

**Final REPORT**-Covering period: November 1<sup>st</sup>, 2006 to Dec. 31<sup>st</sup>, 2011  
**Grant Number: TA-MOU-05-M24-024**

Submitted to the U.S. Agency for International Development; Bureau for Global Programs, Field Support and Research; Center for Economic Growth and Agricultural Development

TITLE OF PROJECT: Integrated Control of the Date Palm Spider Mite

Principal Investigator: Dr. Eric PALEVSKY

Grantee Institution: Agricultural Research Organization (ARO), Inst. of Plant Protection, Department of Entomology, Newe-Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel; Cellular - 972 (0) 50-6-220-111; Fax - 972 (0) 4-983-6936

Email: [palevsky@volcani.agri.gov.il](mailto:palevsky@volcani.agri.gov.il)

Collaborator: Dr. Ilan SHOMER

Institution: ARO, Institute of Technology and Storage of Agricultural Products, Dept. of Food Science, Bet-Dagan 50250, Israel. Tel: +972-3-968-3706; Fax: +972-3-960-4428. Email: [ilan@volcani.agri.gov.il](mailto:ilan@volcani.agri.gov.il)

Collaborator: Dr. Hamutal BOROCHOV-NEORI

Institution: Southern Arava R& D Center, M. P. Hevel Eilat 88820, Israel. Tel: +972-8-635-5747; Fax: +972-8- 635-5730. Email: [hamutalneori@ardom.ardom.co.il](mailto:hamutalneori@ardom.ardom.co.il)

Collaborator: Prof. Uri Gerson

Institution: Department of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, , POB 12, Rehovot 76100, Israel. Tel: +972-8-948-9220; Fax: +972-8-946-6768. Email: [gerson@agri.huji.ac.il](mailto:gerson@agri.huji.ac.il)

Collaborator: Dr. Marwan ABDELWALI

Institution: National Center for Agricultural Research and Extension, Baqa P.O.B 639, Jordan. Tel: +962-6-472-5071; Fax: + 962-6-472-6099. Email: [marwan@ncare.gov.jo](mailto:marwan@ncare.gov.jo)

## Executive Summary

The old world date mite, *Oligonychus afrasiaticus* (McGregor), is a serious primary pest of date palms *Phoenix dactylifera* L. grown in arid regions throughout the Middle East and North Africa. Spider-mite populations on the immature fruit bunch escalate rapidly throughout the early ripening stages, spinning dense webbing and intensively feeding on fruit exocarp. The objectives of our project were: 1) Develop a monitoring tool and a day degree model for predicting date mite infestation on specific date palm varieties. 2) Evaluate native and introduced natural enemies for use in an integrated mite management program. 3) Develop postharvest treatments to mitigate evident mite damage caused to fruits by spider mites. 4) Evaluate date palm varieties for levels of spider mite (*O. afrasiaticus*) resistance. Identify mechanisms of host plant resistance to this pest. 5) Evaluate the effects of indigenous entomo-pathogenic fungi. Below we summarize our project following its objectives.

1) Monitoring the first webbing can be done successfully with reasonable accuracy using good-quality binoculars. In order to minimize scouting efforts, use of the climatic models developed herein is recommended. Clearly, however, more research is needed to refine these models. It is suggested that reducing pesticide use in date palm orchards can lead to a more abundant phytoseiid population, ultimately resulting in a more sustainable agro-system.

2a) *Typhlodromus athiasae* was found to be a good candidate for augmentative biological control of *O. afrasiaticus* due to its drought-tolerance, its natural presence on the bunches, and, most importantly, its reduction of pest populations and accompanying damage. Pollen supply as alternative food was essential for *T. athiasae* establishment and for efficient pest control. However, as the control provided by *T. athiasae* was not adequate, releasing and conservation methods should be further evaluated with additional indigenous phytoseiids.

2b) *Neoseiulus californicus* released from an improved sachet system did not provide adequate control of *O. afrasiaticus*. Due to the extreme arid conditions, the sachets did not serve as a rearing unit. In order to use *N. californicus* in such conditions, sachet design and/or prey species must be improved. Alternatively, *N. californicus* can be released more frequently and in higher numbers, without considering the sachet as a rearing unit.

3) Mite damage is most evident at harvest when the fruits are dry. Postharvest treatments based on fruit hydration under controlled temperature and relative humidity (RH) conditions were developed to mitigate mite damage. Hydration of mite damaged 'Medjool' fruit for 48 h at 47°C/95%RH efficiently restored fruit quality that was largely sustained during 8-month storage and 6-week shelf-life. Hydration of mite affected 'Deglet-Noor' fruit strands for 48 h at 40°C/60%RH improved fruit quality; the latter was mostly maintained during 4-month storage and 1-week shelf-life. The successful quality restoration and adaptation of long term preservation procedures will reduce considerably the economic loss inflicted by the mite.

4) To identify potential constituent mite attractants and repellents in the fruit, we have tested the relationships between date fruit chemical parameters and mite phenology in susceptible ('Medjool', 'Barhi' and 'Deglet-Noor') and insusceptible ('Zahidi') cultivars. Maximal water content (WC) and WC>80% were required for mite ascent and population buildup, respectively. Mineral composition, EC, pH, titratable acidity and total soluble solids and phenolics' contents changed similarly in all cultivars; however, the values and extent and rate of change were cultivar dependent, suggesting possible relation to susceptibility. The observations on chlorophylls *a* and *b* and lutein suggest a role as mite attractants. The findings on hydrocaffeic acid and specific catechin derivatives suggest they all may act as mite repellents.

5) The effect of the Basidiomycota fungi *Meira argovae*, *Meira geulakonigii* and *Acaromyces ingoldii* on the mite *Oligonychus afrasiaticus* was evaluated in the laboratory. The number of mites was significantly reduced by all three fungi. These fungi are indigenous to Israel, so their application out of doors will not be a problem. However, a major drawback could be the very low humidity prevailing in the Arava Valley. That may be overcome by various mechanical means. Follow up field trials will need to be conducted to determine the feasibility and efficacy of field applications.

Grant Number: TA-MOU-05-M24-024

**Section I: Technical Progress**

I. A) Research tasks

Task No.	Research Objectives listed as tasks	Country	Page
1	Developing a monitoring tool and a day degree model for predicting date mite infestation on specific date palm varieties	(J,I)	4
2A	Climatic and nutritional effects on demographic parameters of potential predatory mite species	(I)	20
2B	Evaluation of predators as natural enemies of <i>Oligonychus afrasiaticus</i> in the field.	(I, J)	42
3	Postharvest treatments to mitigate evident damage caused to fruits by spider mites.	(I)	66
4A	Exocarp structure in relation to mite phenology and population density.	(I)	72
4B	Fruit chemistry in relation to mite phenology and population density.	(I)	73
5	Indigenous entomophagous fungi for control of <i>Oligonychus afrasiaticus</i>	(I)	92
	Acknowledgements		94
	References		95

## 1. Developing a monitoring tool and a day degree model for predicting date mite infestation on specific date palm varieties (Israel and Jordan).

### **1.1 Introduction**

Monitoring pest populations in the field is a key factor of IPM (Integrated Pest Management) programs. The ability to accurately predict pest levels in time and space, together with a deep understanding of pest biology and damage, allows the grower to take reasonable decisions regarding pest management. Using pesticides only when it is really necessary leads to a reduction in chemical control, in accordance with the ecological approaches of modern agriculture (Pedigo and Rice 2009).

However, in the case of *Oligonychus afrasiaticus* on date palms, monitoring is a great challenge. As a primary pest with an explosive nature for population increase, *O. afrasiaticus* must be detected before establishment on the bunch; otherwise, it will be too late to prevent fruit damage. In addition, relative to date palm trees, date mites are tiny and have a patchy distribution (Gispert et al. 2001, Palevsky et al. 2003), making it easy to miss them.

Washing samples of strands with ethanol (Palevsky et al. 2003), as done also in this study, or with soapy water (Gispert et al. 2001), can reflect the actual population in the orchard. However, this method is not practical for use by growers, since it is too labor-intensive and demands expensive equipment, such as a good quality stereomicroscope. Some growers inspect the fruit bunches of each individual tree from a close distance, but usually alongside carrying out other agrotechnical practices. When trees grow over 2-3 meters, they use mechanical towers, which are also costly.

Based on tree fruit phenology, the Extension Service of the Ministry of Agriculture recommends that the first application be applied prophylactically on 'Barhi' and 'Medjool' in mid-May and on 'Deglet Noor' in mid-June (O. Uko and S. Dobrinin, personal communication). Additionally, most growers will prophylactically apply one or two more acaricides per season. It was previously suggested that implementation of a simplified scouting method based on a webbing index could be a practical solution for monitoring *O. afrasiaticus* (Palevsky et al. 2004). A prediction model for the infestation dynamics of *O. afrasiaticus* could improve the timing of such scouting efforts and of acaricide treatments, thereby improving pest control.

Degree-Day (DD) models are applied in IPM programs for predicting the timing of a defined pest population threshold and for optimizing pesticide application (Pedigo and Rice 2009). Spider mites, like all other arthropods, are poikilothermic and are thus directly affected by temperature. It has been extensively demonstrated that the accumulation of heat over time from a specific start point (biofix) can predict the timing of expected events in (arthropod) pest phenology with reasonable accuracy (Pedigo and Rice 2009). This biofix can be a specific date (e.g., January 1<sup>st</sup>) or a biological event (e.g., the emergence of the first adult, the start point of cultivar flowering), which is related to pest development and/or its population dynamics. The accumulation of heat from that point, also known as 'physiological time', is reflected by degree-days between the lower and the upper temperature thresholds of pest development. Degree-Day models are also used in control programs of spider mites (Broufas and Koveos 2000, Gotoh et al. 2004a). However, these models are based only on the effect of temperature, whereas the development of many spider mite species is negatively influenced by air humidity. The fecundity, survival and growth rates of the cosmopolitan spider mite pest, *Tetranychus urticae*, are positively related to low humidity (Hazan et al. 1973), such that its control can be achieved by creating a humid environment (Duso et al. 2004a).

The combined effect of temperature and humidity on the life history parameters of *O. afrasiaticus* from Mauritania was evaluated by Coudin and Galvez (1976). In that study, within the temperature and humidity ranges of 23.5-31°C and 38-70% RH, respectively, the authors recorded the best demographic performance at 25.5°C with 38% RH.

*Oligonychus pratensis*, the Banks grass mite, also known as the new world date mite, infests date palm fruit in California and is similar in phenology to *O. afrasiaticus* (Carpenter and Elmer 1978, Gispert et al. 2001). This pest has been widely studied since it is also an important pest of Gramineae crops, such as corn and sorghum, in hot and dry areas in the southwestern United States (Holtzer et al. 1984, Perring et al. 1984b). It has been found that the developmental rate of *O. pratensis* is highly temperature-dependent, especially between 25°C and 37°C, with the maximum rate when approaching 37°C (Perring et al. 1984b). The effect of humidity tends to be smaller in comparison to that of temperature. However, a warm environment and low humidity (higher Vapor Pressure Deficit – VPD) was better for mite development, with a higher intrinsic rate of increase than in more humid conditions (same temperature, low VPD) (Perring et al. 1984a).

The main goals of this chapter were to provide ways of improving the monitoring and control of *O. afrasiaticus* by: (1) evaluating the use of high-powered binoculars as a monitoring tool for *O. afrasiaticus* webbing; and (2) exploring the potential of climatic variables to predict the timing and level of infestation.

## **1.2 Methods**

### 1.2.1 Fast scouting method for detecting *O. afrasiaticus* webbing

This part was divided into three experiments, conducted over a period of three consecutive years:

**2007** – Nine 'Medjool' (8 years old) and twelve 'Barhi' (15 years old) trees in the Israeli SAV (29°49'N; 35°02'E) were sampled fortnightly from mid-April until the mite population declined (23 July for 'Barhi' and 6 August for 'Medjool'): Observations with binoculars (10x42HG L DCF, Nikon, Japan) were made from four sides of each tree at ca. 10m from the tree trunk. Additionally, every two weeks for 'Barhi' and every four weeks for 'Medjool', random samples of 15 strands per tree (3 strands from 5 bunches) were taken and washed in alcohol, and the mites were counted as described in the General Methods section. The number of spider mites found in the ethanol wash at the time that webbing was first observed was considered to be the detection threshold for the binoculars method. When webbing was observed, but no ethanol wash was performed on the same day (occurred only for 'Medjool'), the threshold number was calculated as the mean of two successive mite counts (two weeks before and after the observation).

**2008** – The trial was carried out in the Jordanian SAV on 'Medjool' and 'Barhi', with 16 trees each that were sampled every 10-14 days from late April until harvest time. In each CV, trees were divided into the binoculars and control treatments, with eight trees in each group. In the binoculars treatment group, each tree was sprayed with an acaricide (0.5ml/L abamectin [Vertimec® EC; Syngenta, Basel, Switzerland]) when webbing was first detected with the binoculars (10X42 L IS WP, Canon, Japan). In the control group, each tree was sprayed when the first mite was observed in the alcohol count. Mite counts in alcohol were carried out for all trees at each sampling date, as described above. At harvest, ca. 100 fruits per tree were evaluated for mite damage according to the regional packing house standards (see General Methods). Statistical comparison between the binocular and the control groups was done by a Mann-Whitney U test.

**2009** – In the last trial, the binocular method was compared to naked eye monitoring (the common method applied by growers) in 25 trees from each CV ('Medjool' and 'Barhi'), monitored in Jordan from early May to mid-August (harvest). Every ten days, the mite population of each tree was sampled by three methods: first with the binoculars for webbing detection, as described above; then for webbing detection by the naked eye at a close distance; and finally by counting adult females in an ethanol wash of 15 strands, as described above. The number of mites counted in the ethanol when webbing was observed (through binoculars and/or the naked eye) for the first time was considered as the minimal detected number (threshold) for the respective method in that tree. Additionally, the control efficacy attained with each monitoring method was compared using a paired design, replicated 15 times. Each tree that was monitored with binoculars was sprayed (soluble sulfur 2 mg/L [Thiovit® jet 80WG; Syngenta]) individually each time webbing was detected with the binoculars. In contrast, all trees monitored with the naked eye were sprayed as a plot when webbing was first detected, which is the commercial practice. When webbing was seen following the first spraying in both treatments, a 8x magnifying glass was used to assure mites were alive before spraying again. At harvest, ca. 100 fruit per tree were evaluated for their mite damage.

The mean (and SE) threshold, for either the binocular or the naked eye method, was calculated on the basis of all minimal detection results. However, statistical comparison between the two methods was performed using a paired t-test based only on trees that had minimal detection counts for both (binocular and naked eye). The number of acaricide applications per tree and the damage results were presented for all trees (25 versus 15), but statistical comparison was performed only for the 15 paired arranged trees, using paired t-test and Wilcoxon signed ranks test. The damage percentages were arcsin square-root transformed in order to achieve normality.

#### 1.2.2 The effect of climatic variables on *O. afrasiaticus* population

In order to lay the foundations for developing a prediction model for *O. afrasiaticus*, I evaluated the effect of climatic variables on seasonal pest population levels, measured in Adult (female) Cumulative Mite Days (ACMDs). Air temperature (°C) and relative humidity (%RH) measures (means of ten-minute intervals) were obtained from the weather monitoring station at Yotvata, Ardom R&D, in cooperation with the Israel Meteorological Service. For further evaluation of the effect of humidity on ACMDs, I calculated the Vapor

Pressure Deficit (VPD) as an index for absolute humidity (Ferro and Chapman 1979) (see details in General Methods).

Based on daily means, I established cumulative models for three climatic variables:

- Cumulative temperature was calculated as a simple Degree-Day (DD) model, with 17 °C as the lower threshold. Since the *O. afrasiaticus* developmental curve in relation to temperature is unknown, I took this value (17°C) from the developmental curve of *O. pratensis* (Perring et al. 1984a). For each day:  $DD = [\text{mean temp}] - 17$ .
- 'Relative dryness' (% RD) was defined as the inverse value of relative humidity (100-RH). In order to evaluate the importance of dryness to *O. afrasiaticus* population dynamics based on preliminary trials, I accumulated only daily means above 50% (RH < 50%).
- Cumulative vapor pressure deficit, as a measure of absolute humidity, was calculated on values above 1, 1.5, 2, and 2.5 kPa. These tentative thresholds were chosen since they are 'located' in the mid-range between Mediterranean (VPD of 1 kPa is correspondent to ca. 60% at 20°C) and extreme arid (2.5 kPa ~ 40% RH at 30°C) conditions. Based on literature data for *O. pratensis* (Perring et al 1984a) and *O. afrasiaticus* (Coudin and Galvez (1976), these tentative values could be of promise. The 2 kPa (correspondent to ca. 50% RH at 30°C) threshold yielded the most predictive model, and therefore I will present only this model here.

I examined two biofixes as the starting point for the cumulative climatic variables: March 1<sup>st</sup> and the date when artificial pollination began each year. Following Extension Service guidelines, pollination commenced in 2007, 2008, and 2009 on March 14<sup>th</sup>, March 23<sup>rd</sup>, and March 24<sup>th</sup>, respectively.

*O. afrasiaticus* population levels were taken from the results of three years (2007-2009) of experiments performed on 'Barhi,' Samar in the SAV. This orchard is located 8km south of the weather station and has similar climatic conditions. In order to have comparable results between years, I used the ACMDs from control trees for the period from May through the end of July (the timing of infestation peak ca. two weeks before the harvest). First, differences in seasonal populations between years were analyzed by one-way ANOVA, followed by Tukey HSD multiple comparison post-hoc test. Then, the relations between cumulative temperature, RD or VPD, and *O. afrasiaticus* ACMDs were analyzed

using Pearson correlation and linear regression. Differences in seasonal CMDs between years were analyzed with one-way ANOVA.

### 1.3 Results

#### 1.3.1 Fast scouting method for detecting *O. afrasiaticus* webbing

In the first experiment (Israel, 2007), webbings of *O. afrasiaticus* were seen through binoculars on seven (out of nine) 'Medjool' and 12 (out of 12) 'Barhi' trees. In the second experiment (Jordan, 2008), webbing was detected with binoculars only on two 'Medjool' trees (out of eight) and one 'Barhi' tree (out of eight).

When combining the results from Israel and Jordan, the binocular detection threshold was found to be slightly (but not significantly) lower on 'Medjool' than on 'Barhi' (8.4 and 14.2 mites/strand, respectively; Table 1.1). In over 70% of the trees (8 out of 9 'Medjool' and 8 out of 13 'Barhi'; n = 16), the binoculars threshold was as low as 4.6 ( $\pm$  0.8 SE) mites per strand. In two infested 'Barhi' trees (Jordanian SAV), binoculars viewing could not detect webbing when the pest population was 0.3 ( $\pm$  0.07 SE) mites per strand. Damage percentage in those trees was negligible. Percentages of damaged fruit were higher in the binoculars treatment group as compared to the control group; however, the sample size of the binoculars treatment group was very low (Table 1.2).

Table 1.1: *Oligonychus afrasiaticus* population levels (adult female mites per strand, mean  $\pm$  SE) detected by binocular observation for Israel in 2007 and Jordan in 2008.

Cultivar	Israel		Israel and Jordan	
	n (trees)	Population level	n (trees)	Population level
Barhi	12	13.1 $\pm$ 4.0	13	14.2 $\pm$ 3.8
Medjool	7	10.1 $\pm$ 4.9	9	8.4 $\pm$ 3.9
Barhi+Medjool	19	12.0 $\pm$ 3.0	22	11.8 $\pm$ 2.8

**Table 1.2:** Comparison of fruit damage (mean percentages from picked fruit  $\pm$  SE) caused by *Oligonychus afrasiaticus* between the binocular and control treatments, Rahma, 2008.

Cultivar	Binocular		Control		p <sup>b</sup>
	n	Damage <sup>a</sup> (%)	n	Damage <sup>a</sup> (%)	
Barhi	1	21	5	4 $\pm$ 1.2	ns
Medjool	2	9 $\pm$ 2.0	4	4 $\pm$ 0.9	ns
Barhi+Medjool	3	13 $\pm$ 4.2	9	4 $\pm$ 0.7	0.02

<sup>a</sup> Sum of all levels of damage

<sup>b</sup> Mann-Whitney U test

In the third experiment (Jordan, 2009), where considerably more replicates were conducted (25 vs. 8 replicates in 2008), the population threshold detected by the naked eye method did not differ from that of the binoculars method (Table 1.3). 'Barhi' and 'Medjool' trees that were controlled commercially according to the naked eye method were sprayed five times each throughout the ripening season, while trees that were sprayed individually according to the binocular monitoring method were sprayed less than once (Table 1.4).

**Table 1.3:** Comparison of the *Oligonychus afrasiaticus* detection thresholds (mites per strand, mean  $\pm$  SE) between the binocular and naked eye methods, Rahma, 2009.

Cultivar	Binocular		Naked eye		Stat. analysis <sup>*</sup>	
	n	Population level	N	Population level	n	p
Barhi	13	3.5 $\pm$ 2.3	16	2.8 $\pm$ 1.9	13	ns
Medjool	6	3.3 $\pm$ 1.9	12	2.0 $\pm$ 1.0	6	ns
Barhi+Medjool	19	3.4 $\pm$ 1.6	28	2.4 $\pm$ 1.1	19	ns

<sup>\*</sup> Paired t-test performed on trees with binocular and naked eye threshold values

**Table 1.4:** Mean ( $\pm$  SE) number of seasonal acaricide applications per tree for 'Medjool' and 'Barhi' performed for *Oligonychus afrasiaticus* control, based on binocular viewing or commercial practice, Rahma, 2009.

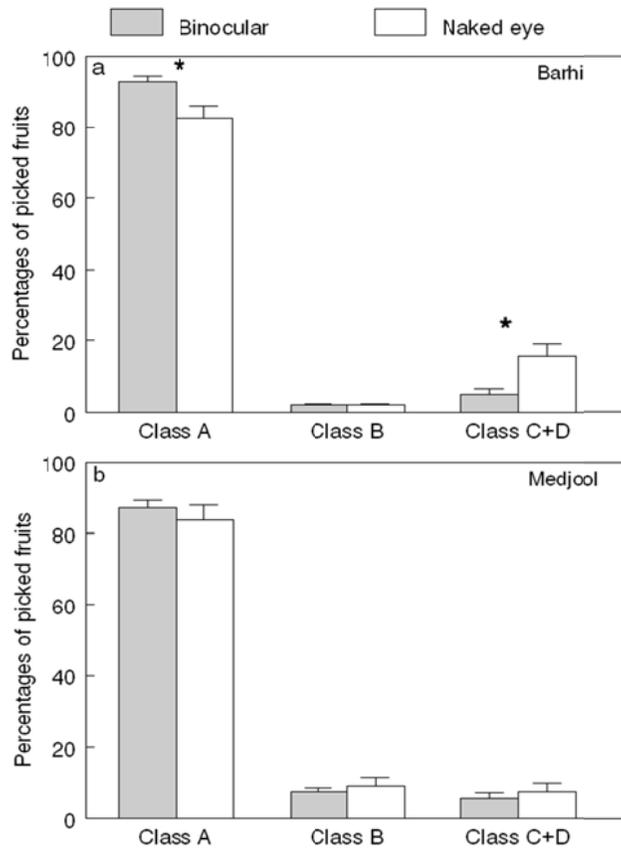
Cultivar	Binocular		Commercial		Stat. analysis <sup>*</sup>	
	n	No. of sprays	n	No. of sprays	n	p
Barhi	15	0.7 $\pm$ 0.2	25	5 $\pm$ 0	15	<0.005
Medjool	15	0.3 $\pm$ 0.1	25	5 $\pm$ 0	15	<0.001

<sup>\*</sup> Wilcoxon signed ranks test on 15 paired trees from both treatments

'Barhi' fruits from the binoculars treatment group were slightly less damaged than fruits from the naked eye treatment group (class A:  $t_{14} = 3.7$ ,  $p < 0.005$ ; class C+D:  $t_{14} = 4.0$ ,  $p < 0.005$ ; Fig. 1.1a), while 'Medjool' fruits showed no difference between the two treatments (class A:  $t_{14} = 0.3$ ,  $p > 0.05$ ; class C+D:  $t_{14} = 0.8$ ,  $p > 0.05$ ; Fig. 1.1b).

### 1.3.2 The effect of climatic variables on *O. afrasiaticus* population

Seasonal CMDs of adult females *O. afrasiaticus* differed between the years of 2007-2009 ( $F_{2,19} = 3.8$ ,  $p < 0.05$ ; Fig. 1.2). The pest population was exceptionally high in 2008, with high damage despite acaricide applications, and exceptionally low, with late increase, in 2009. The infestation level in 2007 was in between.



**Figure 1.1:** Comparison of fruit damage caused by *Oligonychus afrasiaticus* between the binocular and naked eye monitoring treatments, presented as the mean (+ SE) percentage of fruit graded for export (Class A), local market (Class B), and industry/discarded (Class C+D), in (a) 'Barhi' and (b) 'Medjool'; Rahma, 2009. Asterisks above the bars indicate significant differences between treatments (paired t-test,  $n = 15$  paired trees of both treatments,  $p < 0.05$ ).

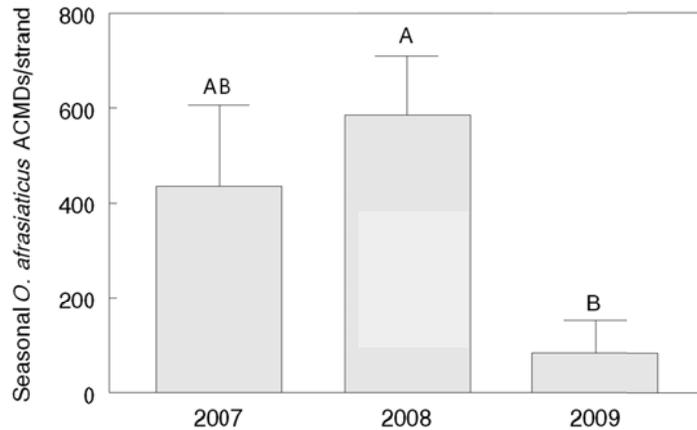
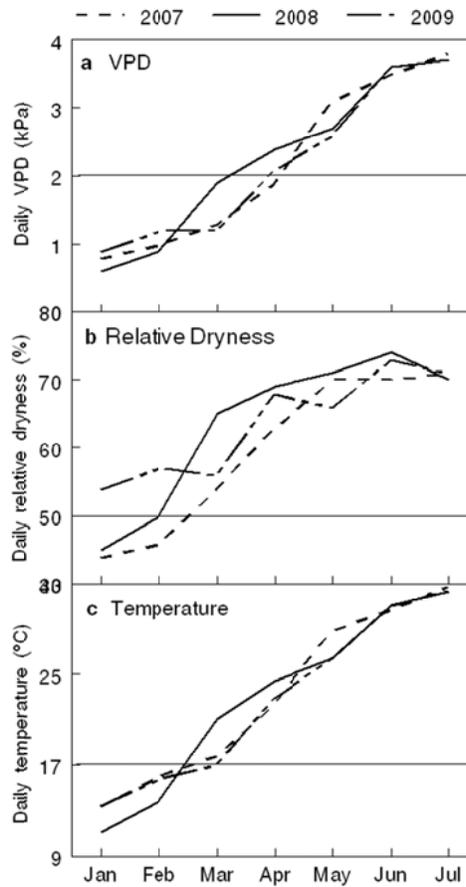


Figure 1.2: Seasonal means (+ SE) of *Oligonychus afrasiaticus* adult-female cumulative mite days (ACMDs) per strand, 'Barhi', Samar. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (Tukey HSD Multiple Comparison Test).

A comparison of climatic variables between the years revealed that 2008 had a warmer spring (March-April) and a dryer spring-summer (March-June) than 2007 and 2009 (Figs. 1.3b, c). The VPD multi-year pattern (Fig. 1.3a) was similar to the pattern of temperature (Fig. 1.3c). Variation between years was shown to be high in spring (March-May) and low in summer (June-July), with curves converging towards July.

Seasonal ACMDs were positively correlated with the degree-days (DD,  $> 17^{\circ}\text{C}$ ) and with cumulative VPD (Vapor Pressure Deficit,  $> 2\text{kPa}$ ), regardless of which biofix was used (starting pollination date or from March 1<sup>st</sup>). When using the log of ACMDs, the Pearson coefficients of these correlations were larger and more significant (Table 1.5). The log of ACMDs also had a positive, though not significant ( $p = 0.055$ ; Fig. 1.4b), trend in relation to the relative dryness (the inverse of relative humidity  $< 50\%$ ) calculated from the pollination start date. Linear regression with cumulative VPD (from pollination start through the end of July) as the predicting variable explained over 50% of the variation in log ACMDs (Fig. 1.4a). This model was slightly better than with degree-days or with both independent variables as the predictors. The accumulation of relative dryness did not improve the model (Fig. 1.4b).

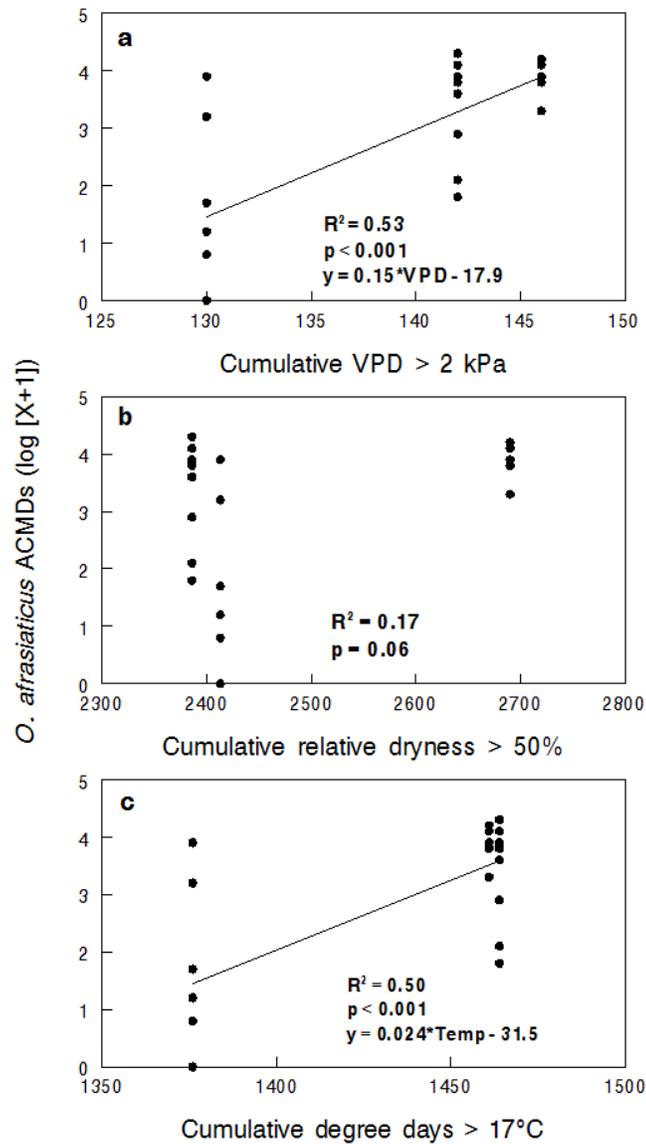


**Figure 1.3:** Daily means (calculated for each month separately) of the climatic conditions in 2007-2009: a) Vapor Pressure Deficit (VPD in kPa); b) relative dryness (inverse of relative humidity, %); and c) temperature (°C). Horizontal lines represent the threshold values used for each variable. Data are from the Israeli Meteorological Service weather station of Ardom R&D at Yotvata, Southern Arava Valley.

**Table 1.5:** Pearson correlation (r) matrix between the seasonal *Oligonychus afrasiaticus* adult cumulative mite days (ACMDs and logACMDs) and the three climatic variables (temperature, relative dryness, and VPD), accumulated from March 1<sup>st</sup> and from pollination (Poll.) start points (biofixes). N = 22 for all combinations.

	Temp (> 17°C)		RD (> 50%)		VPD (> 2 kPa)	
	Poll.	Mar 1 <sup>st</sup>	Poll.	Mar 1 <sup>st</sup>	Poll.	Mar 1 <sup>st</sup>
ACMDs	0.51 *	0.53 *	0.34 <sup>ns</sup>	0.29 <sup>ns</sup>	0.53 *	0.53 *
Log <sub>10</sub> ACMDs	0.71 **	0.71 **	0.42 <sup>ns</sup>	0.35 <sup>ns</sup>	0.73 **	0.72 **

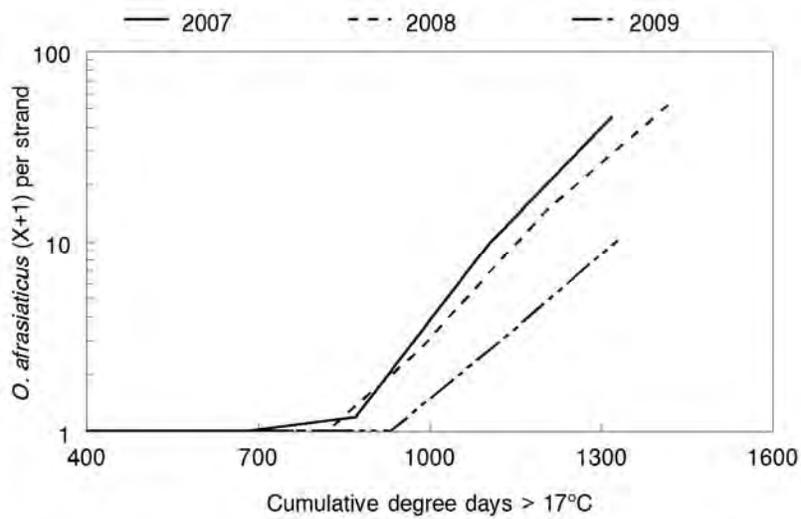
\* 0.01 < p < 0.05; \*\* p < 0.001; <sup>ns</sup> Not significant



**Figure 1.4:** *Oligonychus afrasiaticus* adult cumulative mite days (ACMDs, log X+1) in relation to: a) cumulative VPD above 2 kPa; b) cumulative relative dryness (inverse of relative humidity) above 50%; and c) cumulative degree days above 17°C. Each point represents one 'Barhi' tree from one season, Samar, 2007-2009, n = 22.

The increase of *O. afrasiaticus* population levels began at 870, 980, and 1020 DD (> 17°C) from pollination in 2007, 2008, and 2009, respectively, reaching a level of 10 adult female mites per strand at 1110, 1140, and 1350 DD, respectively (Fig. 1.5).

