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Seasonal pattern of infestation by the carob moth *Ectomyelois ceratoniae* in pomegranate cultivars

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ABSTRACT

Pomegranate (*Punica granatum* L.) orchards in the Middle East are typically composed of a mix of different cultivars in which variation in fruit infestation by carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) has been observed. However, seasonal variation in infestation and adaptation of the carob moth to this cropping system have not been explored. We monitored the progress of fruit infestation in 10 pomegranate cultivars during the growing season of two consecutive years in pomegranate orchards of Iran. Overall, levels of infestation in fruits were strongly correlated with susceptibility to fruit cracking in pomegranate, so that cracked fruits and cracking-susceptible cultivars were infested the most. However, this pattern changed during the season. Infestation was first observed on cracking-susceptible cultivars. At this point almost all cracked fruits were infested. Towards the end of the season, infestation in uncracked fruits and cracking-resistant cultivars increased. Uncracked fruits seem better overwintering sites for carob moth as under simulated winter conditions, survival of insect larvae in uncracked fruits was >3 times higher than in cracked fruits. Taken together, our data reveal that cracked fruits of pomegranate are the better host during the growing season, while uncracked fruits better sustain carob moth population in winter. It seems therefore advisable not to grow cracking-susceptible and cracking-resistant cultivars together in the same area.

1. Introduction

Phytophagous insects synchronize their life cycle with growth stages and phenology of the host plants to enhance fitness, optimize food intake, and minimize the impact of adverse environmental factors such as natural enemies and winter (Kooi et al., 1991; Zvereva, 2002; Schoonhoven et al., 2005; Visser and Both, 2005; da Silva et al., 2016). Synchronization can be achieved when both insects and plants respond to the same environmental conditions or when insects respond to signals that are specific to the phenology of their host plants (Tasin et al., 2005; Proffit et al., 2007).

Variation in suitability and availability causes phytophagous insects to display distinct preferences for particular plant species, cultivars, and even plant growth stages during the season (Jallow et al., 2004; van Asch and Visser, 2007). Heteroecious, host-alternating aphids, for example, switch host plants during the season; they spend winter on tree or bush, but in summer they migrate to herbaceous plants, and at the end of season they return to the trees (Vilcinskas, 2016). Also, population sizes of the thrips *Frankliniella occidentalis* (Pergande) change over a season among chrysanthemum (*Dendranthema grandiflora* Tzvelev) cultivars, because resistance against this insect develops differently among the cultivars (de Kogel et al., 1997).

The carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) is a polyphagous destructive pest worldwide, attacking different fruits before harvest and, as a stored product pest, after harvest. The insect is recognized as the economically most important pest of pomegranate, *Punica granatum* L. (Lythraceae), in almost all pomegranate production areas of the Middle East, causing 30–80% yield loss (Kashkuli and Eghtedar, 1975; Shakeri, 2004; Sobhani et al., 2015). The pest larvae feed on internal parts of the fruit, resulting in contamination with saprophytic fungi,

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which makes the fruit unfit for human consumption and food processing industries, and thus unmarketable (Shakeri, 2004). Female carob moths lay eggs inside the pomegranate crown (calyx) or in the cracks of the fruit peel if they are available. The larvae are capable of burrowing into the fruit from the fruit crown, but likely take advantage of the cracks in the peel to penetrate the fruit. Since egg-laying and larval feeding activities occur within the fruits and are thus hidden from the outside world, commercial insecticides are not efficient and therefore not used against this pest. Carob moths have 3–5 generations per year on pomegranate. The last generation larvae enter diapause inside the fruit at the end of the season (Al-Izzi et al., 1985).

Pomegranate is native to Iran (Morton, 1987), and there is a rich genotypic diversity of this plant species in the area (Sarkhosh et al., 2006, 2011). Pomegranate orchards in most areas of the Middle East are typically composed of a mix of different cultivars. There is also a tendency to increase this genotypic diversity, especially in Iran which is one of the largest producers of this fruit in the world (Shakeri, 2004; Sarkhosh et al., 2011; Sobhani et al., 2015). Fruit peel cracking is a very common phenomenon in pomegranate and is considered as a major disorder of the fruits (Shakeri, 2004; Khalil and Aly, 2013; Galindo et al., 2014; Hoseini et al., 2014; Saei et al., 2014). Recent studies have shown that pomegranate cultivars differ in their susceptibility to fruit cracking (Yuan et al., 2010; Saei et al., 2014). Pomegranate cultivars also exhibit different susceptibility to the carob moth (Moawad et al., 2011; Sobhani et al., 2015). Whether susceptibility of the cultivars to carob moth infestation is related to fruit cracking has been poorly studied. In this study, we monitored seasonal patterns of carob moth infestation and fruit cracking in 10 pomegranate cultivars in the field and determined seasonal variation in association patterns of these two common phenomena in pomegranate. These patterns provide insight into the adaptation of carob moth to pomegranate cultivars in the Middle East, and practical information for management of the pest.

