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Oligonychus afrasiaticus and phytoseiid predators' seasonal occurrence on date palm *Phoenix dactylifera* (Deglet Noor cultivar) in Tunisian oases

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Abstract

The old world date mite *Oligonychus afrasiaticus* (McGregor) (Prostigmata Tetranychidae) is an important spider mite pest of the date palms *Phoenix dactylifera* L. in most of North Africa and the Middle East. This study was conducted between 2004 and 2006 in an orchard of date palm trees located in southwest Tunisia. The objective of this study was the inventory of mites that revolve around the date palm, to quantify abundance of *O. afrasiaticus* in trees over different types of ground cover and to document its dispersal. The starting of *O. afrasiaticus* infestation on fruits varied between years, ranging from the first to the third week of July. Mite populations on pinnae remained low from May through December, not exceeding two mites per pinnae, whereas on fruit they reached peak populations of approximately 14 motile forms of mite per fruit in 2006. Indigenous phytoseiid were not found on fruits between mid-Julys till the end of August, when pest populations peaked. Plants such as *Sorghum bicolor* (L.) Moench, *Solanum melongena* L., and *Cucumis melo* L. contained densities of *O. afrasiaticus* during early spring, and may have formed the source for later date palm infestation. Fruit bunch infestation was not prevented or even alleviated by glue barriers so infestation of date palm by *O. afrasiaticus* may occur via aerial dispersal of motile forms. In ground cover phytoseiid and tetranychid mites were scarce, two of tetranychid species are new to science were founded.

Key words: Tunisia, date palm, *Oligonychus afrasiaticus*, sulfur, migratory flux, Phytoseiidae.

Introduction

In Tunisia, the date palm spider mite, *Oligonychus afrasiaticus* (McGregor) (Prostigmata Tetranychidae), is one of the four major pests of date palms. When present, it can cause very serious damage to fruits (Dhouibi, 1991; Khoualdia *et al.*, 1997).

The bionomics of such mite has not thoroughly studied; however, Hussain (1969) reported that it has 6 generations/year, its peak infestation in Iraq occurs around the middle of July and that it prefers dryer areas. The infestation of this mite usually starts around mid-May to June when it builds dusty, creamy silken webs on date bunches around strands and date fruits, where adults and immature live. Dust and sand grains adhere to the webs on date fruits making the environment under such webbing suitable for reproduction, development and survival of mites. Feeding on the immature green dates causes severe fruit scarring, sometimes so badly that the dates turn brown and have a scabbed appearance. The skin of infested fruit becomes hard and then cracks and shrivels, reducing the grade of the fruit and making such fruits unmarketable (Hussain, 1974; De Montaigne and Fall, 1986; Guessoum, 1986). In several areas, control of *O. afrasiaticus* populations is done mainly by sulfur (Coudin and Galvez, 1976; Guessoum, 1986; Dhouibi, 1991; Djerbi, 1993; Palevsky *et al.*, 2004). Similar programs have been conducted in California for many years for the control of the new world date mite *Oligonychus pratensis* (Banks) (Carpenter and Elmer, 1978).

At present, in Tunisia there is no available research on the behaviour of *O. afrasiaticus* and the relative importance of natural enemies. Thus, the aims of this work are: (1) Study of the occurrence and fluctuation of *O. afrasiaticus*; (2) Determination of dispersal ability and overwintering habitat of *O. afrasiaticus*; (3) To catalogue mites that revolve around the date palm, and (4) To establish the natural enemy species composition that co-occur with this pest.

Materials and methods

Study area and production systems

The study was carried out between 2004 and 2006 in an orchard of 2 ha planted with mixed varieties of date palm trees. This area is located in Segdoud, from the region of Tozeur (Djerid), in southwest of Tunisia. Tozeur belongs to the superior bioclimatic Saharan zone with a temperate winter. The annual mean temperature is about 21.6 °C, while 44 °C in August and 2.5 °C in January are the absolute maximum and minimum temperatures, respectively. The annual average rainfall is about 96 mm.