When calculating cumulative kPa (kPa-Days) above 2 kPa VPD, the pest population began to increase at 70, 70, and 88 kPa-Days in 2007, 2008, and 2009, respectively, reaching a level of 10 adult female mites per strand at 100, 88, and 120 kPa-Days, respectively.



**Figure 1.5:** Dynamics of adult female *Oligonychus afrasiaticus* (X+1) as a function of cumulative temperature (> 17°C) calculated from the first pollination action, 'Barhi', Samar, 2007-9.

## 1.4 Discussion

### 1.4.1 Assessment of the fast scouting tool

The population levels when webbings were first detected through the binoculars were only slightly higher (not significant) than those first detected by the commercial naked eye method. In addition, despite the higher number of spraying events following the naked eye method, the level of fruit damage was found to be similar for 'Medjool' or even lower for 'Barhi' when trees were sprayed according to binocular detection.

The monitoring intervals of 10 days used in the third year of the experiment were more effective than the 14-day intervals used in the first two years. These short intervals are particularly important during the phase when primary establishment of *O. afrasiaticus* and rapid growth of its population are expected. Accordingly, scouts should look for signs of webbing from early May through early July for 'Medjool' and 'Barhi', and from early June through early August for 'Deglet Noor' (Palevsky et al. 2003). It must be noted that in 2009, infestation was relatively low and late in the season, with a moderate rate of

population increase. It is therefore recommended that the time intervals between scouting sessions be shortened to one week.

There was a small and insignificant, but consistent, difference between the threshold populations detected for 'Medjool' and 'Barhi', with higher values found in 'Barhi' for both the binocular and the naked eye methods. I assume that this slight difference is derived from the variation in susceptibility to spider mite attack between the two cultivars (Palevsky et al. 2003). Populations of *O. afrasiaticus* tend to increase more rapidly on 'Barhi' fruit than on 'Medjool' fruit (personal observations). In addition, 'Barhi' is harvested early, in the unripe 'Khalal' stage, when even small mite damage is clearly seen. Consequently, early detection of *O. afrasiaticus* webbing is especially important on 'Barhi'.

Monitoring *O. afrasiaticus* populations is of great importance due to the rapid population growth of this primary pest (Coudin and Galvez 1976, Carpenter and Elmer 1978, Palevsky et al. 2003). A short delay in identifying the initial infestation, leading to a late application of control treatments, can result in substantial fruit and economic damage. Monitoring trees that are higher than 6 meters (ca.10 years and older) with the naked eye technique is laborious, dangerous if done with ladders, and extremely costly when using a mechanical tower. Using binoculars as a scouting tool for detecting *O. afrasiaticus* webbing can both be effective and reduce costs substantially, as each scout only needs to purchase a pair of good-quality binoculars (cost ranging between US\$1200-3000) and all monitoring can be done from the orchard floor.

#### 1.4.2 The effect of climatic variables on *O. afrasiaticus* population

The results of this study show that the seasonal cumulative mite days (ACMDs) is positively related to cumulative DD and to cumulative VPD. The effect of temperature on arthropod populations, including spider mites, is well recognized, and DD models are commonly used for predicting insect and mite pest populations in agro-systems (Broufas and Koveos 2000, Gotoh et al. 2004a, Pedigo and Rice 2009). While the effect of air humidity or VPD on spider mite growth is also known (Hazan et al. 1973, Holtzer et al. 1988), it is hardly used for the prediction of mite population dynamics. Beyond confirming the importance of temperature, the results of this study indicate that relative humidity and VPD also have a considerable effect on the seasonal population levels of *O. afrasiaticus*.

Different climatic effects on *O. afrasiaticus* populations have been reported by Palevsky et al. (2005), following a three-year (2001-2003) study of 'Barhi', 'Medjool', and 'Deglet

Noor' orchards at Yotvata in the SAV. The highest ACMDs were recorded in 2002, the year that had the coolest spring (May), while lower ACMDs were observed in 2003, with the warmest spring. They proposed that a warmer spring expedites fruit development, subsequently shortening the period for *O. afrasiaticus* population build-up (Palevsky et al. 2005). Using the raw data of the 'Barhi' trees (E. Palevsky, personal communication) and relating it to climate variables, I found a significant negative correlation between seasonal ACMDs and cumulative degree-days ( $> 17^{\circ}\text{C}$ ) ( $r = -0.85$ ,  $n = 15$ ,  $p < 0.001$ ), in agreement with their hypothesis. I also found a non-significant positive trend with relative dryness ( $\text{RH} < 50\%$ ).

Although temperature and relative humidity in 2001-3 and 2007-9 were within the same range, other factors found to differ between these two groups of years may have contributed to the above discrepancy. For example, there were huge differences in the ACMDs and infestation timing between the two groups. The pest population in 2001-3 was ca. 2-3-fold higher and started 4-7 weeks earlier than in 2007-9. Differences of agro-technique methods applied to the conventional orchard (Yotvata, 2001-3) in comparison to the organic one (Samar, 2007-9) could also contribute to this inconsistency. However, in 2002, the year with the highest ACMDs of 2001-3, February through April were warmer than in 2003 and dryer than in both 2001 and 2003. In the current study, March-April 2008 were the warmest and driest months of 2007-9. Taking both studies into consideration, it appears that the effects of climatic conditions from February onward had a dramatic influence on pest dynamics, possibly due to the mode of initial *O. afrasiaticus* infestation on the young bunches. These findings suggest that warm and dry weather in early spring enhances the establishment of *O. afrasiaticus* on immature fruit bunches.

Hazan et al. (1973) showed that, in a certain range, the development of the two-spotted spider mite, *Tetranychus urticae*, is accelerated when temperature increases and relative humidity decreases. Outbreaks of this mite in the field were also related to high-temperature and low-humidity conditions (Kumral and Kovanci 2005).

Such effects of temperature and humidity on the life tables of the new world date mite, *O. pratensis*, have been thoroughly studied. While temperature was found to be the major abiotic factor influencing the growth rate of *O. pratensis* (Congdon and Logan 1983, Perring et al. 1984b), relative humidity or VPD (as a measure for absolute humidity) appeared to be of some importance as well, especially when combined with temperature (Perring et al. 1984a). The growth rate was found to increase with temperature up to  $37^{\circ}\text{C}$

and then decline. While the influence of VPD was negligible at low temperatures, higher VPD (lower humidity) was found to increase the rate of development under warmer conditions, with the maximum effect found at 36°C (Perring et al. 1984a, 1984b). The simulation model based on these data showed how important VPD is for the population dynamics of *O. pratensis* in the field (Berry et al. 1991). This model and the above results were also consistent with the field observation that outbreaks of *O. pratensis* are timed with hot and dry weather (Perring et al. 1984a).

*Oligonychus afrasiaticus* seems to have analogous performance on a similar temperature range (Coudin and Galvez 1976, Al-Sweedy et al. 2006). In Mauritania, Coudin and Galvez (1976) recorded higher *O. afrasiaticus* longevity, fecundity, and survival at 25.5°C with 38% RH, in comparison to higher or lower temperature with 60-70% RH. In addition, the intrinsic rate of increase at 31°C with 60% RH was superior than at 25°C with 70% RH (Coudin and Galvez 1976). However, in regard to the effect of humidity in the field, there are only circumstantial clues suggesting that higher demographic values are attained under dry conditions. In Iraq and the Arabian Peninsula, infestations were highest and damage most severe in arid dry regions, whereas the date palms grown adjacent to wells or large bodies of water had a far lower incidence of infestation (Hussain 1969, Talhouk 1991). In Israel, pest outbreaks are common in the extremely arid regions of the Central and Southern Arava Valley, while occurring only sporadically around the Dead Sea and not at all in the more humid date-palm growing regions of the Jordan and Beit-Shean Valleys (Palevsky et al. 2005).

The aerial dispersal of spider mites is also affected by air humidity. Laboratory results and field observations have indicated that low relative humidity stimulates the aerial dispersal of *Tetranychus urticae* (Smitley and Kennedy 1988). It has been suggested that aerial dispersal is also an important component in the life history of *O. pratensis* (Holtzer et al. 1984). In the laboratory, the adult females of *O. pratensis* exhibit an aerial dispersal posture more often when held at low humidities (Margolies 1987). If *O. afrasiaticus* displays similar dispersal behavior in relation to air moisture, then low relative humidity in early spring could advance its initial establishment on fruit bunches and its later distribution from infested to non-infested bunches. Wind has also been found to play an important role in spider mite aerial distribution (Smitley and Kennedy 1988, Lawson et al. 1996), though its effect on *O. afrasiaticus* was not included in this study.

Laboratory studies of temperature and humidity effects on the growth rate of *O. afrasiaticus* are needed to determine developmental rate curves and threshold values. Also needed are the monitoring of population dynamics and climatic data from different date palm varieties during several successive years. This information should provide the foundation for using climate-based prediction models, such as CLIMEX.

#### 1.4.3 Summary

Combining the results from the two parts of this chapter can optimize the monitoring and control of *O. afrasiaticus* in the SAV. The climate model allows us to predict the expected timing of initial infestation, thereby enabling growers to concentrate their binocular monitoring efforts into a short period of time when the mites are expected to establish. For this short period, reducing the time intervals between scouting sessions to one week (instead of ten days) will improve pest detection without increasing overall scouting efforts. Because the results of the DD and cumulative VPD models were similar, relying on the former is suggested for the sake of simplicity. Accordingly, the timing of the initial infestation of *O. afrasiaticus* (1 mite/strand) is 870-1020 DD from the initiation of pollination. It is thus recommended that the binoculars scouting (or other monitoring methods) be started no later than 800 DD. However, while this model is true for the 'Barhi' orchard at Samar kibbutz farm, for other cultivars, as well as in different locations, it must be further adjusted.

## 2A Climatic and nutritional effects on demographic parameters of potential predatory mite species

### **2A.1 Introduction**

Commercially available acarine biocontrol agents (ABAs), primarily predatory mites of the family Phytoseiidae, have been successfully used to control spider mites in the Mediterranean basin and temperate regions. However, the application of these ABAs to agricultural systems in arid regions has been fraught with difficulties (Gerson and Weintraub 2007, Walzer et al. 2007). In general, dry ambient conditions enhance spider mite development (Hazan et al. 1973, Perring et al. 1984a), while suppressing phytoseiid populations (Sabelis 1985, Bakker et al. 1993). Phytoseiids, like many terrestrial arthropods, have evolved physiological as well as behavioral attributes in order to maintain their water balance and to cope with moisture limitations (Hadely 1994). Their motile stages can uptake water from their prey (Sabelis 1985), actively absorb water vapor from surrounding air (Gaede 1992, Yoder 1998), reduce water loss through body surface and respiration (Yoder 1998), and escape from sunlit, warm and dry habitats to darker, cooler and more moist ones (Bernstein 1983, Auger et al. 1999). Certain species are even able to take water directly from their host plant (Nomikou et al. 2003, Porres et al. 1975). Gravid females can also choose an oviposition site where humidity is likely to be higher so as to assure egg survival (Grostal and O'Dowd 1994). However, the egg itself is more vulnerable, since it cannot escape or absorb water vapor from the environment, and is therefore considered to be the most drought-sensitive phytoseiid life stage (Sabelis 1985, Bakker et al. 1993).

While specialist phytoseiids can provide fast and effective control in protected crops, generalist phytoseiids may be more suitable for long-term control in orchard agrosystems (McMurtry 1992, McMurtry and Croft 1997). The diet of generalist phytoseiids includes pollen, fungi, and other food types, thus allowing them to cope with low prey densities (McMurtry and Croft 1997). Indigenous generalist phytoseiids, as opposed to exotic species, are adapted to the local environment and prey (McMurtry 1992, Gerson and Vacante 1993, Escudero and Ferragut 2005). Furthermore, before importation and release of a generalist exotic species, lengthy procedures, such as risk assessments and product registrations, may be required, depending on the country and quarantine practices (Van Lenteren et al. 2006).

*Oligonychus afrasiaticus* causes its most extensive damage to date palms in arid regions throughout North Africa and the Middle East (Palevsky et al. 2003). The pest establishes itself rapidly on fruit bunches from May through July, when the mean air humidity in the Southern Arava Valley (SAV) is as low as 30-35% (IMS meteorological station at SAV R&D). The Banks grass mite, *O. pratensis*, is a major pest of field crops (Perring et al. 1984a, 1984b) and date palms (Gispert et al. 2001) in North America. Its population dynamics and damage to date palm fruit in California are similar to those of *O. afrasiaticus*. Perring et al. (1984a) examined *O. pratensis* performance on corn at different temperatures and Vapor Pressure Deficit (VPD = SD) values and found that its optimal conditions are 36°C with 17% RH (VPD = 4.8 kPa). These conditions were consistent with the field observation that Banks grass mite outbreaks are timed with hot and dry weather (Perring et al. 1984a). Although the optimal range of temperature and humidity for *O. afrasiaticus* development is unknown, it is likely to have a similar range. None of the (commercial) biological control agents, particularly phytoseiids, are known to be effective under such extreme arid conditions. It therefore seems promising to use the local generalist phytoseiids found in date palm orchards (Palevsky et al. 2009) or other dry-adapted species inhabiting this region.

The aim of this chapter was to evaluate the potential of five species and strains of indigenous phytoseiids inhabiting the date palm cultivating region of the Arava in order to control the old world date mite. This was accomplished by: 1) determining the effect of humidity on egg hatching and developmental time, and 2) assessing the ability of these indigenous phytoseiids to establish a population and efficiently control the pest on date palm bunches in the SAV.

## **2A.2 Methods**

### 2A.2.1 Phytoseiid cultures

Three species of phytoseiid mites, *Typhlodromus athiasae* Porath & Swirski, *Cydnoseius negevi* (Swirski & Amitai), and *Neoseiulus longilaterus* (Athias-Henriot) were collected from a variety of plant species taken from within and around date palm orchards in the Dead Sea and the Arava Valleys in Israel (see Table 2A.1 for collection details). For establishing cultures, predatory mites were collected by beating plant material over a

collection tray and by extraction with Berlese funnels. A northern strain of *T. athiasae* was collected from an organic grapefruit (*Citrus paradise*) orchard at Beit Shearim in the Jezreel Valley. The mites were reared on black plastic coasters (12cm diameter), placed upside down on poly-acryl gel, serving both as a water source and as a barrier (Argov et al. 2006). A mixture of pollen of oak (*Quercus ithaburensis*), cattail (*Typha domingensis*), and maize (*Zea mays*), was provided twice a week. After the populations were established, cultures were identified according to Swirski et al. (1998). In order to evaluate another dry-adapted species also inhabiting the Arava Valley (Swirski and Amitai 1997), *Euseius scutalis* (Athias-Henriot) was collected from avocado orchards in the western Galilee and reared on *Solanum nigrum* with oak pollen. Two other species, *Neoseiulus barkeri* Hughes and *Typhlodromus hierochunticus* Amitai & Swirski, were also collected from the Dead Sea rift (Table 2A.1). However, their lab cultures were too small for further evaluation.

Three experiments were carried out, one in the lab and two in the field. The field experiments were conducted on young (< 10 years old) trees, 'Medjool' cultivar (CV), at Grofit date palm farm in Israel's Southern Arava Valley.

**Table 2A.1:** Sites and host plants of the phytoseiid species/strains collections

Species/strain	Site	Host plant	Location
<i>Typhlodromus athiasae</i> Arava	Neot Smadar <sup>a</sup>	<i>Cynodon dactylon</i>	30°00'N; 35°05'E
<i>T. athiasae</i> Jezreel Valley	Beit-Shearim <sup>b</sup>	<i>Citrus paradisi</i>	32°42'N; 35°11'E
<i>Neoseiulus longilaterus</i>	Ein-Tzin <sup>c</sup>	<i>Juncus acutus</i>	30°53'N; 35°09'E
<i>Cydnoseius negevi</i>	Samar <sup>a</sup>	<i>C. dactylon</i> , <i>Phragmites australis</i>	29°49'N; 35°02'E
	Neot smadar <sup>a</sup>	<i>C. dactylon</i>	30°00'N; 35°05'E
	Eilot <sup>a</sup>	<i>P. australis</i> , <i>Echinochloa</i> sp.	29°37' N; 34°59'E
	Grofit <sup>a</sup>	<i>C. dactylon</i>	29°56'N; 35°04'E
	Elifaz <sup>a</sup>	<i>Echinochloa</i> sp.	29°48'N; 35°02'E
<i>Euseius scutalis</i>	Gaaton <sup>d</sup>	<i>Persea americana</i>	30°00'N; 35°10'E
<i>Neoseiulus barkeri</i> <sup>e</sup>	Neot Smadar <sup>a</sup>	<i>Cynodon dactylon</i>	30°00'N; 35°05'E
<i>Typhlodromus hierochunticus</i> <sup>e</sup>	Ein-Gedi <sup>a</sup>	<i>Salvadora persica</i>	31°27'N; 35°23'E

<sup>a</sup> Date palm orchard

<sup>b</sup> Organic citrus orchard

<sup>c</sup> Oasis

<sup>d</sup> Avocado orchard

<sup>e</sup> Culture was too small for further evaluation

### 2A.2.2 Egg hatching of indigenous phytoseiids at different humidity levels

Even-aged (< 18h) cohorts of eggs were obtained by placing adult gravid females of *C. negevi*, *N. longilaterus* and the two strains of *T. athiasae* on plastic plates surrounded by filter paper immersed in water, with glass coverslips plus synthetic fibers beneath, as oviposition sites. *Euseius scutalis* females were placed on *S. nigrum* leaves floated on poly-acryl-amide gel. A mixture of oak, cattail, and maize pollen was supplied every 2-3 days. Newly laid eggs were collected twice a day and kept at 4°C on a wet filter paper in a closed Petri dish. The experimental arenas, 1.5cm diameter holes in plexiglass slides (10cm X 4cm X 0.5cm), were laser cut in order to avoid cracking and drilling imperfections. The arenas were sealed at the bottom with fine mesh glued to the plexiglass slide and at the top with a glass microscope slide fastened to the plexiglass with a rubber band.

The eggs were set on the mesh screen, with 8-20 eggs/arena, and the arenas were placed in small humidity chambers constructed from plastic boxes. Relative humidities within these chambers were maintained by saturated salt solutions of MgCl<sub>2</sub>\*6H<sub>2</sub>O (32.5%); Ca(NO<sub>3</sub>)<sub>2</sub>\*4H<sub>2</sub>O (47%); Mg(NO<sub>3</sub>)<sub>2</sub>\*6H<sub>2</sub>O (52%); NaNO<sub>2</sub> (63%); NaCl + KCl (71%); and KCl (84.5%) (Winston and Bates 1960). The plastic boxes were placed in 30 ± 1.5°C (resembling the Arava mean temperature in May-July) and 16L:8D. Climate conditions were monitored by temperature and humidity data loggers (Hobo U-14-001, Onset®, MA, USA).

For comparison sake, humidity levels are presented both in relative humidity (% RH) and absolute humidity values (Vapor Pressure Deficit [VPD] = Saturation Deficit [SD]). VPD was calculated based on the temperature (30°C) and relative humidity (see General Methods).

Every 24h up to three days (almost no egg hatched beyond the third day), egg state (i.e., shriveled, hatched, not hatched) was examined, larvae were removed, and egg developmental time was recorded. Eggs that were damaged due to handling were excluded. For each humidity level and species/strain, 30 – 200 eggs were examined. When no eggs hatched (100% mortality) after examining at least 30 eggs at a specific humidity level, egg hatching was considered as 0%.

### *Statistical analysis*

The effect of species/strain and relative humidity on egg hatching probabilities was analyzed with two-way ANOVA (with each arena being a replicate). Probit analysis with natural log transformation was performed in order to compare the effect of humidity on the hatching probability curve among species/strains and also to compute RH<sub>50</sub> and VPD<sub>50</sub> values (when 50% of the eggs hatched). Comparison of egg development time among species/strains (within RH) and among RH (within species/strain) was performed using Kruskal-Wallis, followed by pairwise Mann-Whitney tests with Bonferroni correction.

### 3.2.3 Establishment of released indigenous phytoseiids on date palm bunches

Establishment of three released indigenous phytoseiid species was assessed in 2008 on 20 young trees, 'Medjool' cultivar (CV), bearing four isolated bunches. Each bunch served as one sampling unit, with each tree forming a block, arranged in a (randomized) split-plot block design. All fruit stalks were applied with sticky barriers, consisting of Rimifoot paste (Rimi Chemicals Co. Ltd, Petach Tikva, Israel) and Petroleum jelly (Vaseline™), in order to prevent ambulatory mites from leaving/accessing the fruit bunches. Thirty adult females of *T. athiasae*, *C. negevi*, and *N. longilaterus* were released on May 2008 during the 'kimri' fruit stage on three bunches of each tree, with one species per bunch, while the fourth bunch served as a control. Two days before release, groups of thirty adult females were collected from the lab culture with an aspirator constructed from a pipette tip (200 µl), which served as a ventilated cage. The tips contained a small piece of wet filter paper as a water/humidity source and maize pollen as food and were kept in an ice box (~5-10°C, ~50% RH) until release.

In the field, for each bunch one tip of the respective species was partially cut and placed in an open Eppendorf 1.5ml tube, previously attached to the bunch. In order to assess the importance of pollen as an alternative food source, maize pollen was applied weekly to all bunches of half the trees (10 of the 20 trees, randomly chosen) using an electrostatic sprayer following Gan-Mor et al. (2003) as described in the General Methods section. After four and six weeks, 5 and 10 strands, respectively, were sampled from each bunch and washed in 70% ethanol. All phytoseiids were then collected and identified.

At harvest, mite damage was assessed by sorting and grading approximately 100 fruit per bunch according to the standard packing house practice for 'Medjool' fruits (see General Methods). If the bunches had less than 100 fruit, then all the fruit were sorted.

#### *Statistical analysis*

The effect of species and pollen variables on fruit damage was analyzed by split-plot ANOVA. Since the number of predatory mites included many zeros and did not have a normal distribution, the differences between the three species and the control within pollen treatments were analyzed with the Friedman test, and the differences between pollen treatments within the predatory species were analyzed by the Mann-Whitney U-test.

#### 3.2.4 Establishment and pest control of released indigenous phytoseiids on date palm trees

The ability of the Arava strain of *T. athiasae* and *E. scutalis* to establish and control *O. afrasiaticus* populations and damage on the tree level was evaluated on 42 young 'Medjool' trees bearing 4-6 bunches. In a random block design, every six trees (constituting a block) received all combinations of species treatment (three levels: *T. athiasae*, *E. scutalis*, and control) and pollen treatment (two levels: with and without pollen), repeated seven times. Predatory mites were mass reared at Bio-Bee (Sde-Eliyahu, Israel).

In mid-May 2009, during the 'kimri' fruit stage, three applications of 120-150 predatory mites per bunch were carried out, with a fortnight interval between applications (*E. scutalis*: ca. 410 per bunch and 1600-2400 per tree; *T. athiasae*: ca. 360 per bunch and 1200-2000 per tree). Predatory mites + rearing medium within handmade net bags were attached to fruit strands on all bunches. The releasing medium of *T. athiasae* consisted of its rearing medium, which included bran and the prey mite *Carpoglyphus lactis* L. On the other hand, *E. scutalis* was collected from plants and mixed with vermiculite for the release. Before each release session, counts of mites per gram in the releasing material were performed using Berlese funnels, while collecting the individuals in 70% ethanol. This was followed by species verification under a compound microscope. Maize pollen was applied every fortnight, as described in the General Methods section.

At the beginning of June, in order to even out the pest infestation level, all bunches that were not naturally infested were manually infested by attaching infested fruit to the uninfested bunches (mean numbers of mites released varied from 35 to 480 mites/bunch).

Pest and predator mite population levels were monitored every fortnight by sampling eight strands from each tree (two strands from four randomly chosen bunches) and washing them in 70% ethanol. *Oligonychus afrasiaticus* cumulative mite days (CMDs) calculations, phytoseiids counts, and the evaluation of fruit damage on 100 fruits per tree, were done as described in the General Methods section.

### *Statistical analysis*

Since the number of predatory mites included many zeros and did not have a normal distribution, the differences between the two species and the control within pollen treatments were analyzed with the Kruskal-Wallis test, followed by multiple Mann-Whitney U-tests with Bonferroni correction. The differences between pollen treatments within the predatory species were analyzed by the Mann-Whitney U-test. The comparisons of *O. afrasiaticus* CMDs and fruit quality on harvest between species and pollen treatments were analyzed by two-way ANOVA with blocks. The relations between phytoseiid numbers, pest CMDs, and fruit damage were analyzed by Spearman non-linear correlation coefficient.

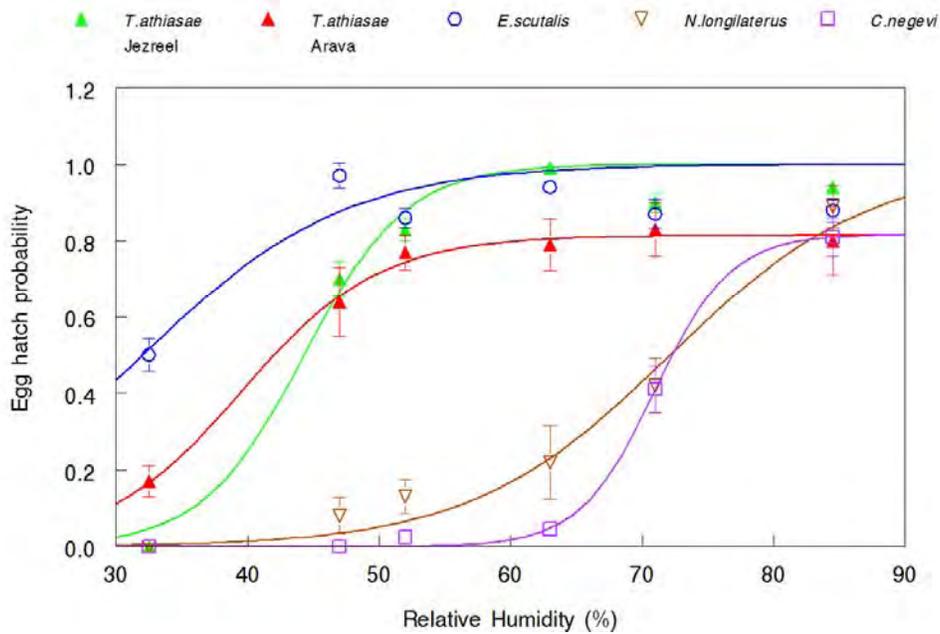
## **2A.3 Results**

### 2A.3.1 Egg hatching of indigenous phytoseiids at different humidity levels

#### 2A.3.1.1 Hatching probability

Egg hatching probability was affected by species/strain ( $F_{4,206} = 155$ ,  $p < 0.001$ ), by the level of relative humidity ( $F_{5,206} = 103$ ,  $p < 0.001$ ), and by the interaction between species/strain and humidity ( $F_{20,206} = 14.7$ ,  $p < 0.001$ , Fig. 2A.1). At 84.5% RH (0.66 kPa), all species/strains showed the highest hatching probability. Below this level, the hatching probability of *C. negevi* and *N. longilaterus* decreased sharply, with RH of less than 25% egg hatching at 63% RH (1.57 kPa). Egg hatch reduction of the two strains of *T. athiasae* and *E. scutalis* was only initiated below 52% RH (2.03 kPa). At the lowest RH level (32%, 2.86 kPa), 53% and 16% of the eggs of *E. scutalis* and *T. athiasae*-Arava, respectively, still hatched, while the eggs of the northern *T. athiasae* strain did not.

Because the hatching probability curves were not parallel ( $\chi^2 = 214$ ,  $df = 4$ ,  $p < 0.001$ ), each species/strain was analyzed separately (Probit analysis, Fig. 2A.1, Table 2A.2). The humidity values allowing 50% survival of the eggs ( $RH_{50}/VPD_{50}$ ) ranged from 25.9 % RH (3.14 kPa) for *E. scutalis* to 69% RH (1.31 kPa) and 73.9% RH (1.11 kPa) for *C. negevi* and *N. longilaterus*, respectively, with the two strains of *T. athiasae* having similar intermediate values (ca 44.5% RH, 2.35 kPa) (Table 2A.2). According to confidence level (95%) overlaps the  $RH_{50}/VPD_{50}$  values of *E. scutalis* and *T. athiasae* (both strains) were significantly different from those of *C. negevi* and *N. longilaterus*. At 32-71% RH (2.86-1.23 kPa), shriveling was observed among the non-hatched eggs of *C. negevi* and *N. longilaterus*, but not among *T. athiasae* and *E. scutalis* eggs.



**Figure 2A.1:** Observed (symbols) and predicted (lines) egg hatching proportions of phytoseiid species and strains after three days at 30°C at different levels of relative humidity. Each symbol represents the mean ( $\pm$  SE) hatching proportion per arena. The curve fitting was based on the egg as the sample unit.

Table 2A.2: Probit analysis results: RH<sub>50</sub> (%), VPD<sub>50</sub> (in parentheses, in kPa), confidence intervals (95%), equation parameters and  $\chi^2$  values of egg hatching of different phytoseiid species/strains.

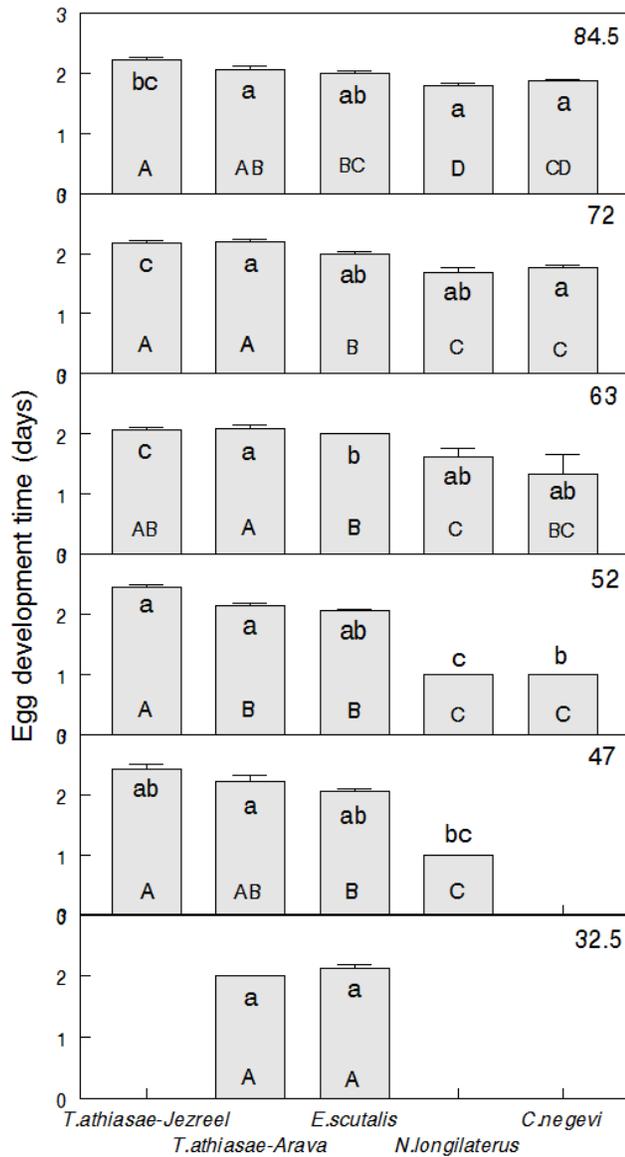
		<i>T. athiasae</i> Arava	<i>T. athiasae</i> Jezreel Valley	<i>E. scutalis</i>	<i>N. longilaterus</i>	<i>C. negevi</i>
RH <sub>50</sub>		44.7 (2.34)	44.2 (2.36)	25.9 (3.14)	69.0 (1.31)	73.9 (1.11)
95% Confidence level		29.6-54.8	12.7-55.8	0-39.1	63.4-76.6	70.5-77.9
Equation parameters*	B	2.15	3.60	1.37	4.58	6.54
	Intercept	-8.18	-13.65	-4.46	-19.36	-28.11
$\chi^2$		16.9	114.1	20.3	10.7	10.7

\* Equation formula: Probit (egg hatch) = Intercept + B\*ln (RH)

#### 2A.3.1.2 Egg development time

Egg development time differed according to species/strain ( $\chi^2 = 287$ , df = 4, p < 0.001) and humidity levels ( $\chi^2 = 74$ , df = 5, p < 0.001) (Fig. 2A.2). The development time of *T. athiasae*-Jezreel was always longer than that of *C. negevi* and *N. longilaterus*, whereas those of *T. athiasae*-Arava and *E. scutalis* were intermediate. The development time of the surviving eggs of *C. negevi* and *N. longilaterus* was significantly shorter at lower RH values (*C. negevi*:  $\chi^2 = 22.3$ , df = 3, p < 0.001; *N. longilaterus*:  $\chi^2 = 37.8$ , df = 4, p < 0.001). The development time of the surviving eggs of *T. athiasae*-Jezreel was significantly longer at lower RH values ( $\chi^2 = 53.6$ , df = 4, p < 0.001). *E. scutalis* had a smaller, but similar, trend ( $\chi^2 = 12.5$ , df = 4, p < 0.05), whereas the egg development time of *T. athiasae*-Arava remained without change at all humidity levels ( $\chi^2 = 5.9$ , df = 4, p > 0.05).

The effect of RH on egg development time among the different species and strains is clearer when presented graphically as the proportion of eggs hatched on each observation day (Fig. 2A.3). At the higher humidities (84.5%, 72% and 63% RH), 13-23% of *T. athiasae*-Jezreel eggs hatched on the third day, while at 52% and 47% RH, that proportion increased to 50% and 43%, respectively. Between 47-84.5% RH, the proportion of hatching eggs on the third day remained nearly constant for *T. athiasae*-Arava and *E. scutalis* (6-21% and 0-5%, respectively).



**Figure 2A.2:** Mean (+ SE) egg development time (in days) of the different species/strains at different humidity levels. Same letters on the bars indicate non-significant ( $p \geq 0.05$ ) differences, with upper-case letters indicating comparison between species/strains within RH and lower-case letters indicating comparison between RH within species/strains (multiple pairwise Mann-Whitney U tests with Bonferroni correction).