2. Material and methods

Field experiments were conducted in a pomegranate orchard located in the Research Station of the College of Agriculture and Natural Resources, University of Tehran, Alborz Province, Iran (35°46′34″N, 50°55′46″E and 1254 m elevation) during the cropping season of 2013 and 2014. In this orchard, 10 high-yield Iranian pomegranate cultivars were planted (cultivars specified in Fig. 1), so that all cultivars were grown under the same environmental conditions, and all were of the same age (8 years old in 2013). There were no pesticide treatments in the study area during the experimental period. Pomegranate cultivars were planted in a randomized complete block design in 4 blocks, with a total of 40 (4 × 10) plots. Each plot contained 5 trees of the same cultivar.

To determine the development of infestation and fruit cracking, 2 trees in 2013 and 1 tree in 2014 were selected randomly in each plot, and checked weekly for infested and cracked fruits, which thus amounted to a total of 8 and 4 trees per cultivar in 2013 and 2014, respectively. From the start of the season in which signs of carob moth infestation on pomegranate became visible (i.e., late August), referred to as growing season, infested and cracked fruits on and under the trees were recorded, marked, and left in the orchard. At the end of the season, when the fruits had developed to marketable stage (i.e., in mid-October), all unmarked infested fruits, i.e., the fruits that showed infestation on the last day of monitoring, were harvested and taken to the laboratory where the number of larvae in each fruit was determined.

To determine survival rates of carob moth larvae under simulated winter conditions, 46 cracked-infested and 46 uncracked-infested fruits were randomly picked from the orchard at harvest time (October 2014), and kept in a fridge at 8 °C and 80% RH for 100 days, after which the fruits were dissected and numbers of dead and alive larvae were recorded.

A linear mixed model (LMM) was used to determine differences among cultivars in terms of the percentage of total fruit infestation, percentage of total fruit cracking, the number of fruits per tree, and the number of larvae per infested fruit. Differences between means were determined using Tukey's HSD test with a 95% confidence interval. Differences between cracked and uncracked fruits in terms of the percentage of infestation and the number of larvae per infested fruit were also determined using a LMM. Cultivars and cracking status of the fruit were included in the models as fixed effects and replicate blocks as random effects. To stabilize the variance, percentages of fruit cracking were first arcsine $\sqrt{X}$ transformed (where X is the fruit cracking rate), whereas percentages of fruit infestation were square root transformed. A general linear model (GLM) was used to determine differences between the two years of the study in term of the number of fruits per tree with year of study included as fixed effect. Correlation analyses were performed between percentage of total fruit infestation, percentage of total fruit cracking, number of fruits per tree, and number of larvae per infested fruit. The survival rate of carob moth larvae in cracked and uncracked fruits after 100 days at 8 °C was also analyzed using GLM, with cracking status of fruits included as fixed effect. All analyses were conducted in R version 3.2.3 (R Core Team. 2015).

3. Results

Average number of fruits per tree differed significantly between the two growing seasons; 83.61 ± 6.43 (mean ± standard error) fruits in 2013 versus 20.10 ± 1.88 in 2014 ($F = 75.66$; df = 1, 54; $P < 0.001$). The number of fruits per tree was also significantly different across cultivars in 2013, but not in 2014 (Table 1). In both years, the cultivars differed further in terms of percentage of total fruit cracking, percentage of total fruit infestation, and the number of larvae per infested fruit (Table 1, Fig. 1).

Fruit infestation was significantly correlated with fruit cracking ($r = 0.71$, $P < 0.001$) and the number of insect larvae per infested fruit ($r = 0.60$, $P < 0.001$), but fruit infestation ($r = -0.08$, $P = 0.37$) and fruit cracking ($r = 0.16$, $P = 0.96$) were not correlated with the number of fruits per tree.

In both years, the number of larvae per infested fruit and total infestation were significantly higher in cracked fruits than in uncracked fruits (Table 1, Fig. 2).

In both years, at the start of the growing season the most susceptible pomegranate cultivars were A (Tabestaneh-Torsh) and B (Alak-Torsh), both in terms of infestation and fruit cracking (Fig. 1). At the end of season, all of the cultivars showed infestation, except cultivar J (Poust-Sefid-Bihasteh) in 2013. Cracked fruits were mostly infested early in the season, while uncracked fruits were primarily infested late in the season (Fig. 1). Throughout the growing season in both years, the number of cracked-infested fruits did not increase, but fruit cracking did increase dramatically, as well as infestation of uncracked fruits (Figs. 1 and 3).

