, predatory mite abundance in avocado orchards

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ABSTRACT

A biological control strategy aimed at enhancing the performance in the field of two predatory mites that are natural enemies of an invasive pest of avocado orchards was applied during two consecutive years. Unexpected poor pest control led us to analyse additional factors involved in the dynamics of the community such as summer environmental conditions, which were very hot and dry. Non-linear regressions considering biotic and abiotic effects suggested that high temperatures during the second half of the summer were central to predator/prey population dynamics. Harsh environmental conditions were therefore a direct cause of biological control disruption. We believe that, in the future, the success of biological control strategies will be limited by the natural enemy's capacity to respond adaptatively to rapid climate changes, and that research aimed at evaluating the evolutionary potential of natural enemies to rapid climate change should be the focus of future investigations.

1. Introduction

Several studies have documented recent vertebrate, invertebrate, and plant range shifts that are associated with global warming (Letcher, 2009). However, fewer studies have addressed the potential effects of climate change on the relationships within communities such as those represented in biological pest control. Scenarios where organisms will be increasingly exposed to extreme high temperatures may become relevant for the future of biological pest control, as most of the natural enemies presently used as biocontrol agents are arthropods, in which many of the key processes related to their survival, reproduction and other fitness-related parameters depend on environmental conditions (Beveridge et al., 2010). Furthermore, sensitivity to temperature increase intensifies with trophic level (Voigt et al., 2003), a bottom-up amplification effect that often occurs to trophic webs subjected to disturbances of any kind (Cagnolo et al., 2002). Therefore, natural enemy efficiency or life-history negatively affected by environmental warming may dampen trophic cascades and profoundly affect pest control. Indeed, recent works have attributed herbivore control disruption in some agro-ecosystems to severe environmental conditions (Montserrat et al., 2012; Stavrinides et al., 2010).

In the coastal areas of the provinces of Malaga and Granada, in the region of Andalusia (Spain), avocado (*Persea americana* Mill., Lauraceae) is the third most abundant non-citrus fruit tree, after almond and olives (MARM, 2011). Avocado orchards in Andalusia had been free from most of the pests present in other avocado producing areas in the world until the detection, in 2004, of the persea mite, *Oligonychus perseae* Tuttle, Baker and Abatiello (Acari: Tetranychidae) (Vela et al., 2007). All the developmental stages of this tetranychid species inhabit dense silken nests built at the underside of avocado leaves, and their feeding activity causes necrotic spots on leaves. Nests protect mites against attack from some species of natural enemies, and also against adverse environmental conditions (Montserrat et al., 2008). In these coastal avocado orchards two species of phytoseiid mites have been found co-occurring with this herbivorous mite (González-Fernández et al., 2009): *Euseius stipulatus* (Athias-Henriot), an omnivore that attacks animal prey and also forages on pollen (Ferragut et al., 1987); and *Neoseiulus californicus* (McGregor), a specialist predator of tetranychid mites. Both species prey on the persea mite (González-Fernández et al., 2009; Montserrat et al., 2008) and are strong candidates to be considered in biological control programs against this pest, although only *N. californicus* is commercially available.

The population dynamics of phytoseiid mites in avocado trees in Spain typically show two population maxima, the first in spring and the second in summer. In spring, when the populations of persea mites are still small, phytoseiid populations increase as individuals feed on pollen deposited on the surface of leaves, whereas, in summer, phytoseiid populations respond numerically to the abundance of the persea mite (González-Fernández et al., 2009; Montserrat et al., 2012). Based on the mite community dynamics,

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a strategy to control this pest consisting of making maize pollen available in avocado orchards, which was released from intercropped maize plants, was recently tested (González-Fernández et al., 2009). Results obtained were very encouraging, as those trees that were closer to the maize pollen sources harboured more phytoseiid and less herbivore mites (González-Fernández et al., 2009). However, differences between treatments diluted with time because maize plants ceased to release pollen.

This study was aimed at enhancing the performance in the field of the two predatory mites, *E. stipulatus* and *N. californicus*, which behave as natural enemies of the persea mite. In the case of the non-commercial omnivore *E. stipulatus*, it was intended to indirectly increase its populations by prolonging the presence of pollen in the system through direct supply of stored pollen to the trees, at the beginning of summer. Enhancement of *N. californicus* performance would be achieved through releases of these mites during mid-summer, when populations of the persea mite start to increase. It was expected that the combination of both predator species supplied with an alternative food source would intensify the effects observed in a previous work (González-Fernández et al., 2009). However, due to unexpected results, we also attempted to identify whether environmental conditions in summer, which were very hot and dry, could have been an important driver of the observed dynamics of the community.