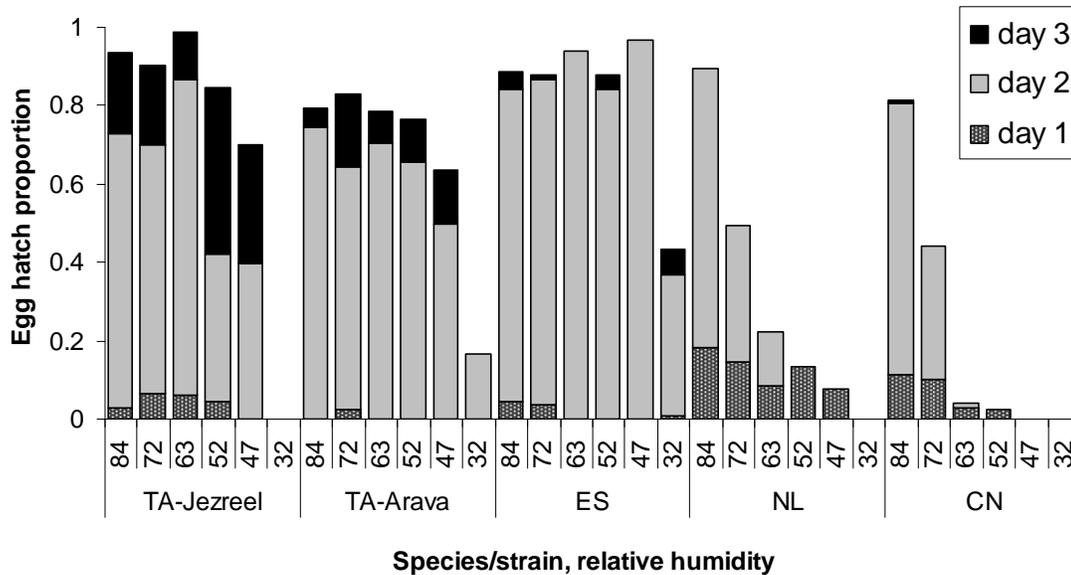
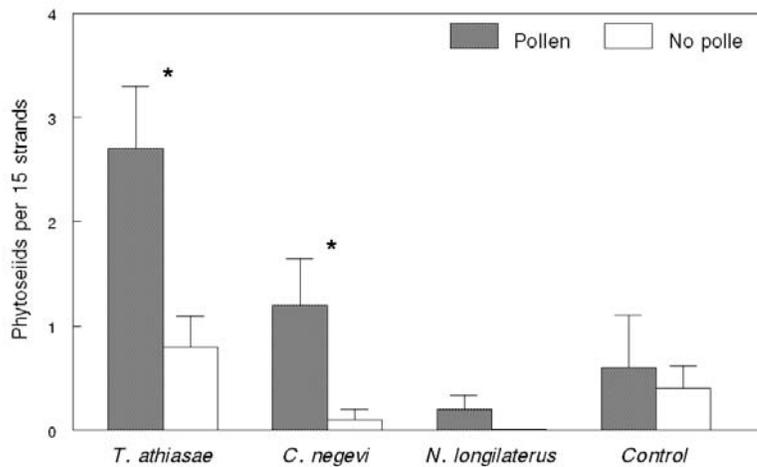


Figure 2A.3: Egg cumulative hatch proportion at different relative humidity levels, after three days, with an indication of the proportion hatched on each day. TA – *T. athiasae*; ES – *E. scutalis*; NL – *N. longilaterus*; CN – *C. negevi*.

### 2A.3.2 Establishment of indigenous phytoseiids on date palm bunches

Among the three species evaluated, *T. athiasae* showed the highest establishment with and without the provision of pollen (with pollen:  $\chi^2_3 = 14.2$ ,  $p < 0.05$ ; without pollen:  $\chi^2_3 = 7.7$ ,  $p = 0.053$ ; Fig. 2A.4). In addition, over 25% of its specimens were identified as immature stages (larvae, protonymph or deutonymph). However, more individuals were counted on bunches that received pollen applications for *T. athiasae* (Mann-Whitney U-test,  $Z = 2.44$ ,  $n = 20$ ,  $p < 0.05$ ) and also for *C. negevi* ( $Z = 2.11$ ,  $n = 20$ ,  $p < 0.05$ ) (Fig. 2A.4).



**Figure 2A.4:** Mean (+ SE) number of phytoseiid individuals per 15 strands, separately sampled from each bunch treatment, with and without maize pollen, 'Medjool', Grofit, 2008. Asterisks indicate significant differences ( $p < 0.05$ ) between pollen treatments within predator species (Mann-Whitney U test). All specimens found on the control bunches were identified as *T. athiasae*. For all columns,  $n = 10$  trees.

Extrapolating from sample size (15 strands) to the bunch level (ca. 50 strands per bunch), *T. athiasae*'s recovery with pollen was 30% of the releasing population (30 females per bunch), whereas the recovery without pollen and recovery in the other two species was less than 15% (Table 2A.3). No effect of either species or pollen was found in the fruit damage, as evaluated at the end of the season (Table 2A.4).

**Table 2A.3:** Mean ( $\pm$  SE) number of phytoseiid individuals per bunch (extrapolated from the number per sample), 'Medjool', Grofit, 2008. In parentheses are the percentages in relation to the initial released population.

Treatment/species	<i>T. athiasae</i>	<i>C. negevi</i>	<i>N. longilaterus</i>
With pollen	9.0 $\pm$ 2.0 (30.0)	4.0 $\pm$ 1.5 (13.3)	0.7 $\pm$ 0.4 (2.2)
Without pollen	2.7 $\pm$ 1.0 (8.9)	0.3 $\pm$ 0.3 (1.1)	0

Table 2A.4: Mean ( $\pm$  SE) percentages at each level of fruit damage for all treatment combinations (n = 10 trees) in the establishment experiment, 'Medjool', Grofit, 2008.

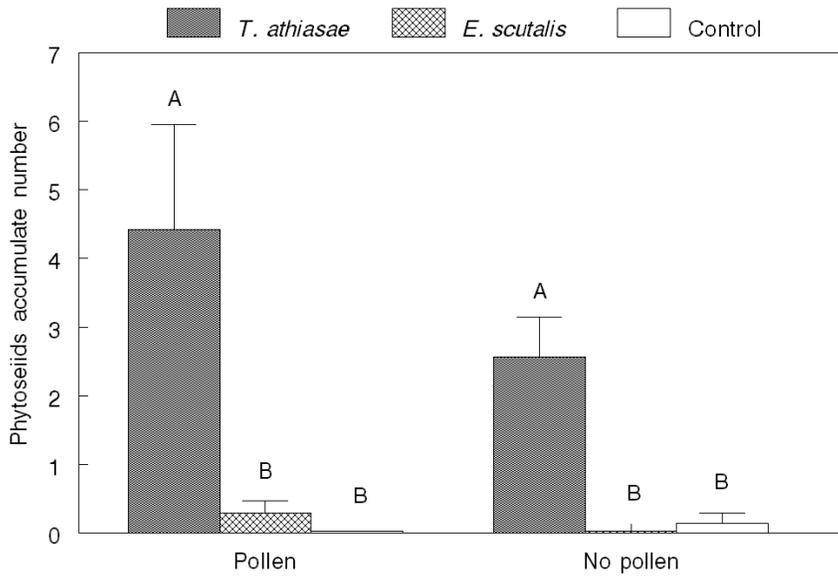
Fruit class <sup>a</sup>	Treatment	<i>T. athiasae</i>	<i>C. negevi</i>	<i>N. longilaterus</i>	Control	p <sup>b</sup>
A	Pollen	75.9 $\pm$ 9.4	75.1 $\pm$ 9.1	66.0 $\pm$ 11.5	72.9 $\pm$ 7.5	ns
	No pollen	73.5 $\pm$ 9.4	69.7 $\pm$ 8.9	68.2 $\pm$ 7.8	54.1 $\pm$ 13.0	
B	Pollen	12.2 $\pm$ 4.3	10.4 $\pm$ 2.4	12.1 $\pm$ 3.3	21.8 $\pm$ 4.9	ns
	No pollen	24.4 $\pm$ 9.3	23.0 $\pm$ 6.1	24.4 $\pm$ 4.5	22.0 $\pm$ 8.1	
C+D	Pollen	11.9 $\pm$ 9.9	14.5 $\pm$ 9.8	21.9 $\pm$ 13.0	5.4 $\pm$ 3.5	ns
	No pollen	2.1 $\pm$ 1.0	7.3 $\pm$ 4.4	7.3 $\pm$ 4.5	4.9 $\pm$ 3.1	

<sup>a</sup> A – Export; B – Local market; C+D – Industrial and discarded fruits

<sup>b</sup> Split-plot Two-way ANOVA

### 2A.3.3 Establishment and pest control of indigenous phytoseiids on date palm trees

In comparison to *E. scutalis* and the control, *T. athiasae* showed the highest establishment with and without the provision of pollen (with pollen:  $\chi^2 = 12.3$ , df = 2, p = 0.002; without pollen:  $\chi^2 = 13.8$ , df = 2, p = 0.001; Fig. 2A.5). Throughout the experiment, 51 specimens were identified as *T. athiasae* (one in the control–pollen treatment, one in *E. scutalis*+pollen, and all the rest in *T. athiasae* treatments), and two were identified as *E. scutalis* (in *E. scutalis* +pollen). Although the phytoseiid population size in the *T. athiasae* treatment was higher on the trees provisioned with pollen, the trend was not significant (Wilcoxon Ranks test: Z = 1.4, df = 6, p = 0.17; Fig. 2A.5). Pest CMDs and level of damage did not differ between predators and pollen treatments (Table 2A.5).



**Figure 2A.5:** Mean (+ SE) phytoseiid numbers per eight strands (accumulated count per tree) of 'Medjool', Grofit, 2009. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (multiple Mann-Whitney U tests with Bonferroni correction).

**Table 2A.5:** Mean ( $\pm$  SE) of *Oligonychus afrasiaticus* Cumulative Mite Days (CMDs) per strand and percentages at each level of fruit damage for all treatment combinations ( $n = 7$  trees) in the establishment and control experiment, 'Medjool', Grofit, 2009

			<i>T. athiasae</i>	<i>E. scutalis</i>	Control	$p^b$
<i>O. afrasiaticus</i> - CMDs		Pollen	686 $\pm$ 299	869 $\pm$ 312	474 $\pm$ 160	ns
		No pollen	441 $\pm$ 208	717 $\pm$ 294	537 $\pm$ 347	
Damage evaluation <sup>a</sup>	Class A	Pollen	84.7 $\pm$ 5.3	79.5 $\pm$ 5.3	80.8 $\pm$ 2.6	ns
		No pollen	75.4 $\pm$ 7.4	72.4 $\pm$ 7.5	77.2 $\pm$ 5.7	
	Class B	Pollen	14.3 $\pm$ 4.8	19.0 $\pm$ 4.7	17.2 $\pm$ 2.4	ns
		No pollen	23.4 $\pm$ 7.3	24.4 $\pm$ 6.1	21.1 $\pm$ 4.9	
	Class C+D	Pollen	1.0 $\pm$ 0.6	1.6 $\pm$ 0.9	1.9 $\pm$ 0.6	ns
		No pollen	1.2 $\pm$ 0.7	3.2 $\pm$ 1.7	1.7 $\pm$ 0.8	

<sup>a</sup> A – Export; B – Local market; C+D – Industrial and discarded fruits

<sup>b</sup> Two-Way ANOVA with blocks

During July, when infestation was highest just before harvest, the *O. afrasiaticus* CMD was negatively correlated with the lower damage classes A+B ( $r_s = -0.32$ ,  $n = 42$ ,  $p = 0.039$ ). There was no correlation between the phytoseiid numbers and pest CMDs. However, when considering only the *T. athiasae* trees ( $n = 14$ ; in the other trees, phytoseiid

numbers were mostly zero), the phytoseiid numbers had non-linear correlations with fruit damage: positive with class A ( $r_s = 0.58$ ,  $p = 0.029$ ) and negative with class B ( $r_s = -0.57$ ,  $p = 0.035$ ) and class C ( $r_s = -0.61$ ,  $p = 0.022$ ).

## 2A.4 Discussion

Drought tolerance is a key factor for establishing a predatory mite population on date palm bunches. Not only is the environment extremely hot and dry, but also the fruit and strands are smooth, lacking pubescence/hairs or domatia. Such plant structures are positively related to phytoseiid retention (Loughner et al. 2010) by serving as an oviposition shelter (Walter 1996), capturing wind-born food (Roda et al. 2003), or protecting predatory mites from desiccation (Grostal and O'Dowd 1994, Rowles and O'Dowd 2009). Thus, their absence is a major obstacle for phytoseiid establishment on date palms.

### 2A.4.1 Drought tolerance of the egg in indigenous phytoseiid species

#### 2A.4.1.1 Egg hatching probability

The egg is the most vulnerable developmental stage to low humidity (Sabelis 1985, Bakker et al. 1993, Castagnoli and Simoni 1994, Walzer et al. 2007), since it is exposed to the environment without the ability to move to more favorable sites, especially in arid environments. In contrast, in their motile stages, phytoseiids can obtain water, either directly by drinking or by prey consumption (Sabelis 1985, Van Dinh et al. 1988, Yoder 1998), as well as by the uptake of water vapor from the atmosphere (Gaede 1992).

In a series of studies evaluating the effect of humidity on egg hatching of 49 strains from 21 phytoseiid species, the range of  $VPD_{50}$  ( $VPD$ : Vapor Pressure Deficit) was 0.39-1.82 kPa (Bakker et al. 1993, Croft et al. 1993, Castagnoli and Simoni 1994, Schausberger 1998, De Courcy Williams et al. 2004a, Walzer et al. 2007, Ferrero et al. 2010). At the upper edge of the range are found species that are considered to have drought tolerance, e.g., *Neoseiulus idaeus* Denmark & Muma (1.51-1.77 kPa); *Galendromus occidentalis* (Nesbitt) (1.67 kPa); *Phytoseiulus longipes* Evans (1.82 kPa); and *Typhlodromus athiasae* (1.81 kPa).

In the present study, only *Cydnoseius negevi* (1.11 kPa) and *Neoseiulus longilaterus* (1.31 kPa) entered this range with values similar to phytoseiids from temperate regions, suggesting that these species do not have any adaptations for coping with aridity, at least in their egg stage. Furthermore, at 71% RH (1.23 kPa) and below, all of their non-hatched eggs (which comprised the majority) had shriveled within the three days of the experiment. Since air humidity in the SAV in the summertime hardly exceeds 70%, it is clear why *C. negevi* and *N. longilaterus* recovery on the date palm bunches was very low as compared to the *T. athiasae*. In contrast, *C. negevi* is very common within date palm orchards on Bermuda grass (*C. dactylon*) and other Gramineae species that grow around the tree trunk, apparently due to the irrigation system of the orchard. These grasses capture moisture between the leaf blade, leaf sheath and culm, creating a humid micro-climate (Gaede 1992) where hatching probability is presumably higher (Van Dinh et al. 1988). Diurnal fluctuations of air moisture with higher values in the early morning may also have a positive effect (De Vis et al. 2006) on *C. negevi* and *N. longilaterus*.

On the upper end of egg drought tolerance in the current study, the two *T. athiasae* strains and *Euseius scutalis* have a higher VPD<sub>50</sub> in comparison to the range found in the literature (see above). This is especially true for the latter's value (3.14 kPa), which is far beyond that range (but note that the VPD<sub>50</sub> is slightly out of the range of relative humidity evaluated here). *E. scutalis* is widely distributed throughout North Africa, the Mediterranean basin and the Middle East, in Mediterranean as well as in hot and dry climates (Bounfour and McMurtry 1987, Swirski and Amitai 1997, Kreiter et al. 2006). Bounfour and McMurtry (1987) recorded the egg hatching of *E. scutalis* collected from arid areas in Morocco at humidity levels of 19% RH. Based on their results, I have calculated the RH<sub>50</sub> and VPD<sub>50</sub>, which turned out to be 32.2% and 2.15kPa (25°C), respectively. In my experiment, *E. scutalis* eggs had a considerably higher VPD<sub>50</sub> of 3.14 kPa (calculated from RH<sub>50</sub> of 25.9% at 30°C), which, to the best of my knowledge, is the most drought-tolerant phytoseiid species ever recorded.

The VPD<sub>50</sub> of the *T. athiasae* from Jezreel Valley was almost identical to that from the Arava Valley (2.34 and 2.36 kPa, respectively). However, the eggs of the latter showed some drought adaptation, since they still hatched (16%) at 32.5% RH (2.86 kPa), whereas the eggs of the former did not. Intra-specific variation with respect to the effect of humidity on egg hatching was reported for several phytoseiid species, among them *Neoseiulus*

*californicus* McGregor (Walzer et al. 2007); *Phytoseiulus persimilis* Athias-Henriot (Perring and Lackey 1989); *Euseius finlandicus* Oudemans; *Typhlodromus pyri* Scheuten (Schausberger 1998); and *Typhlodromalus limonicus* (Garman & McGregor) (Bakker et al. 1993). The latter authors concluded that humidity tolerance level is probably a strain-specific trait more than a species-specific one and that it is determined by the climatic characteristics of the habitat.

In our case, the difference between the two *T. athiasae* strains in hatching probability at low humidity can be related to the air moisture differences among their natural habitats. The mean annual air humidity in the Jezreel Valley is 62.3%, while it is 45% in the Southern Arava Valley (Israel Meteorological Service). Using VPD values (Ferro and Chapman 1979), the difference between the two habitats is found to be even more pronounced because the SAV is warmer than the Jezreel Valley (mean annual is 23.2°C and 19.5°C, respectively). Bakker et al. (1993) speculated that the benefit of being tolerant to a wide range of saturation deficits is offset by some cost factor. In my experiment, the Arava strain of *T. athiasae* showed consistent lower egg hatching (ca. 0.8) at all humidities above 50% in comparison with its northern counterpart (usually above 0.9). Possibly, the cost factor of its ability to hatch at a very low humidity (high VPD) is the lower hatching probability under more moist conditions.

In a previous study of *T. athiasae* collected from the same *Citrus* orchard at Jezreel Valley (Ferrero et al. 2010), the authors reported similar RH<sub>50</sub> as found here (43%). Surprisingly, however, their calculated VPD<sub>50</sub> (1.81 kPa) was somewhat different, due to different temperatures used in the calculation (25°C versus 30°C here). The same discrepancy was also noted for *N. californicus* by Castagnoli and Simoni (1994). When they compared egg hatching of the same population at 21°C and 29°C, they found very similar RH<sub>50</sub> (73.7 and 74.7, respectively), but considerably different VPD<sub>50</sub> (0.62 and 0.98 kPa, respectively). Also notable are the similar hatching probabilities between the Moroccan *E. scutalis* (32.2% RH<sub>50</sub> at 25°C, calculated from Bounfour and McMurtry 1987), and the *E. scutalis* evaluated here (25.9% at 30°C) in comparison to the corresponding VPD values (2.15 and 3.14 kPa, respectively). Moreover, Bakker et al. (1993) found that among climate characters of the phytoseiids' habitat, the egg VPD<sub>50</sub> is only correlated with average relative humidity and not with vapor pressure deficit.

In all of the above examples, the VPD<sub>50</sub> increased with temperature within the species' optimal range of temperatures. It could be that egg tolerance to air drought rises because temperature by itself catalyzes physiological processes, including mite development (Sabelis 1985), thereby shortening exposure time. It is therefore suggested that although VPD<sub>50</sub> is the acceptable and conventional measure for comparing different experimental setups (Ferro and Chapman 1979), the RH<sub>50</sub> and the temperature should not be ignored. It is also recommended that future research be carried out at the same temperature of previous studies in order to be more comparable.

#### 2A.4.1.2 Egg development time

Egg development time also differed between the drought-sensitive species (*N. longilaterus* and *C. negevi*) and the drought-tolerant ones (*T. athiasae* and *E. scutalis*), with a shorter time required for the former. This could be a strategy for increasing survival by shortening exposure to the harsh ambient conditions. Egg retention occurs in phytoseiids as a reaction to predation threat (Montserrat et al. 2007), and perhaps also in response to abiotic constraints. However, oviposition in my experiment took place in high humidity (70-80%), indicating that this short egg development time is not an induced response of the gravid female reaction to dry ambient conditions, but rather is species/strain-specific. Considering also the quick shriveling of *N. longilaterus* and *C. negevi* eggs at moderate and low humidities, where the eggs of the other three species/strains kept their original form, it is more reasonable to claim that both phenomena (short development time and shriveling) are related to the egg shell structure.

Phytoseiid eggs, like other terrestrial arthropods, face a tradeoff between exchanging metabolic gases (O<sub>2</sub> and CO<sub>2</sub>) with the external environment and conserving water (Hadely 1994, Woods 2010), especially in an arid environment. The rate of development rises with an increase in metabolic gas exchange, but also with higher water loss to the environment. Unlike the motile stages, and with some exceptions (Yoder and Denlinger 1992), arthropod eggs do not absorb atmospheric vapor water (Hadely 1994, Woods 2010). Therefore, their key survival tactic is to maximize water conservation, which depends on the egg's permeability to gas exchange and water vapor and on air moisture (Hadely 1994, Woods 2010). This was also found to be true for the eggs of some ticks, including the lone star tick, *Amblyomma americanum* (Yoder et al. 2004). In their motile life stages, phytoseiids do absorb water in sub-saturated air (Gaede 1992), an ability which is enhanced in arid-

adapted species (Yoder 1998). In *Phytoseiulus persimilis*, gas exchange takes place throughout the entire egg stage (Thurling 1980), with a probable loss of some egg moisture, but nothing is known about the water absorption of phytoseiid eggs. I hypothesize that the egg shell of *N. longilaterus* and *C. negevi* is more permeable to gas exchange than that of *T. athiasae* and *E. scutalis*. As a result, the embryonic development rate would be faster, but the rate of desiccation would be also higher. In contrast, the egg shell of the more drought-tolerant *T. athiasae* and *E. scutalis* is less permeable to gas exchange, resulting in higher egg survival at low humidity, but later hatching. Further investigation is needed to evaluate this phenomenon.

The effect of RH on egg development time in phytoseiids has not been deeply analyzed in previous studies. The egg development time of *N. longilaterus* and *C. negevi* was found to be significantly shorter at low humidity, but only a very small proportion of the eggs survived at low humidity, and all of them hatched in the first day of the experiment. However, while the egg development time of *T. athiasae*-Arava and *E. scutalis* had only a slight extension as RH decreased, it was significantly prolonged for *T. athiasae*-Jezreel - from 2.06 days at 63% RH to more than 2.4 days at 47% and 52% RH, where more than 65% of the eggs still hatched.

The trend of prolonged egg development time in phytoseiids at low RH (summarized in Table 2A.6) has also been observed in several strains of *P. persimilis* (Perring and Lackey 1989, Santi and Maccagnani 2000, De Courcy Williams et al. 2004a); *N. californicus* (Castagnoli and Simoni 1994, De Courcy Williams et al. 2004a, Walzer et al. 2007); *T. athiasae* (Reuveny et al. 1996); *E. scutalis* (Bounfour and McMurtry 1987); *N. fallacis* (Kramer and Hain 1989); and *P. longipes* (Badii and McMurtry 1984). In some cases, the differences in egg development time between humidity levels were not statistically significant or were not analyzed (indicated in Table 2A.6 legend). However, in 17 of the 24 species and strains reviewed, the above trend was found. It seems that this phenomenon has no association with phytoseiid life type. A similar pattern of retarded development time at low humidity is also found in many insects (Bursell 1974) and other mites (Sanchez-Ramos et al. 2007).

The water permeability of arthropod eggs changes throughout their development (Hadely 1994). For example, egg shell of the sphingid moth *Manduca sexta* changes its gas and water flux in reaction to a change of ambient oxygen pressure, ambient temperature, and

embryonic metabolic demand (Woods 2010). It is not known whether air moisture can stimulate similar changes in egg shell conductance. However, although the mechanism is unclear, relative humidity does induce egg hatching of the cicada *Cryptotympana facialis* (Moriyama and Numata 2006) and the haematophagous bug *Rhodnius prolixus* (Schilman et al. 2009). Therefore, it is possible that phytoseiid eggs are able to sense ambient air humidity and react accordingly. When experiencing low humidity, which is close to their survival limit, they may actively reduce egg permeability in order to conserve water content. As a result, the rate of gas exchange also decreases and development time becomes longer.

**Table 2A.6:** Egg development time in relation to relative humidity change in different phytoseiid species and strains. Life types from McMurtry and Croft (1997).

Species	No. of strains evaluated	Egg development time in relation to RH: Negative (-); Positive (+); or None (0)	Temp range (°C)	Humidity range (%RH)	Life type	Reference
<i>P. persimilis</i>	1	–	20	60-90	I	De Courcy Williams et al. 2004a
	2	– <sup>a</sup>	26.7-32.2	73-87		Perring and Lackey 1989
	1	– <sup>b</sup>	26	50-90		Santi and Maccagnani 2000
<i>P. longipes</i>	1	– <sup>a</sup>	21-33	30-90	I	Badii and McMurtry 1984
<i>G. occidentalis</i>	1	+ <sup>c</sup>	14	35-95.5	II	Mangini and Hain 1991
<i>N. fallacis</i>	1	+ <sup>c</sup>	14	35-95.5	II	Mangini and Hain 1991
	1	– <sup>a</sup>	20-32	63-93		Kramer and Hain 1989
<i>N. californicus</i>	5	–	25	64-72.4	II-III	Walzer et al. 2007
	2	0	25	64-72.4		Walzer et al. 2007
	1	–	21-33	70-100		Castagnoli and Simoni, 1994
	1	–	20	60-90		De Courcy Williams et al. 2004a
<i>N. cucumeris</i>	1	0	20	60-90	III	De Courcy Williams et al. 2004a
<i>T. athiasae</i> – Upper Galilee	1	– <sup>a</sup>	25	40-90	III	Reuveny et al. 1996
<i>T. athiasae</i> – Jezreel	1	–	30	32.5-84.5		This study
<i>T. athiasae</i> – Arava	1	0	30	32.5-84.5		This study
<i>I. degenerans</i>	1	0	20	60-90	IV	De Courcy Williams et al. 2004a
<i>E. scutalis</i>	1	– <sup>d</sup>	25	19-100	IV	Bounfour and McMurtry 1987
	1	–	30	32.5-84.5		This study

<sup>a</sup> There was a trend, but differences between humidities were not statistically significant.

<sup>b</sup> Egg development time was elongated in relation to exposure time to low RH (50%).

<sup>c</sup> Experiment included only low VPDs (0.1-1kPa).

<sup>d</sup> Differences between humidities were not statistically analyzed.

In most studies on phytoseiid eggs, including the present one, development time was calculated on the basis of daily observations, whereas the total egg development time is mostly in the range of 1.5-3 days. In order to improve the resolution, observation intervals must be shortened. It will also allow accurate examination of the prolonged egg development time at low humidity among phytoseiid species/strains.

#### 2A.4.2 Indigenous phytoseiid establishment and pest control on date palms

Drought tolerance is a necessary, but not exclusive, precondition for being a successful biological control agent on the bunches in the Arava's arid environment. The host plant, among many other factors, plays an important role in determining the probability of the phytoseiid to establish (Walter 1996, Beard and Walter 2001). Structural and chemical plant traits affect the phytoseiid population, either directly or indirectly, through their food and environmental resources (e.g., Walter 1996, Gerson and Weintraub 2007).

*Euseius scutalis* did not establish on the bunches even when pollen was provisioned. It is somehow surprising, since *E. scutalis* has a high reproductive rate when feeding on pollen (Nomikou et al. 2001), including maize pollen (Maoz et al. 2011), and has drought tolerance (Bounfour and McMurtry 1987). In addition, a few specimens of *E. scutalis* have been found on date palms in Tunisia (Kreiter et al. 2006). The negligible number of *E. scutalis* in the samples of the present study is probably due to its host-plant sensitivity. Lab rearing of *E. scutalis* always demands special attention and is possible only on a plant substrate (Nomikou et al. 2001) or on a very special artificial system (developed for *Euseius victoriensis* (Womersley) and *Euseius elinae* (Schicha); Argov et al. 2002). Mass rearing of *E. scutalis* has also encountered great difficulties.

Nomikou et al. (2003) concluded that *E. scutalis* feeds on plant sap, since its survival on leaves from insecticide-treated plants was ten times lower than on leaves from untreated plants. Mortality on treated leaves has been observed both in the absence and the presence of pollen, suggesting that plant feeding of *E. scutalis* is indispensable (Nomikou et al. 2003). In *Euseius hibisci* (Chant), Porres et al. (1975) showed that its ability to use plant fluid depends on the plant species, as it extracted plant material from avocado leaf, but not from lemon leaf. Perhaps the establishment of *E. scutalis* is limited by the hard exocarp of the premature date fruits, which increases its resistance to penetration as the fruit matures (Palevsky et al. 2005), and by its fibrous strands and fruit stalk, which make it impossible to uptake plant sap.

In contrast to *E. scutalis*, *T. athiasae* is easily reared on various plastic substrates (see method section and also Swirski et al. 1967, Reuveny et al. 1996, Nomikou et al. 2001), suggesting that this species is not obligated to consume plant material. Reuveny et al. (1996) found that the highest fecundity of *T. athiasae* is obtained at 25°C with 70% RH

and decreases when the temperature is raised to 29°C or the RH is lowered to 40%. The latter are closer to the Arava's summer conditions (means of 30°C and 30%). However, they evaluated *T. athiasae* that was collected from apple orchards in northern Israel, thereby being less adaptive to arid conditions than the local Arava strain. It should also be noted that living on plant tissue, instead of a plastic rearing unit, was shown to dramatically enhance the phytoseiid survival under the same ambient conditions (Castagnoli and Simoni 1994).

In my lab culture, rearing *T. athiasae* on bean leaf was more successful than rearing it on plastic substrate (personal observation). The natural, and almost exclusive, presence of *T. athiasae* on date palm bunches (of different cultivars) in the SAV, together with its establishment success in the bunch-scale experiment, are indicative of *T. athiasae*'s ability to manage in the tough micro-habitat. The negative relation between its population size and the fruit damage proves that it can affect the spider mite population and the level of damage. However, despite the large numbers of predators released *T. athiasae* did not significantly reduce the pest population and fruit damage in comparison to the control treatments. Possibly, the pest population level following (deliberate) infestation, performed to homogenize pest populations on all experimental trees, was too high for the predatory mite to control. These results resemble the outcome of *T. athiasae* releases in apple orchards in northern Israel for controlling the European Red Mite *Panonychus ulmi* (Palevsky 1997). The pest population was significantly reduced in two out of three apple varieties, but exceeded the damage threshold in all cultivars. This control failure was partially related to the low reproduction rate of *T. athiasae* (Palevsky 1997).

#### 2A.4.3 Conclusions - Potential for biological control

There is no doubt that date palm fruit and bunch present an unfavorable habitat for most phytoseiids. However, among the four species evaluated here, *T. athiasae* showed the highest potential for being a successful biological control agent of the old world date mite, since it is drought-tolerant to some extent and has the ability to establish on the date palm bunch. It seems that the *T. athiasae*-Arava strain is slightly more drought-tolerant than the Jezreel strain and therefore has a better chance of being effective in extremely arid regions. However, despite the high number of predators released, the control outcome was inadequate.

## **2B. Evaluation of predators as natural enemies of *Oligonychus afrasiaticus* in the field**

### **2B.1 Introduction**

Biological control of *O. afrasiaticus* on date palms faces three major constraints. First, *O. afrasiaticus* is a primary pest, causing direct damage to the fruit. Second, it has an explosive population growth. Third, it causes the worst damage in extreme arid conditions. In order to efficiently reduce the pest population, the suitable natural enemy must be present on the bunch before the pest arrives and throughout the entire infestation period. It must also perform efficiently in this extreme dry environment. Among the different strategies of biological control based on natural enemies, there are two main approaches to controlling an indigenous pest (Hajek 2004): 1) Introduction of an exotic natural enemy categorized as a 'new association of classical biological control' (Eilenberg et al. 2001); and 2) reliance on indigenous species, using conservation and/or augmentation methodologies.

Employing the first approach, *Neoseiulus californicus* (McGregor) is a type II-III (Croft et al. 1998) phytoseiid that is available from commercial insectaries. It is naturally found in many regions with a hot and dry climate (Castagnoli and Simoni 2003) and is widely used as a biological control agent in protected crops and perennials (Castagnoli and Simoni 2003, Gerson and Weintraub 2007). Its relatively high fecundity (ca. 3 eggs/female/day) was recorded while feeding on two-spotted spider mites (*Tetranychus urticae*), though *N. californicus* is known to feed and reproduce on a variety of mites, insects, and pollen (Swirski et al. 1970, McMurtry and Croft 1997, Argov et al. 2006, Ragusa et al. 2009). It can develop and reproduce when feeding on *T. urticae* at a temperature range of 15–35°C (Gotoh et al. 2004b), with the highest spider mite consumption at 35°C (Ahn et al. 2010). Its ability to survive long periods of starvation (Palevsky et al. 1999) may indicate that *N. californicus* can be effective even after extended periods of prey deprivation.

Releases of the European mass-reared *N. californicus* on established populations of *O. afrasiaticus* in date palms in southern Tunisia significantly reduced the pest levels, but the damage reduction was not satisfactory (Othman et al. 2001). The authors suggested that further studies should focus on early releases of this predatory mite prior to pest colonization. In a recent European multi-institutional project, two *N. californicus* strains adapted to dry ambient conditions were identified (Walzer et al. 2007, Palevsky et al. 2008). These strains were more efficient than *Phytoseiulus persimilis* in the suppression of

*T. urticae* and persisted longer on pepper and strawberry at low humidity levels (50% RH) (Palevsky et al. 2008). The recent introduction of slow-release sachets for *N. californicus* (Koppert Biological Systems, the Netherlands, KNL) are designed to serve as a temporal rearing unit with a surplus of prey (mite), allowing for a steady flow of *N. californicus* to the target plant.

Another way to manage *O. afrasiaticus* is by relying on its indigenous natural enemies. Developing conservation and/or augmentation techniques for the utilization of native predators could lead to effective control methods because the predators are expected to be adapted to these extreme arid conditions (Gerson and Vacante 1993, Escudero and Ferragut 2005). Furthermore, indigenous species have no potential negative effects on the environment as compared to exotic predators, and releases do not require a registration dossier, which is now a prerequisite for utilizing exotic species in North America and many European countries (Bigler et al. 2005, Loomans 2007).

Several species of spider mite predators are found in date palm orchards in the SAV. The ladybird beetle *Stethorus gilvifrons* (Coleoptera: Coccinellidae) is occasionally observed in large numbers on heavily infested bunches during the summer (personal observation). While this species is well known for its ability to rapidly reduce spider mite populations, it is usually effective only when large numbers of prey are present (Sohrabi and Shishebhori 2007). Lacewings (*Chrysoperla* sp. [Neuroptera: Chrysopidae]) nymphs are also seen feeding on *O. afrasiaticus*, but their numbers are quite low (personal observation).