Larval survival under simulated winter conditions differed significantly between cracked and uncracked fruits, both in terms of percentage survivors (79.32 ± 5.26% in uncracked versus 14.58 ± 4.46% in cracked fruit) ($F = 87.99$; df = 1, 90; $P < 0.0001$) and the number of survivors (1.8 ± 0.19 in uncracked versus 0.58 ± 0.16 in cracked fruit) ($F = 24.66$; df = 1, 90; $P < 0.0001$).

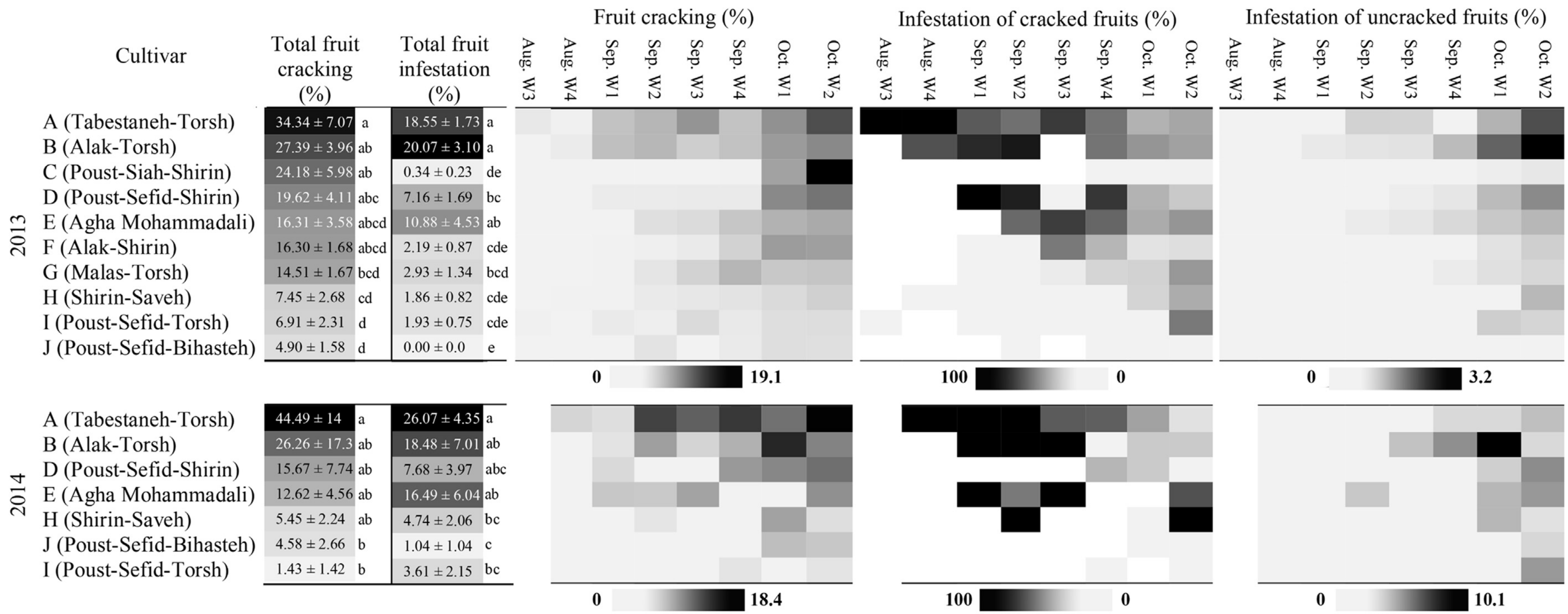

Fig. 1. Heat map, showing the average percentages (±SE) of total fruit cracking and total fruit infestation by the carob moth, and seasonal pattern of fruit cracking and fruit infestation in different pomegranate cultivars during the growing seasons of 2013 and 2014. The gradation of gray depicts the averages from high (black) to low (light gray). For each year, cultivars are ordered according to their susceptibility to fruit cracking. Means with different letters (a–d) are significantly different ($\alpha = 0.05$). Blanks indicate no infested and cracked fruit. W = week.

Table 1
Linear mixed model analysis of the variation in pomegranate fruit infestation, fruit cracking, number of fruits per tree, and number of carob moth larvae per infested fruit in different pomegranate cultivars in 2013 and 2014.

