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The role of *Euwallacea* nr. *fornicatus* (Coleoptera: Scolytinae) in the wilt syndrome of avocado trees in Israel

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Abstract The polyphagous shot hole borer (PSHB), *Euwallacea* nr. *fornicatus* (Coleoptera; Scolytinae) has become a serious threat to the avocado industry and several shade tree species in Israel. Branch wilting and tree mortality is the outcome of PSHB galleries. Understanding the relationship between avocado trees and the PSHB is required for considering management strategies. In Israel, 52 tree species from 26 botanical families were attacked by the PSHB, but only 12 species were suitable for beetle reproduction. All examined avocado cultivars were attacked, but 'Hass' most severely. Large and medium diameter avocado branches were more resistant to PSHB, compared to thin branches. Effectively, gallery density increased as branch diameter decreased. Concomitantly, in large and medium diameter branches, extensive sugar exudation occurred and beetle attack rarely progressed to the formation of natal galleries, whereas minimal sugar exudation was observed in thin branches. This was more evident in those that were weakened by repeated attacks followed by successful beetle colonization. PSHB prefers and successfully colonized branches that had been previously attacked by its conspecifics, and reproduction was much higher in these branches, as opposed to initial attacks. Lesion frequencies increased from late spring (April) until late summer (September). Avocado branches at the early stages of beetle colonization may be identified by sugar exudation at the base of the thin branches. The main approach for reducing damage caused by the PSHB is sanitation, achieved by the removal of colonized branches and intact infested pruned slash.

Keywords Ambrosia beetle · *Euwallacea* · Avocado · Colonization · Damage

Introduction

Avocado production in Israel is a major industry covering an area of approximately 8000 ha. An estimated 100,000 tons were produced during the 2015/16 season, with about 65% of the crop being exported (Noy 2016). The first commercial orchards were planted in the early 1950s (Homsky 1995), with 70% of the plantations cultivated along the Coastal Plain, and the rest located in the Northern inland valleys. An average local avocado plantation ranges from one to 40 ha. and is surrounded by other agricultural crops or ornamentals.

Surveys of local avocado pests began in the late 1940s (Avidov and Ben Haim 1950). Although many arthropod species may inflict damage to avocado trees in Israel, for decades careful management due to restriction of

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frequent, prophylactic or responsive pesticide sprays have benefited the avocado industry. Over the years, chemical treatments in adjacent cotton fields disrupted biological control in avocado orchards; however, the situation has been resolved by pesticide regulations in cotton. The activity of invasive arthropod pests (e.g. the long-tailed mealybug, *Pseudococcus longispinus,* the greenhouse thrips, *Heliothrips haemorrhoidalis*, the Japanese bayberry whitefly, *Parabemisia myricae,* the pyriform scale *Protopulvinaria pyriformis,* and the persea mite *Oligonychus perseae*) were effectively restrained over the years by introduced and local natural enemies (Swirski et al. 1988; Maoz et al. 2011). However, two important pests that still require attention of the avocado growers, are the giant looper, *Boarmia selenaria* and the orchid thrips, *Chaetanaphothrips orchidii*, yet, the damage they cause is moderate and there is no need to apply synthetic insecticides for their management.

An ambrosia beetle, *Euwallacea* sp. near *fornicatus* (Scolytinae), recently termed in California as the polyphagous shot hole borer (PSHB), was first recorded in Israel in 2009 in an avocado plantation in the central coastal area (Glil Yam) and by early 2016 the beetle had spread to nearly all the avocado cultivation areas in the country (Mendel et al. 2012a, b, 2016). The most conspicuous symptom of the beetle attack on avocado trees, besides branch wilting, is the exudation of white powdery 7-carbon sugars, mannoheptulose and perseitol (Liu et al. 1999), which pinpoints the new penetration spots of the pest (Mendel et al. 2012a, b) (Fig. 1b, c and e). The PSHB has become a serious threat to the industry, which requires growers to consider integrated pest management schemes and other host plants on which the beetle proliferates. To date, the two main plant protection threats to avocado cultivation in Israel are branch dieback and mortality of young avocado plants caused by two fungal pathogens, *Lasiodiplodia theobromae* and *L. pseudotheobromae* (Shtienberg et al. 2015), and branch wilting associated with the PSHB (Mendel et al. 2016).

Euwallacea fornicatus (Eichhoff) sensu *lato*, is a complex of several cryptic species (O'Donnell et al. 2015; Stouthamer et al. 2017). The beetle populations that have invaded the Los Angeles area of California, South Africa and Israel were previously given the

Fig. 1 **a** - PSHB attack on the base of a large sized branch of avocado (cv. Hass) that eventually caused its wilting; **b** - typical sugar exudation at the base of an avocado branch (cv. Hass) successfully colonized by PSHB; **c** - lesion developed on a main branch (cv. Hass); **d** - removal of the cortex and part of the xylem at the point of the lesion (in C) exposes staining of the xylem; **e** - large size lesions on mature trees (cv. Reed); **f** - artificial colonization using Eppendorf tubes, two PSHB inserted into each one; **g** - artificially colonized branches, one month after induced attack

common name, PSHB (Eskalen et al. 2013). The presumed native range of this species includes Northern Thailand, Vietnam, China, Taiwan and Okinawa (Stouthamer et al. 2017). The presence of three symbiotic fungi: *Fusarium euwallaceae*, *Graphium euwallaceae* and *Paracremonium pembeum* was detected in the larvae and adult PSHBs and from the brood galleries of the beetles in four tree species (Freeman et al. 2013; Freeman et al. 2016; Lynch et al. 2017). Isolations from female beetle mandibular mycangia recovered mainly *G. euwallaceae* and *P. pembeum* during adult maturation, whereas isolations from the heads of mature adult beetles detected *F. euwallaceae*, almost exclusively (Freeman et al. 2016). Lately, the evolution of a clade within *Fusarium* associated exclusively and with specificity to each ambrosia beetle species in the genus *Euwallacea* has been revealed (Kasson et al. 2013; O'Donnell et al. 2015).

