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CHARACTERISTICS OF DATE FRUIT AND ITS INFLUENCE ON POPULATION DYNAMICS OF Oligonychus afrasiaticus McGregor (Acari: Tetranychidae) IN THE SOUTHERN OF TUNISIA

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Summary: Oligonychus afrasiaticus, the old world date mite, is a serious pest of dates in Tunisia. The infestation of O. afrasiaticus begins and increased during the Kimri stage, characterised by the green colour of fruit. The aim of this study is to compare the susceptibility of three cultivars of date palm (Phoenix dactylifera) from Tunisia: ‘Deglet Noor’, ‘Alig’ and ‘Bessr’, at different ripening periods, to feeding by O. afrasiaticus. The ‘Deglet Noor’ cultivar was the most susceptible to O. afrasiaticus. The largest population of O. afrasiaticus on ‘Deglet Noor’, ‘Alig’ and ‘Bessr’ were recorded when the water content reached 83 %. Highest acid activity >2 mEq/100g, prevented mite establishment on the tree cultivars. Later in the season, when total sugar levels reached >420 mg/g fresh fruit, total soluble solids increased above 10% and fruit moisture decreased to < 80 %, mite populations declined. Total phenolics and hardness of the exocarp were not key factors in mite establishment.

Introduction

The date palm (Phoenix dactylifera L.) has always played an important part of the economic and social life of the people from arid and semi arid regions. Tunisia is one of the major date producing countries, the number of palm trees being estimated to be over 4 millions with production around 110,000 tons per year (FAOSTAT, 2005).

The date mite Oligonychus afrasiaticus McGregor (Acari: Tetranychidae) is a serious pest in Tunisia (Khoualdia et al., 2001) often responsible of serious damage to the fruits (Dhouibi, 1991).

During formation and ripening, the date fruit passes through four distinct stages named by their Arabic denominations; kimri, khalaal, rutab and tamr. Each of them is distinguished by one or more characters, of physical and/or chemical nature. The infestation of O. afrasiaticus begins and increases in summertime, i.e, during the Kimri stage (characterised by the green coloration of the fruit, rapid increase in size, weight, and reducing sugars). At this stage, the moisture content and the acid activity are at the highest (Barreveld, 1993). By feeding on the immature green dates, O. afrasiaticus causes severe fruit scarring and a reddish-brown colored fruit.

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Mite populations begin to decline with change of fruit color to yellow or red at the Khalaal stage, when the weight gain is slow whereas saccharose content increases, moisture content goes down, and tannins start to precipitate and lose their astringency.

At the Rutab stage, the fruit weight continues to decrease by moisture loss, and mite individuals become rare. When fruits turn into Tamr, the mite is absent: in this stage the fruit moisture is ≤25% (Barreveeld, 1993).

Seasonal declining of O. afrasiaticus populations on date palm fruits seems to be a phenomenon occurring everywhere not depending on climatical or geographical zones. This decrease has been noted from USA (Mauk et al., 2005), Middle East (Palevsky, 2005; Hussain, 19974; Talhouk, 1991) and North Africa (Khoualdia et al., 2001; Guessoum, 1986). It is often related to the chemical-change in the dates (Palevsky, 2005). Differences in varietal susceptibility have been reported from several areas, in Libya, the varieties ‘Asabir’, ‘Aurig’, ‘Bestian’, ‘Apel’ and ‘Talise’ were found to be most susceptible while ‘Tafsirt’ was found to be less susceptible (Edongali et al., 1988). Cultivars ‘Hilali’, ‘Gibri’ and ‘Khanazani’ in Oman were infested by O. afrasiaticus during April, whereas other cultivars were attacked later in the season (Elwan, 2000). In Israel, Palevsky (2005) reported that cultivar ‘Deglet Noor’ were more attacked than ‘Medjool’ and ‘Barhi’ cultivars. The Iraqi variety “Sayer” is relatively resistant to mite attack (Hussain, 1974).

In the USA, the Banks grass mite Oligonychus pratensis Banks causes direct damage on immature green date fruit by puncturing cells and sucking the juice from the surface of the fruit (Mauk et al., 2005). Spider mites penetrate the leaf surface with their stylets to a depth of 70-120 μm and suck out the cell contents (Tomezyk and Kropczynska, 1985). The ability of chelicers to penetrate the fruit exocarp is of crucial importance to mite feeding. So, leaf hardness has been correlated with host plant resistance to the Tetranychus urticae (Koch) in cotton (Sadrats et al., 1998). We suppose that characteristics of exocarp are related to cheliceral penetration and that the access to feeding sites differs between more and less susceptible varieties.