The oases in this area are continental and of the modern type with a mean planting space of 10 x 10 m. The principal vegetation was date palm trees with a predominance of the Deglet Noor variety and very few market-gardening or fodder practices for farmer consumption. Plot had historical outbreaks of *O. afrasiaticus* and has received 4 sprays of wettable sulfur on June 22nd, July 18th, July 25th and August 1st in 2004. In 2005, it received 2 sprays of sulfur on July 3rd and August 15th. In 2006, it received 3 sprays of wettable sulfur on July 2nd, July 24th and August 1st. Every year, the rate of sulfur was about 100 - 150 g per palm tree.

Sampling mites on fruits and pinnae

Date fruits and pinnae of the Deglet Noor variety were used to assess seasonal abundance of *O. afrasiaticus.* Samples were collected weekly from 23 May to 10 October in 2004; from 30 April to 25 October in 2005 and from 15 April to 4 November in 2006. Mite populations were monitored twice a month during the rest of the years of this study.

Ten trees were tagged for subsequent sampling. These trees were chosen at random to assure coverage of the orchard, 2 trees located in the North, South, West, East and in the middle of oases.

Samples of 10 fruits and 10 pinnae were taken per palm tree. From the beginning of the fructification until harvest, fruits were taken from bunches in four locations (NSWE). The ten pinnae were sampled as follows: five in the central crown (which are less than 1 year old) and five in the base (which are the oldest fronds). Fruits and pinnae were individually placed in plastic bags, kept in an insulated cooler with ice, and brought to the laboratory and stored at 4 °C. Mites were collected and counted under a stereomicroscope. We determined the mean number of eggs and motile forms of *O. afrasiaticus* and predator for 100 fruits and pinnae, per sampling date.

At each sampling date, the percentage of fruits with mite damage was calculated as number of infested fruits (at least by 1 tetranychid) divided by the total number of fruits sampled (n = 100). Weekly all trees were observed for general appearance and presence of webbing. The frequency of total date palm infestation was calculated as number of total infested trees divided by the total number of date palm trees, of the Deglet Noor variety (n = 102).

Sampling mites in ground cover vegetation

To determine plant species served as hosts for phytophagous mites and refuge for natural enemies, volume of approximately 1 l of leaves or 100 leaves of a given plant species collected randomly under sampling trees were carefully clipped. Samples were individually placed in plastic bags, kept in an insulated cooler with ice, and brought to the laboratory and stored at 4 °C. Mites were directly collected on leaves with a fine paint brush using a stereoscopic microscope or by using the leaves dipping-shaking-washing method (Boller 1984), mites being collected on a filter at the end of the process. Mites were then transferred with a fine paint brush into small plastic vials containing 70° alcohol for later identification. A total of 20 plant species were monitored in all years. Sampling dates in 2004-2006 were synchronized with those for date palm fruits and pinnae.

Assessments of mites leaving or migrating to the bunch

Date bunches extend from the tree on stalks, allowing each bunch to stand alone. The ambulatory mite that wants to reach a bunch would have to walk up this stalk. To determine the migration of *O. afrasiaticus* to the bunches 5 cm wide bands of masking tape coated with Tangle trap® were applied to the base of the fruit stalks.

Five trees (not trees used to monitor seasonal abundance) were randomly selected. Two bunches were treated with glue barriers on each sampling tree. In 2004, sticky bands were placed on May 2nd and were replaced at approximately 2-week intervals until October 25th. In 2005, bands were fastened on April 27th and were replaced at 2 week intervals until October 15th. In 2006 bands were setup on April 15th and were replaced at 2-week intervals until October 21st. Bands being transferred to and from orchards were temporarily covered with plastic food wrap to prevent them from sticking to other objects. On removal dates, bands were brought to the laboratory where mites were counted under a stereomicroscope. Separate records were kept for mites caught near the bottom margin of bands (crawling up the tree) and those caught along the top margin (crawling downwards). Because mites were never seen further than a few millimetres from either edge of the bands, there was no overlap between upward and downward crawling individuals.