Nine species of generalist phytoseiids have been detected in date palm orchards in the SAV (Palevsky et al. 2009). Specimens of different species were found in the ground cover grass, on date palm fronds, and on the fruit bunch. Besides feeding on various phytophagous mites and insects, generalist phytoseiids can also feed on pollen (McMurtry and Croft 1997). Naturally occurring pollen in the field has been associated with phytoseiid abundance (Roda et al. 2003, Duso et al. 2004b). It has also been shown that pollen application can augment released (Van-Rijn et al. 1999, Nomikou et al. 2002) as well as naturally occurring phytoseiids (Onzo et al. 2005, Maoz et al. 2011). I therefore hypothesized that applying pollen onto the fruit bunches would increase the indigenous phytoseiid populations, resulting in a reduction in the *O. afrasiaticus* population and its associated damage.

*Typhlodromus athiasae* Porath and Swirski is a type III-generalist phytoseiid (McMurtry and Croft 1997) that feeds on a variety of food types, such as spider mites and pollen (Swirski et al. 1967). *Typhlodromus athiasae* is common on many cultivated crops, as well as on natural plants in Israel (Swirski and Amitai 1997), and is widespread throughout the Mediterranean basin (De Moraes et al. 2004). It also inhabits date palm orchards of the SAV, where it has sporadically been found on the trees and the ground cover grass (Palevsky et al. 2009). Its low reproductive rate is comparable to other generalist phytoseiids, such as *Typhlodromus pyri*, and is similar when feeding on spider mites and on pollen (Swirski et al. 1967, Reuveny et al. 1996, Nomikou et al. 2001). Although the optimal conditions for *T. athiasae* are 25°C and 70% relative humidity (RH), it can also hatch, achieve adulthood, and reproduce at 29°C (70% RH) or at 40% RH (25 °C) (Reuveny et al. 1996). The RH<sub>50</sub> of *T. athiasae* egg hatching is 44% RH (30°C), but hatching still takes place at a relative humidity as low as 32.5% (Arava strain) (see Chapter 3). In my preliminary field study in 2007, this was the main species present on fruit bunches in the Arava. These two findings suggest that *T. athiasae* has potential in an augmentative biological control program in the SAV.

In this chapter, I evaluated in commercial orchards: (I) the effect of pollen provision, applied before and throughout pest establishment on the fruit bunches, on the population levels of the indigenous phytoseiid species and the subsequent pest control achieved; (II) the effect of augmentative releases of the indigenous generalist predator *T. athiasae*, with and without pollen provision; and (III) the effect of inundative sequential releases of an exotic dry-adapted strain of *N. californicus* on the control of *O. afrasiaticus*.

## **2B.2 Methods**

In order to evaluate the different biological control strategies described above, five field experiments in the Israeli and Jordanian SAV were carried out between 2007-2010. In addition, phytoseiid species were surveyed in two date palm orchards in the SAV and one orchard in the Dead Sea area. All experiments were performed in commercial date palm orchards in the SAV. The conservation experiment and *T. athiasae* releases were conducted in a mature organic 'Barhi' orchard in Kibbutz Samar, Israel. The *N. californicus* release experiments were conducted in conventional orchards of 'Barhi' and 'Medjool' in Rahma, Jordan.

In all of the experiments, the tree was the sampling unit. In the biological control experiments, pest and predatory mite population levels were monitored every 10-14 days by sampling 15 strands from each tree (3 strands from 5 random bunches per tree), which were washed in 70% ethanol. *Oligonychus afrasiaticus* cumulative mite days (CMDs) calculations and phytoseiid counts were done as described in the General Methods section. Mite damage at harvest was assessed by sampling ca. 100 fruits per tree, which were randomly picked from the marked bunches and sorted according to the standard packing house classification (see General Methods).

The experiments were divided into three parts:

#### 2B.2.1 Conservation of indigenous phytoseiid populations on date palm trees, Israel 2007

The potential of the conservation approach in supporting indigenous phytoseiids for biological control of *O. afrasiaticus* was evaluated in two steps. First, the presence of indigenous generalist predators was surveyed in the vicinity of fruit strands in three plots by sampling 24, 14, and 6 trees at Mitzpe-Shalem ('Barhi'), Grofit ('Medjool'), and Samar ('Barhi'), respectively. One bunch from each tree was cut from the fruit stalk (peduncle) base adjacent to the trunk. Then the stalk part was separated from the strands part, and both parts were washed in 70% ethanol. Predator counts and identification were done as described in the General Methods section. At Grofit and Samar, the same trees were sampled twice, after the fruit set and just before the pest population was expected to increase, whereas at Mitzpe-Shalem, samples were taken only once, after the fruit set.

Second, the effect of pollen applications on the conservation and enhancement of indigenous phytoseiid populations was evaluated on 'Barhi' in the spring-summer of 2007. Using a block design, 22 trees were divided into three pollen application treatments: date palm pollen (n = 7); maize pollen (n = 7); and a non-treated control (n = 8). Sampling sessions of pest and predator populations were carried out every fortnight, from mid-April through the end of July, following the application of the respective pollen, as described in the General Methods section.

#### 4.2.2 Augmentative releases of the indigenous *Typhlodromus athiasae*, Israel 2008-2009

The effect of an indigenous dry-adapted generalist predator, with and without alternative food, was examined on 'Barhi' in the spring-summer of 2008 and 2009. Since *T. athiasae*

was almost the only phytoseiid species that was found in samples of the conservation experiment (see results), and based on its ability to establish a population on date bunches (see Chapter 3), I decided to evaluate its efficacy as a biological control agent. Thus, *T. athiasae* was collected from an organic citrus orchard at Beit-Shearim, in the Jezreel Valley of northern Israel, and mass reared at Bio-Bee, Israel.

**2008** – In a block-design experiment, 18 trees received one of three treatments: *T. athiasae* releases (*T. athiasae* treatment, hereafter); *T. athiasae* releases with weekly application of maize pollen (*T. athiasae*+pollen treatment, hereafter); and a negative control (no predator releases and without pollen application), with six replicates per treatment. In mid-May, during the 'kimri' fruit stage, two applications of approximately 2000 predatory mites per tree were carried out, with a fortnight interval between applications. The mites were released on 10 marked bunches per tree, with approximately 200 per bunch per release, making for a total of 400 per bunch and 4000 per tree. Mites within rearing medium were put in a paper cup, which was stapled and attached to the base of the bunch. Before each releasing session, the mites per gram in the releasing material were counted, using Berlese funnels that extracted the individuals in 70% ethanol. Then slides were prepared and species identification was verified under a compound microscope. Samples were taken after each fortnight.

**2009** – Releases were repeated in the same plot. In a block design, 16 trees received one of two treatments: *T. athiasae* releases with fortnightly applications of pollen (*T. athiasae*+pollen), and a negative control (no pollen or predators), with eight replicates per treatment. In mid-May, during the 'kimri' fruit stage, three applications of 1000-3000 predatory mites per tree were carried out, with a fortnight interval between applications. The mites were released on 10 marked bunches per tree, with approximately 700 per bunch and 7000 per tree. In order to improve the releasing method, mites + rearing medium in handmade net bags were attached to the base of the bunch. In so doing, the *T. athiasae* are 'forced' to leave the releasing vessel because the unprotected medium dries quickly (in comparison to the vessel used in 2008). Predator counts and species verification were done before each session, as described above. Samples were taken and maize pollen was applied every fortnight.

#### 2B.2.3 Inundative releases of the exotic *Neoseiulus californicus*, Jordan 2009-2010

The effect of an arid-adapted strain of commercial *N. californicus* (Walzer et al. 2007) was evaluated by sequential releases on 'Medjool' and 'Barhi' in Rahma, the Jordanian SAV. Five releasing sessions were performed once every three weeks from the beginning of May until the end of July. Samples were taken every 10 days.

**2009** – Release (n = 10) and control trees (n = 10) for each CV were randomly chosen to comply with a paired *t*-test experimental design. Slow-release sachets (Spical, Koppert Biological Systems), containing bran and other nutrients, the prey mites (*Carpoglyphus lactis*), and the predators, were used. In each release session, two sachets containing 250 mites each (500 mites total) were hung on each fruit bunch, with 8-10 bunches per tree.

**2010** – The objective of the experiment this year was to evaluate the effects of different predator release rates on phytoseiid and spider mite population levels and subsequent damage. The effect of different prey mite species (in the same sachet, but without *N. californicus*) on the indigenous phytoseiid population was also examined. For each CV, the following seven treatments were compared in a randomized block Latin Square design: (1-3) one, two, or four sachets per bunch with *N. californicus*; (4) four sachets per bunch with the prey mite *C. lactis*; (5) four sachets per bunch with the prey mite *Lepidoglyphus destructor*; (6) a sprayed control (0.5ml/L abamectin [Agri-Mek® EC; Syngenta, Basel, Switzerland]); and (7) no treatment control. Each treatment was repeated seven times in seven tree rows (i.e., blocks). An improved slow-release sachet (Spical-Plus, Koppert Biological Systems), containing the prey mite *L. destructor*, was used. This type of sachet was designed for the continuous emergence of approximately 1000 predators per sachet within a six-week period (Koppert Biological Systems 2009).

The above described emergence rate has been tested in temperate experimental greenhouses, but had yet to be examined in an arid region outdoors. In order to evaluate the efficiency of this sachet in the SAV arid environment, an emergence experiment was coupled with each releasing session. Nine ventilated cages (50cm X 50cm X 50cm) were located in an outdoor shaded area in the Arava (Tsafi Research Station, Jordan). In each cage, eight sachets were hung on a stand on the day of the field release. The stand was placed on black sticky tape in such a way that all *N. californicus* emerging from the sachets could not avoid getting stuck on the tape. After seven days, the strips of black tape were removed and the phytoseiids were counted under a stereomicroscope. Data loggers (Easylog EL-USB-2, Lascar electronics) were used to monitor temperature and relative

humidity near the cages, as well as near the plots where the releasing experiments took place.

#### 2B.2.4 Statistical analysis

Comparisons of the numbers of phytoseiids between the fruit stalks and the strands were done by the Wilcoxon rank test, while the dependence of that difference on sampling time was analyzed with cross-tabs. Comparisons between treatments of the *O. afrasiaticus* CMDs, the numbers of phytoseiids, and fruit quality on harvest in the conservation and *T. athiasae* release (2008) were analyzed by block-design ANOVA. Fruit percentages of the *T. athiasae* release (2008) were arc-sin ( $\sqrt{p}$ ) transformed to achieve normality before being analyzed. Comparisons between release and control trees in the *T. athiasae* (2009) and *N. californicus* (2009) releasing experiments were analyzed by paired t-test or by Wilcoxon rank test (when the assumption of normal distribution was not fulfilled). Correlations between the *T. athiasae* total number, *O. afrasiaticus* population variables, and damage percentages were analyzed by Pearson and Spearman tests. Spearman was also used to correlate emerged *N. californicus* numbers with ambient conditions, including temperature, relative humidity, and vapor pressure deficit (VPD, see General Methods), and non-linear regression was applied to estimate the relation curve.

### **2B.3 Results**

#### 2B.3.1 Conservation of indigenous phytoseiid populations on date palm trees

After fruit set, phytoseiids were found on the strands or fruit stalks of most of the bunches in all sites that were sampled (Tables 2B.1, 2B.2), though generally in low numbers. At Grofit, but not in the other study sites, the numbers of predatory mites on the stalk as compared to the strands was found to be dependent on the sampling time ( $\chi^2 = 8.9$ ,  $df = 1$ ,  $p = 0.003$ ). After fruit set, the phytoseiid population was higher on the stalk, whereas six weeks later the opposite was found to be the case (Table 2B.1). *Typhlodromus athiasae* was the most common phytoseiid found at all sites (Table 2B.2). At the northern site of Mitzpe Shalem, non-phytoseiid Mesostigmata and some *Cydnoseius negevi* (Phytoseiidae) were also found, mostly on the fruit stalk. At least some of these mites were seen on the base of the fruit stalk, adjacent to the trunk. In the SAV, the numbers of phytoseiids on

bunches tended to decline between the first (end of March) and second sample, six weeks later (Wilcoxon rank test:  $Z = 1.87, n = 20, p = 0.06$  for stalk and strand together; Table 2B.2).

**Table 2B.1:** Mean ( $\pm$  SE) phytoseiid number per bunch on fruit stalks and on strands, with Wilcoxon signed ranks for each site and date, 2010.

Site	CV <sup>a</sup>	Fruit phenology <sup>b</sup>	Date	N bunches	Fruit stalks	Strands	Wilcoxon Z	p
M. Shalem	B	FS	11 Mar	24	0.96 $\pm$ 0.31	0.79 $\pm$ 0.26	0.36	0.72
Grofit	M	FS	25 Mar	14	1.79 $\pm$ 0.42	0.71 $\pm$ 0.22	2.33	0.02
Grofit	M	In	4 May	14	0.43 $\pm$ 0.20	1.10 $\pm$ 0.34	1.22	0.22
Samar	B	FS	25 Mar	6	0.67 $\pm$ 0.42	0.33 $\pm$ 0.33	0.58	0.56
Samar	B	In	4 May	6	0.17 $\pm$ 0.17	0.17 $\pm$ 0.17	0	1.0

<sup>a</sup> Cultivars: B – 'Barhi'; M – 'Medjool'

<sup>b</sup> FS – after fruit set; In – small green fruit just before infestation begins

**Table 2B.2:** Number of Mesostigmata and phytoseiid mites per site and percentages of *Typhlodromus athiasae* (TA) and *Cydnoseius negevi* (CN) from identified phytoseiids.

Site	CV <sup>a</sup>	Fruit phenology <sup>b</sup>	Date	N bunches	Mesostigmata	phytoseiids	TA (%)	CN (%)
M. Shalem	B	FS	11 Mar	24	68 <sup>c</sup>	42	74	26 <sup>d</sup>
Grofit	M	FS	25 Mar	14	36	35	100	0
Grofit	M	In	4 May	14	21	20	100	0
Samar	B	FS	25 Mar	6	6	6	67	0
Samar	B	In	4 May	6	2	1	100	0

<sup>a</sup> B – 'Barhi'; M – 'Medjool'

<sup>b</sup> FS – after fruit set; In – small green fruit just before infestation begins

<sup>c</sup> All non-phytoseiid Mesostigmata mites were found on the fruit stalk

<sup>d</sup> > 85% of them were found on the fruit stalk

In the pollen application experiment, the population size of *O. afrasiaticus* throughout the ripening season was highly variable within treatments, and no differences were found between treatments (Table 2B.3). Fifty-three predatory mites (in seven sample sessions) were found in the samples, with no difference between treatments. All mounted specimens (n = 50) were identified as *T. athiasae*.

**Table 2B.3:** Mean ( $\pm$  SE) *Oligonychus afrasiaticus* cumulative mite days (CMDs) per strand of adult female and phytoseiid sum per tree in the maize pollen, date pollen, and control treatments, 'Barhi', Samar, 2007.

	Control (n = 8)	Date pollen (7)	Maize pollen (7)	F <sub>2,12</sub> <sup>*</sup>	P <sup>*</sup>
<i>O. afrasiaticus</i> - female CMDs	436 $\pm$ 170	661 $\pm$ 353	300 $\pm$ 135	0.89	0.44
Phytoseiids - sum per tree	0.9 $\pm$ 0.3	5.1 $\pm$ 2.1	1.6 $\pm$ 0.7	2.95	0.09

\* One-way ANOVA with blocks

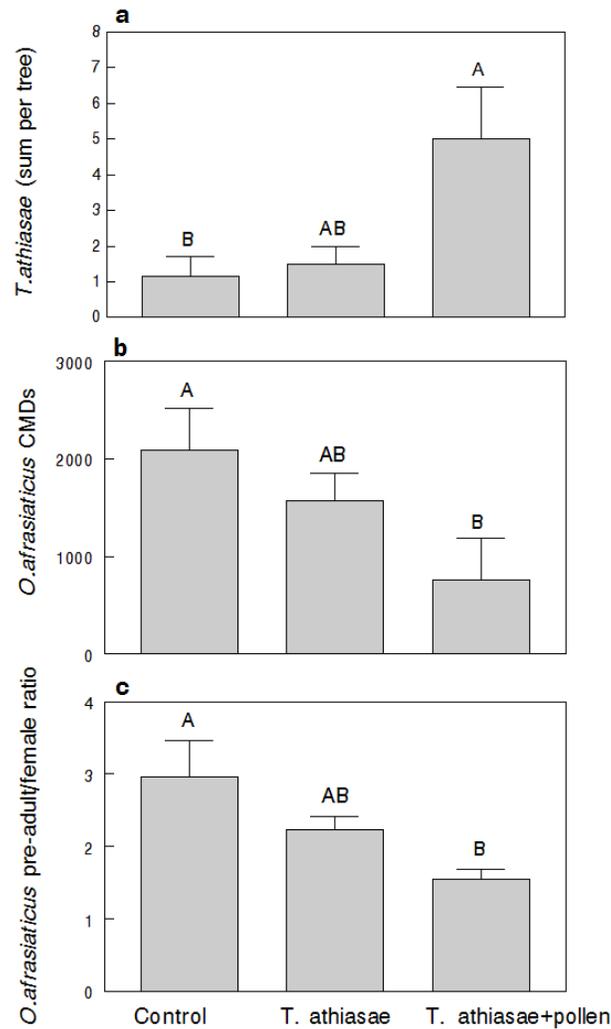
### 2B.3.2 Augmentative releases of the indigenous *Typhlodromus athiasae*

In 2008, even though the population levels of *T. athiasae* were generally very low (total of 42 individuals in five sampling sessions of 18 trees), they were significantly higher in the *T. athiasae*+pollen treatment in comparison to the *T. athiasae* release alone and the control treatments ( $F_{2,10} = 5.21$ ,  $p = 0.028$ ). There was no difference found between the *T. athiasae* and the control treatments (Fig. 2B.1a).

At the infestation peak during the second half of July, when pest infestation was more evenly spread just before harvest, the *O. afrasiaticus* population level (as measured by cumulative mite days, CMDs) in the *T. athiasae*+pollen treatment was significantly lower than in the control treatment ( $F_{2,10} = 4.4$ ,  $p = 0.04$ ). The level for the *T. athiasae* treatment alone was found to fall in between (Fig. 2B.1b). Not only did the population levels differ between treatments, but also the population structure, namely the ratio of all pre-adult CMD to adult female CMD ( $F_{2,10} = 5.8$ ,  $p = 0.02$ ). In the *T. athiasae*+pollen treatment, there were significantly less larvae and nymphs per each adult female than in the control treatment (Fig. 2B.1c).

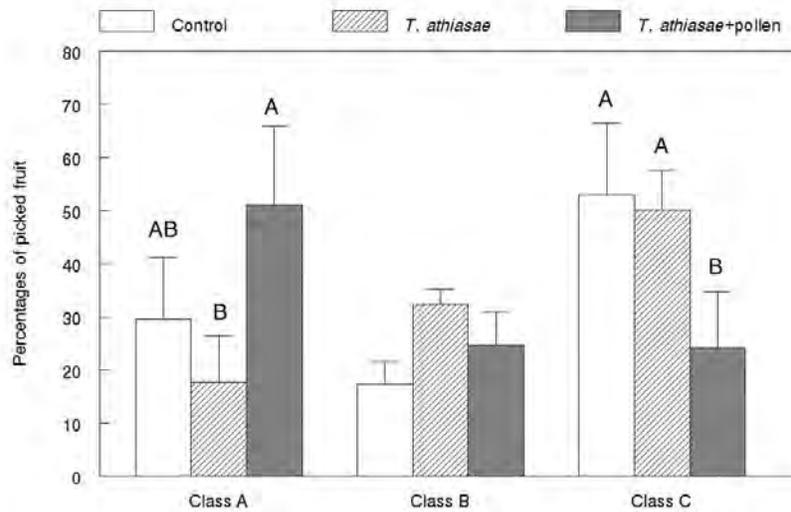
Extremely high fruit damage was detected in the control trees and the trees that only received *T. athiasae* release without pollen application. Fifty percent or more of their fruit yields were classified into the lowest class C (fruit with no commercial value) and were thus discarded. In contrast, the release trees supplied with pollen had substantially less fruit graded as class C ( $F_{2,10} = 6.68$ ,  $p < 0.05$ ). In the *T. athiasae*+pollen treatment, approximately 50% of the fruit was designated for export (class A), as compared to only 18% and 30% in the *T. athiasae* and control treatments, respectively ( $F_{2,10} = 4.45$ ,  $p < 0.05$ ; Fig. 2B.2).

Taking all the trees together, there was a negative correlation between CMDs at the infestation peak (14-28 of July) of *O. afrasiaticus* (all life stages) and the percentage of class A fruit upon harvest ( $r = -0.75$ ,  $p < 0.001$ ,  $n = 18$ ). In addition, there was a positive correlation of the CMDs with the percentage of class C fruit upon harvest ( $r = 0.84$ ,  $p < 0.001$ ,  $n = 18$ ). The *O. afrasiaticus* pre-adult to female adult CMD ratio was negatively correlated with the sum per tree of *T. athiasae* (non-linear relation:  $r_s = -0.498$ ,  $p = 0.035$ ,  $n = 18$ ).



**Figure 2B.1:** Comparison between control (n = 6), *Typhlodromus athiasae* release (6), and *T. athiasae* release + pollen application (6) treatments, 'Barhi', Samar, 2008. Mean (+ SE) of (a) total number of *T. athiasae*; (b) CMDs (cumulative mite days) of *Oligonychus afrasiaticus* (all life stages) per strand at infestation peak (14-28 of July); and (c) pre-adult CMD to female adult CMD ratio. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (Tukey HSD Multiple Comparison Test).

That is to say, where there were more predators, there were less pre-adults per spider mite female. The total number of *T. athiasae* per tree was not correlated with the CMDs of *O. afrasiaticus* (all life stages) ( $r = -0.1$ ,  $p = 0.7$ ,  $n = 18$ ) or with the percentages of fruit damage (class A:  $r = 0.38$ ,  $p = 0.13$ ,  $n = 18$ ; class C:  $r = -0.29$ ,  $p = 0.25$ ,  $n = 18$ ). In 2009, the total numbers of *T. athiasae* found in the samples were extremely low. However, the majority of those found was counted in samples from the treated trees (Table 2B.4).



**Figure 2B.2:** Mean (+ SE) percentage of graded fruit (class A-export; class B-local market; class C-no commercial value) for the control (n = 6), *Typhlodromus athiasae* release (6), and *T. athiasae* release + pollen application (6) treatments, 'Barhi', Samar, 2008. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (Tukey HSD Multiple Comparison Test). Statistical analysis was not performed for the B class results, since they have inter-relations with the class A and C results – the numbers of each treatment sum up to 100 percent.

*Oligonychus afrasiaticus* CMDs were relatively low and did not differ between treatments, though there was a tendency towards larger populations in the control treatment (Table 2B.4). Although none of the trees were treated with acaricides, the level of damage was exceptionally low, with no differences found between treatments. In both treatments, over 90% of the fruit was graded as A class (Table 2B.4).

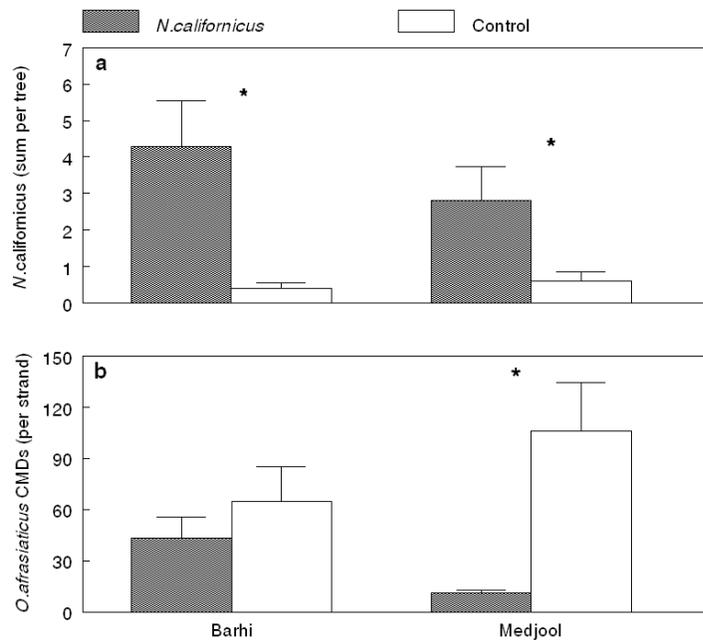
**Table 2B.4:** Mean ( $\pm$  SE) *Oligonychus afrasiaticus* cumulative mite days (CMDs) per strand, *Typhlodromus athiasae* sum per tree, and damage evaluation for the *T. athiasae* release + maize pollen application and control treatments, 'Barhi', Samar, 2009.

		Control (n = 8)	<i>T. athiasae</i> + Maize pollen (8)	Z*	P*
<i>O. afrasiaticus</i> - CMDs		658 $\pm$ 461	57 $\pm$ 34	0.49	0.62
<i>T. athiasae</i> - sum per tree		0.13 $\pm$ 0.13	0.75 $\pm$ 0.25	1.67	0.10
Damage evaluation (%)	Class A	90.3 $\pm$ 6.4	90.5 $\pm$ 5.5	0.14	0.89
	Class B	5.7 $\pm$ 3.1	7.2 $\pm$ 4.0	0.41	0.69
	Class C	4.0 $\pm$ 3.7	2.4 $\pm$ 1.5	0.14	0.89

\* Wilcoxon signed ranks test

### 2B.3.3 Inundative releases of the exotic *Neoseiulus californicus*

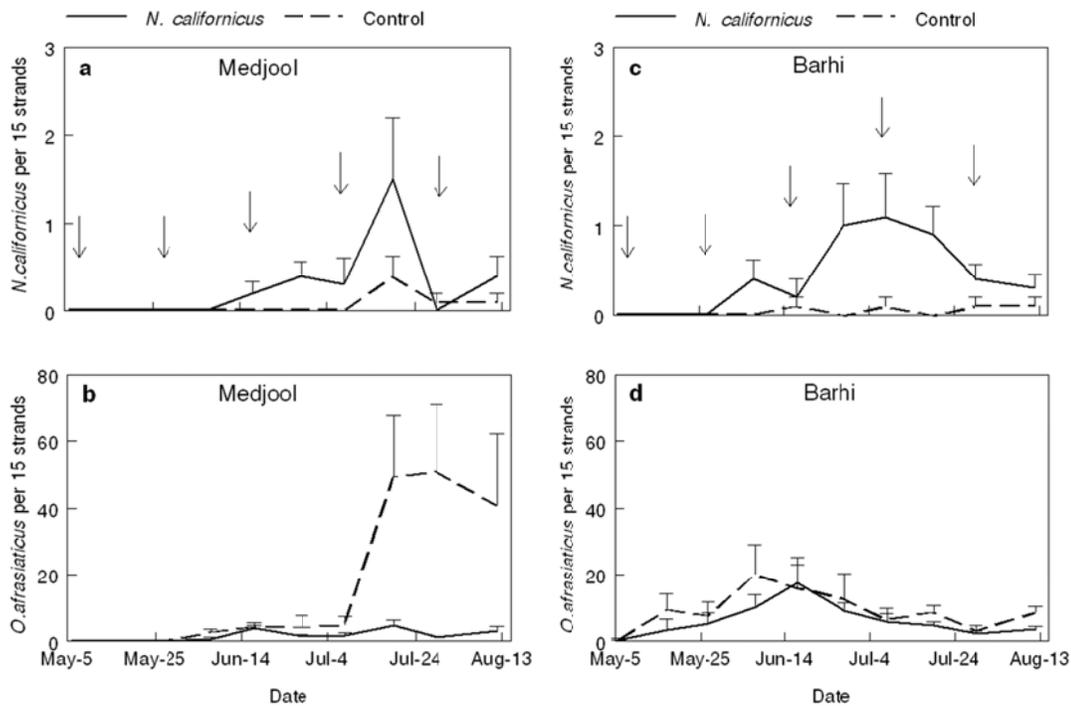
The predator releases in 2009 significantly increased the *N. californicus* population levels on 'Barhi' (Wilcoxon signed ranks test,  $Z = -2.68$ ,  $n = 10$ ,  $p = 0.007$ ) and 'Medjool' ( $Z = -2.4$ ,  $n = 10$ ,  $p = 0.016$ ; Fig. 2B.3a). However, the spider mite populations were negatively affected on 'Medjool' ( $t_9 = 3.29$ ,  $p = 0.009$ ), but not on 'Barhi' ( $t_9 = 1.4$ ,  $p = 0.2$ ; Fig. 2B.3b), though the trend was similar.



**Figure 2B.3:** Mean (+ SE) of (a) total number of *Neoseiulus californicus* per tree, and (b) CMDs (cumulative mite days) of *Oligonychus afrasiaticus* (all life stages) per strand in Barhi and 'Medjool', Rahma, 2009. Asterisks above the bars indicate significant differences between treatments (Wilcoxon signed ranks test,  $p < 0.05$ ).

After *N. californicus* was first detected on 'Medjool' trees (mid-June), the population level was always (except once) higher for the release than for the control trees (Fig. 2B.4a). In addition, whereas the *O. afrasiaticus* populations started to increase on the control trees (at the beginning of July), they remained at low levels with the release treatment (Fig. 2B.4b). The *N. californicus* populations on 'Barhi' were also higher for the treated trees throughout the experiment, whereas the pest populations were similar for both the release and the control treatments (Figs. 2B.4c, 2B.4d).

The fruit damage was significantly lower for the *N. californicus* release than for the control trees on 'Medjool' (Fig. 2B.5a), but not on 'Barhi' (Fig. 2B.5b). In comparison to the control trees, the treated 'Medjool' trees had 10% more fruit graded as class A ( $t_9 = 2.28$ ,  $p = 0.048$ ) and two times less fruit graded as classes C+D ( $t_9 = 2.38$ ,  $p = 0.041$ ). The trend between treatments was in the same direction for the 'Barhi' trees, but was not significant (for class A:  $t_9 = 0.78$ ,  $p = 0.46$ ; C+D:  $t_9 = 0.46$ ,  $p = 0.66$ ).



**Figure 2B.4:** Population dynamics (mean + SE per 15 strands) of (a) *Neoseiulus californicus* and (b) *Oligonychus afrasiaticus* in release and control treatments on 'Medjool'; and (c) *N. californicus* and (d) *O. afrasiaticus* in release and control treatments on 'Barhi'; Rahma, 2009. Arrows indicate *N. californicus* releases in the release treatments.

In 2010, the infestation level was extremely low, even lower than that of 2009. *Oligonychus afrasiaticus* CMDs per strand for the control treatment in 2009 and 2010 were 106 ( $\pm 28$  SE) and 3.1 ( $\pm 1.3$ ), respectively, on 'Medjool' ( $t_{9,04} = 3.63$ ,  $p = 0.005$ ) and 65 ( $\pm 20$ ) and 31 ( $\pm 13$ ), respectively, on 'Barhi' ( $t_{13,8} = 1.45$ ,  $p = 0.17$ ). There were no differences between treatments in infestation levels (*O. afrasiaticus* CMD), phytoseiid numbers (sum per tree), or fruit damage for both CVs (Tables 2B.5, 2B.6).

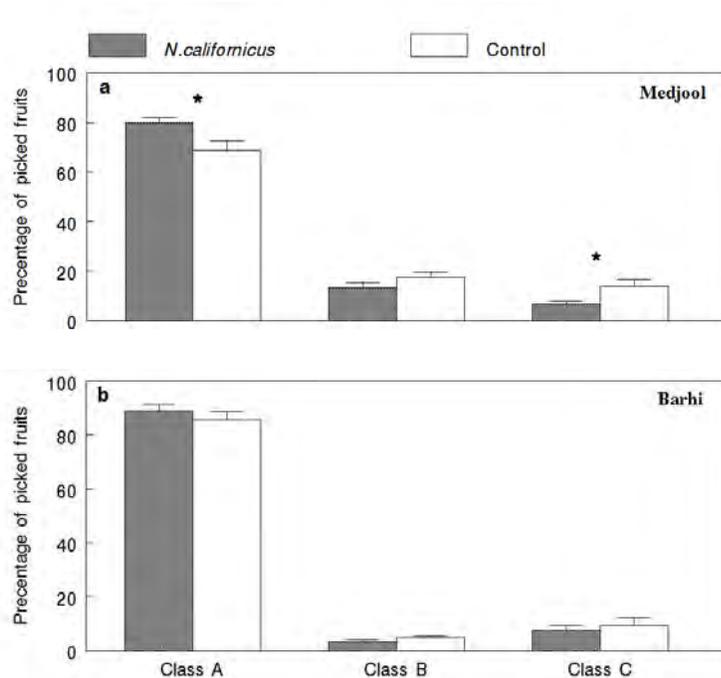


Figure 2B.5: Damage comparison between *Neoseiulus californicus* release and control treatments, presented as the mean (+ SE) percentage of fruit graded for export (Class A), local market (Class B), and industry/discarded (Class C), in (a) 'Medjool' and (b) 'Barhi', Rahma, 2009. Asterisks above the bars indicate significant differences between treatments (paired t-test,  $p < 0.05$ ).

The total number of *N. californicus* that emerged from one sachet after seven days was very low, with between 4.5–22.5 predators per sachet (Table 2B.7). These numbers differed significantly between shipments ( $F_{4,40} = 24.3$ ,  $p < 0.001$ ), being higher in the first two shipments than in the last three (Table 2B.7). These emergence numbers had a non-linear correlation with the mean ambient temperature of the evaluation week ( $r_s = -0.79$ ,  $n = 45$ ,  $p < 0.001$ ), the mean relative humidity ( $r_s = 0.35$ ,  $n = 45$ ,  $p = 0.02$ ), and the mean VPD ( $r_s = -0.81$ ,  $n = 45$ ,  $p < 0.001$ ). Non-linear regression of the emergence numbers as dependent on VPD revealed a sigmoid curve accounting for 84% of the variance in the emergence numbers (Fig. 2B.6). The 95% confidence levels of the four parameters (b1-b4) do not contain zeros, indicating a significant estimation (Table 2B.8).