Tetranychids are sensitive to the chemical composition of the host plant. Essential nutrients levels including soluble proteins and carbohydrates have been associated with performance of T. urticae by several authors (Kielkiewicz & Van de Vrie, 1990), and several studies on T. urticae have demonstrated a positive correlation between population growth and leaf sugar concentration of several host plants (Rodriguez et al., 1960; Rodriguez & Cambell, 1961).

Other data illustrated the variation in abundance of T. urticae on mature leaves of Gerbbera: greater contents of reducing sugars were found in young leaves than in mature one and young leaves seem to be protected by this level of concentration in reducing sugars (Kielkiewicz, 1995). On chrysanthemum, the highest density of spider mites on the older leaves was correlated with low level of phenolic substances. Low content in mono and polyphenols could protect young leaves, concentration of these substances increases after spider mite feeding, implying an induced defence system (Kielkiewicz, 1995).

An over abundance of amino acids in the diet can be detrimental to T. urticae as high rate of amino acids induces excessive osmotic pressure in the hemolymph (Sun, 1963).

To understand the relation between spider mite density and fruit chemistry, detailed chemical analysis of date fruit during maturation in several varieties is needed. The goal of this study was to compare the susceptibility of different date palms varieties: ‘Deglet Noor’, ‘Alig’ and ‘Bessr’ to O. afrasiaticus, and to determine if exocarp hardness or and nutritional quality of the dates may explain the differences in O. afrasiaticus abundance on these fruits.

**Materials and methods**

**Mite monitoring and damage assessment**

During the 2006 season three varieties of date: ‘Alig’, ‘Bessr’ and ‘Deglet Noor’, were selected from a single plot of mixed cultivars in Segdoud (southern Tunisia). Trees were sprayed with sulfur twice on 24/7/2006 and on 1/8/2006.
*O. afrasiaticus* was monitored on ten trees of each variety, located in the North, South, West, and East, or in the middle of plot. Samples were taken at random of 10 date fruits per palm tree, every week from June through September to determine the dynamic of *O. afrasiaticus*. In laboratory, all stages of mites were counted directly with the aid of a microscope.

**Measure of exocarp resistance to penetration**

To measure penetration resistance of dates to mites we used a texture analyser (model LLOYD INSTRUMENTS TA Plus) equipped with a puncture probe of 4 mm diameter. Penetration resistance was monitored during the season: first when only variety Deglet Noor was infested and second when all three cultivars were infested. To evaluate if the hardness of exocarp prevents from no mite establishment, five fruits were sampled per cultivar. The parameter recorded was force of resistance (in Newton) as a function of penetration depth (2mm).

**Chemical analyses**

At each stage of fruit development, around 20 fruits /tree were collected from a selection of bunches chosen at random trees growing at the same location. Fruits of uniform size, free of physical damage and injury from insects and fungal infection, were selected and used for subsequent experiments.

On each occasion, the fruits were brought to the laboratory on the day of harvesting and fruits were weighted, after the removal of a sub-sample for moisture determination, were stored at -20°C for subsequent analysis.

Mono and disaccharides were extracted successively with 15 ml of methanol (90%) and 10 ml of methanol (80%). After centrifugation, the supernatant was filtered and the clear filtrate was brought to dryness under reduced pressure at 40°C in a rotary evaporator. The residue obtained was stored in a freezer (-20°C).

The concentrations of sucrose, glucose and fructose were measured by high-performance liquid chromatography analysis (HPLC) Shimadzu, equipped with Knauer NH2 column. The products were separated by isocratic elution with water at a flow rate of 0.8 ml/min and detected with a refractive index detector (SHIMADZU, RID-10A). Quantification of mono-and disaccharides carried out using acetonitril as standards at 85%.

The moisture was determined by drying samples of dates and seeds in a vacuum oven set at 65°C for 48 h and water content calculated (Moisture (%) = [(initial weight-final weight)/initial weight] ×100)

Total soluble solids (TSS) values are indicative of the extent of solute accumulation, and relate physical properties (osmoticum, viscosity) of the sap. TSS was measured in fruit juice with a refractometer (optical technology K7121, Abbe Mod. RMT).

Titratable acidity was determined in juice by titrating with sodium 0.1 N sodium hydroxide, results expressed as a mEq/100 g.