Year	Variable	Cultivar			Cracking status of fruit		
		Df	χ^2	*P*	Df	χ^2	*P*
2013	Total fruit infestation (%)	9	177.54	<0.001	–	–	–
	Total fruit cracking (%)	9	76.38	<0.001	–	–	–
	Number of fruits per tree	9	60.94	<0.001	–	–	–
	Number of larvae per infested fruit	8	19.81	0.011	1	19.69	<0.001
	Infestation (in cracked/uncracked fruits) (%)			–	1	30.61	<0.001
2014	Total fruit infestation (%)	6	36.11	<0.001	–	–	–
	Total fruit cracking (%)	6	18.38	0.005	–	–	–
	Number of fruits per tree	6	6.92	0.328	–	–	–
	Number of larvae per infested fruit	6	39.77	<0.001	1	48.42	<0.001
	Infestation (in cracked/uncracked fruits) (%)	–	–	–	1	30.93	<0.001

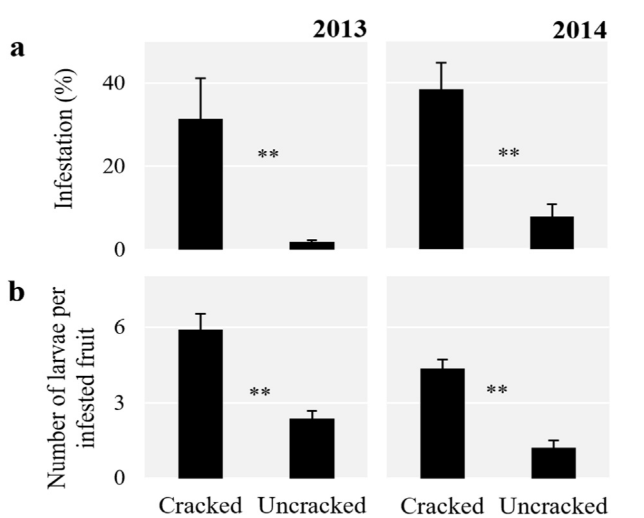

Fig. 2. Average (+SE) percentage of infestation (a) and the number of carob moth larvae per infested fruit (b) in cracked and uncracked pomegranate fruits. ** indicates significant difference at 0.01 confidence level.

4. Discussion

Field monitoring during the growing season of two years in a pomegranate orchard with 10 different cultivars showed that fruit infestation by the carob moth is significantly affected by the level of fruit cracking. Although in total only ~20% of the fruits were cracked in a year, total infestation rate was 5–15 times higher in cracked fruits than in uncracked fruits. We also found that different pomegranate cultivars are differentially susceptible to fruit cracking, confirming previous studies (Yuan et al., 2010; Saei et al., 2014), as well as to fruit infestation by the carob moth (Moawad et al., 2011; Sobhani et al., 2015). As the number of larvae per cracked-infested fruit was higher than that in uncracked fruit, more eggs were likely to be laid on cracked than on uncracked fruits, or more larvae can survive in cracked fruits. Female carob moths normally lay eggs inside the pomegranate crown and also in the cracks of the fruit (Shakeri, 2004; Talaiee et al., 2010). To get inside the fruit, carob moth larvae need to make a hole and pass the fruit peel inside the crown. Cracks on fruit seem to ease the access of larvae into the fruit. In our field observations, we found that first instars and most second instars cannot enter the fruit through the crown, while in cracked fruits first instars are found deep inside the