**Table 2B.5:** Mean ( $\pm$  SE) *Oligonychus afrasiaticus* CMDs (cumulative mite days) per strand, phytoseiid cumulative number per tree, and damage evaluation on ‘Barhi’, Rahma, 2010. Significance refers to block-design One-way ANOVA for each dependent variable. The number of sachets per bunch per release in every treatment is indicated in parentheses.

		Treatment							
		<i>N. californicus</i>	<i>N. californicus</i>	<i>N. californicus</i>	<i>L. destructor</i>	<i>C. lactis</i>	Abamectin	Control	p
		(1)	(2)	(4)	(4)	(4)			
<i>O. afrasiaticus</i> (CMDs)		90.8 $\pm$ 71.0	59.9 $\pm$ 24.0	71.4 $\pm$ 30.0	44.7 $\pm$ 19.0	60.4 $\pm$ 26.0	42.4 $\pm$ 31.0	30.5 $\pm$ 12.0	ns
Phytoseiids (cumulative number)		1.1 $\pm$ 0.4	1.0 $\pm$ 0.7	2.0 $\pm$ 0.9	0.1 $\pm$ 0.1	1.1 $\pm$ 0.6	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	ns
Fruit damage evaluation (%) <sup>*</sup>	Class A	48.1 $\pm$ 5.1	53.6 $\pm$ 5.7	57.3 $\pm$ 6.1	50.9 $\pm$ 7.9	48.3 $\pm$ 4.0	58.3 $\pm$ 5.4	46.4 $\pm$ 7.0	ns
	Class B	31.1 $\pm$ 4.5	26.9 $\pm$ 5.9	27.0 $\pm$ 5.9	29.6 $\pm$ 5.4	27.9 $\pm$ 4.5	24.0 $\pm$ 5.5	31.3 $\pm$ 6.0	ns
	Class C	13.3 $\pm$ 2.3	10.6 $\pm$ 1.8	9.3 $\pm$ 1.0	13.3 $\pm$ 3.4	15.9 $\pm$ 1.9	10.0 $\pm$ 2.2	13.4 $\pm$ 2.5	ns
	Class D	7.7 $\pm$ 0.9	8.6 $\pm$ 1.4	6.7 $\pm$ 1.1	6.0 $\pm$ 1.1	8.1 $\pm$ 1.6	8.9 $\pm$ 1.4	8.9 $\pm$ 1.4	ns

<sup>\*</sup> A – Export; B – Local market; C – Industrial; D – Discarded fruits

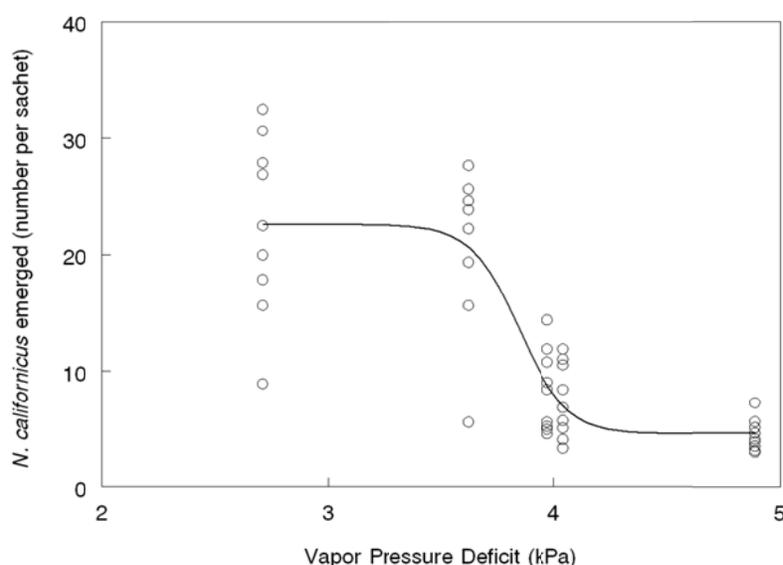
**Table 2B.6:** Mean ( $\pm$  SE) *Oligonychus afrasiaticus* CMDs (cumulative mite days) per strand, phytoseiid cumulative number per tree, and damage evaluation on ‘Medjool’, Rahma, 2010. Significance refers to block-design One-way ANOVA for each dependent variable. The number of sachets per bunch per release in every treatment is indicated in parentheses.

		Treatment							
		<i>N. californicus</i>	<i>N. californicus</i>	<i>N. californicus</i>	<i>L. destructor</i>	<i>C. lactis</i>	Abamectin	Control	p
		(1)	(2)	(4)	(4)	(4)			
<i>O. afrasiaticus</i> (CMDs)		3.4 $\pm$ 1.0	1.9 $\pm$ 0.5	2.8 $\pm$ 0.5	2.7 $\pm$ 0.8	2.2 $\pm$ 0.8	3.4 $\pm$ 1.3	3.1 $\pm$ 1.3	ns
Phytoseiids (cumulative number)		1.0 $\pm$ 0.3	1.1 $\pm$ 0.5	0.6 $\pm$ 0.2	1.1 $\pm$ 0.3	1.3 $\pm$ 0.4	1.0 $\pm$ 0.7	0.7 $\pm$ 0.3	ns
Fruit damage evaluation (%) <sup>*</sup>	Class A	76.3 $\pm$ 4.9	74.0 $\pm$ 7.2	71.7 $\pm$ 7	69.6 $\pm$ 6.5	73.3 $\pm$ 5.8	78.3 $\pm$ 5.1	72.1 $\pm$ 8.3	ns
	Class B	22.4 $\pm$ 5.1	24.3 $\pm$ 7	26.0 $\pm$ 6.2	27.9 $\pm$ 6.1	25.6 $\pm$ 5.7	19.9 $\pm$ 4.9	25.7 $\pm$ 7.7	ns
	Class C	1.1 $\pm$ 0.4	1.1 $\pm$ 0.6	2.1 $\pm$ 1.4	2.4 $\pm$ 0.9	1.1 $\pm$ 0.4	1.4 $\pm$ 0.7	1.7 $\pm$ 0.7	ns
	Class D	0.3 $\pm$ 0.3	0.6 $\pm$ 0.2	0.1 $\pm$ 0.1	0 $\pm$ 0	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	ns

<sup>\*</sup> A – Export; B – Local market; C – Industrial; D – Discarded fruits

**Table 2B.7:** Mean ( $\pm$  SE) numbers of emerged *Neoseiulus californicus* per sachet, and mean temperature, RH (relative humidity), and VPD (vapor pressure deficit) values (calculated from temperature and RH) at the week of each evaluation, Tsafi, 2010. Same letters indicate non-significant ( $p \geq 0.05$ ) differences between shipments (Tukey HSD multiple comparison test).

Shipment	Evaluation dates	Emerged <i>N. californicus</i>	Temp (°C)	RH (%)	VPD (kPa)
1	3-10.5	22.5 $\pm$ 2.6 a	29.4	33.8	2.71
2	30.5-6.6	20.8 $\pm$ 2.2 a	34.5	33.7	3.62
3	20-27.6	7.4 $\pm$ 1 b	35.7	30.8	4.04
4	11-18.7	8.3 $\pm$ 1.2 b	37.1	37.0	3.97
5	1-8.8	4.5 $\pm$ 0.5 b	39.5	31.8	4.89



**Figure 2B.6:** *Neoseiulus californicus* emergence (each circle represent one sachet) during seven days in relation to the mean week Vapor Pressure Deficit (VPD), Tsafi, 2010. Equation:  $y = 4.66 + 19.7 / (1 + \exp^{-(\text{VPD} - 3.84) / -0.097})$ .

**Table 2B.8:** Estimate and 95% confidence intervals of the parameters of the non-linear regression equation:  $y = b1 + b2 / (1 + \exp^{-(\text{VPD} - b3) / b4})$  (VPD= Vapor Pressure Deficit). Since the 95% confidence intervals of all parameters do not contain 0, the parameters are significant.

Parameter	Value	95% confidence interval
b1	4.66	2.19 – 7.14
b2	19.7	16.0 – 23.4
b3	3.84	3.73 – 3.95
b4	-0.097	-0.15 – (-0.04)

## 2B.4 Discussion

### 2B.4.1 Conservation biological control of *O. afrasiaticus* in date palms

It is now acknowledged that the introduction of exotic biological control agents may have negative effects on the environment and therefore should be done with great care (Van Lenteren et al. 2003, 2006). This awareness has led to the development of guidelines and restrictions in order to regulate the importation and release of invertebrate biological control agents (Bigler et al. 2005, Loomans 2007). Due to both the awareness and the legal restrictions, more efforts are now being made to develop conservation techniques, for enhancing indigenous natural enemy populations, with the aim of achieving long-term sustainable pest control (Tscharntke et al. 2007).

Despite the presence of generalist phytoseiids in the Arava date palm orchards, in the ground cover grass (Palevsky et al. 2009), and on the fruit stalks and strands early in the season, the provision of alternative food did not have an effect on predator population size on the bunches or on the population size of *O. afrasiaticus* and its subsequent damage. The date bunch presents a harsh habitat for phytoseiids. From May through August, the minimum and maximum relative humidity means are 10-20% and 50-60%, respectively, while the minimum and maximum temperature means are 20-25°C and 34-40°C, respectively. Another constraint for phytoseiid survival is the lack of domatia on the smooth surface of the date fruit, as domatia can improve their performance in low humidity (Grostal and O'Dowd 1994, but see Norton et al. 2001). Furthermore, the availability of food resources for predators on the bunch is unpredictable.

Thus, regardless of pollen provision, only very low phytoseiid numbers were seen on the bunch. In contrast, they were easily found in the ground cover grass (personal observation) and on fruit stalk, at least early in the ripening season. A tendency towards higher numbers was found on the trees supplied with date pollen than with maize pollen. It is somewhat surprising given that the main phytoseiid found in this experiment, *T. athiasae*, feeds and reproduces on maize pollen (Swirski et al. 1967, and in my lab culture) but could not be maintained on date palm pollen as the only food source (preliminary results). Whereas maize pollen is known to be suitable for the reproduction and survival of other generalist phytoseiids (Bruce-Oliver et al. 1996, Gnanvossou et al. 2005), date pollen is suitable for some (e.g., *Proprioseiopsis aetus* (Chant) [Fouly 1997] and *Euseius yousefi* [Momen 2001]), but not for others (e.g., *Neoseiulus barkeri* [Momen 1995]).

*Typhlodromus athiasae* was by far the most dominant predatory mite found on bunches in the fruit stalks/strands survey, as well as in the conservation experiment (on the control as well as the treated trees). The same predominance has also been observed in other 'Medjool' and 'Barhi' orchards in the SAV (personal observation). These findings suggest that *T. athiasae* is able to cope with the bunch conditions as well as the harsh environment (see also Chapter 3). Yet, the decreasing number of *T. athiasae* on the fruit bunches from March to May, as the heat and dryness increase, demonstrates that this ability is limited. *Cydnoseius negevi* and other mites belonging to the order Mesostigmata have been found in the more humid region of the Dead Sea (Mitzpe Shalem), mainly on fruit stalks (Tables 2B.1,2B.2). These mites are frequently seen in the more protected part close to the trunk (personal observation), possibly an indication of their low to moderate adaptation to dry environments (see also Chapter 3 regarding *C. negevi* egg hatch).

#### 2B.4.2 The efficacy of *T. athiasae* augmentative releases

*Typhlodromus athiasae* is a generalist phytoseiid common to arid and semi-arid Mediterranean regions in Israel (Swirski and Amitai 1997) and other countries in the Middle East (De Moraes et al. 2004). Despite its dominance in citrus (Swirski et al. 1967) and apple (Palevsky 1997) orchards, as well as its ability to feed on a variety of pest insects and mites (Swirski et al. 1967, Palevsky 1997, Nomikou et al. 2001), only a few attempts to use it as a biological control agent have been conducted. While augmentative releases of *T. athiasae* in apple orchards have been shown to significantly lower the population size of the European red mite, *Panonychus ulmi*, they have failed to prevent economic damage (Palevsky 1997).

The results presented here (2008) indicate that augmentative releases of *T. athiasae* can reduce the population size of *O. afrasiaticus* and the damage caused to the date palm fruit. In addition, this control was achieved only when maize pollen was supplied. The positive role of pollen as an alternative food for pest control by generalist phytoseiids has been demonstrated for many generalist phytoseiids, both in the lab (McMurtry and Scriven 1966, Van-Rijn et al. 1999, Nomikou et al. 2002) and in the field (Onzo et al. 2005, Gonzalez-Fernandez et al. 2009, Maoz et al. 2011).

*Typhlodromus pyri* Scheuten is a congeneric species that shares many traits with *T. athiasae* (Reuveny et al. 1996). Its success in controlling *Panonychus ulmi* to levels below the economic threshold in apple orchards in North America is partially related to the abundance of alternative

food sources within its habitat (Walde et al. 1992, Hardman et al. 1997). The quantity of alternative food available early in the spring, before densities of *P. ulmi* begin to increase, may have a dramatic effect on the ability of *T. pyri* to successfully suppress spider mites (Walde et al. 1992, Hardman et al. 1997, Addison et al. 2000). Roda et al. (2003) found that applied and naturally occurring pollen captured by the apple leaf enhances *T. pyri* populations. For *Euseius fustis*, fecundity was found to be higher when feeding on a combination of pollen and spider mites in comparison with either of them alone (Bruce-Oliver et al. 1996). For *Amblyseius hibisci*, despite the reduction (25.7%) of *Oligonychus punicae* consumption when pollen was offered, the reproduction rate increased more (63%) (McMurtry and Scriven 1966), thereby allowing better spider mite control. *Typhlodromus athiasae* survive and reproduce when feeding on maize pollen (Swirski et al. 1967), which was also used in this study to rear it in the lab. On the treated trees, maize pollen enabled *T. athiasae* to establish on the bunch before the pest arrived and perhaps diversified its diet afterwards.

The ability of *T. athiasae* to complete its life cycle and reproduce on the bunch was shown in Chapter 3. Still, though hundreds of *T. athiasae* per bunch were released, only very low numbers were detected in the samples, suggesting that the conditions on date bunches in the SAV are sub-optimal for population development. Reuveny et al. (1996) showed that the optimal conditions for *T. athiasae* are 25°C with 70% RH, which are far from the ambient conditions of the SAV date bunch. Rather, they are closer to the conditions of the Bermuda grass ground cover, where the humidity is enhanced by irrigation, allowing for substantial populations of *T. athiasae* and other phytoseiids. Considering that the egg stage is the most sensitive stage to low humidity, egg hatching experiments have shown that *T. athiasae* eggs hardly hatch, if at all, below 43% RH (Ferrero et al. 2010) (see Chapter 3).

Although *T. athiasae* significantly reduced the population size of *O. afrasiaticus* and its damage to date fruit, it did not control the pest. *Typhlodromus athiasae* is a generalist type III phytoseiid (McMurtry and Croft 1997), which may have difficulty entering dense webbing. It is commonly associated with *P. ulmi* in apple orchards (Palevsky 1997) and *P. citri* in citrus (Porath and Swirski 1965). These two species of spider mites belong to the 'little web type' group (subgroup LW-c), spinning only simple webs (Saito 1985) that can be easily penetrated by *T. athiasae*. In contrast, *O. afrasiaticus* has a 'complicated web type' (CW, subgroup 'p', 'r', or 'u'; Saito 1985)

with greater web density, making it more difficult for *T. athiasae* to reach their prey and prevent damage to the fruit.

Furthermore, similar to *T. pyri* and other generalist phytoseiids belonging to the genus *Typhlodromus*, the reproduction rate of *T. athiasae* when feeding on pollen and various preys is ca. 0.6-1.5 eggs/female/day (Swirski et al. 1967, Reuveny et al. 1996, Nomikou et al. 2001). With such a low rate of increase, it cannot track the rapid population growth of *O. afrasiaticus*. As in this study, augmentative releases of *T. athiasae* for control of *P. ulmi* on apple trees did achieve a reduction in the pest population, but not below the damage threshold (Palevsky 1997). In that case, even though 1000 predators per tree were applied, it still took three weeks for *T. athiasae* to lower the pest level, by which time the cumulative population level of *P. ulmi* was above the damage threshold.

Another constraint for *T. athiasae* is probably the smoothness of the fruit. Its counterpart, *T. pyri*, is known to be positively affected by apple leaf pubescence (Roda et al. 2003). *T. pyri* numbers on grape leaves were also positively related to the abundance of non-glandular leaf trichomes (Loughner et al. 2008) and domatia (English-Loeb et al. 2002), which are lacking in date fruit. In contrast, higher humidity, domatia, and an abundance of alternative food for *T.athiasae* can be found at the base of the fruit stalk and in the date palm trunk.

Another possible explanation for the low *T. athiasae* numbers detected in this study could be diurnal movement between the bunch and the trunk, using the fruit stalk as a walkway. During the daytime, which is when the mites were sampled, the predators could be hiding at the base of the fruit stalks. Before sunrise, when the humidity increases, the predators may graze the bunches, moving back as the humidity drops (Zundel et al. 2007).

Despite the low numbers of *T. athiasae* detected in the samples, the *T. athiasae* + pollen treatment did reduce the pest population size and thereby its damage by 50% in 2008. This trend was observed in the following year (2009) as well, but the effect was not significant. Predator numbers were extremely low, and the level of damage did not differ between treatments. Additionally, the infestation in 2009 was exceptionally low and late in the season, leading to a very low damage level in the non-spray controls (over 90% were graded for export in 2009 vs. 30% in 2008). This low infestation might be related to the climatic conditions of 2009 (see Chapter 5).

The lower immature to adult female ratio in the *T. athiasae*+pollen treatment, as compared to the control treatment, implies that *T. athiasae* tends to feed on the younger spider mite stages, including egg, larvae and nymphs, over the adult. Furuichi et al. (2005) showed that the specialist phytoseiid *Amblyseius womersleyi* preferentially feeds on eggs rather than adults of the spider mites *Tetranychus urticae* and *T. kanzawai*. This preference was related to higher oviposition when feeding on spider mite eggs as compared to adults (Furuichi et al. 2005). The generalist *Euseius hibisci* favored the immature life stages of *T. urticae*, eating more eggs, larvae and protonymphs than deutonymphs and adult females (Badii et al. 2004).

#### 2B.4.3 The efficacy of *N. californicus* inundative releases

*Neoseiulus californicus* is a successful biological control agent of spider mites in the Mediterranean and other semi-arid regions (Castagnoli and Simoni 2003). The dry-adapted strain, which was recently evaluated (Walzer et al. 2007, Palevsky et al. 2008) and is now commercially used, extends the potential use of *N. californicus* into dryer regions. However, its effect in desert areas has not been extensively studied. In a study carried out in Tunisia, Othman et al. (2001) examined the effect of *N. californicus* releases in sachets on *O. afrasiaticus* population levels and accompanying damage. They released *N. californicus* on infested date palm trees, from mid-July until mid-August, and reported a significant reduction in the pest population and level of damage, which was clearer on heavily infested trees (Othman et al. 2001).

In the current study, *N. californicus* succeeded in reducing the size of the *O. afrasiaticus* population and the subsequent damage, even though the level of infestation and damage caused in the summer of 2009 was very low (see also results of the 2009 *T. athiasae* releases). Some individuals were found on the bunch 10 and 20 days post-release (one and two sampling intervals, respectively; see Figs. 4a, 5a), suggesting that they can survive the extreme dry conditions of the Arava Valley. Because egg adaptations to cope with dry climate are more strain-specific than species-specific (Bakker et al. 1993), the use of a dry-adapted strain can partially explain this survival. In addition, Castagnoli and Simoni (1994) found that *N. californicus* larvae are more tolerant to an arid temperature-humidity regime than the eggs, enabling this species to survive even in habitats which are not suitable for its egg development.

It was also shown that the humidity experienced by *N. californicus* on a living plant, within its boundary layer, is much higher than on a plastic arena (Gaede 1992, Castagnoli and Simoni 1994). However, the date bunch in the SAV is exposed to frequent dry winds, probably resulting

in low moisture compensation by the plant. Moreover, dispersal behavior is one of the strategies used by *N. californicus* to avoid unfavorable ambient conditions, such as high temperature, high light intensity, and low humidity (Auger et al. 1999) – the conditions that characterize the SAV. It is also known that *N. californicus* can survive several days without food (Palevsky et al. 1999), which may explain how *N. californicus* survived on the bunch even when the pest level was negligible. However, lack of water further reduced the *N. californicus* survival without food from 9.8 days with water to 3.6 days without water (De Courcy Williams et al. 2004b), providing a plausible explanation for the low predator numbers in the samples of the current study.

The slow-release technique based on a small rearing unit using prey mites of the order Astigmata was first introduced for *Neoseiulus cucumeris* (Oudemans) by Ramakers (1990) to improve its establishment when prey or pollen was scarce. Since then, this methodology has been adopted for releasing *N. cucumeris*, *A. swirskii*, and most recently for *N. californicus*. The sequential use of slow-release sachet in the current study enables a small, but constant, flow of predators, protecting the bunch from newly arriving spider mites. However, the limited food (before arrival of the pest) and water, together with the extreme arid environment, constrain the ability of *N. californicus* to survive on the bunch out of the sachet. As such, the predatory mite served in our case as a short-term 'living pesticide'.

In 2010, despite the use of newly designed sachets (Spical-plus), no effect of the treatment on the population size was found for either the predator or the pest, nor was there any impact on the level of subsequent damage. The *O. afasiaticus* populations were extremely low, even lower than in 2009. Similarly, in many other orchards throughout the Arava region pest populations in 2010 were extremely low and damage was negligible, even when acaricides were not applied (A. Greenberg and S. Dobrinin, personal communication). The numbers of predators detected in the samples were also lower than those of 2009, even with the high application rate of four sachets per bunch per release. These new sachets are designed to serve as small rearing units, supplying *N. californicus* with shelter and food for its multiplication. They allow 50-100 mites at the time of release to 'produce' at least 1000 dispersing mites during a six-week period (Koppert Biological Systems 2009). In contrast, the sachets we used in 2009 (Spical) were less effective as rearing units and thus were filled with 250 *N. californicus* mites per sachet.

Perhaps, due to the extreme ambient conditions in the SAV, *N. californicus* reproduction cannot take place in both types of sachets, such that the number of effective *N. californicus* is

determined at the release point. Therefore, the 2009 release rate (500 mites per bunch in two sachets) could actually have been higher than the highest rate in 2010 (200-400 mites per bunch in four sachets). In addition, several sachets were found torn (probably by rodents, which apparently fed on the bran) ten days after they were placed in the trees. In the emergence experiment that was conducted only for the new type of sachet, the dispersing numbers per sachet in the first week post-release did not exceed 22, as compared to approximately 100 under optimal conditions (Koppert Biological Systems 2009). These findings emphasize the difficulty of using *N. californicus* as a biological control agent in the date palms of the SAV.

Ship and Wang (2003) evaluated slow-release sachets of *N. cucumeris* (1000 mites per sachet) against the western flower thrips, *Frankliniella occidentalis*, in tomato plants. They showed that thrips control was achieved when more than 100 emerging predators per sachet per week were dispersed on each tomato plant. *Amblyseius swirskii* is also commercially available in slow-release sachets. Chow et al. (2010) counted 200-400 *A. swirskii* dispersed from one slow-release sachet (Koppert Biological Systems, The Netherlands) in four weeks at 24°C (RH was not listed). The number of predatory mites per week increased from 20-50 in the first week, to its peak (130-200) in the second or third week, and then declined (Chow et al. 2010). Examination of the performance of the same type of sachets under different levels of air humidity (25°C) revealed that the humidity level did not affect the total number of *A. swirskii* emerging from each sachet, but did affect the dispersal rate (Luczynski 2008). At 85% RH, the ca. 1300 mites emerged at a similar rate over the first four weeks, while at 65% and 75% RH, over 95% of the mites had left the sachet within the first two weeks. This pattern was related to the dramatic decline of the prey mite (*Carpoglyphus lactis*) population in the sachet during the first week of the experiment in these low-humidity treatments (Luczynski 2008).

In the current study, the prey mites of *N. californicus* were *C. lactis* in 2009 and *Lepidoglyphus destructor* in 2010. Both prey mites are drought-sensitive, with an optimal humidity range of 84-94% for the former (Chmielewski 1971, Okamoto 1984) and 85-100% for the latter (Danielsen et al. 2004). Therefore, it can be assumed that their population decreased immediately after exposure to the SAV environment due to death and dispersal. As a result, the *N. californicus* population not only suffered directly from the harsh ambient conditions, but also indirectly through lack of its prey as the food and water resource. This prey drought sensitivity also did not

allow us to evaluate the establishment of indigenous phytoseiids in the treatments where the prey alone was applied in excess.

The sigmoid curve of the emergence numbers in relation to vapor pressure deficit (VPD) implies that above 3.6-3.9 kPa VPD, *N. californicus* performance decreased sharply to even lower levels, at which only a few motiles were dispersed from every sachet within seven days. *Neoseiulus californicus* is among the more dry-adapted phytoseiids that are commercially available, occurring in warm and relatively dry regions of California, South America, North Africa, and the Mediterranean basin (De Moraes et al. 2004). It is tolerant to a wide range of temperature, up to 33°C (Castagnoli and Simoni 1994, 2003). While the mean summer temperature in the SAV is 35°C, the phytoseiids are frequently exposed to much higher temperatures ( $\geq 40^\circ\text{C}$ ) at midday. In addition, air moisture combined with temperature (absolute humidity, which is expressed as VPD) is more critical than temperature alone. For instance, *N. californicus* larvae survival was very high at 29°C with 70-100% RH, whereas at 33°C it remained high only at 95-100% RH (Castagnoli and Simoni 1994). The present study was carried out with a dry-adapted strain of *N. californicus*, its advantage was evaluated for survival and reproduction at 25°C with 64% RH (Walzer et al. 2007) and for spider mite control on living plants at 25-26°C with 50% RH (Palevsky et al. 2008). However, these two climate conditions correspond to VPD of 1.1 and 1.6-1.7 kPa, respectively, which are far below the SAV summer range of water deficit (2.5-5 kPa).

In summary, although the presence of phytoseiids was observed on the fruit bunch/stalk, their population levels were found to be very low, and pollen applications were not adequate to conserve an effective population size. Nevertheless, the inundative releases of the two predatory mites, the indigenous *T. athiasae* and the exotic *N. californicus*, were partially successful in controlling the old world date mite. For further comparison between these two systems, see the General Discussion section.

### 3 Develop postharvest treatments to mitigate evident mite damage caused to fruits by spider mites.

Mite damage is most evident at harvest when the fruits are dry. Customary re-hydration and cooling treatments were efficient in mitigating evident mite damage on ‘Medjool’ date fruits (Palevsky *et al.*, 2004). Long-term preservation of semi-dry ‘Medjool’ date fruits was achieved in controlled freeze-temperature regimes (Shomer *et al.*, 1998). Successful adaptation of the preservation procedures to quality-restored mite-damaged fruits will reverse much of the economic loss from mite damage.

#### MATERIALS AND METHODS

**Hydration** - ‘Medjool’ fruit (2007-2008) and ‘Deglet Noor’ fruit strands (2008-2009, 2010-2011) with evident mite damage from acaricide untreated trees were collected and graded. Fruit and fruit strands with up to 40% damage were placed in trays. Half of the trays were hydrated for 48 hours in a temperature and humidity controlled room set as specified under “Results” During this period other trays were stored at ~25°C (control).

**Long-term preservation and shelf-life** - Control and hydrated ‘Medjool’ fruit tightly wrapped in polyethylene film were stored at -18°C for 8 months and removed to 25°C for 6-week shelf-life period. Control and hydrated ‘Deglet Noor’ fruit strands were tightly wrapped in polyethylene film and stored in a cold room (T~4.5°C and RH~71%). After storage the trays were transferred for shelf-life at 25°C. Storage and shelf-life periods are specified under “Results”.

Fruit quality was assessed after storage and shelf-life periods by customary date packing house practices.

#### RESULTS

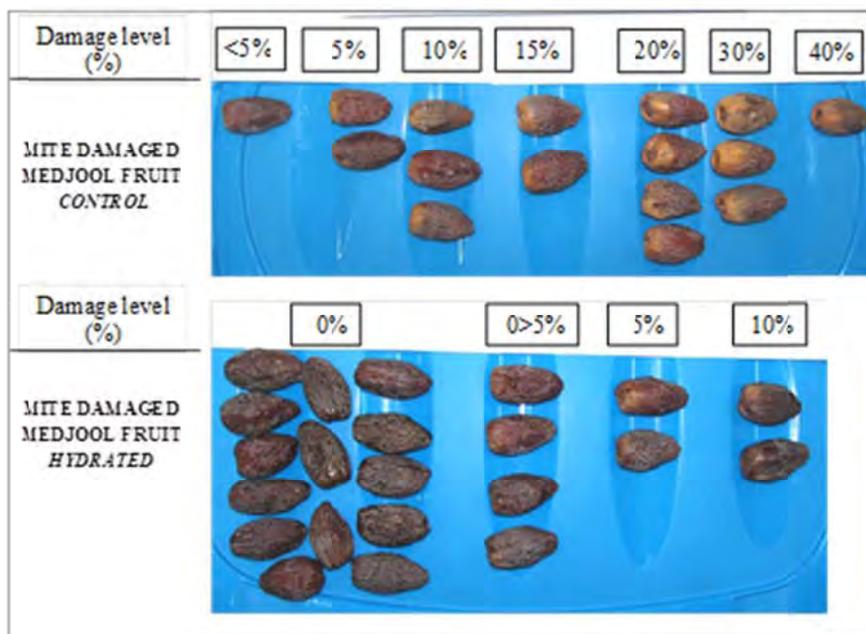
‘Medjool’ fruit hydration was carried out at 47°C and 95% air relative humidity. Hydration treatment increased fruit weight and water content and markedly reduced the apparent damage inflicted by the mite (Table 3.1). Fruit quality parameters after the storage and shelf-life periods are presented in Table 3.2 and Picture 3.1. The increased fruit weight and water content as well as the improved quality by hydration were maintained during storage and shelflife.

**Table 3.1:** Characterization of control and hydrated mite-damaged 'Medjool' fruit.

Treatment	Fruit Weight (g)	Water Content (%)	Fraction of fruit with the indicated damage level (%)							
			none	<5%	5%	10%	15%	20%	30%	40%
 Control	19.8 ±0.4	21.9 ±0.3	/	4.6 ±2.6	12.6 ±0.3	15.7 ±6.0	14.9 ±4.2	25.6 ±7.7	17.1 ±6.3	9.6 ±3.4
 Hydrated	21.3 ±0.6	25.9 ±0.7	61.9 ±13.3	19.9 ±7.2	10.1 ±4.6	8.1 ±4.7	/	/	/	/

**Table 3.2:** Quality parameters of control and hydrated mite-damaged 'Medjool' date fruit after 8-month storage at -18°C and 6-week shelf-life at 25°C.

Treatment	Fruit weight (g)	Water content (%)	Fraction of fruit with the indicated damage level (%)						
			0	<5%	5%	10%	20%	30%	40%
Control	19.7±0.5	20.5±0.4	/	6±2	13±1	16±6	26±8	17±6	10±3
Hydrated	20.8±0.3	24.1±0.3	68±15	20±7	10±5	8±5	/	/	/



**Picture 3.1:** Control and hydrated mite damaged ‘Medjool’ date fruit after 8-month preservation at  $-18^{\circ}\text{C}$  and 6-week shelf life at  $25^{\circ}\text{C}$ .

‘Deglet Noor’ hydration in 2008 was carried out at  $43\text{--}46^{\circ}\text{C}$  and 94% air humidity. The hydration process increased fruit weight and water content from  $7.4\pm 0.2$  and  $21.9\pm 0.4$  to  $7.8\pm 0.3$  g and  $25.5\pm 0.4$  %, respectively, led to darker color and reduced the apparent damage inflicted by the mite (Picture 3.2).



**Picture 3.2:** ‘Deglet Noor’ fruit strands prior to (control) and following hydration (hydrated).

Fruit quality parameters after 8-month cold storage (Picture 3.3) are summarized in Table 3.3. During the 8-month cold room storage, the average water content increased by approximately 15% with a concomitant increase of approximately 4% in fruit weight. These increases were larger than the ones we have measured in export-quality "wet" 'Deglet Noor' strands stored under similar conditions. It is likely that the porous skin of the mite affected fruit allows for a more extensive water uptake from the surrounding humid atmosphere. Long term cold storage resulted in damage development, more in control than in the hydrated fruit.



**Picture 3.3:** Hydrated and control 'Deglet Noor' fruit strands after 8 month cold storage.

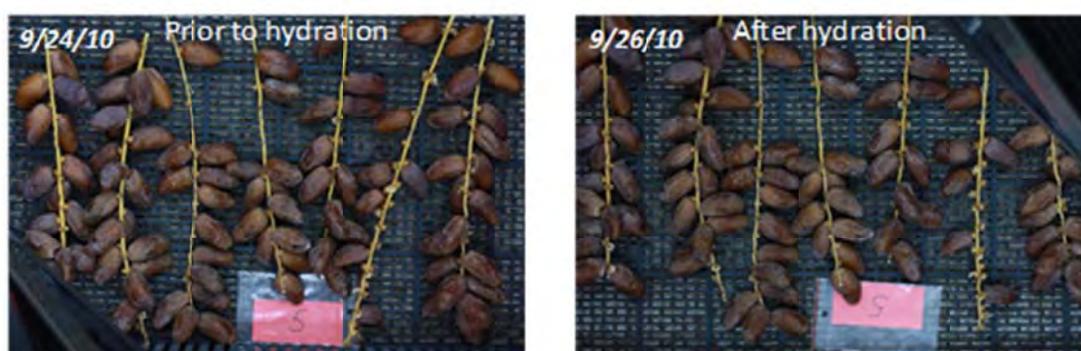
**Table 3.3:** Quality parameters of control and hydrated mite-damaged 'Deglet Noor' date fruit before and after 8-month storage at ~4°C.

Time	Treatment	Fruit weight (g)	Water content (%)	Affected fruit (% of total)		
				Blistering	Discoloration	Microbial contamination
Prior to storage	Control	10.1±0.6	22.2±0.8	High quality fruit were selected		
	Hydrated	10.3±1.1	26.1±2.5			
After storage	Control	10.4±1.2	26.7±1.8	25	12	14.7
	Hydrated	10.8±0.9	30.5±0.6	27	7.8	7.6

Unlike export-quality "wet" 'Deglet Noor' strands, the mite affected fruit were susceptible to yeast and mold development already during the cold storage. Microbial infestation intensified

rapidly at 25°C and shortly after removal from the cold room most of the fruit of both treatments was no longer acceptable for human consumption.