Total phenolics were extracted from crushed flesh and skin of date fruit, in hot methanol by 1-hour homogenisation, then centrifuged (5000 rpm, 15 min). These operations were replicated by 30-min homogenisation at second and third time. The total phenolic content in the methanol extracts were determined calorimetrically using Folin-Ciocalteu reagent.

In a tube 500 ml of distilled water, 100 ml of diluted sample (10-1) and 250 ml of Folin–Ciocalteu reagent were added and vortexed. After 3 min, 1.25 ml of aqueous sodium carbonate (20%) was added, and the mixture was vortexed and allowed to stand at room temperature with exclusion of light, for 60 min. The absorbance was read at 760 nm. The total phenol concentration was calculated from the calibration curve, using gallic acid as a standard, and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g fresh fruit.

**Statistical analysis**

Experiments were analyzed by ANOVA followed by pair wise comparisons according to Tukey (SPSS 10.0).
Fig. 1: Mean ± resistance of penetration (measured in Newton) to the depth of 2mm in to the exocarp of date cultivars ‘Deglet Noor’, ‘Alig’ and ‘Bessr’ in July and in August at Segdoud, South of Tunisia, 2006.

RESULTS

Exocarp resistance to penetration

As illustrated by Fig. 1, resistance to penetration change between cultivars, ‘Deglet Noor’ was more resistant to penetration than ‘Alig’ and ‘Bessr’ in July and in August ($F = 13.770$, $d.f. = 2.29$; $P < 0.001$). This resistance to penetration decreased from June to August for each cultivar. Decrease was significantly for ‘Alig’ ($F = 226.165$, $d.f. = 1.8$; $P < 0.001$).

Water content, acidity and TSS in relation to mite phenology

The colonization by *O. afrasiaticus* occurred only in the ‘Deglet Noor’ cultivar during the first week of July. On that date, water content was similar in three cultivars: ‘Deglet Nour’ (86.5%), ‘Alig’ (87.1%) and ‘Bessr’ (87.2%) ($F = 2.7$, $d.f. = 2.8$; $P > 0.0001$). During the third week of July, spider mite density increased on ‘Deglet Noor’, individuals appeared on ‘Alig’. The water content was similar (respectively 84.78% and 83.94%), but less than the value measured on ‘Bessr’ (86.49%) ($F = 123.455$, $d.f. = 2.8$; $P < 0.0001$).

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*O. afrasiaticus* on ‘Alig’ were recorded when the water content was 83.38%. Mite establishment was detected on ‘Bessr’ cultivar when the fruit moisture decreased significantly (first week of August: $<84.99$%).

During the first week of September, lower fruit moisture was noted on the three cultivars ($<80.6$%), and coincides with the decrease in mite populations on ‘Deglet Noor’, ‘Alig’ and ‘Bessr’ dates.

The effects of fruit acidity on mite populations are shown in Fig. 2C. In the third week of June there were no significant differences in the acidity of three cultivars ($P > 0.0001$); data indicate highest acid activity in the tree cultivars.

The mite population development on ‘Deglet Noor’ coincided with the decrease of fruit acidity from 2.2 mEq/100g to 0.4 mEq/100g. The acidity was similar on the fruits of ‘Bessr’ (0.83 mEq/100g) and ‘Alig’ (0.8 mEq/100g), significantly higher than in ‘Deglet Noor’ ($F = 392.5$, $d.f. = 17$; $P < 0.0001$).

The density of *O. afrasiaticus* peaked on 23rd July on the ‘Deglet Noor’ when fruit acidity was 1.37mEq/100g.

The different date fruit cultivars present a TSS ascending with ripening stage. During the second week of August, the ‘Bessr’ cultivar reached the highest value, followed by ‘Alig’ and ‘Deglet Noor’. These results showed that mite populations decreased significantly when TSS increased above 10%.

As illustrated by Fig. 3, the total sugar level during the third week of June differs between three cultivars: ‘Deglet Noor’ (190.71 mg/g FW), ‘Alig’ (209.49 mg/g FW) and ‘Bessr’ (164.3 mg/g FW). Mites were detected earlier in ‘Deglet Noor’ than in the two other cultivars. In early July we recorded the first infestation. The absence of mites at July 7th was due to the elimination of dates attacked by a farmer. When the levels of fructose, sucrose and glucose were 409.32 mg/g FW, the maximum of the infestation was reached with 1419 motile forms/100 dates.