On each sampling date, up to 30 mites per band were removed and placed in Hoyer's medium on microscopic slides for later identification under a compound microscope. To do this, it was first necessary to cut out a small piece of plastic food wrap covering the portion of the sticky band housing each mite. Then a drop or two of commercial organic solvent, Varsol® was placed on the band to wash away Tangle trap® embedding the mite. Next the mite was transferred by a single-hair paintbrush to a glass slide and washed again in Varsol® to remove any remaining Tangle trap so that the mounting medium could penetrate and clear the mite for identification.

Identification of spider mites

Samples of extracted spider mites, from all habitats (ground cover, sticky bands, fruits and pinnae) were mounted in Hoyer's medium on microscopic slides and identified using a phase and interferential contrast microscope. The strains were identified and morphologically characterized at Montpellier SupAgro (France).

Data analysis

The parameter cumulative mite days (CMDs) were used to assess the effect of mite density over time on fruit damage. Mite days were defined according to Beers and Hull (1990) as one mite present per fruit strand for 1 day and calculated as the mean of both successive motile forms counts multiplied by the number of intervening days. The fortnight values were summed over the season to give the CMDs.

Experiments were analyzed by a paired t-test or ANOVA followed by pair wise comparisons according to LSD, by using SPSS 10.0 (SPSS, 1999).

Results

Mite in ground cover vegetation

Table 1 lists the flower species sampled. The table demonstrates, firstly, that on the sampled flowers *O. afrasiaticus* was the most frequent among its congener. From 2004 to 2006, *O. afrasiaticus* was obtained mostly

Table 1. Mites sampled collection data (2004-2006), with reference to host plants, number of specimens and sample dates.

Species	Host plant	Dates sampled (No. Specimens)
Euseius scutalis (Athias-Henriot)	*Abelmoschus esculentus* L.	November 2006 (4)
Neoseiulus cucumeris (Oudemans)	*Sorghum bicolor* L. *Setaria* (Beauv) sp.	June (18) and October 2006 (11) June2006 (3)
Typhlodromus athenas (Swirski et Ragusa)	Deglet Noor (trunk) Deglet Noor (pinnae) *Solanum melongena* L. leaves	November 2005 (2) from June to November: 2005 (43), 2006 (17) April2006 (13)
Oligonychus afrasiaticus (McGregor)	*Solanum melongena* L. *Cucumis melo* L. leaves *Sorghum bicolor* L. leaves *Solanum melongena* L. leaves	April 2006 (8) August 2006 2004 (518), 2005 (44), 2006 (39) 2006 (1825)
Petrobia pseudotetranychina Auger et Flechtmann	*Atriplex* sp.	July 2006 (4)
Tetranychus urticae Koch	*Cucumis melo* L. leaves *Citrullus lanatus* (Thunb.) leaves	August 2005 (13) June 2005 (8) , September 2005 (16)
Petrobia carthagensis Auger et Flechtmann	*Cynodon dactylon* L.	July 2006 (5)

from herbaceous vegetables *Cucumis melo* L., *Solanum melongena* L. and *Sorghum bicolor* (L.) Moench. Two new species *Petrobia carthagensis* (Auger et Flechtmann) and *Petrobia pseudotetranychina* (Auger et Flechtmann) were founded (Auger *et al*., 2009). Lastly, four of the predatory mites namely *Neoseiulus cucumeris* (Oudemans), *Typhlodromus* (*Anthoseius*) *athenas* (Swirski and Ragusa) and *Euseius scutalis* (Athias-Henriot) were recorded with low densities.

Seasonal abundance of *O. afrasiaticus*

In 2004, over-wintering females of *O. afrasiaticus* began colonizing fruits in the second week of July (figure 1c). Spider mite density increased rapidly during the Kimri stage, characterized by green fruit colour. *O. afrasiaticus* population's levels dropped following sulfur applications on July 18th, July 25th and August 1st. Only one week after this decrease, there were a get back of populations (> 8 motile forms per fruit). From late August, with the colour change of fruit to yellow, counts of *O. afrasiaticus* in fruits decreased gradually and remained relatively low for the rest of the season. No mites were detected crawling up bunches. Counts of downward mites caught per bunch sticky band exceeded 44 motile forms at the end of August (figure 1b). Low densities of *O. afrasiaticus* were detected on pinnae from mid-September till late November (< 2 per leaf) (figure 1c). Samples taken from ground-cover plants showed detectable numbers of mites on sorghum from mid August to late October (figure 1a).