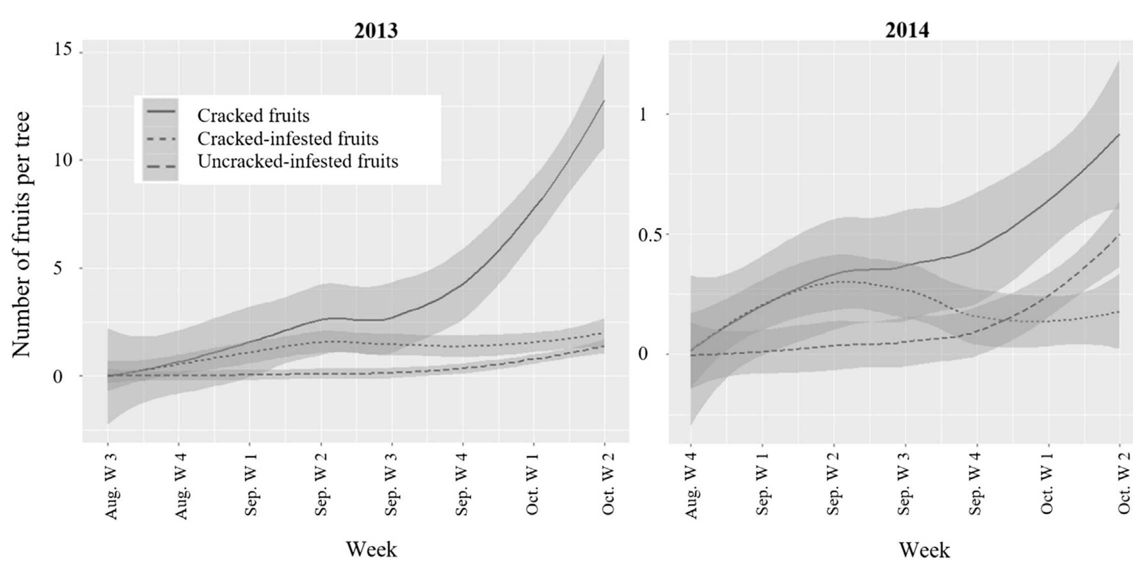

Fig. 3. Progress of fruit cracking and infestation by carob moth of cracked and uncracked pomegranate fruits in the experimental orchard during the cropping seasons of 2013 and 2014. Solid lines are fitted to the number of fruits per tree and the shaded areas represent 95% confidence intervals.

fruits. Furthermore, we usually observed larvae of different stages in a fruit, suggesting that females oviposited on already infested fruits.

Even though the fruit quantity differed significantly across the cultivars and between the two study years, this did not affect the patterns of fruit infestation and fruit cracking. In both years, infestation started on the highest cracking-susceptible cultivars. At this point, almost all of the cracked fruits were infested by the carob moth. However, towards the end of the growing season, infestation in uncracked fruits increased even though many more cracked-uninfested fruits were available than in early and mid season, so that also the more fruit-cracking resistant cultivars became infested. Cold winters have been reported to cause high mortality in overwintering populations of carob moth (Shakeri, 1993; Mehrnejad, 2002). Under simulated winter conditions, we found that >3 times more carob moth larvae survived in uncracked than in cracked fruits. Thus, the increased infestation in uncracked fruits and in cracking-resistant cultivars at the end of season may be related to the survival of insect larvae in uncracked fruits, which better protect the carob moth larvae against unfavorable winter conditions.

Natural enemies may also affect the seasonal pattern of infestation of pomegranate by carob moth. At the start of the season, ovipositing females and their offspring are probably more protected from predators and parasitoids in the cracks than in the crown where common predators, such as spiders (Araneae), ladybird beetles (Coccinellidae), true bugs (Heteroptera), the green lacewing, *Chrysoperla carnea* (Stephens), as well as parasitic wasps (Hymenoptera) are generally present (Shakeri, 2004; Mehrazin et al., 2016). However, carob moths are reported to be attacked by larval parasitoids mostly at the end of the season (Kishani-Farahani et al., 2010; Sobhani et al., 2015; Mehrazin et al., 2016). Cracks on fruit peel can make carob moth larvae inside more vulnerable to parasitism by facilitating the access of ovipositing female parasitoids to the inside of the fruit, which may explain the finding of larvae mostly in uncracked fruits in the late season.

Since cracked pomegranates were much more susceptible to carob moth infestation than uncracked fruits, agricultural control of fruit cracking is likely to suppress the carob moth population below economic injury levels. Control of fruit cracking may be feasible by using resistant cultivars or through recommended horticultural operations and treatments e.g., management of irrigation and pruning, and application of gibberellic acid and benzyladenine (Khalil and Aly, 2013; Galindo et al., 2014; Saei et al., 2014). However, as discussed above, uncracked fruits appear to play an important role in the establishment and growth of the insect population in the beginning of the next growing season. Even though collecting and destroying infested pomegranates at the end of the cropping season was already recommended for the control of the carob moth in the mid seventies (Kashkuli and Eghtedar, 1975; Shakeri, 2004), this method has recently been reported to have important negative effects, in terms of population sizes and species diversity, on larval parasitoids of carob moths (Kishani-Farahani et al., 2012). Cracked fruits are likely to host higher species diversity and more abundant larval parasitoids, as cracks on the fruit peel most likely facilitate the access of these natural enemies to the carob moth larvae inside at the end of season. Thus, destroying only uncracked infested fruits after harvest appears to be a good alternative pest control method, as this will conserve populations of natural enemies.

Another lesson that can be learned from the observed seasonal pattern of pomegranate fruit infestation by the carob moth is that for IPM programs it seems better not to grow cracking-susceptible and cracking-resistant pomegranate cultivars in the same area, as cracked and uncracked fruits differently sustain the pest population in different seasons. This is important in the Middle East, especially in Iran, where pomegranate orchards are typically composed of a mix of cultivars that differ in cracking level.

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