For the ‘Alig’ cultivar, mite populations increased with the increase of the total sugar, at the end of July we noted 1139 motile forms of mites per 100 dates when total sugars were 323.25 mg/g FW. The colonization by *O. afrasiaticus* of ‘Bessr’ dates was late; on early August the density increased rapidly and reached 861 mites/100 dates when total sugars were 403.96 mg/ g FW.
Fig. 2: Variation in fruit weight, acidity, total soluble solids and water content in relation to *O. afrasiaticus* number, at Segdoud, South of Tunisia, 2006, on date cultivars ‘Deglet Nour’, ‘Alig’ and ‘Bessr’:

(a) motile forms number of *O. afrasiaticus* per 100 dates fruits; (b) mean ± SE fruit weight (g); (c) mean of total acidity (mEq/100 g); (d) mean ± SE TSS (%) and (e) mean ± SE water content (%).

In August, after the passage to the Khalal stage, sugar content rises fast as mite densities declined on these three cultivars we noted that mite level on the ‘Deglet Noor’ cultivar was higher than on the two other cultivars (Mid August, 360 mites/100 dates and concentration in total sugars of 425.87 mg/g FW) Concentration in sugars was higher on ‘Alig’ (466.53 mg/g FW) as on Bessr (606.29 mg/g FW).

On June 18, there was no significant difference in the concentration of phenolics among ‘Alig’ (446.594203 mg GAE/100 g FW) and ‘Deglet Noor’ (458.846377 mg GAE/100 g FW). But these concentrations were higher than in ‘Bessr’ (61.210171 mg GAE/100 g FW) (Fig. 4). During July, the concentration in phenolic compounds was different according to the cultivar: ‘Alig’ had the highest rate in total phenolics, followed by ‘Deglet Noor’ and ‘Bessr’.
Fig. 3: Mean ± SE sucrose, glucose and fructose levels and number of motile forms of *O. afrasiaticus* per 100 fruits at Segdoud, Tunisia, 2006 on date cultivars ‘Bessr’ (a), ‘Alig’ (b) and ‘Deglet Noor’ (c).

Thus, it seems that total phenolics can not be seen as an important factor, explaining lower *O. afrasiaticus* performance on ‘Bessr’ cultivar and higher attacks on ‘Alig’ and ‘Deglet Noor’.

**DISCUSSION**

The ‘Deglet Noor’ cultivar was the more susceptible to *O. afrasiaticus*. Hardness of the exocarp does not appear to be a factor affecting mite establishment because resistance to penetration was highest on ‘Deglet Noor’ in June and in August. On the date cultivars ‘Medjool’, ‘Barhi’ and ‘Deglet Noor’, resistance to *O. afrasiaticus* penetration was highest in July when mite populations peaked (Palevsky, 2005).

The nutritional requirements of phytophagous mites are still not fully understood. Numerous studies on various tetranychids on various hosts have shown that the density and the fecundity of these mites depended on plant quality. Tetranychids pierce the parenchyma tissue of leaves with their stylets and siphon out the cells’ contents (van der Geest, 1985; Jeppson et al., 1975). Consequently, mite nutrition is directly affected by the chemical composition of ingested fluids. Results suggest that the observed difference of ‘Deglet Noor’, ‘Alig’ and ‘Bessr’ cultivars susceptibility to *O. afrasiaticus* were due to seasonal differences in nutritional quality of cultivars.

Increase on sugar levels (glucose, fructose and sucrose), is necessary to the mite establishment on three cultivars. Bessr cultivar was the less susceptible to the *O. afrasiaticus* infestation did not occur before the end of July when concentration of sugars was below 400 mg/g FW. However, population declined when an increase in total sugar levels during date ripening occurred. This increase coincided with the passage of fruit dates to the Khalal stage. This
explains the decline of mites on the three cultivars with the increase of the Total soluble solids (TSS) values during the date ripening.

In recent studies, fruit dates of cultivars: ‘Barhi’, ‘Deglet Noor’ and ‘Mdjool’ were analysed, it demonstrated that performance of O. afrasiaticus varied greatly depending on the sugars levels on dates (Palevsky and al., 2005). Carbohydrates play an important role in the development of mites, but with higher levels, they could also be a source of resistance used by plants against mites. Studies conducted by De Angelis et al. (1983) underline a significance of soluble carbohydrates in injured peppermint leaves has been explained as osmotic adjustment in response to mite-induced water stress. Three weeks of T. urticae feeding caused a significant increase in reducing sugar contents, in young and mature Gerbera leaves (Kielkiewicz, 1995).