In 2005, data showed that the active period of *O. afrasiaticus* on fruits occurred throughout the period from the third week of July until mid August (figure 2c). The mean number of mites reached its maximum on the 3rd of August with 6 motile forms per fruit. Sulfur treatments were applied on July 3rd and August 15th, seems not affecting *O. afrasiaticus* populations. As in 2004, no mites were detected crawling up bunches. However

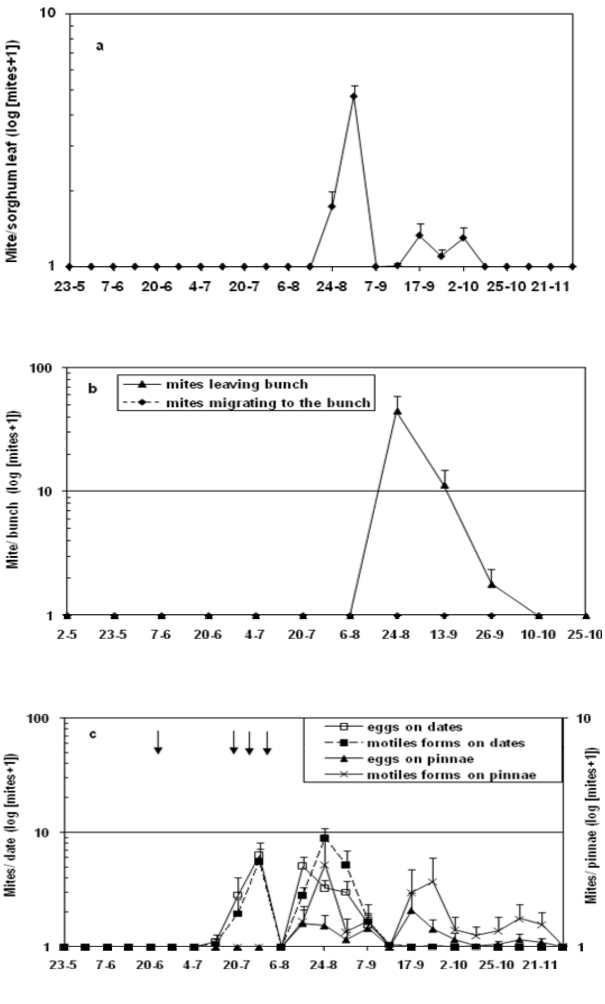

Figure 1. Mean (± 95% CL) counts of *O. afrasiaticus* in 2004 per sorghum leaf (a), upward and downward crawling *O. afrasiaticus* on bunch (b) and *O. afrasiaticus* densities per fruit and per pinnae (c). Arrows indicate dates of sulfur application.