In an attempt to reduce microbial infestation in the treated fruit, the hydration of ‘Deglet Noor’ strands in 2010 was carried out under milder conditions, 40°C and 60% relative humidity. The hydration process increased fruit weight and water content from 11.0±0.6 and 25.9±2.1 to 11.9±0.4 g and 27.5±3.6 %, respectively, and reduced the apparent mite effected damage by approximately 27%; as observed in our earlier trial, the hydration process was accompanied by fruit color darkening (Picture 3.4).



**Picture 3.4:** Mite effected ‘Deglet Noor’ fruit strands prior to and following hydration.

After 4-month cold-storage the trays were transferred to a 25°C-room for 1-week shelf-life. Fruit quality parameters are summarized in Table 3.4.

**Table 3.4:** Quality parameters of control and hydrated mite-damaged ‘Deglet Noor’ date fruit before and after 4-month storage at ~4°C and 1-week shelf life at 25°C.

Treatment	Fruit weight (g)	Water content (%)	Fraction of fruit with the indicated damage level (%)						
			0%	<5%	5%	10%	20%	30%	40%
Control	11.0 ±0.6	25.9 ±2.1	78 ±11	3.2 ±2.1	3.2 ±2.5	4.9 ±3.5	6.3 ±5.1	2.9 ±3.0	1.7 ±2.2
Hydrated	11.9 ±0.4	27.4 ±3.6	84 ±11	5.4 ±5.1	3.5 ±2.3	4.1 ±4.7	1.8 ±2.2	1.2 ±1.6	0.2 ±0.4
Control, + storage	11.6 ±1.0	30.6 ±2.5	87 ±18	0.9 ±1.3	0.9 ±1.3	3.6 ±5.1	7.3 ±10.3	0.0	0.0
Hydrated, + storage	12.4 ±0.1	31.1 ±0.3	86 ±17	0.0	2.9 ±4.1	5.7 ±6.1	2.5 ±3.5	2.1 ±2.9	0.4 ±0.6
Control,	11.9	26.5	72	12.9	6.7	5.6	2.2	0.0	0.0

+ storage	±0.7	±1.0	±24	±3.7	±9.4	±7.9	±3.1		
+ shelf life									
Hydrated,	11.5	27.5	82	8.6	3.9	3.2	0.8	1.2	0.0
+ storage	±0.1	±1.8	±13	±2.6	±2.4	±4.6	±1.1	±1.7	
+ shelf life									

---

Similarly to the earlier study on 'Deglet Noor' strands, fruit water content increased significantly during cold storage with a moderate increase in fruit weight and reduction in mite damage in both control and hydrated fruit. During shelf-life fruit water content, weight and apparent mite damage returned to the levels measured prior to storage. Microbial infestation during cold storage and shelf-life was very small.

### Summary

Hydration of mite damaged 'Medjool' fruit for 48 h at 47<sup>0</sup>C and 95% relative humidity was highly efficient in restoring fruit quality that was largely sustained during long term storage (up to 8 months) and shelf-life (up to 6 weeks).

Hydration of mite affected 'Deglet Noor' fruit strands at 40<sup>0</sup>C and 60% relative humidity was beneficial in mitigating mite inflicted damage. However, more work is required to assure quality preservation of the hydrated fruit. Further research should concentrate on the refinement of the hydration process with respect to temperature, humidity and exposure period. In addition, optimization of long-term storage of the hydrated fruit with respect to duration and temperature regimes is needed.

#### 4A. Exocarp structure in relation to mite phenology and population density

Fruit exocarp was prepared for light and electron microscopy according to Shomer et al. (1998). Sections were cut with a LKB Pyramitome (2  $\mu$  width) and stained with toluidine blue. Samples were taken every 3 weeks from June to September 2008 and examined using an Olympus BX61 microscope. Structural components of the exocarp (cuticle, epidermis, parenchyma and sclerotic stone cells) were observed from clean and infested Medjool and Deglet Noor fruits. In infested fruits cell walls were penetrated by mite chelicera tearing the cuticle and causing damage to the epidermal cells (Figures 4A.1 and 4A.2).

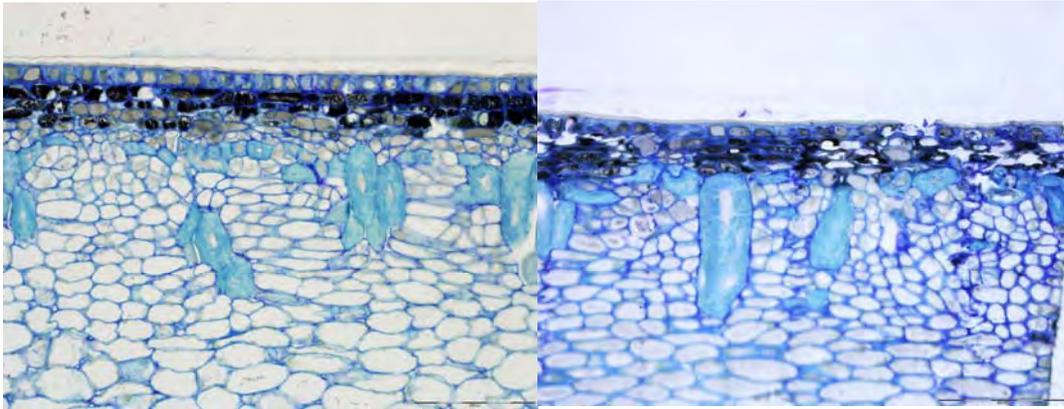


Figure 4A.1: Medjool green fruit infested (left) and control (right). Arava, June 2008 (400 x).

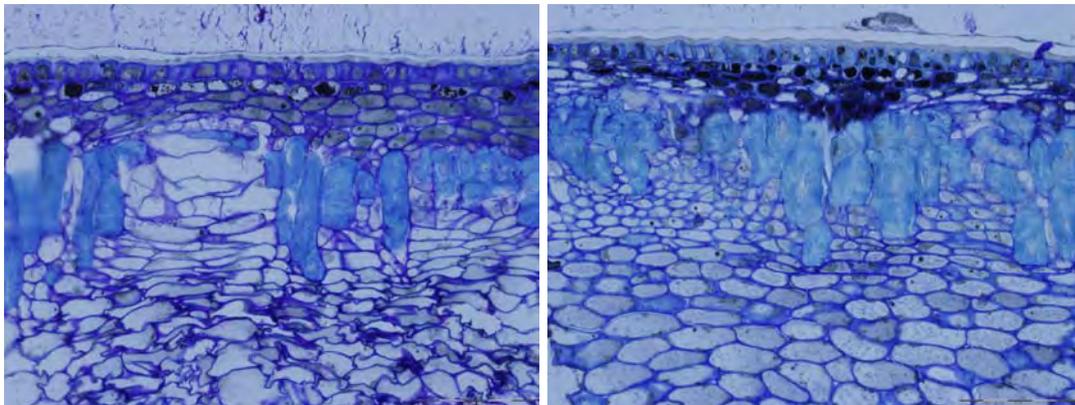


Figure 4A.2: Deglet Noor green fruit infested (left) and control (right). Arava, July 2008 (400 x).

**Objective 4B:** Relationship between fruit chemistry and mite phenology and population density.

In compliance with Objective 4B, date fruit chemical composition was monitored during fruit development in the mite susceptible CVs 'Medjool', 'Barhi' and 'Deglet Noor' and insusceptible CV 'Zahidi'. Sampling period coincided with the mite life cycle on the date fruit, as established in our earlier studies (Palevsky *et al*, 2004, 2005). Plant chemical defense against insects is largely ascribed to plant secondary metabolites (Rosenthal and Berenbaum, 1991) and, to a lesser extent, primary metabolites (Slansky, 1990). Our current study tests for relationships between date fruit chemical parameters, mite population density and susceptibility/resistance to the spider mite; the aim being to identify potential constituent attractants and repellents in the fruit.

#### MATERIALS AND METHODS

**Plant material.** The study was conducted in date orchards situated in the Israeli southern Arava Valley (SAV) (29°33' N, 34°57' E). Four date cultivars (CVs) were studied; 'Medjool' and 'Deglet Noor' from "Grofit orchard" and 'Barhi' and 'Zahidi' from "Samar orchard". Medjool, Barhi and Deglet Noor are susceptible to the date spider mite whereas 'Zahidi' is an insusceptible CV. Five commercial and five acaricide-untreated trees of each CV, from different locations within each orchard, were sampled fortnightly from the beginning of the kimri stage to fruit color change from green to yellow ('Medjool', 'Barhi' and 'Zahidi') or red ('Deglet Noor'). Sampling periods were 5/13 to 8/6, 5/29 to 8/6, and 6/10 to 8/29 for 'Medjool', 'Barhi'/'Zahidi' and 'Deglet Noor', respectively. Each tree was a distinct replicate; five fruit strands from five central bunches were removed and used in fruit chemical analysis.

**Water content (WC) and dry weight (DW).** Ten grams of thinly sliced date fruit tissue were arranged in a single layer on drying plates and placed in a laboratory air circulating oven (Heraeus Instruments, Model UT6, Germany) set at 60°C. After 24 h the plates were weighed twice daily and removed from the oven after the same weight was recorded in two consecutive measurements. WC and DW were calculated as follows:

$$\text{WC (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100; \quad \text{DW (\%)} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

**Mineral analysis.** Mineral content of date fruit tissue samples was analyzed by the analytical chemistry laboratory of the southern Arava Research and Development following

protocols the Israeli Ministry of Agriculture Extension Service. Sodium, potassium, calcium and magnesium were measured in oven-dried tissue digested to clarity with hot concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 30%). For iron, zinc and manganese measurements, ash was prepared from oven dried fruit tissue (5 hours at  $550^\circ\text{C}$  in a laboratory furnace, BIFA Laboratory Furnaces, Model MS8-36) and digested to clarity with 6N hydrochloric acid (HCl). Sodium and potassium were measured by a flame photometer (JENWAY, PFP7); calcium, magnesium, iron, zinc and manganese were analyzed by an atomic absorption spectrometer (Perkin Elmer, 3100).

**Fruit processing.** Fruits were washed with water and dried at  $42^\circ\text{C}$  for 20 minutes to remove the excess surface water. The fruits were pitted and the mesocarp and exocarp tissues were further processed in a hard-fruit juice extractor (Hard Fruit Juice Extractor, Magimix, Le Duo, France). The resulting liquid was centrifuged (10,000 rpm for 10 min at  $4^\circ\text{C}$ , Sorvall Instruments RC5C, USA, rotor number SS-34) and the clear supernatant (date juice) was chemically analyzed.

**Total soluble solids (TSS), electrical conductance (EC), pH and titratable acidity (TA).** TSS (in %) was measured in date juice with a hand-held refractometer (ATAGO, ATC-1E, Brix 0-32%, Japan, calibrated with distilled water). A conductometer (Metrohm, 644, Switzerland) equipped with 1-cm measuring cell was used to measure EC (in  $\text{mS cm}^{-1}$ ). The pH was measured using a specialized food electrode (pH 211 microprocessor pH-meter and FC 200B food electrode, Hanna Instruments, USA). TA (in mequiv. acid  $\text{dL}^{-1}$ ) was determined colorimetrically by titration with 0.1N NaOH using the pH indicator phenol phtalein.

**Total soluble phenolics content.** Date juice was extracted in 80% methanol supplemented with 2 mM sodium fluoride (NaF), at a ratio of 1:2 (v/v). The extracts were centrifuged as described earlier and the clear supernatant was diluted 10 to 100-fold with DDW (depending on CV and sampling date). Concentration of total soluble phenolics was measured colorimetrically with Folin-Ciocalteu 2N phenol reagent (SIGMA Chemical Co, USA) according to Singleton and Rossi (29). Aliquots of 100  $\mu\text{L}$  were added to 900  $\mu\text{L}$  reaction solution consisting of 200  $\mu\text{L}$  freshly prepared 10-fold diluted Folin-Ciocalteu reagent, 100  $\mu\text{L}$  20%  $\text{Na}_2\text{CO}_3$  and 600  $\mu\text{L}$  DDW. Tannic acid (SIGMA Chemical Co, USA) was used for the calibration curve (0-100  $\mu\text{g mL}^{-1}$ ). The absorbance at 765 nm was measured with a spectrophotometer (SHIMADZU Corporation, UV-1650PC, Kyoto, Japan) after 1-hour incubation, and the results, after

subtracting the contribution of glucose, fructose and sucrose to the readings were expressed in tannic acid equivalents, mg kg<sup>-1</sup> date tissue.

**RP-HPLC partial analysis of soluble phenolics in date fruit.** RP-HPLC partial analysis of soluble phenolics in date extracts was performed as described earlier (Rock et al., 2009). Methanolic extracts of date fruit juice were prepared as described earlier. After centrifugation the supernatant was filtered through a 0.45µm filter. Samples of 20 µL were analyzed using the LaChrom Merck Hitachi HPLC system, consisting of Pump L7100, Column oven L7350, Mixer-degasser L-7614 and Manual Injector Rheodyne, coupled with a diode array detector (DAD) with 3D feature (Multiwavelength Detector, Jasco MD-2010 Plus), interface (Jasco LC-Net II / ADC) and scientific software (EZChrom *Elite*<sup>TM</sup> Client/Server version 3.1.6 build 3.1.6.2433) that provides data acquisition in real time and post run data manipulation and integration facilities. An endcapped Purospher®Star RP-18 column (250 x 4 mm LichroCART® cartridge, 5 µm particle size) with endcapped Lichrospher®100 RP-18 guard column (4 x 4 mm LichroCART® cartridge, 5 µm particle size) were used. The binary mobile phase consisted of phosphoric acid in DDW (0.1%, pH 2.4) (solvent A), and acetonitrile (solvent B). Elution was carried out at 1 mL/min with the following scheme: 15% solvent B for the first 5 min; 15% solvent B to reach 100% by 15 min; The column was then washed and re-equilibrated by 5- and 10-min post runs with 100% and 15% solvent B, respectively.

Every run was monitored in real time by three display modes simultaneously: contour plot, chromatogram display at a chosen wavelength (usually 280 nm) and UV spectra. The oven temperature was set at 40°C, and the pressure was 169 atm. Acetonitrile was HPLC grade (LiChrosolv Merck); Column-filtered water was further distilled by Corning Megapure System, MP-6A, and passed through a 0.20µm Nylon membrane. Phosphoric acid and NaF were of analytical grade.

**Phenolics' tentative identification and concentration assessment.** Chromatogram individual peaks were tentatively assigned on the basis of the phenolics' standard library constructed as follows: Each standard (50-100 µg mL<sup>-1</sup> in methanol) was injected separately and the acquired data of UV-VIS absorption spectra and retention times were incorporated into the system phenolic standard library. The library included the following phenolics' standards: hydroxybenzoic (salicylic) acid, quercetin-3-β-glucoside, ellagic acid (Fluka, Israel), catechin, epicatechin, catechin gallate, epicatechin gallate, galocatechin, epigallocatechin, galocatechin

gallate, epigallocatechin gallate, naringin, pyrogallol, gallic-, p-coumaric-, cinnamic-, chlorogenic-, caffeic-, hydrocaffeic-, ferulic-, sinapic- and tannic acid (Sigma-Aldrich, Israel). Peak identification was performed by the software. Each peak was tested for purity by three point purity test and for similarity by a library search comparing the peak spectrum to that of the standards. A high similarity index together with a common retention time was considered positive identification; a similar UV-VIS absorption spectrum but a different retention time implied partial identification (e.g. derivative of the phenolic compound with the similar absorption spectrum). Relative standard deviation (RSD) for the retention times in repetitive runs of date extracts was in the range of 0.2-1.6%.

The software also calculates the peak area. For phenolic quantitative evaluation, extracts were run repeatedly at several dilutions to assure linearity between peak area and sample concentration. Epicatechin and caffeic acid standards were used for the quantitative evaluation of individual phenolic peaks. Calibration curves (linear,  $R^2=0.9999$ ) were constructed with the standards at four concentrations (0.01, 0.10, 0.25 and 0.50 mg mL<sup>-1</sup>). RSD for peak areas in multiple runs was within 0.4-3.2%. A detection limit (minimal peak area) was set at 15,000. Total concentrations of catechins and phenolic acids were calculated by summation of the concentrations of the corresponding individual phenolics..

**Chlorophylls and carotenoids analysis.** Pigment extraction was carried out following Fish *et al* (2002). 10 g date juice were homogenized on ice in the dark with 370 ml extraction solution consisting of hexane:acetone:ethanol:water (10:5:4.75:0.25, v/v) and 0.01% butylated hydroxytoluene (BHT). The homogenate was left in the dark for 15 min at room temperature to settle. The upper phase (hexane) was collected and the hexane evaporated under vacuum. The dry pellet was dissolve in 0.5 ml acetone. Twenty  $\mu$ L of the acetone pigment solution were analyzed according to Yuan and Chen (1998) using the HPLC system described earlier. The mobile phases consisted of dichloromethane:methanol:acetonitrile:water, 5:85:5.5:4.5, v/v, (solvent A), and dichloromethane:methanol:acetonitrile:water, 22:28:45.5:4.5, v/v, (solvent B). The elution was set to go with 100% solvent A for 15 min, gradient from 100% to 50% solvent A between 15 to 35min and from 50% to 0% solvent A between 35 to 60 min. Each run was followed by 10 min equilibration step with 100% solvent A. The flow rate was 1 ml/min. Column temperature was set at 35°C and the pressure was 136 atm. Pigment standard library was

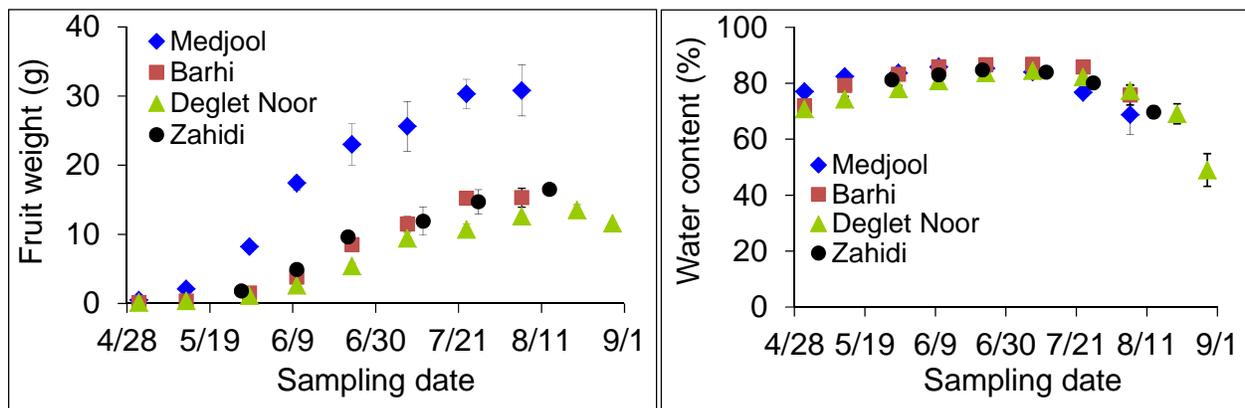
constructed using chlorophyll a, chlorophyll b, lutein,  $\beta$ -carotene and lycopene (all were a generous gift from Algatechnologies, Ltd., Israel).

**Statistical Analysis.** Data are reported as means  $\pm$  their respective standard deviations.

## RESULTS

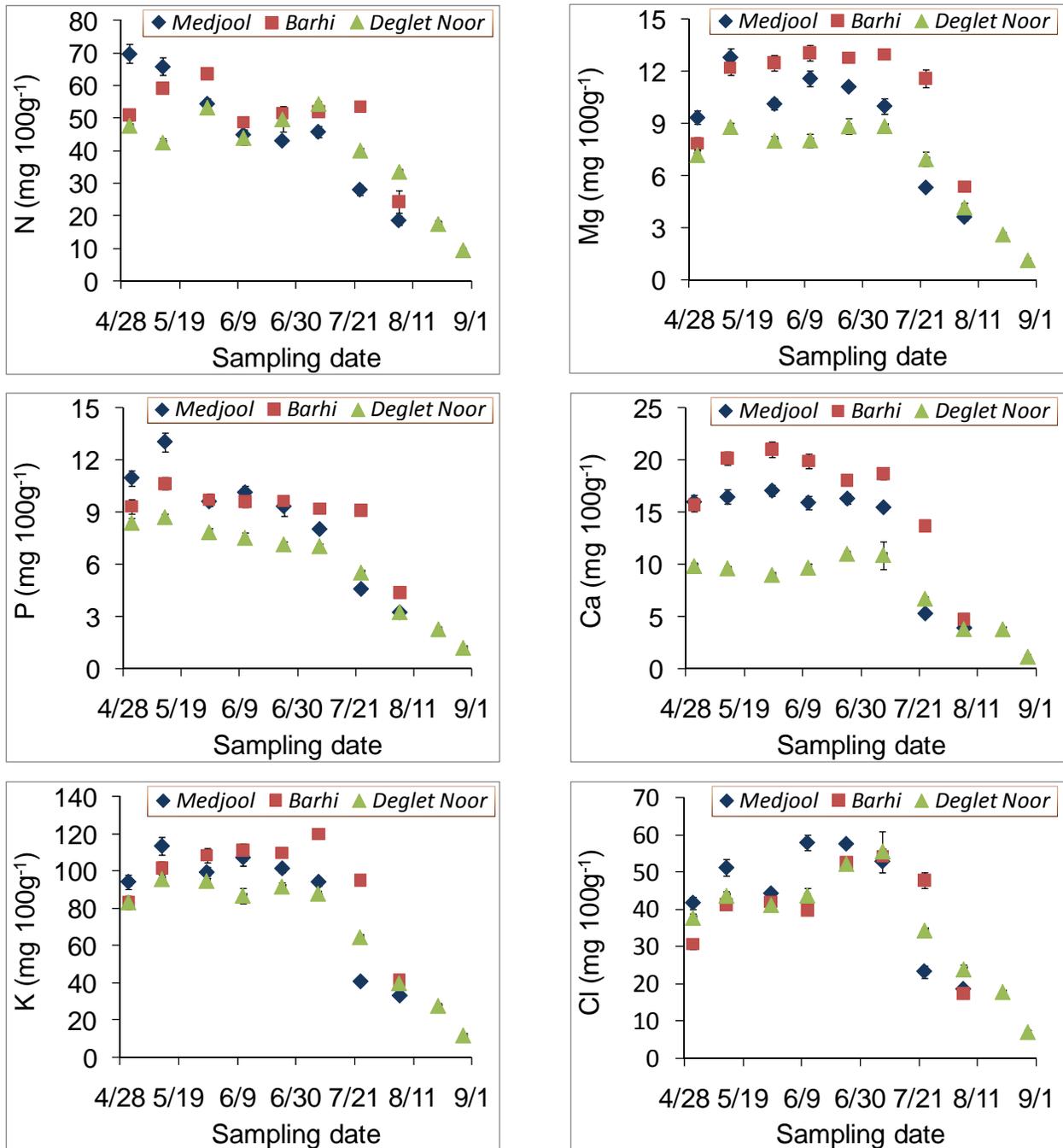
### Physico-chemical properties of dates during fruit development.

Figure 4B.1 presents the average fruit weight and water content in the four CVs during the time period relevant to the mite ascent, population buildup and descent. In agreement with earlier findings (Palevsky *et al*, 2005) during date fruit development water content initially increased (**phase 1**), remained high for a certain period (**phase 2**) and decreased on later dates (**phase 3**). A specific set of time and period of maximal fruit water content characterized each of the susceptible cultivars and may be related to the cultivar's characteristic mite phenology.



**Figure 4B.1:** Fruit weight and water content during fruit development in four date cultivars.

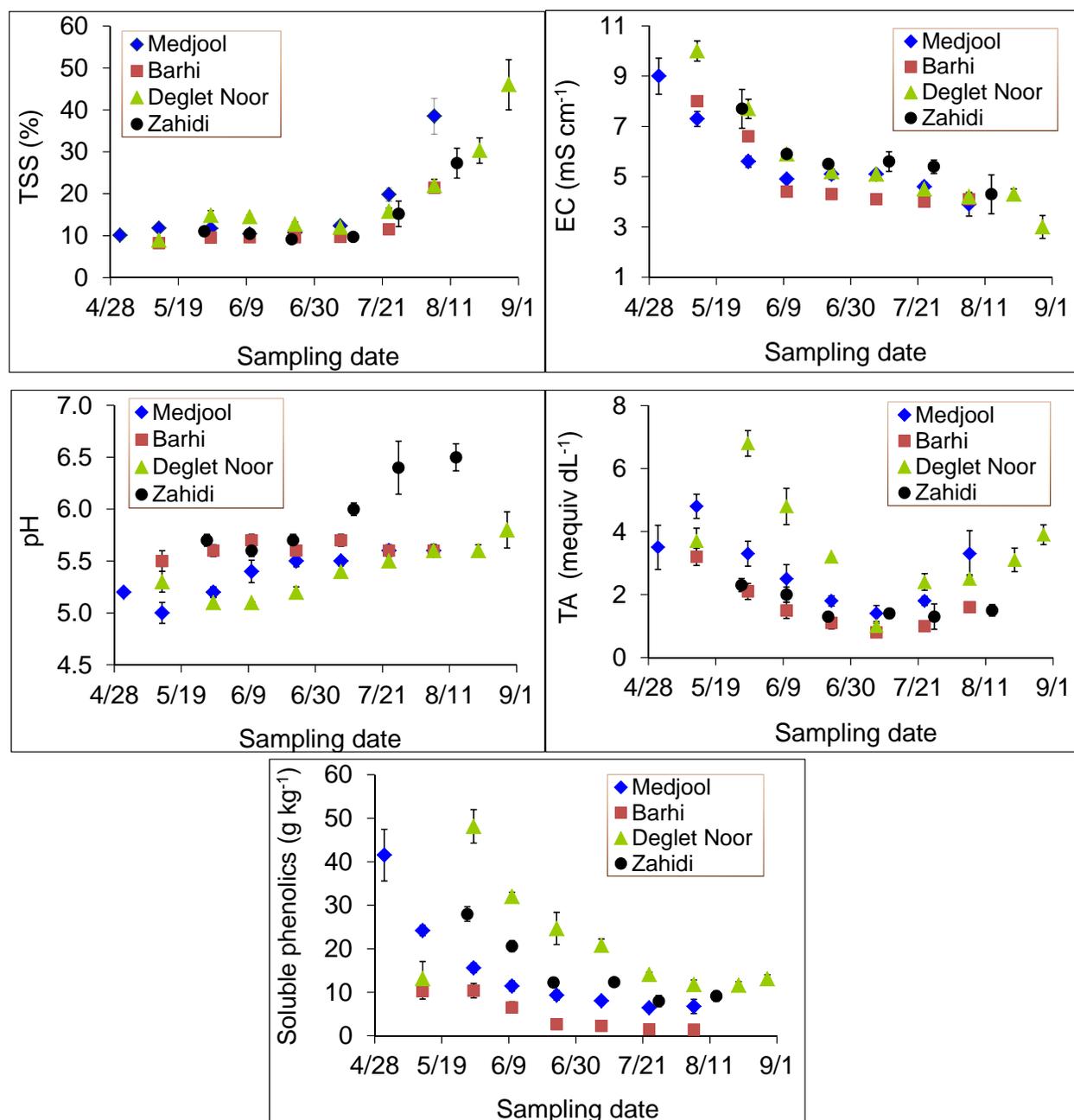
Mineral content in fruit of the three susceptible CVs, 'Medjool', 'Barhi' and 'Deglet Noor', is shown in Figure 4B.2. The content (in mg per 100g DW) of the analyzed minerals (N, P, K, Ca, Mg and Cl) decreased during the entire studied period, however, at a faster rate along phases 1 and 3 compared to phase 2. Sodium level was always below flame photometry resolution, i.e.,  $\leq 0.02\%$ . Mineral content sharply decreased parallel to the decrease in water content.



**Figure 4B.2:** Mineral content during fruit development in three mite susceptible date cultivars.

It should be noted that fruit weight and water content as well as mineral composition were not significantly different between fruit sampled from acaricide treated or untreated trees.

The juice extracted from the fruit of the four date cultivars was analyzed with respect to total soluble solids (TSS) concentration, electrical conductivity (EC), pH, titratable acidity (TA) and total soluble phenolics content. The results are presented in Figure 4B.3.



**Figure 4B.3:** Chemical parameters of "fruit juice" during fruit development in four date cultivars.

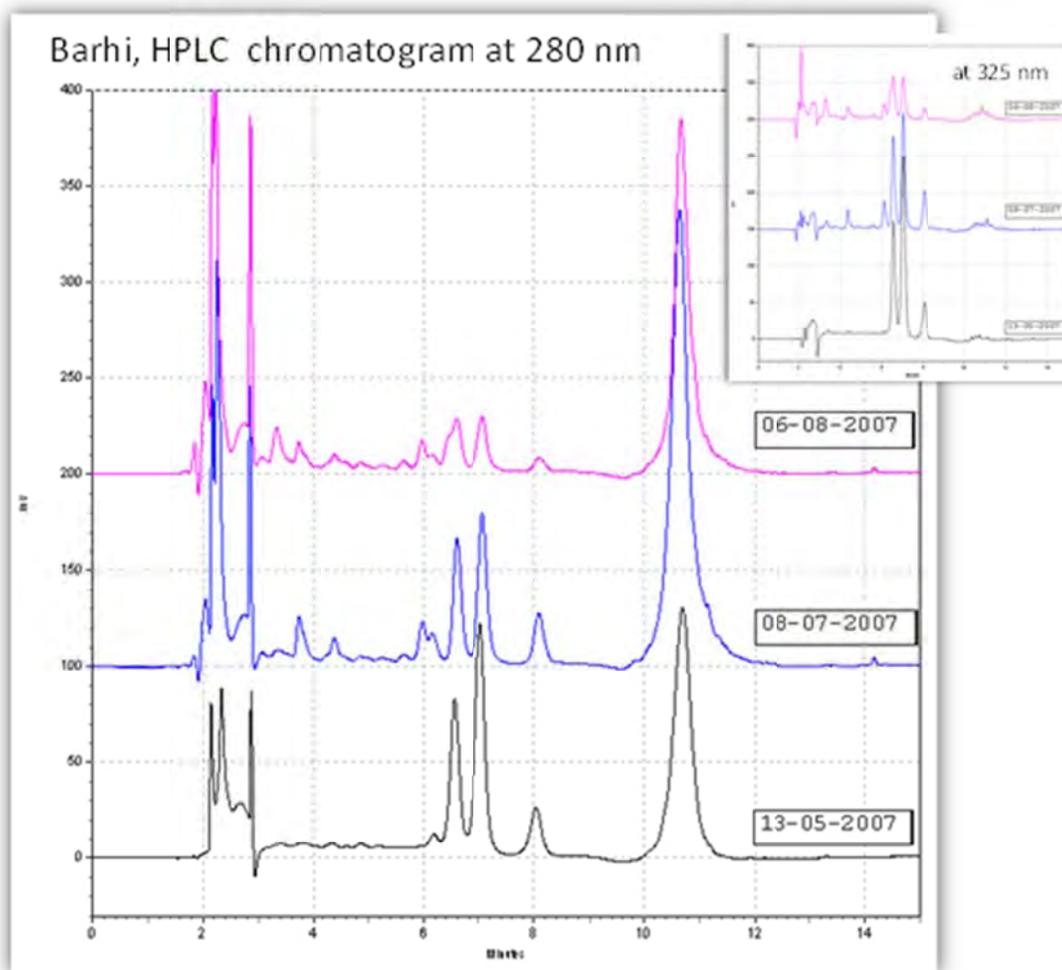
The trend of change in juice chemical composition during fruit development was similar in fruit of the four CVs; however, the absolute values and rates of change were cultivar dependent and may be related to the cultivar susceptibility to the mite. TSS was relatively low and practically constant during phases 1 and 2, and rapidly increased during phase 3. Juice EC decreased during the entire sampling period, however, at a faster rate along phase 1 and 3 compared to phase 2. Notably, during phase 2 the EC in 'Zahidi' was higher than in the susceptible cultivars, especially in later dates. Juice pH in 'Medjool' and 'Deglet Noor' fruit increased by close to 1 pH unit during the sampling period reaching pH 5.5-5.6; in 'Barhi', on the other hand, the pH was 5.5-5.6 during the entire period. The highest pH was measured in 'Zahidi', reaching a value of 6.5 toward the end of the sampling term. The content of TA in date fruit juice decreased initially, with the minimal value coinciding with maximal water content. Later on, TA increased parallel to TSS and inversely to the water content, except for 'Zahidi' where TA remained low. The content of soluble phenolics decreased at a rapid pace during phase 1 and at a slower pace during phase 2; the level remained virtually unchanged during phase 3.

As earlier noted for the variations in water content and mineral composition during date fruit development no significant differences between acaricide treated and untreated fruit were measured in the fruit juice chemical composition.

#### **Partial analysis of date soluble phenolics during fruit development.**

The content of soluble phenolic compounds in the date fruit sap varied markedly during the period relevant to mite ascent and population buildup (Fig. 3). Phenolics are secondary metabolites that may act as mite attractants and repellants. A detailed qualitative and quantitative analysis of phenolics in the developing date fruit was carried out in all four cultivars by means of reverse phase HPLC, as described under "Materials and Methods". Representative HPLC chromatograms of 'Bahri' fruit juice phenolics extracts are displayed in Figure 4B.4. Chromatograms at 280 nm (all phenolics) and 325 nm (phenolic acids) are presented for three sampling dates corresponding to three phases during mite population cycle: 13-05-2007 – preceding mite ascent; 08-07-2007 – during population buildup; 06-08-2007 – during mite descent. Analogous chromatograms were obtained with the four CVs on all sampling dates. It should be noted that very similar chromatograms were obtained from acaricide treated and untreated trees. Also noteworthy is that the analogous chromatograms of the four CVs were

comparable but differences in peaks' intensities and some disparity in minor peaks were observed.