Same results were founded for Tetramychus cinnabarinus feeding on tomato plants (Kielkiewicz, 1990). Kielkiewicz and Van de Vrie (1990) demonstrated that mite density was lower on young chrysanthemum leaves than mature one. These authors reported that young chrysanthemum leaves appeared to be protected against T. urticae by a higher concentration of mono and polyphenols, although they contained higher levels of nutrients than mature ones.

Colonization of dates by O. afrasiaticus occurred at the earliest time in the first week of July on ‘Deglet Noor’ cultivar, mites attains its maximum when the water content was under 85%.

Similar results were observed with ‘Alig’ and ‘Bessr’ cultivars. The decline in moisture content during maturation is shown clearly in all varieties. The mite population decreased on the three cultivars mid August, i.e. when water content was less than 80%. Earlier, and particularly during the Khalal stage, the water levels in the fruits was high enough to allow fungi to proliferate at the expense of available sugars (Imad, 1995). Decrease in water content with elevated sugar contents, render the date extremely resistant to fungal spoilage (Imad, 1995) and to O. afrasiaticus (Palevsky and al., 2005). Chemical analysis of infested and fully matured dates showed that in infested dates, water soluble substances as sugar are less (Hussain, 1974).

In avocado trees, attacked by O. perseae sugar and starch concentration are maximal from late-winter to early-spring and decrease throughout summer (Cameron and Borst, 1938; Scholefield, 1985; El-Hamalawi and Menge, 1995). Development of mite populations was negatively correlated with carbohydrate contents in the avocado trees. Similar starch and sugar cycles have been observed on susceptible and resistant cultivars, suggesting that cycling carbohydrate contents may not be the unique factor responsible for differences in susceptibility to O. perseae (Kerguelen, 2000).

Acidity of the dates can be an important factor. The host plant-mediated impact of simulated acid rain (pH 3.0, 3.5, 4.0, 5.6, and 6.8) on the behavior, the development, and the reproduction of carmine spider mite, Tetramychus cinnabarinus (Boisduvals) were evaluated. The adults of carmine spider mite prefer to aggregate on pH 4.0 acid rain-treated eggplant leaves. The developmental times of nymph, larva and the pre-oviposition period of the mite on kidney bean leaves were significantly affected by acid rain treatments. The mites feeding on acid rain-treated leaves (pH 3.0–5.6) had significantly best reproduction and increased longevity than those feeding on deionized water-treated leaves. The results implied that the population growths of the mite were enhanced by application of acid rain (pH 3.0–5.6) on host plants (Wang, 2004). Strong acid rain (pH < 3.0) inhibited the growth of both host plant and mite (Wang, 2005).

Lower content in phenolics was observed for ‘Bessr’, a resistant cultivar to O. afrasiaticus. Total phenolics content in ‘Alig’ and ‘Deglet Noor’ has been reported to decrease from a maximum level in June to a minimum level in beginning July an increasing again in July. In August and September we noted a decrease in total phenolics. These variations are not consistent with studies that indicated that phenolics are probably toxic to the mite. Larson & Barry (1984) reported that high level of phenolics in the young lateral leaves of peppermint may explain why only a few TSSM were found. Other studies underlined that in young and mature Gerbera leaves, significant increases in concentration of total phenolics were solely observed when the plants were infested. In the same studies there were no significant
difference in the phenolics concentration among no infested young and mature Gerbera leaves (Kielkiewicz, 1995).

Establishment of *O. afrasiaticus* was not delayed by fruit phenolics. On ‘Bessr’ fruit, during ripening stages lower phenolics content was measured and a lower performance of *O. afrasiaticus*. The ability of ‘Deglet Noor’ and ‘Alig’ to support populations of *O. afrasiaticus* did not depend on the phenolics contents, suggesting these compounds cannot be a limiting factor for mite.

In conclusion, our studies demonstrated that:

— The resistance to penetration and the concentration in phenolics did not affect mite infestation on ‘Alig’, ‘Bessr’ and ‘Deglet Noor’ cultivars.

— The water content, the sugar concentration, the acidity and TSS are the factors involved, they determined the favourable or not condition for the mite infestation.

For developing efficient pest control programs, estimation of difference in susceptibility of varietals is crucial. Less susceptible cultivars can be left no-sprayed, or sprayed at a low threshold. Identifying characteristics that enhance resistance to spider damage will enable plant breeders to favour resistant varieties, and to avoid the most susceptible. On other hand, increase in cultivar diversity in orchards should be considered as a low cost strategy to reduce damage and associated yield reductions due to the infesting mite *O. afrasiaticus*.

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