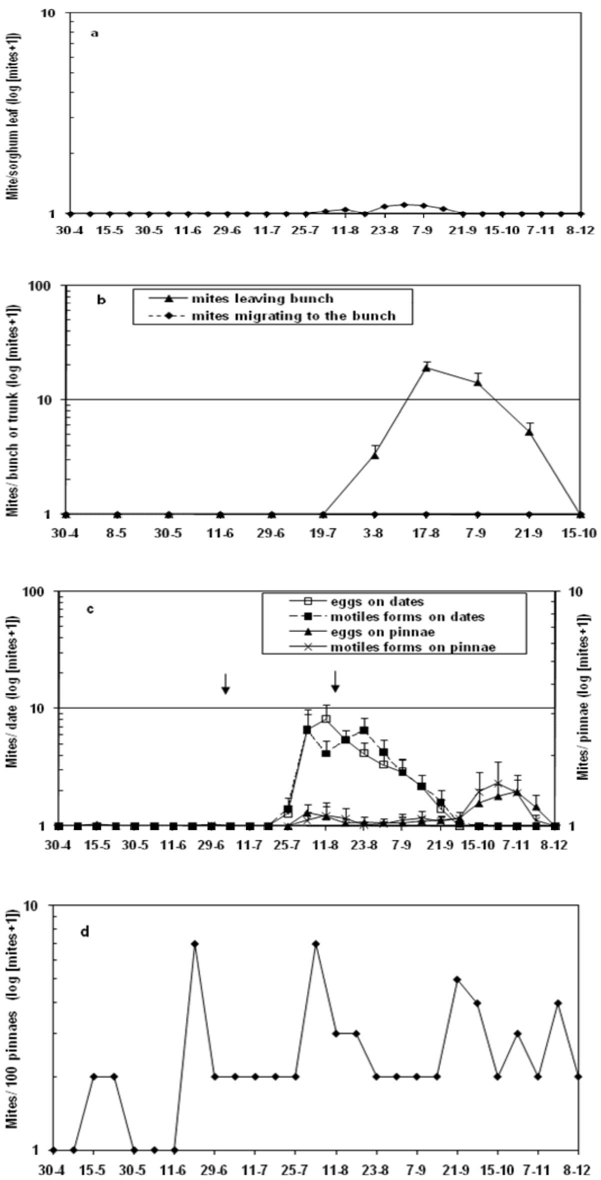

Figure 2. Mean (± 95% CL) counts of *O. afrasiaticus* in 2005 per sorghum leaf (a), upward and downward crawling *O. afrasiaticus* on bunch (b), *O. afrasiaticus* densities per fruit and per pinnae (c) and *T. athenas* densities per 100 pinnae (d). Arrows indicate dates of sulfur application.

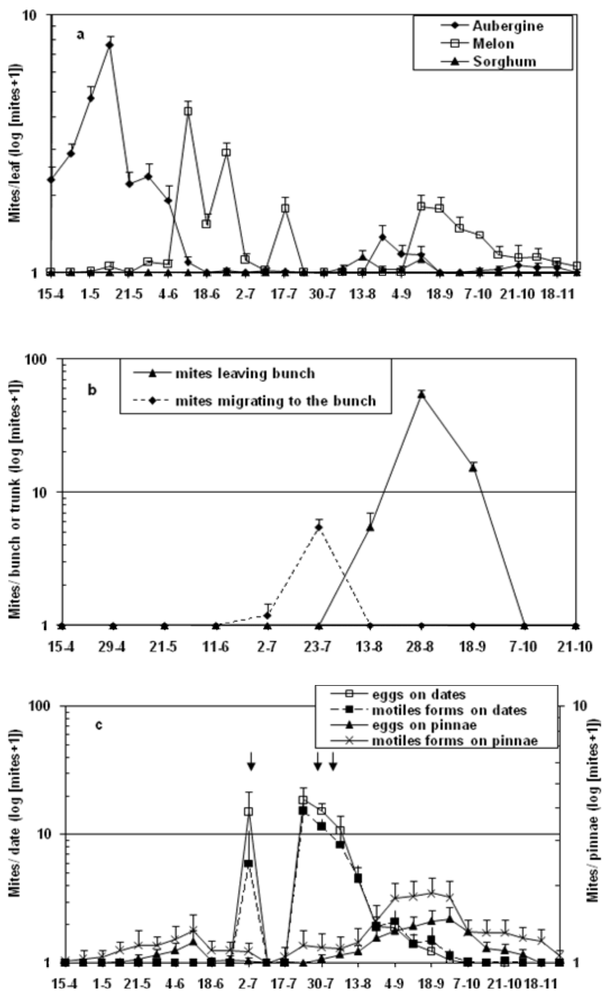

Figure 3. Mean (± 95% CL) counts of *O. afrasiaticus* in 2006 per sorghum leaf (a), upward and downward crawling *O. afrasiaticus* on bunch (b) and *O. afrasiaticus* densities per fruit and per pinnae (c). Arrows indicate dates of sulfur application.

densities of *O. afrasiaticus* leaving bunch peaked at 18.2 / bunch (figure 2b). Mite populations (figure 2c) on pinnae were present from April to December. Low densities of *O. afrasiaticus* (≤ 1) were present on sorghum leaves in August and September (figure 2a).