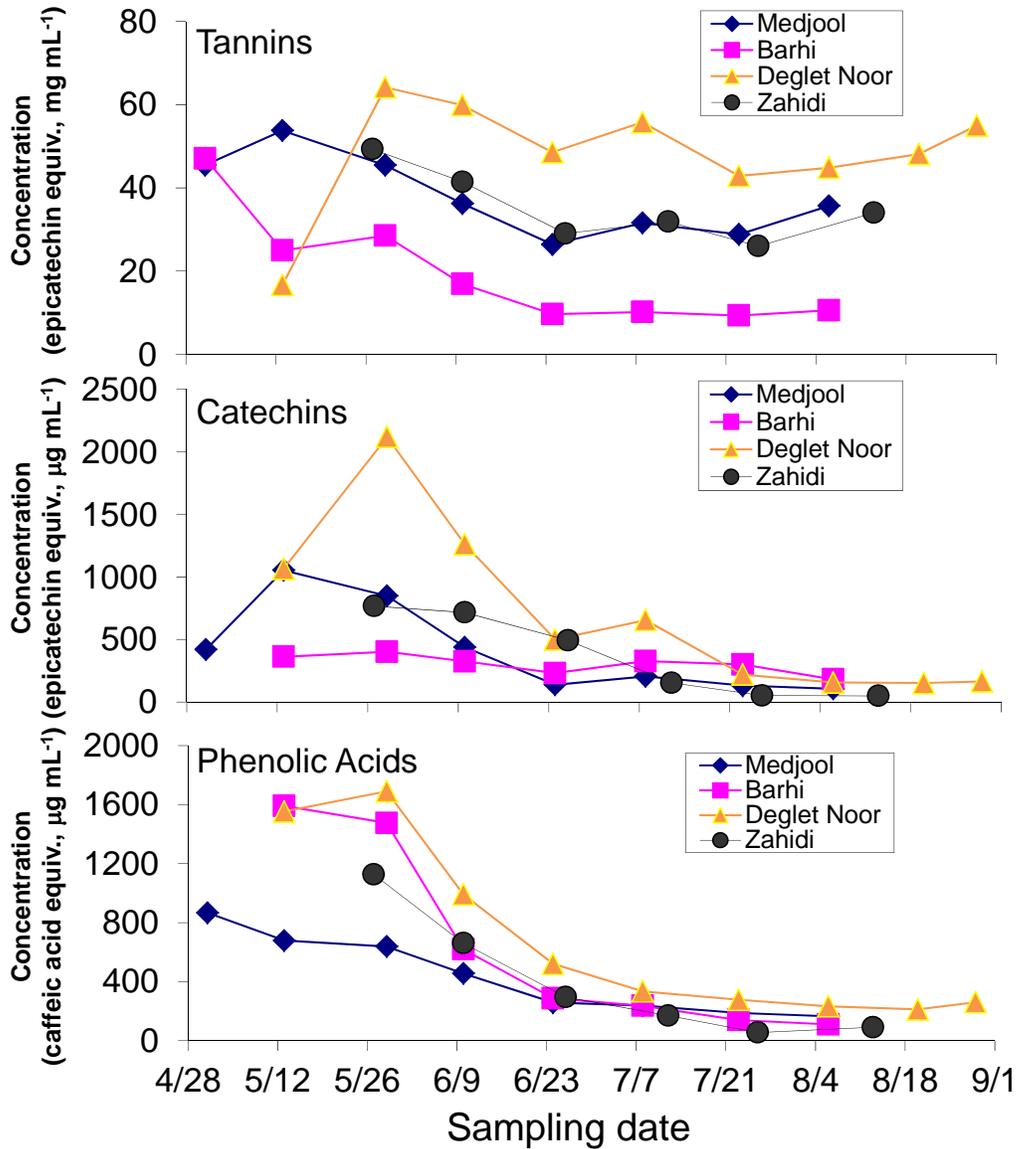
**Figure 4B.4:** HPLC chromatograms of phenolics' extracts from 'Barhi' date fruits along fruit development. Sampling dates correspond to the spider mite phenology: 13-05-2007 – preceding ascent; 08-07-2007 – during population buildup; 06-08-2007 - during descent. Chromatograms at 280 (all phenolics) and 325 nm (insert, mostly phenolic acids) are presented.

It is apparent from Fig. 4 that during date fruit development (the period relevant to the date spider mite life cycle on the fruit) not only the content of individual phenolics declined (as reflected by the decrease in peak size) but the profile of the phenolic compounds changed as well.

The major phenolics in the date fruit extracts were of three types: tannins, phenolic acids and catechins. The largest proportion of the soluble phenolics, manifested in the chromatograms by the large peak at 10.6 min, is tentatively comprised of tannins, i.e. oligomeric phenolic compounds. The earlier phase of the chromatograms (i.e., retention times < 10 min) accommodates peaks relating to derivatives of phenolic acids and catechins.

Figure 4B.5 displays the contents of tannins and total catechin and phenolic acid derivatives along fruit development in the fruit juice of the four CVs. The highest levels of all three types of phenolics were measured on the early sampling dates in all CVs. The level of tannins on later dates declined significantly in 'Bahri', less so in 'Medjool' and 'Zahidi' and to an even lesser extent in 'Deglet Noor'. Tannin concentration was in fact high all along the sampling period in 'Medjool', 'Deglet Noor' and 'Zahidi'. The low level of tannins in the fully developed unripe 'Barhi' date fruit is likely to be the reason for its relatively low astringency. The content of catechin derivatives decreased significantly during fruit development in 'Medjool', 'Deglet Noor' and 'Zahidi'. 'Bahri' contained a relatively low level of catechins already early in development. Catechins concentration was low in all four CVs at the end of the sampling period. The content of phenolic acid derivatives decreased greatly during the sampling period in all four CVs, reaching low levels at the end of the sampling term. The content of the three types of phenolics was consistently higher in 'Deglet Noor' compared to other CVs.

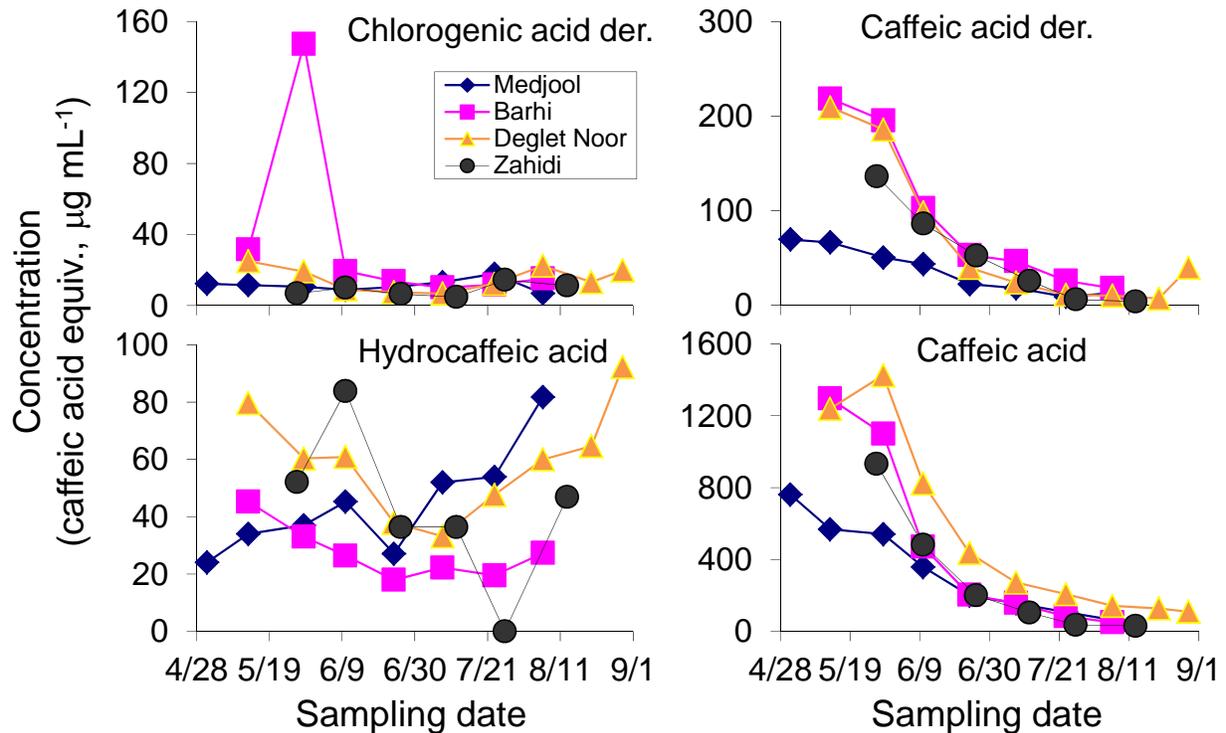
The tannins are unlikely to act as mite attractants or repellents considering the big difference in their content between 'Barhi' and the two other mite-susceptible CVs during the entire sampling period. Moreover, a high level of tannins was maintained in 'Medjool' and 'Deglet Noor' fruit at all times. The significant changes in phenolic acids and catechins concentrations in the fruit sap during the period relevant to mite population establishment point at a potential role for molecules of these phenolics types as mite attractants or repellants. Four types of phenolic acids (chlorogenic acid derivatives, hydrocaffeic acid, caffeic acid derivatives and caffeic acid) and seven catechin derivatives (labeled as Cat-1 through Cat- 7) were consistently detected and therefore quantified.



**Figure 4B.5:** Concentration of tannins, total catechins and total caffeic acids in fruit juice of four date cultivars during fruit development.

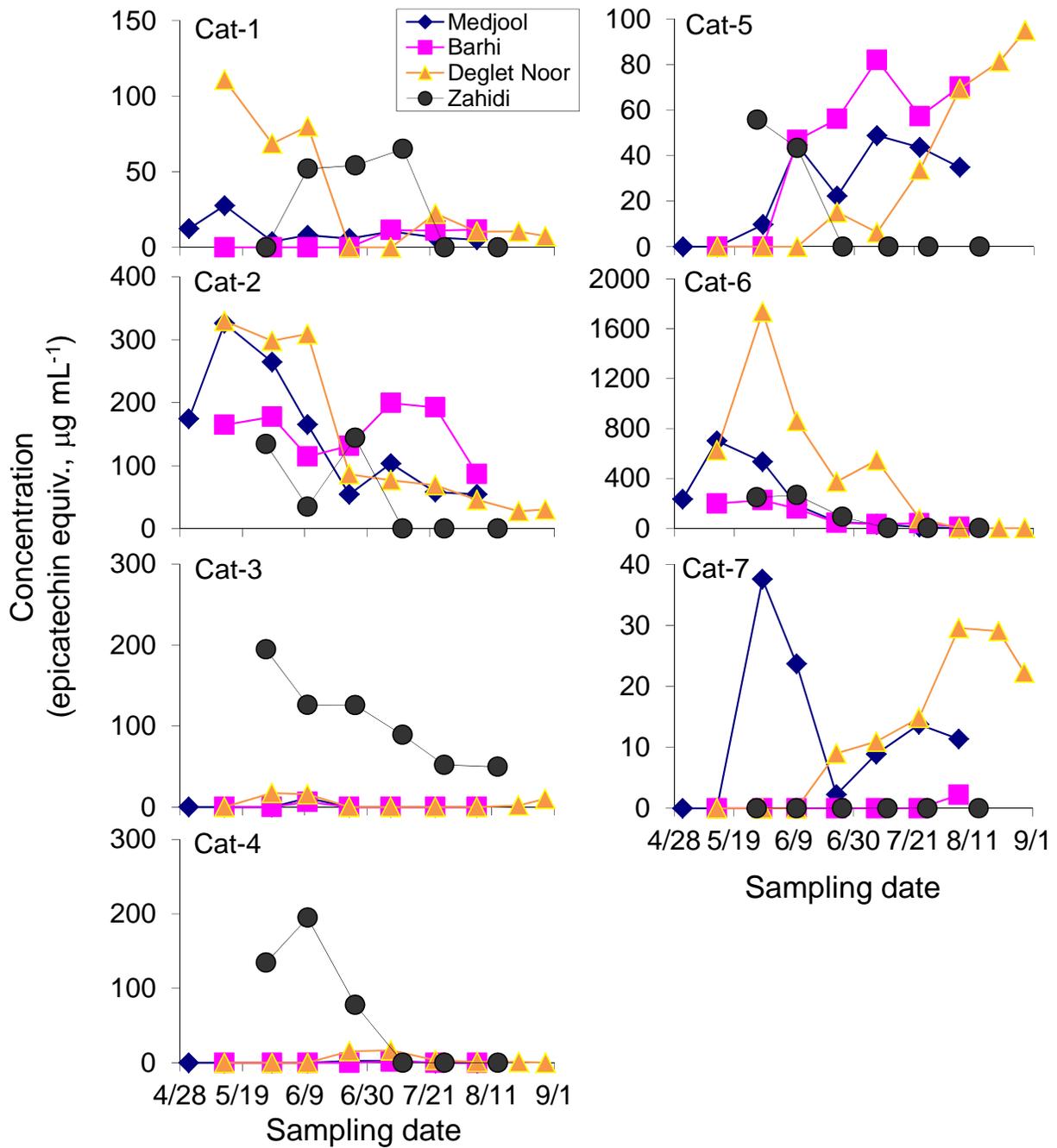
Figures 4B.6 and 4B.7 present the content of the individual catechins and phenolic acids, respectively, during fruit development in the four date CVs. Caffeic acid and its derivatives were the major phenolic acids (Fig. 6) and decreased sharply along fruit development in all CVs. The levels were low during periods related to both mite population buildup and descent. The concentration of chlorogenic acid derivatives was low all along fruit development in all CVs, with the exception of an early date in 'Bahri'. The change in hydrocaffeic acid during early

stages of fruit development was cultivar dependent. During the period relevant to mite population buildup the concentration was relatively low and increased in later dates. The pattern suggests a possible role for hydrocaffeic acid as a mite repellent.



**Figure 4B.6:** The concentration of individual phenolic acids during fruit development in four date cultivars.

Unlike the phenolic acids, the content and pattern of change in certain catechins during fruit development was significantly different in 'Zahidi' compared to the mite susceptible CVs (Fig. 7): The concentration of Cat-1 was significantly higher during the period relevant to mite population buildup, Cat-3 level was much larger during periods relevant to both mite ascent and population buildup and Cat-4 content was markedly higher during the period corresponding to mite ascent and initial population establishment. The change in Cat-5 level during fruit development was in the opposite direction in 'Zahidi' compared to the mite sensitive CVs, starting low and increasing significantly in the latter and starting high and decreasing to nil in 'Zahidi'.



**Figure 4B.7:** The concentration of individual phenolic acids during fruit development in four date cultivars.

The level and pattern of change in Cat-2 and Cat-7 during fruit development were cultivar dependent. The concentration of Cat-6, the major catechin in all CVs, changed similarly in all

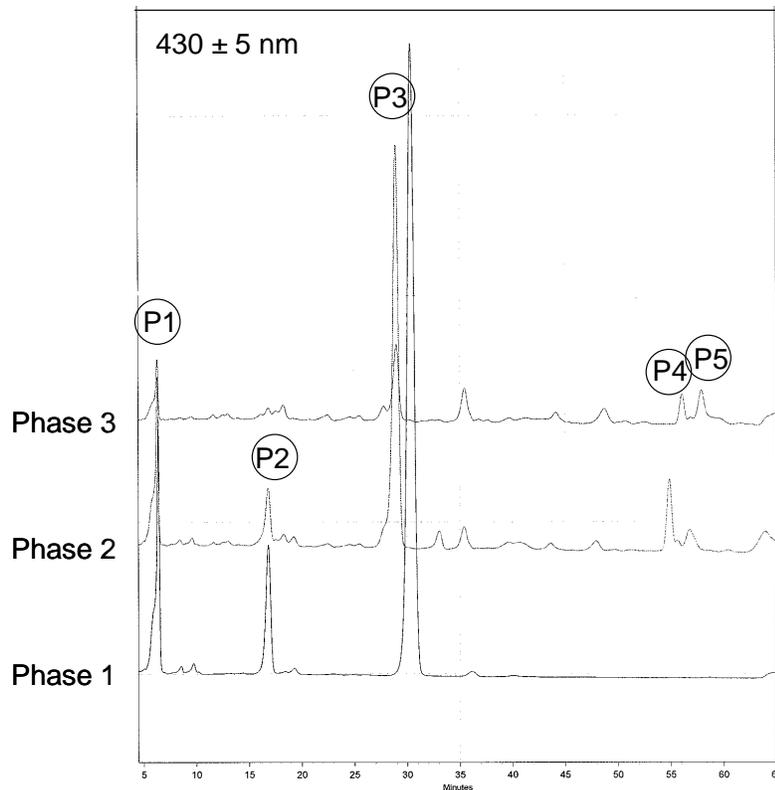
four CVs; it was high early in fruit development and decreased later on reaching very low or zero levels during the periods relevant to the end of mite population buildup and mite descent.

### Analysis of date chlorophylls and carotenoids during fruit development.

The hydrophobic pigments (chlorophylls and carotenoids) in date fruit were analyzed on three sampling dates during fruit development corresponding to three phases in mite population establishment (Palevsky *et al*, 2005): (1) preceding ascent, (2) extensive population build-up and (3) during descent. The pigments were extracted and analyzed by HPLC as described under “Materials and Methods”.

Figure 4B.8 presents HPLC chromatograms at 430 nm of the pigments in 'Deglet Noor' fruit from sampling dates relating to phases 1 to 3.

Deglet Noor – fruit chlorophyll and carotenoid HPLC chromatogram

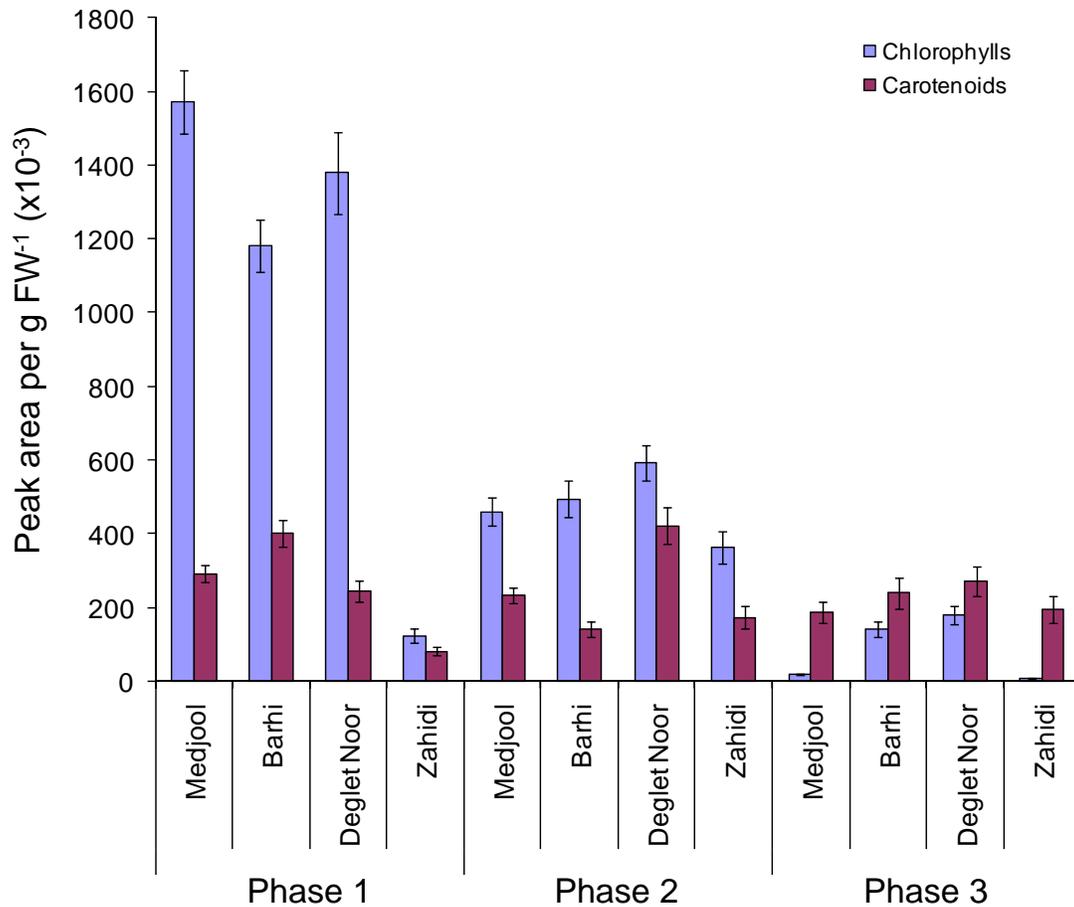


**Figure 4B.8:** HPLC chromatogram at 430 nm of hydrophobic pigments extracted from 'Deglet Noor' fruit on dates relating to 3 phases in mite population establishment: (1) beginning

ascent, (2) during population build-up and (3) along descent. Peak identification: P1- lutein; P2- chlorophyll b; P3- chlorophyll a; P4-  $\beta$ -carotene; P5- pheophytin-like.

Similar chromatograms were obtained with fruits of 'Medjool', 'Barhi' and 'Zahidi'; peak intensity and occurrence of certain peaks were however cultivar dependent. The major peaks corresponded to chlorophyll *a* and *b* and their degradation product (**pheophytin**), lutein,  $\beta$ -carotene and several unidentified carotenoids (denoted as C1 to C10). It is evident from Figure 4B.8 that pigment composition changed markedly during the course of fruit development.

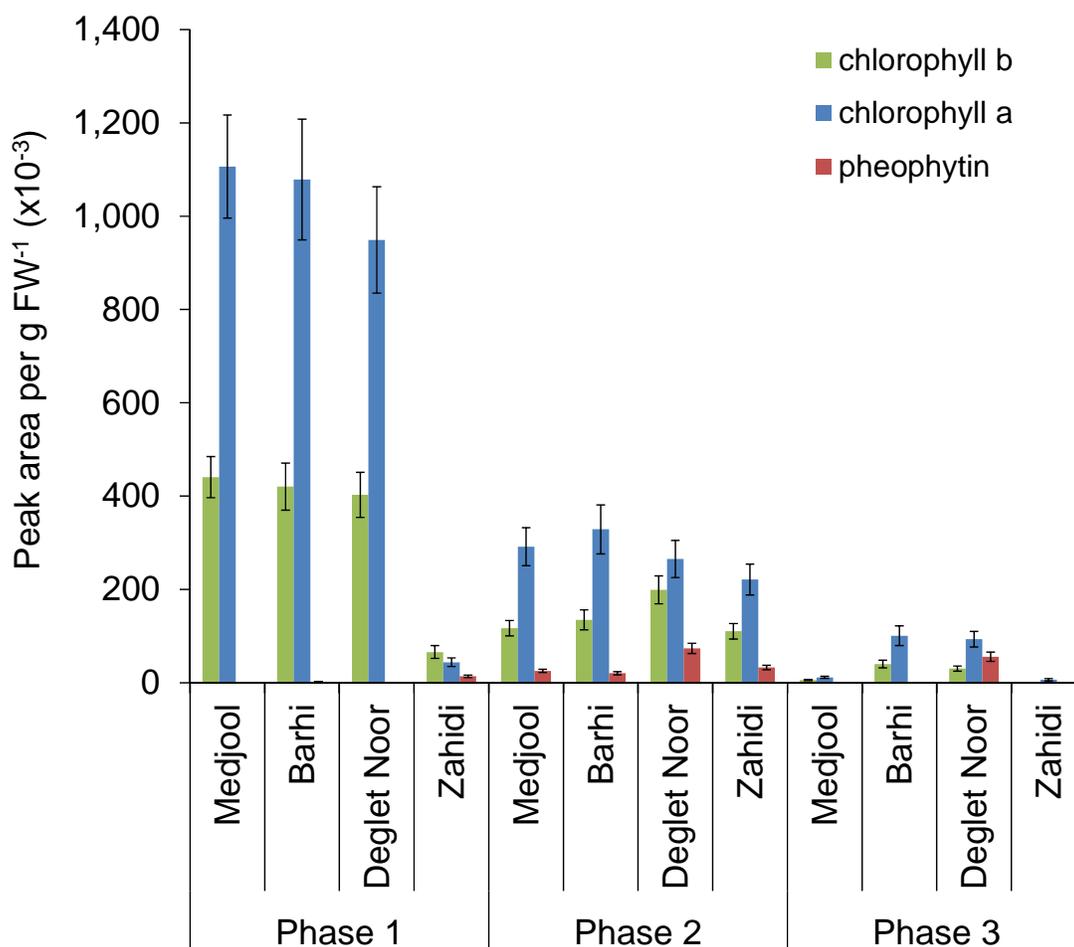
Figure 4B.9 presents the total contents of chlorophylls and carotenoids in fruit of the four date CVs sampled during phases 1 to 3. In phase 1 mite susceptible CVs contained high levels of chlorophylls whereas the concentration in 'Zahidi' was low. 'Zahidi' also contained the lowest level of carotenoids. The content of chlorophylls decreased significantly in phase 2 in the mite susceptible CVs, but increased in 'Zahidi', reaching a similar level to that of other CVs.



**Figure 4B.9:** Chlorophylls and carotenoids content during fruit development in four date cultivars.

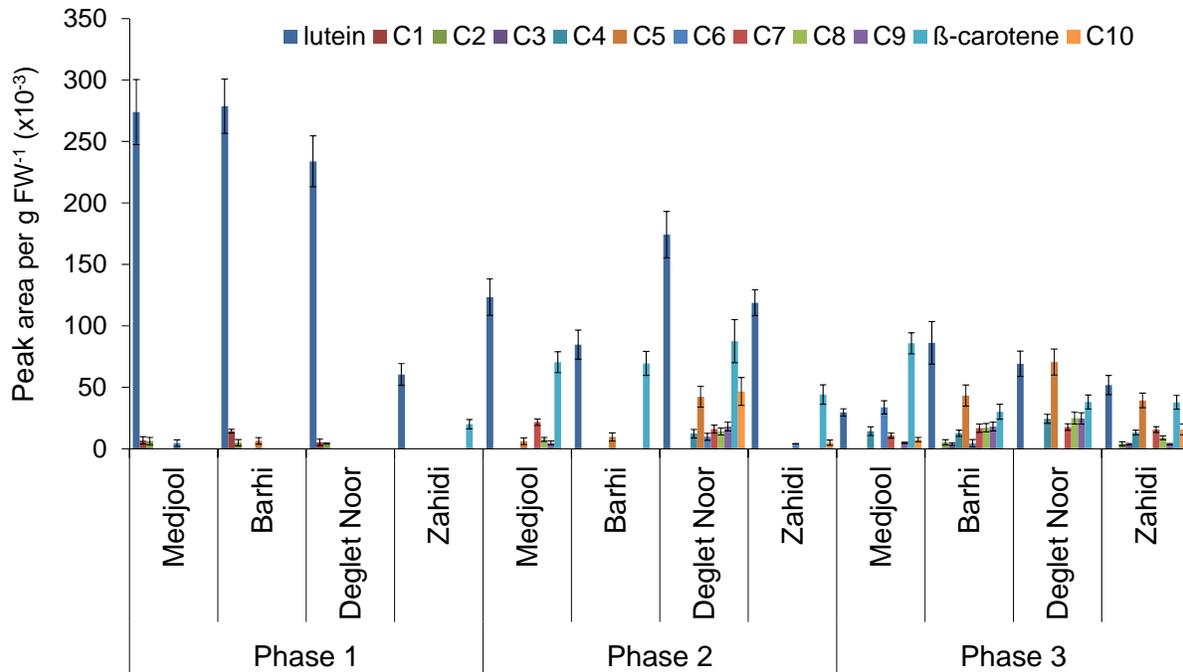
The lowest chlorophylls' concentrations were measured in phase 3 in all four CVs. Carotenoids content varied along fruit development in a cultivar dependent manner.

Detailed analysis of the chlorophylls in the fruit of the four date CVs sampled at three developmental phases is shown in Figure 4B.10. In phase 1 the concentrations of both chlorophyll *a* and *b* were high in mite susceptible CVs and low in 'Zahidi'. Pheophytin-like pigment was only present in 'Zahidi', in a low quantity though. The level of both chlorophyll *a* and *b* decreased in mite susceptible CVs and increased in 'Zahidi' in phase 2. Pheophytin-like pigment was present in all four CVs. The content of each chlorophyll pigment decreased to its lowest level in phase 3 in all CVs.



**Figure 4B.10:** Content of individual chlorophylls during fruit development in four date cultivars.

Figure 4B.11 summarizes the levels of the individual carotenoids in fruit of the four date CVs sampled at the three developmental phases. Lutein was the major carotenoid in fruit from phase 1. Its content was high in mite susceptible CVs and low in ‘Zahidi’. Minor peaks of unidentified carotenoids were detected in the susceptible CVs but not in ‘Zahidi’. On the other hand, ‘Zahidi’ fruit contained  $\beta$ -carotene which was absent in other CVs. In phase 2 the concentration of lutein decreased in the susceptible CVs but increased in ‘Zahidi’. Significant levels of  $\beta$ -carotene were present in fruit of all CVs though at a lower concentration in ‘Zahidi’. Several peaks of unidentified carotenoids developed, most significantly in ‘Deglet Noor’. In phase 3 each of the four CVs had a unique profile of carotenoids consisting of different levels of lutein,  $\beta$ -carotene and unidentified carotenoids.



**Figure 4B.11:** Content of individual chlorophylls during fruit development in four date cultivars.

## Summary

Our report summarizes the analyses of date fruit chemical parameters during the period in fruit development that correspond to the date spider mite life cycle on the fruit (ascent, population buildup and descent) as established earlier (Palevsky *et al*, 2005). The study included three mite susceptible CVs, 'Medjool', 'Barhi' and 'Deglet Noor' and one insusceptible CV, 'Zahidi'. The examined fruit chemical parameters comprised mineral composition (including total nitrogen, phosphate, potassium, calcium, magnesium, sodium and chloride), water content, juice TSS, EC, pH, TA, total soluble phenolics' content and secondary metabolites profiles (including polyphenolics, chlorophylls and carotenoids).

Mineral composition, TSS, EC, pH, TA and total soluble phenolics' concentration changed during fruit development in similar trends in all four CVs; however, the absolute values as well as the degree and rate of change of each parameter were cultivar dependent, indicating that these properties may relate to cultivar susceptibility to the mite. The content of certain secondary metabolites (chlorophylls, carotenoids and polyphenolics) varied during seasonal changes in mite phenology and population density in a manner suggesting that they may play a role as constitutive attractants/repellents. Especially, considering the marked differences in these metabolites between the mite susceptible CVs, 'Medjool', 'Barhi' and 'Deglet Noor', and the

insusceptible CV, 'Zahidi'. Chlorophyll *a* and *b* as well as lutein may act as mite attractants, as they are very high in the susceptible CVs and low in 'Zahidi' preceding mite ascent. The change in  $\beta$ -carotene content during fruit development is consistent with the possibility that it has a nutritional value in the mite diet. The pattern of change in hydrocaffeic acid and Cat-5 contents during fruit development and the remarkably higher contents of Cat-1, Cat-3, and Cat-4 in 'Zahidi' compared to the susceptible CVs suggest they all may act as mite repellents.

## 5 – Indigenous entomophagous fungi for control of *Oligonychus afrasiaticus*

### ***The effect of the Basidiomycota fungi Meira argovae, Meira geulakonigii and Acaromyces ingoldii on the mite Oligonychus afrasiaticus in the laboratory.***

In the past we tested the effects of these fungi on several species of spider mites (Gerson *et al.*, 2008), and obtained variable, albeit always significant, control results. Notwithstanding several inherent problems (to be discussed below), we thus decided to assay these fungi also against *O. afrasiaticus*, a major pest of date palms. Due to the phenology of this pest (which infests and injures dates during the hottest summer months, Palevsky *et al.*, 2005) the experiment could take place only during that period. Also, technical constraints required us to conduct the experiment in two parts. In the first, only the effect of the fungi *Meira argovae* and *Meira geulakonigii* was explored, whereas in the second, the effect of *M. argovae* was compared with the effect of *Acaromyces ingoldii*.

All three fungi were grown in the dark on their respective media for one week at 25°C. *Meira argovae* and *M. geulakonigii* were maintained on 2.4% w/v potato dextrose broth (PDB), in Erlenmeyer flasks. Samples from these flasks were then taken to obtain suspensions of  $10^8$  and  $10^9$  blastoconidia /ml in water. *Acaromyces ingoldii*, which does not form blastoconidia in liquid media, was grown on boiled wheat seeds. Sterile water was then added and the required amount of blastoconidia obtained by centrifugation.

The experimental set-up for both experiments was identical. Strands of heavily mite infested dates (CV Deglet Noor) were collected on July 25<sup>th</sup>, 2011, and on August 8<sup>th</sup> at Samar, in the Southern Arava Valley. The strands, each with at least 30 fruits, were randomly divided into five groups, for the five treatments. In the first part these were: Control (water), *Meira argovae* and *Meira geulakonigii*, the two fungi being applied at concentrations of  $10^8$  and  $10^9$  spores /ml in water. The strands, each with at least 6-7 fruits, were hung from sticks placed on the margins of containers, so that the dates from each strand did not touch each other. The containers' bottoms were padded with humid filter paper. All strands with the dates that were exposed to each of the five fungi were placed in the same container. Treatments were applied by spraying on July 27<sup>th</sup>, 2011; the containers with the strands were then kept at 30°C for 1 week and examined. The results are in Figure 1.

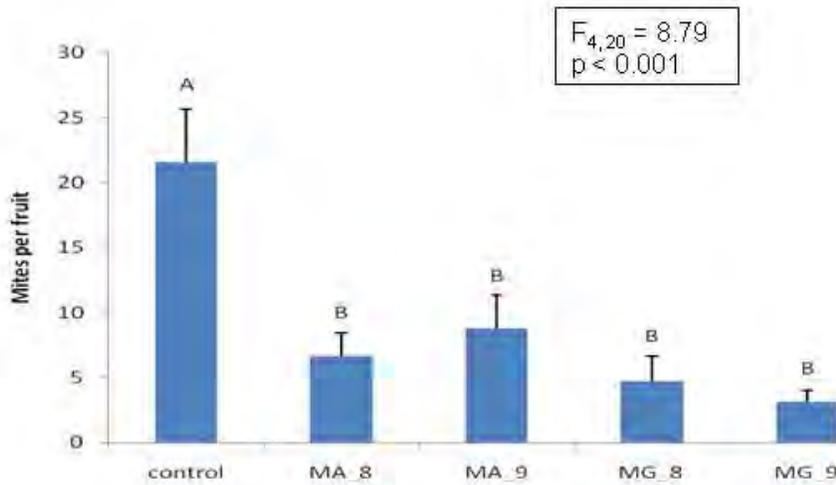


Figure 1 – Mean (+SE) mites per fruit in the five treatments at the 3<sup>th</sup> of August trial: control; *M. Argovae* at  $10^8$  (MA\_8) and  $10^9$  (MA\_9); *M. geulakonigii* at  $10^8$  (MG\_8) and  $10^9$  (MG\_9). Statistic results from One-Way ANOVA. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (Bonferroni multiple comparison).