2. Methods

Cultures of the predatory mite *E. stipulatus* started in 2007 from ca. 300 individuals collected from avocado trees located in the area of study. *Euseius stipulatus* was cultured in a climate chamber at 25 ± 1 °C, 65 ± 5% HR and 16:8 L:D. Rearing units consisted of sponges (30 cm × 20 cm × 5 cm, approx.) covered with cotton wool, with a plastic sheet (27 cm × 17 cm, approx.) on top, and placed inside water-containing trays (8 l, 42.5 cm × 26 cm × 7.5 cm). Three bean (*Phaseolus vulgaris*) plants (6–10 leaves old) were positioned vertically with the stems in contact with three of the walls of the sponge, the roots in contact with the water of the trays, and the aerial parts touching each other forming a tent-like three-dimensional structure, where mites could easily walk from one plant to the other. Some of the leaves contained cotton threads that served as oviposition sites for the mites. Mites were fed ad libitum twice a week with pollen of *Carpobrotus edulis* that was spread on leaves with a fine brush. When required, new rearings were made either by transferring the cotton threads filled with eggs or by transferring adult females (ca. 150), to a new unit. Pollen of *C. edulis* was obtained from male flowers that were dried in a stove at 37 °C for 48 h, and then sieved (350 μm). *Neoseiulus californicus* used in the field experiments were kindly supplied by Koppert Biological Systems, The Netherlands.

2.1. Commercial pollen as alternative food

The aim of this experiment was to determine if commercial bee pollen dissolved in water is a suitable food source for the omnivore mite, *E. stipulatus*. Commercial bee pollen was chosen because it is inexpensive and easily available. Commercial pollen in pellet form was obtained from a local shop, and comprised 60–80% *Cistus* spp. (Cistaceae) pollen; the remaining pollen was from various species of Boraginaceae, Rosaceae and Fabaceae.

Experiments were carried out in a climate chamber (600 l) at 25 ± 1 °C, 65 ± 5% HR and 16:8 L:D. The experimental set-up consisted of plastic arenas (3.5 cm Ø) placed on a layer of water-soaked cotton wool inside plastic containers (100 ml, 6.7 cm high, lower diameter 5.1 cm, upper 6.5 cm). The experimental arenas were allocated to the following treatments: (i) arenas supplied with commercial bee pollen ($N = 12$): 40 (approx.) pellets of commercial bee pollen were dissolved in 100 ml of tap water, and one drop of the solution was applied on the experimental arenas; (ii) arenas supplied with maize pollen ($N = 10$) as positive control, because, in previous field experiments (González-Fernández et al., 2009), predatory mites showed a numerical response to the presence of maize pollen that resulted in a decrease on the abundance of the pest; and iii) arenas with no food, as a negative control ($N = 12$). One gravid *E. stipulatus* female (10–15 d old since egg stage) was placed in each of the arenas using a fine brush. After 24, 48, and 78 h the number of eggs laid by the female was counted. To ensure that only the effect of the treatment was measured, only the average number of eggs laid during the second and third day was used in the analyses. The number of eggs/*d* was analysed with a one-factor ANOVA, with type of pollen as main factor. Means were separated with the Tukey Unequal N HSD test.

2.2. Pollen application and predator release

This experiment was designed to evaluate whether the combination of alternative food supply at the beginning of summer with artificial releases of *N. californicus* in mid-summer would result in a better control of the persea mite, *O. perseae*, through higher total numbers of predatory mites.

Preliminary experiments carried out in the laboratory revealed that, on the one hand, patches of commercial pollen were easily created on the leaves when avocado leaves were sprayed with 2 g/l of commercial pollen dissolved in water, from a distance of 1.5 m (Fig. A, supplementary material). On the other hand, patches contained pollen consumable by mites – who pierce individual grains with their mouthparts and suck their contents – as most of the sprayed pollen grains did not explode (Fig. B, supplementary material).

Field experiments were carried out in a 2 ha 'Hass' avocado orchard located at the IHSM La Mayora (Malaga, Spain) during two consecutive years (2009 and 2010) from the beginning of June to the end of September. The experimental field was divided East-to-West into five blocks, each containing five groups of three adjacent trees, which were separated by several additional avocado trees. Each of the groups per block was randomly assigned to one of the following treatments: (a) Pollen: 14 g of pollen (acquired at the same shop and from the same stock as the one described above) dissolved on 7 l of water were applied to the tree canopy using a spray gun trolley equipped with a piston pump; (b) water: 7 l of water were applied to the canopies using the same device as in (a), as control treatment of pollen application; (c) *N. californicus* release: 1000–3500 individuals of *N. californicus* were released per tree (see below); (d) Pollen + *N. californicus*: Trees were supplied with the same amount of pollen and number of *N. californicus* as in the treatments with either only pollen supply (a) or only predator release (c); (e) control: trees with no treatment applied. For each treatment, only the tree located in the middle of the group of three trees was used for samplings.

Prior to the start of the experiments, the abundance of phytoseiids and persea mites was assessed in May, when pollen counts are at their maximum, to ensure that phytoseiid abundance was high, and herbivore abundance low, as expected. At the end of May of 2009 there was an average of 1.16 ± 0.08 phytoseiid mites/leaf, and 0.048 ± 0.017 nests of persea mites/leaf. In 2010 these values averaged 1.42 ± 0.99 and 0.336 ± 0.172, respectively. Because abundance of pollen starts decreasing at the end of May, the first treatments involving pollen applications and the control were done within the first week of June. Subsequent treatments with pollen were decided upon actual abundances of phytoseiids recorded in the field. As a rule of thumb, the treatments were made when populations of predators decreased considerably during two consecutive