In 2006, mite populations were detected in the ground-cover, in spring (April-May) before being seen on the fruits. From April to mid June, mites were found on egg-plant and melon leaves (figure 3a). From July till August, the absence of mites in the ground-cover coincided with its appearance on the fruit. On fruits, (figure 3c) infestations occurred in the beginning of July. Densities of maximum motile instar registered in 2006 were 2.5 times higher than in 2005, despite both sulfur treatments applied on July 24th and August 1st. Counts of *O. afrasiaticus* on pinnae were low during May and June, but by early September they increased (figure 3c). During the years of observation no specialized spider mite predators for *O. afrasiaticus* were found on Deglet Noor fruits. The most common and abundant predator was *T. athenas*, collected on Deglet Noor pinnae was consistently seen in low numbers (less than one per pinnae) (figure 2 d).

Mean monthly temperatures (maximum and minimum) of the month of July and August 2005 (35.3 °C) were approximately (2 °C) above the long-term mean in 2004 (33.3), similar to the mean in 2006 (33.7), and considerably higher (approximately 3 °C) than the long-term mean. During the rest of the season, climatic conditions were approximately similar in all three years. Densities of *O. afrasiaticus* increased from 2004 to 2006, although mean seasonal CMDs (F = 0.42; df = 2, 33; $P > 0.05$) (table 2) and the percent of damaged fruit were not significantly different. Damage caused by feeding of *O. afrasiaticus* was noted on 35.29, 25.49 and 21. 56% trees respectively on 2004, 2005 and 2006.

Table 2. Mean seasonal CMDs (mobile forms/date) and percent of date fruit collected with mite damage (n = 10 fruit per tree) on the cv. Deglet Noor.

	CMDs	Fruit with mite damage (%)	Total of infested trees (%)
2004	165.59 ns	12.07 ns	35.29
2005	192.28 ns	16.27 ns	25.49
2006	296.24 ns	15.00 ns	21.56

ns = P > 0.05 (ANOVA).

Discussion

Seven mite species in total were found during the different surveys. Among them two species of Tetranychidae are new to science and previously described by Auger *et al*. (2009), namely the species *P. carthagensis* and *P. pseudotetranychina*. The only mite pest of date fruit in Segdoud oasis is *O. afrasiaticus*. Investigators have speculated that dynamics of *O. afrasiaticus* is a function of climate (Lepesme, 1947; De Montaigne and Fall, 1986; Dhouibi, 1991; Palevsky, 2003). Our research supports these results, fruit infestation started during the first three weeks of July. Greater heat and a mid-summer drought caused *O. afrasiaticus* population growth in fruits. Monthly mean temperatures of 32 °C in July and August in Tozeur seemed to be very favourable for an increase in the pest mite population according to a life table study (Ben Chaaban *et al*., 2008). Populations of *O. afrasiaticus* responds to the decrease of temperature in autumn, by leaving dates bunches and migrating to the pinnae and ground cover. Literature reported that the dust mite migrates to rejections, fibres, palms, inflorescence, male date palms and infertile date palms, on grasses as *Cynodon dactylon* L. and *Lolium* sp. (Lepesme, 1947; Hussain, 1969; Guessoum, 1986; Dhouibi, 1991; Palevsky, 2003). Also moving on cucumber, watermelon, fig, grapevine (Guessoum, 1986).

Not only climatic conditions seem to be the cause for the distribution of tetranychid mites, but it also appears to be a response to food shortage (Helle and Sabelis, 1985; Margolies and Kennedy, 1985; Frlexner *et al*., 1991). Previous results revealed that performance of *O. afrasiaticus* varied greatly depending on the chemical composition of fruits (Palevsky, 2005; Aldosari and Ali, 2007; Ben Chaaban and Chermiti, 2009). In fact, the fruit infestation begins and increases during the Kimri stage, characterized by the green colour of fruit, rapid increase in size, weight, and reducing sugars. At this stage, moisture content and acid activity are at the highest (Barreveld, 1993; Palevsky *et al*., 2005; Aldosari and Ali, 2007; Ben Chaaban and Chermiti, 2009). Mite populations begin to decline with colour change of fruit to yellow or red at the khalal stage. The decreased in water content with elevated sugar contents at this stage, render the date extremely resistant to *O. afrasiaticus* (Palevsky *et al*., 2005; Aldosari and Ali, 2007; Ben Chaaban and Chermiti, 2009).