In the second part the effect of the most efficient concentration of *M. argovae* blastoconidia ( $10^9$ ) was compared with a similar concentration of *A. ingoldii*. As noted, the dates were collected on August 8<sup>th</sup> at Samar, and the experiment was set-up as above. The strands were treated on August 8<sup>th</sup> and examined one week later (August 18<sup>th</sup>). The results are shown in Figure 2.

**Discussion:** The growth in the number of mites per fruit (as seen in the controls) was most likely due to the natural increase in their numbers, which peak in August (Palevsky *et al.*, 2005). The number of mites was significantly reduced by all three fungi, whose effect, although variable, was only insignificantly different. These result show that all three fungi may reduce the numbers of another pest mite, albeit, so fae, only under laboratory conditions. Can the fungi thus be used to control *O. afrasiaticus* infesting dates in the Southern Arava Valley? The fungi are indigenous to Israel, so their application out of doors will not be a problem. However, a major drawback could be the very low humidity prevailing in that part of the country. That may be overcome by various mechanical means, but only field trials can provide a clear answer.

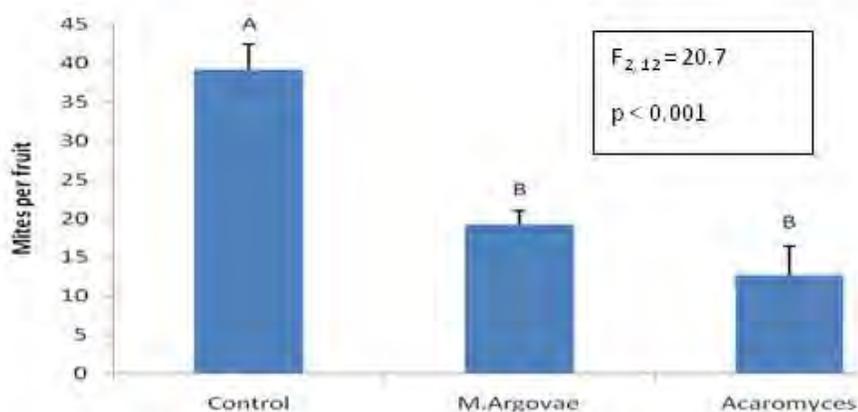


Figure 2 - Mean (+SE) mites per fruit in the three treatments at the 18<sup>th</sup> of August trial: control; *Acaromyces* at  $10^9$ ; *M. geulakonigii* at  $10^9$ . Statistic results from One-Way ANOVA. Same letters above the bars indicate non-significant ( $p \geq 0.05$ ) differences between treatments (Bonferroni multiple comparison).

Acknowledgements: We are very grateful for the expert technical assistance of Mrs. Shira Gal, Mrs. Sylvi Judeinstein, Ms. Aviva Gafni and Eng. Jihad Haddadin. We acknowledge the efforts of Dr. Alon Lotan who successfully conducted his PhD within the framework of this project and was responsible for all the statistical analyses. We wish to thank Amnon Greenberg of the Ardom R&D for his administrative support and Dr. Shimon Steinberg, Shiya Kaminsky and the R&D staff of Bio-Bee, Sde-Eliyahu, for their technical support.

## REFERENCES

- Addison, J. A., J. M. Hardman, and S. J. Walde. 2000. Pollen availability for predaceous mites on apple: spatial and temporal heterogeneity. *Experimental and Applied Acarology* **24**:1-18.
- Ahn, J. J., K. W. Kim, and J. H. Lee. 2010. Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves. *Journal of Applied Entomology* **134**:98-104.
- Al-Sweedy, T. M., I. J. Al-Jboory, and T. R. Ahmad. 2006. Age-specific fecundity of old world date mite *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae). *Arab Journal of Plant Protection* **24**:14-19.
- Argov, Y., S. Amitai, G. A. C. Beattie, and U. Gerson. 2002. Rearing, release and establishment of imported predatory mites to control citrus rust mite in Israel. *Biocontrol* **47**:399-409.
- Argov, Y., M. Berkeley, S. Domeratzky, E. Melamed, P. Weintraub, and E. Palevsky. 2006. Identification of pollen for small scale mass rearing of *Neoseiulus californicus* and a novel method for quality control. *IOBC/wprs Bulletin: Integrated Control in Protected Crops, Mediterranean Climate* **29**:127-132.
- Auger, P., M. S. Tixier, S. Kreiter, and G. Fauvel. 1999. Factors affecting ambulatory dispersal in the predaceous mite *Neoseiulus californicus* (Acari : Phytoseiidae). *Experimental and Applied Acarology* **23**:235-250.
- Badii, M. H., E. Hernandez-Ortiz, A. E. Flores, and J. Landeros. 2004. Prey stage preference and functional response of *Euseius hibisci* to *Tetranychus urticae* (Acari : Phytoseiidae, Tetranychidae). *Experimental and Applied Acarology* **34**:263-273.
- Badii, M. H. and J. A. McMurtry. 1984. Life-history of and life table parameters for *Phytoseiulus-longipes* with comparative studies on *Phytoseiulus-persimilis* and *Typhlodromus-occidentalis* (Acari, Phytoseiidae). *Acarologia* **25**:111-123.
- Bakker, F. M., M. E. Klein, N. C. Mesa, and A. R. Braun. 1993. Saturation deficit tolerance spectra of phytophagous mites and their phytoseiid predators on Cassava. *Experimental and Applied Acarology* **17**:97-113.
- Beard, J. J. and G. H. Walter. 2001. Host plant specificity in several species of generalist mite predators. *Ecological Entomology* **26**:562-570.
- Bernstein, C. 1983. Some aspects of *Phytoseiulus-persimilis* [Acarina, Phytoseiidae] dispersal behavior. *Entomophaga* **28**:185-198.
- Bernstein, Z. 2004. The Date Palm. The Plant Production and Marketing Board, Tel-Aviv, Israel (in Hebrew).
- Berry, J. S., T. O. Holtzer, and J. M. Norman. 1991. MiteSim - a simulation-model of the Banks grass mite (Acari, Tetranychidae) and the predatory mite, *Neoseiulus-fallacis* (Acari, Phytoseiidae) on maize: model development and validation. *Ecological Modelling* **53**:291-317.
- Bigler, F., J. S. Bale, M. J. W. Cock, H. Dreyer, R. Greatrex, U. Kuhlmann, A. J. M. Loomans, and J. C. Van Lenteren. 2005. Guidelines on information requirements for import and release of invertebrate biological control agents in European countries. *Biocontrol News and Information* **26**:115-123.
- Bounfour, M. and J. A. McMurtry. 1987. Biology and ecology of *Euseius-scutalis* (Athias-Henriot) (Acarina, Phytoseiidae). *Hilgardia* **55**:1-23.

- Broufas, G. D. and D. S. Koveos. 2000. Threshold temperature for post-diapause development and degree-days to hatching of winter eggs of the European red mite (Acari : Tetranychidae) in Northern Greece. *Environmental Entomology* **29**:710-713.
- Bruce-Oliver, S. J., M. A. Hoy, and J. S. Yaninek. 1996. Effect of some food sources associated with cassava in Africa on the development, fecundity and longevity of *Euseius fustis* (Pritchard and Baker) (Acari: Phytoseiidae). *Experimental and Applied Acarology* **20**:73-85.
- Bursell, E. 1974. Environmental Aspects-Humidity. in M. Rockstein, editor. *The Physiology of Insecta*. Academic Press, New-York.
- Carpenter, J. B. and H. S. Elmer. 1978. Pests and diseases of the date palm. *Agriculture Handbook*. Science and Education Administration, Department of Agriculture, USA.
- Castagnoli, M. and S. Simoni. 1994. The effect of different constant humidities on egg and larvae of *Amblyseius californicus* (McGregor) (Acarina Phytoseiidae). *Redia* **77**:349-359.
- Castagnoli, M. and S. Simoni. 2003. *Neoseiulus californicus* (McGregor) (Acari Phytoseiidae): Survey of biological and behavioural traits of a versatile predator. *Redia* **86**:153-164.
- Chmielewski, W. 1971. Morphology, biology and ecology of *Carpoglyphus lactis* (L., 1758) (Glycyphagidae, Acarina). *Prace Naukowe Instytutu Ochrony Roslin* **13**:64-166.
- Chow, A., A. Chau, and K. M. Heinz. 2010. Compatibility of *Amblyseius (Typhlodromips) swirskii* (Athias-Henriot) (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) for biological control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on roses. *Biological Control* **53**:188-196.
- Congdon, B. D. and J. A. Logan. 1983. Temperature effects on development and fecundity of *Oligonychus pratensis* (Acari, Tetranychidae). *Environmental Entomology* **12**:359-362.
- Coudin, B. and F. Galvez. 1976. Biologie de l'acarier du palmier-dattier *Oligonychus afrasiaticus* (McGregor) en Mauritanie. *Fruits* **31**:543-550.
- Croft, B. A., R. H. Messing, J. E. Dunley, and W. B. Strong. 1993. Effects of humidity on eggs and immatures of *Neoseiulus-fallacis*, *Amblyseius-andersoni*, *Metaseiulus-occidentalis* and *Typhlodromus-pyri* (phytoseiidae) - implications for biological-control on apple, caneberry, strawberry and hop. *Experimental and Applied Acarology* **17**:451-459.
- Croft, B. A., L. N. Monetti, and P. D. Pratt. 1998. Comparative life histories and predation types: Are *Neoseiulus californicus* and *N-fallacis* (Acari : Phytoseiidae) similar type II selective predators of spider mites? *Environmental Entomology* **27**:531-538.
- Danielsen, C., L. S. Hansen, G. Nachman, and C. Herling. 2004. The influence of temperature and relative humidity on the development of *Lepidoglyphus destructor* (Acari : Glycyphagidae) and its production of allergens: a laboratory experiment. *Experimental and Applied Acarology* **32**:151-170.
- De Courcy Williams, M. E., L. Kravar-Garde, J. S. Fenlon, and K. D. Sunderland. 2004a. Phytoseiid mites in protected crops: the effect of humidity and food availability on egg hatch and adult life span of *Iphiseius degenerans*, *Neoseiulus cucumeris*, *N. californicus* and *Phytoseiulus persimilis* (Acari : Phytoseiidae). *Experimental and Applied Acarology* **32**:1-13.
- De Courcy Williams, M. E., L. Kravar-Garde, J. S. Fenlon, and K. D. Sunderland. 2004b. The relationship between dietary specialism and availability of food and water on cannibalistic interactions among predatory mites in protected crops. *Experimental and Applied Acarology* **33**:31-44.
- De Moraes, G. J., J. A. McMurtry, H. A. Denmark, and C. B. Campos. 2004. A revised catalog of the mite family Phytoseiidae. *Zootaxa*:1-494.

- De Vis, R. M. J., G. J. De Moraes, and M. R. Bellini. 2006. Effect of air humidity on the egg viability of predatory mites (Acari : Phytoseiidae, Stigmaeidae) common on rubber trees in Brazil. *Experimental and Applied Acarology* **38**:25-32.
- Duso, C., F. Chiarini, L. Conte, V. Bonora, L. D. Monta, and S. Otto. 2004a. Fogging can control *Tetranychus urticae* on greenhouse cucumbers. *Journal of Pest Science* **77**:105-111.
- Duso, C., V. Malagnini, A. Paganelli, L. Aldegheri, M. Bottini, and S. Otto. 2004b. Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. *Biocontrol* **49**:397-415.
- Eilenberg, J., A. Hajek, and C. Lomer. 2001. Suggestions for unifying the terminology in biological control. *Biocontrol* **46**:387-400.
- Elwan, A. A. 2000. Survey of the insect and mite pests associated with date palm trees in Al-Dakhliya region, Sultanate of Oman. *Egyptian Journal of Agricultural Research* **78**:653-664.
- English-Loeb, G., A. P. Norton, and M. A. Walker. 2002. Behavioral and population consequences of acarodomatia in grapes on phytoseiid mites (Mesostigmata) and implications for plant breeding. *Entomologia Experimentalis Et Applicata* **104**:307-319.
- Escudero, L. A. and F. Ferragut. 2005. Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari : Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari : Tetranychidae). *Biological Control* **32**:378-384.
- Ferrero, M., C. Gigot, M. S. Tixier, Y. M. van Houten, and S. Kreiter. 2010. Egg hatching response to a range of air humidities for six species of predatory mites. *Entomologia Experimentalis Et Applicata* **135**:237-244.
- Ferro, D. N. and R. B. Chapman. 1979. Effects of different constant humidities and temperatures on two-spotted spider-mite egg hatch. *Environmental Entomology* **8**:701-705.
- Fish, WW, Perkins-Veazie, P and Collins, JK. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *J. Food Comp. Anal.* **15**:309-317.
- Fouly, A. H. 1997. Effects of prey mites and pollen on the biology and life tables of *Proprioiseiopsis aetus* (Chant) (Acari, Phytoseiidae). *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* **121**:435-439.
- Furuichi, H., K. Oku, S. Yano, A. Takafuji, and M. Osakabe. 2005. Why does the predatory mite *Neoseiulus womersleyi* Schicha (Acari : Phytoseiidae) prefer spider mite eggs to adults? *Applied Entomology and Zoology* **40**:675-678.
- Gaede, K. 1992. On the water-balance of *Phytoseiulus-persimilis* A-H and its ecological significance. *Experimental & Applied Acarology* **15**:181-198.
- Gan-Mor, S., A. Bechar, B. Ronen, D. Eisikowitch, and Y. Vaknin. 2003. Electrostatic pollen applicator development and tests for almond, kiwi, date, and pistachio - An overview. *Applied Engineering in Agriculture* **19**:119-124.
- Gerson, U. and V. Vacante. 1993. The use of indigenous acarine predators to control citrus mite pests. *OILB/SROP Bulletin* **16**:115-119.
- Gerson, U. and P. G. Weintraub. 2007. Mites for the control of pests in protected cultivation. *Pest Management Science* **63**:658-676.
- Gerson U., A. Gafni, Z. Paz and A. Szejnberg. 2008. A tale of three acaropathogenic fungi in Israel: *Hirsutella*, *Meira* and *Acaromyces*. *Experimental and Applied Acarology* **46**: 183-194.
- Gispert, C., C. Farrar, T. M. Perring, R. B. Halliday, D. E. Walter, H. C. Proctor, R. A. Norton, and M. J. Colloff. 2001. Seasonal abundance of the Banks grass mite *Oligonychus*

- pratensis* (Banks) (Prostigmata: Tetranychidae) and a predatory mite, and their response to sulfur treatment on commercial date palms *Phoenix dactylifera* L. in southern California. *Acarology: proceedings of the 10th International Congress*:403-408.
- Gnanvossou, D., R. Hanna, J. S. Yaninek, and M. Toko. 2005. Comparative life history traits of three neotropical phytoseiid mites maintained on plant-based diets. *Biological Control* **35**:32-39.
- Gonzalez-Fernandez, J. J., F. de la Pena, J. I. Hormaza, J. R. Boyero, J. M. Vela, E. Wong, M. M. Trigo, and M. Montserrat. 2009. Alternative food improves the combined effect of an omnivore and a predator on biological pest control. A case study in avocado orchards. *Bulletin of Entomological Research* **99**:433-444.
- Gotoh, T., A. Suwa, Y. Kitashima, and H. A. Rezk. 2004a. Developmental and reproductive performance of *Tetranychus pueraricola* Ehara and Gotoh (Acari : Tetranychidae) at four constant temperatures. *Applied Entomology and Zoology* **39**:675-682.
- Gotoh, T., K. Yamaguchi, and K. Mori. 2004b. Effect of temperature on life history of the predatory mite *Amblyseius (Neoseiulus) californicus* (Acari : Phytoseiidae). *Experimental and Applied Acarology* **32**:15-30.
- Grostal, P. and D. J. O'Dowd. 1994. Plants, mites and mutualism - leaf domatia and the abundance and reproduction of mites on *Viburnum tinus* (Caprifoliaceae). *Oecologia* **97**:308-315.
- Hadely, N. F. 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, New York.
- Hajek, A. 2004. *Natural Enemies - An Introduction to Biological Control*. Cambridge University Press, Cambridge, UK.
- Hardman, J. M., M. L. Rogers, S. O. Gaul, and E. D. Bent. 1997. Insectary rearing and initial testing in Canada of an organophosphate/pyrethroid-resistant strain of the predator mite *Typhlodromus pyri* (Acari : Phytoseiidae) from New Zealand. *Environmental Entomology* **26**:1424-1436.
- Hazan, A., U. Gerson, and A. S. Tahori. 1973. Life history and life tables of the Carmine spider mite. *Acarologia (Paris)* **15**:414-440.
- Holtzer, T. O., J. M. Norman, T. M. Perring, J. S. Berry, and J. C. Heintz. 1988. Effects of microenvironment on the dynamics of spider mite populations. *Experimental & Applied Acarology* **4**:247-264.
- Holtzer, T. O., T. M. Perring, and M. W. Johnson. 1984. Winter and spring distribution and density of Banks grass mite (Acari, Tetranychidae) in adjacent wheat and corn. *Journal of the Kansas Entomological Society* **57**:333-335.
- Hussain, A. A. 1969. Biology of *Paratetranychus afrasiaticus* McGr., infesting date palms in Iraq. *Bulletin of the Society of Entomology of Egypt* **33**:221-225.
- Koppert Biological Systems. 2009. Koppert Biological Systems News, December.
- Kramer, D. A. and F. P. Hain. 1989. Effect of constant-humidity and variable-humidity and temperature regimes on the survival and developmental periods of *Oligonychus-ununguis* (Acarina, Tetranychidae) and *Neoseiulus-fallacis* (Acarina, Phytoseiidae). *Environmental Entomology* **18**:741-746.
- Kreiter, S., M. S. Tixier, P. Auger, and K. L. Grissa. 2006. Phytoseiid mites (Acari : Mesostigmata) of southern Tunisia. *Acarologia (Paris)* **46**:5-12.
- Kumral, N. A. and B. Kovanci. 2005. Seasonal population dynamics of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari : Tetranychidae) under acaricide constraint on eggplant in Bursa Province (Turkey). *Acarologia (Paris)* **45**:295-301.

- Lawson, D. S., J. P. Nyrop, and T. J. Dennehy. 1996. Aerial dispersal of European red mites (Acari: Tetranychidae) in commercial apple orchards. *Experimental & Applied Acarology* **20**:193-202.
- Loomans, A. J. M. 2007. Regulation of invertebrate biological control agents in Europe: review and recommendations in its pursuit of a harmonised regulatory system. Report EU project REBECA [Regulation of Biological Control Agents].
- Loughner, R., K. Goldman, G. Loeb, and J. Nyrop. 2008. Influence of leaf trichomes on predatory mite (*Typhlodromus pyri*) abundance in grape varieties. *Experimental and Applied Acarology* **45**:111-122.
- Loughner, R., K. Wentworth, G. Loeb, and J. Nyrop. 2009. Leaf trichomes influence predatory mite densities through dispersal behavior. *Entomologia Experimentalis Et Applicata* **134**:78-88.
- Loughner, R., K. Wentworth, G. Loeb, and J. Nyrop. 2010. Influence of leaf trichomes on predatory mite density and distribution in plant assemblages and implications for biological control. *Biological Control* **54**:255-262.
- Luczynski, A. 2008. Influence of relative humidity on the release pattern of *Amblyseius swirskii* and *Carpoglyphus lactis* from slow release sachets - Internal report. Koppert Biological Systems.
- Maoz, Y., Gal, S., Argov, Y., Coll, M and Palevsky, E. 2011. Evaluation of an exotic spider mite predator and an indigenous pollen feeder for the biocontrol of *Oligonychus perseae*. *Biological Control* **59**: 147–157
- Margolies, D. C. 1987. Conditions eliciting aerial dispersal behavior in Banks grass mite, *Oligonychus-pratensis* (Acari, Tetranychidae). *Environmental Entomology* **16**:928-932.
- McMurtry, J. A. 1992. Dynamics and potential impact of generalist phytoseiids in agroecosystems and possibilities for establishment of exotic species. *Experimental and Applied Acarology* **14**:371-382.
- McMurtry, J. A. and B. A. Croft. 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* **42**:291-321.
- McMurtry, J. A. and G. T. Scriven. 1966. Influence of pollen and prey density on number of prey consumed by *Amblyseius hibisci* (Acarina - Phytoseiidae). *Annals of the Entomological Society of America* **59**:147-&.
- Momen, F. M. 1995. Feeding, development and reproduction of *Amblyseius-barkeri* (Acarina, Phytoseiidae) on various kinds of food substances. *Acarologia* **36**:101-105.
- Momen, F. M. 2001. Biology of *Euseius yousefi* (Acari: Phytoseiidae) life tables and feeding behaviour on different diets. *Acta Phytopathologica et Entomologica Hungarica* **36**:411-417.
- Montserrat, M., C. Bas, S. Magalhaes, M. W. Sabelis, A. M. de Roos, and A. Janssen. 2007. Predators induce egg retention in prey. *Oecologia* **150**:699-705.
- Moriyama, M. and H. Numata. 2006. Induction of egg hatching by high humidity in the cicada *Cryptotympana facialis*. *Journal of Insect Physiology* **52**:1219-1225.
- Nomikou, M., A. Janssen, and M. W. Sabelis. 2003. Phytoseiid predator of whitefly feeds on plant tissue. *Experimental and Applied Acarology* **31**:27-36.
- Nomikou, M., A. Janssen, R. Schraag, and M. W. Sabelis. 2001. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Experimental and Applied Acarology* **25**:271-291.

- Nomikou, M., A. Janssen, R. Schraag, and M. W. Sabelis. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Experimental and Applied Acarology* **27**:57-68.
- Norton, A. P., G. English-Loeb, and E. Belden. 2001. Host plant manipulation of natural enemies: leaf domatia protect beneficial mites from insect predators. *Oecologia* **126**:535-542.
- Okamoto, M. 1984. Studies on the environmental factors for the life cycle of *Carpoglyphus lactis*: 1. The effect of relative humidities on individual rearing. *Medical entomology and zoology* **35**:269-275.
- Onzo, A., R. Hanna, K. Negloh, M. Toko, and M. W. Sabelis. 2005. Biological control of cassava green mite with exotic and indigenous phytoseiid predators - Effects of intraguild predation and supplementary food. *Biological Control* **33**:143-152.
- Othman, K., A. Rhouma, Belhadj R., Alimi E., Fallah H., Kreiter P., L. C., and B. J. 2001. Lutte biologique contre un acarien ravageur des dattes: essai d'utilisation de *Neoseiulus californicus* contre *Oligonychus afrasiaticus* dans les palmeraies du Djerid (Sud tunisien). *Phytoma* **540**:30-31.
- Palevsky, E. 1997. Development of a program for integrated management of the European red mite, *Panonychus ulmi*, for the main apple cultivars in Israel. Hebrew University of Jerusalem.
- Palevsky, E., H. Borochoy-Neori, and U. Gerson. 2005. Population dynamics of *Oligonychus afrasiaticus* in the southern Arava Valley of Israel in relation to date fruit characteristics and climatic conditions. *Agricultural and Forest Entomology* **7**:283-290.
- Palevsky, E., S. Gal, and E. A. Ueckermann. 2009. Phytoseiidae from date palms in Israel with descriptions of two new taxa and a key to the species found on date palms worldwide (Acari: Mesostigmata). *Journal of Natural History* **43**:1715-1747.
- Palevsky, E., H. Reuveny, O. Okonis, and U. Gerson. 1999. Comparative behavioural studies of larval and adult stages of the phytoseiids (Acari : Mesostigmata) *Typhlodromus athiasae* and *Neoseiulus californicus*. *Experimental and Applied Acarology* **23**:467-485.
- Palevsky, E., O. Ucko, S. Peles, S. Yablonski, and U. Gerson. 2003. Species of *Oligonychus* infesting date palm cultivars in the Southern Arava Valley of Israel. *Phytoparasitica* **31**:144-153.
- Palevsky, E., O. Ucko, S. Peles, S. Yablonski, and U. Gerson. 2004. Evaluation of control measures for *Oligonychus afrasiaticus* infesting date palm cutlivars in the Southern Arava Valley of Israel. *Crop Protection* **23**:387-392.
- Palevsky, E., A. Walzer, S. Gal, and P. Schausberger. 2008. Evaluation of dry-adapted strains of the predatory mite *Neoseiulus californicus* for spider mite control on cucumber, strawberry and pepper. *Experimental and Applied Acarology* **45**:15-27.
- Pedigo, L. P. and M. E. Rice. 2009. *Entomology and Pest Management*. 6<sup>th</sup> edition. Pearson Prentice Hall, New Jersey, USA.
- Perring, T. M., T. O. Holtzer, J. A. Kalisch, and J. M. Norman. 1984a. Temperature and humidity effects on ovipositional rates, fecundity, and longevity of adult female Banks grass mites (Acari, Tetranychidae). *Annals of the Entomological Society of America* **77**:581-586.
- Perring, T. M., T. O. Holtzer, J. L. Toole, J. M. Norman, and G. L. Myers. 1984b. Influences of temperature and humidity on pre-adult development of the Banks grass mite (Acari, Tetranychidae). *Environmental Entomology* **13**:338-343.

- Perring, T. M. and L. J. Lackey. 1989. Temperature and humidity effects on mortality and pre-adult development of two *Phytoseiulus persimilis* strains (Acari: Phytoseiidae). *International Journal of Acarology* **15**:47 - 52.
- Porath, A. and E. Swirski. 1965. A survey of phytoseiid mites (Acarina:Phytoseiidae) on citrus, with a description of one new species. *Israel Journal of Agricultural Research* **15**:87-100.
- Porres, M. A., J. A. McMurtry, and R. B. March. 1975. Investigations of leaf sap feeding by 3 species of phytoseiid mites by labeling with radioactive phosphoric-acid  $H_3^{32}PO_4$ . *Annals of the Entomological Society of America* **68**:871-872.
- Ragusa, E., H. Tsolakis, and R. J. Palomero. 2009. Effect of pollens and preys on various biological parameters of the generalist mite *Cydnodromus californicus*. *Bulletin of Insectology* **62**:153-158.
- Ramakers, P. M. J. 1990. Manipulation of phytoseiid thrips predators in the absence of thrips. *SROP/WPRS Bulletin* **13**:169-172.
- Reuveny, H., E. Palevsky, and U. Gerson. 1996. Laboratory life history studies of the predaceous mite *Typhlodromus athiasae* (Acari: Phytoseiidae). *Systematic and Applied Acarology* **1**:45-53.
- Rock, W., M. Rosenblat, H. Borochoy-Neori, N. Volkova, S. Judeinstein, M. Elias, and M. Aviram. 2009. Effects of Date (*Phoenix dactylifera* L., Medjool or Hallawi Variety) consumption by healthy subjects on serum glucose and lipid levels and on serum oxidative status: A pilot study. *Journal of Agricultural and Food Chemistry* **57**:8010-8017.
- Roda, A., J. Nyrop, and G. English-Loeb. 2003. Leaf pubescence mediates the abundance of non-prey food and the density of the predatory mite *Typhlodromus pyri*. *Experimental and Applied Acarology* **29**:193-211.
- Rosenthal, G.A. and Berenbaum, M.R. (eds.) 1991. *Herbivores – Their Interactions with Plant Secondary Metabolites*. Volume 1, Academic Press, San Diego, 468 pp.
- Rowles, A. D. and D. J. O'Dowd. 2009. Leaf domatia and protection of a predatory mite *Typhlodromus doreenae* Schicha (Acari: Phytoseiidae) from drying humidity. *Australian Journal of Entomology* **48**:276-281.
- Sabelis, M. W. 1985. The Phytoseiids - Development. Pages 43-53 in W. Helle and M. W. Sabelis, editors. *Spider Mites: Their biology, Natural Enemies and Control* Elsevier, Amsterdam, The Netherlands.
- Saito, Y. 1985. Life types of spider mites. Pages 253-264 in W. Helle and M. W. Sabelis, editors. *Spider Mites: Their biology, Natural Enemies and Control*
- Sanchez-Ramos, I., F. Alvarez-Alfageme, and P. Castanera. 2007. Effects of relative humidity on development, fecundity and survival of three storage mites. *Experimental and Applied Acarology* **41**:87-100.
- Santi, F. and B. Maccagnani. 2000. Influence of the humidity on mortality rate and embryonic development time of two strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). *Bollettino dell'Istituto di Entomologia "Guido Grandi" della Universita degli Studi di Bologna*:1-11.
- Schausberger, P. 1998. The influence of relative humidity on egg hatch in *Euseius finlandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari, Phytoseiidae). *Journal of Applied Entomology* **122**:497-500.

- Schilman, P. E., S. A. Minoli, and C. R. Lazzari. 2009. The adaptive value of hatching towards the end of the night: lessons from eggs of the haematophagous bug *Rhodnius prolixus*. *Physiological Entomology* **34**:231-237.
- Shomer, I., H. Borochoy-Neori, B. Lutzki, and U. Merin. 1998. Morphological, structural and membranal alterations in frozen tissues of Madjhoul date (*Phoenix dactylifera* L.) fruits. *Postharvest Biol. and Technol.* **14**: 207-215.
- Singleton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents." *Am. J. Enol. Vitic.*, **16**:144-58.
- Slansky, F. Jr. 1990. Insect nutritional ecology as a basis for studying host plant resistance. *Fla. Entomol.* **73**: 359-378.
- Smitley, D. R. and G. G. Kennedy. 1988. Aerial dispersal of the two-spotted spider-mite (*Tetranychus urticae*) from field corn. *Experimental & Applied Acarology* **5**:33-46.
- Sohrabi, F. and P. Shishebhoh. 2007. Functional and numerical responses of *Stethorus gilvifrons* Mulsant feeding on strawberry spider mite, *Tetranychus turkestani* Ugarov and Nikolski. *Pakistan Journal of Biological Sciences* **10**:4563-4566.
- Swirski, E. and S. Amitai. 1997. Annotated list of phytoseiid mites (Mesostigmata: Phytoseiidae) in Israel. *Israel Journal of Entomology* **31**:21-46.
- Swirski, E., S. Amitai, and N. Dorzia. 1967. Laboratory studies on feeding development and oviposition of predaceous mite *Typhlodromus athiasae* P. and S. (Acarina - Phytoseiidae) on various kinds of food substances. *Israel Journal of Agricultural Research* **17**:213-&.
- Swirski, E., S. Amitai, and N. Dorzia. 1970. Laboratory studies on the feeding habits, post-embryonic survival and oviposition of the predaceous mites *Amblyseius chilensis* Dosse and *Amblyseius hibisci* Chant (Acarina: Phytoseiidae) on various kinds of food substances. *Entomophaga* **15**:93-106.
- Swirski, E., S. Ragusa Di Chiara, and H. Tsolakis. 1998. Keys to the Phytoseiid mites (Parasitiformes, Phytoseiidae) of Israel. *Phytophaga* **8**:85-154.
- Talhouk, A. S. 1991. On the management of the date palm and its arthropod enemies in the Arabian peninsula. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* **111**:514-520.
- Thurling, D. J. 1980. Metabolic rate and life stage of the mites *Tetranychus cinnabarinus* Boisd (Prostigmata) and *Phytoseiulus persimilis* A-H (Mesostigmata). *Oecologia* **46**:391-396.
- Tscharntke, T., R. Bommarco, Y. Clough, T. O. Crist, D. Kleijn, T. A. Rand, J. M. Tylianakis, S. van Nouhuys, and S. Vidal. 2007. Conservation biological control and enemy diversity on a landscape scale. *Biological Control* **43**:294-309.
- Van-Rijn, P. C. J., Y. M. Van Houten, and M. W. Sabelis. 1999. Pollen improves thrips control with predatory mites. *IOBC/WPRS Bulletin* **22**:209-212.
- Van Dinh, N., M. W. Sabelis, and A. Janssen. 1988. Influence of humidity and water availability on the survival of *Amblyseius idaeus* and *A. anonymus* (Acarina: Phytoseiidae). *Experimental and Applied Acarology* **4**:27-40.
- Van Lenteren, J. C., D. Babendreier, F. Bigler, G. Burgio, H. M. T. Hokkanen, S. Kuske, A. J. M. Loomans, I. Menzler-Hokkanen, P. C. J. Van Rijn, M. B. Thomas, M. G. Tommasini, and Q. Q. Zeng. 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biocontrol* **48**:3-38.
- Van Lenteren, J. C., J. Bale, E. Bigler, H. M. T. Hokkanen, and A. M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology* **51**:609-634.

- Walde, S. J., J. P. Nyrop, and J. M. Hardman. 1992. Dynamics of *Panonychus-ulmi* and *Typhlodromus-pyri* - factors contributing to persistence. *Experimental and Applied Acarology* **14**:261-291.
- Walter, D. E. 1996. Living on leaves: Mites, tomenta, and leaf domatia. *Annual Review of Entomology* **41**:101-114.
- Walzer, A., M. Castagnoli, S. Simoni, M. Liguori, E. Palevsky, and P. Schausberger. 2007. Intraspecific variation in humidity susceptibility of the predatory mite *Neoseiulus californicus*: Survival, development and reproduction. *Biological Control* **41**:42-52.
- Winston, P. W. and D. S. Bates. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* **41**:232-237.
- Woods, H. A. 2010. Water loss and gas exchange by eggs of *Manduca sexta*: Trading off costs and benefits. *Journal of Insect Physiology* **56**:480-487.
- Yoder, J. A. 1998. A comparison of the water balance characteristics of *Typhlodromus occidentalis* and *Amblyseius finlandicus* mites (Acari : Phytoseiidae) and evidence for the site of water vapour uptake. *Experimental and Applied Acarology* **22**:279-286.
- Yoder, J. A., J. B. Benoit, and A. M. Opaluch. 2004. Water relations in eggs of the lone star tick, *Amblyomma americanum*, with experimental work on the capacity for water vapor absorption. *Experimental and Applied Acarology* **33**:235-242.
- Yoder, J. A. and D. L. Denlinger. 1992. Water-vapor uptake by diapausing eggs of a tropical walking stick. *Physiological Entomology* **17**:97-103.
- Yuan, J-P and Chen, F. 1998. Chromatographic separation and purification of trans-astaxanthin from the extracts of *Haematococcus pluvialis*. *J. Agric. Food Chem.* **46**: 3371-3375.
- Zundel, C., R. Hanna, U. Scheidegger, and P. Nagel. 2007. Living at the threshold: Where does the neotropical phytoseiid mite *Typhlodromalus aripo* survive the dry season? *Experimental and Applied Acarology* **41**:11-26.