During May and June, fewer or no motile forms were detected crawling towards bunches. But despite this, there were infestations of fruits. Mites infest dates by not reaching stalks coming from fronds but by airborne infestation. Similar results were founded by Palevsky *et al*. (2004), they demonstrated that fruit bunch infestation was not prevented or even alleviated by glue barriers or dense netting covering bunches. Gispert *et al*. (2001), who studied the temporal and spatial distributions of *O. pratensis* on Deglet Noor, showed fruit bunch infestation to be generally clumped, with heavily infested bunches being adjacent to non-infested ones, and suggested that infestation resulted from random arrivals of individual mites. In the case of *O. afrasiaticus* and *O. pratensis*, even one mated female is enough to establish a colony that will rapidly infest an entire bunch (Coudain and Galvez, 1976; Gispert *et al*., 2001; Palevsky *et al*., 2004).

Throughout the assays there was considerable variability between replicates and time of sulfur application. These summer treatments apparently reduced *O. afrasiaticus* populations and reduced its impact. Sulfur varies from being non-toxic to highly toxic to spider mites in laboratory assays (Blumel and Hausdorf, 2002; Guichou *et al*., 2002). Under laboratory conditions, the acaricidal effect of wettable sulfur is influenced by climatic conditions and the stage of development of *Tetranychus urticae* (Koch). Its ovicidal effect results from the combined action of temperature and relative humidity. Wettable sulfur becomes effective at 27.5 °C and 75% RH. Beyond this threshold, the acaricidal effect increases with rising temperature or humidity, to become complete at a temperature of 35 °C and 90% RH (Auger *et al*., 2003).

In this study, the occurrence of phytoseiids was sporadic. Predator mites were not found on fruits. Very few *T. athenas* were collected from pinnae, but never founded in association with *O. afrasiaticus*. Three factors low prey numbers, dry conditions and application of sulfur were the most likely causes of the scarcity of phytoseiids on fruits. Dry conditions prevented phytoseiids to establish themselves. Sabelis (1985) mentioned that among factors that determine the effectiveness of phytoseiids, humidity appears to be of major importance. The egg stage is especially sensitive to low humidity. A second probable factor was the application of sulfur. Earlier findings revealed that sulfur can initially suppress pest mite populations (Croft, 1990), mite densities often increase after applications cease, apparently because sulfur increases mortality, and decreases fecundity, of predators (Van de Vrie *et al*., 1972; Hanna *et al*., 1997; James *et al*., 2002; Costello, 2007). Another reason for the scarcity of phytoseiids on fruits was probably the presence of other food sources on the date palm. In fact, *T. athenas* motile forms and eggs were founded under *Parlatoria blanchardi* (Targioni) scales on pinnae. Previous studies suggested that generalist predator feeding on various species of tetranychidae, tarsonemidae, pollen, fungi, and other mites (McMurtry and Croft, 1997; Luh and Croft, 2001). Natural enemies must be active in the early stages pest infestations, in order to have a regulatory impact (Van Driesche and Bellows, 1996). In California, the biological control of *O. pratensis* on date palms has been associated with the indigenous phytoseiid *Galendromus mcgregori* (Chant), whose presence coincides with that of the pest during the summer months (Gispert *et al*., 2001). These authors suggested that inundative releases early in the season could delay pest population increase and reduce damage. Khoualdia *et al*. (2001) released the phytoseiid *Neoseiulus californicus* (McGregor) in south Tunisia to control *O. afrasiaticus* and obtained significant reductions of the pest's populations. These authors then recommended releases earlier in the season.

The number of known species of phytoseiid mites for the Tunisian fauna is now 25 (Kreiter *et al*., 2008). Oases are probably habitats with high endemism. New surveys in oasis of Tunisia would consequently be needed.

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