Fungi and Coleoptera are among the most evolutionarily successful and diverse heterotrophic organisms in the world. Due to their unique adaptive capacities, fungi and beetles co-occur and interact in various terrestrial habitats (Schigel 2012). The habit of feeding on fungi cultured in the xylem makes it possible for ambrosia beetles to use a great variety of plant taxa; the extreme resource of host plants, in conjunction with the colonization advantage conferred by haplodiploidy and inbreeding, may have promoted their rapid diversification (Jordal et al. 2000).

Ambrosia beetles spend much of their life cycle within the xylem or phloem of hosts. Therefore, they are easily introduced into new ecosystems through the intra- and intercontinental movement of untreated logs, lumber and solid wood packing material or ornaments (Haack 2006; Marini et al. 2011). Some of the most significant problems over the last century, associated with invasive ambrosia beetles, have emerged on healthy trees, inflicted by these beetles and the typically benign fungi they carry (Ploetz et al. 2013).

In their natural habitats, ambrosia beetles are known to attack weak, dying or dead trees. Both abiotic and biotic factors could predispose trees to attack by ambrosia beetles and plants that seem vital may actually be physiologically stressed which makes them more susceptible to the beetle attack (Ranger et al. 2010). Hulcr and Dunn (2011) suggested that the sudden emergence of pathogenicity on healthy trees by the ambrosia beetles is due to the interaction between these new exotic symbionts and the 'naïve' host trees. These authors related damage of the trees to (i) the ability of the symbiotic fungus to either overcome resistance of naive hosts, or (ii) trigger a suicidal over-reaction as happened in the case of *X. glabratus* and laurel trees (native *Persea* spp.,) in Eastern USA, and (iii) an 'olfactory mismatch' in the insect, whereby a subset of live trees is perceived as dead or physiological suitable for colonization. Volatiles produced by the fungal symbiont may contribute to this syndrome by attracting the beetle partner (Hulcr et al. 2011).

The genus *Euwallacea* Hopkins includes 54 recognized species (Alonso-Zarazaga and Lyal 2009); the general diagnosis and description of the congeners was given by Hulcr and Smith (2010) and is the only beetle genus known to cultivate ambrosia Fusaria, sometimes in addition to microascalean and ophiostomatalean fungi. The Fusaria associated with *Euwallacea* form a monophyletic group (Ambrosia *Fusarium* Clade or AFC) within the *F. solani* species complex (Kasson et al. 2013). The *Fusarium* – *Euwallacea* mutualism represents one of 11 known evolutionary origins of fungiculture by Scolytinae ambrosia beetles (O'Donnell et al. 2015). Information on life and seasonal histories, or about the host plant interaction, has been revealed for only a few species. However, two species of *Euwallacea fornicatus* Eichhoff sensu *lato* have received much attention, since they evolved as serious pests.

The best-known is the tea shot hole borer (TSHB), which has been subjected to intensive studies by many entomologists for more than 100 years, mainly in Sri Lanka; the earliest studies were probably conducted by Green (1903) and Speyer (1917a, b). The other is the PSHB that has recently received much attention after being discovered as a serious pest of avocado and several ornamental tree species in California and Israel (Eskalen et al. 2012; Mendel et al. 2012a, b). The adult TSHB and PSHB appear quite similar; Chen et al. (2016) studied morphometric characteristics and the cuticular hydrocarbons of both beetles and showed that despite significant differences in head width, pronotom width and body length of females there are overlapping ranges of these measurements, whereas both species differ in their hydrocarbon profiles. Both species display multivoltinous development. Recently, Cooperband et al. (2016) compared the development and progeny production of both species reared on artificial diet and found that both completed their development in 22 days at 24 °C. Several studies conducted in Sri Lanka examined the rate of development of the

TSHB reared on tea branches or on an artificial diet (temperature was not always indicated). In these studies the average development of the immature stages last 24 to 36 days while 40 to 55 days were needed for the entire life cycle (Speyer 1917a; Gadd 1941, 1949; Sivapalan and Sivanandarajah 1977; Walgama and Zalucki 2007). The average rate of development of the PSHB in Israel from egg to egg at 25 °C, lasts 45 days (Freeman et al. 2012). Furthermore, both beetle species have a strict preference for each of their Fusaria symbiotic diets; e.g. TSHB larvae could not complete their life cycle on *F. euwallaceae* from avocado while PSHB could not survive on the *F. ambrosium* symbiont originating from tea (Freeman et al. 2013). There is little doubt that both beetle species differ in their host range as early information regarding the list of host plants of *Euwallacea fornicatus* sensu *lato* was probably related to the plant hosts of different congeners. However, concerning avocado, the TSHB in Florida and Sri Lanka does not attack live trees (personal communication, Jorge Peña and Keerthi Mohotti, respectively).

Currently, the management options for PSHB are limited. Since members of the tribe Xyleborini do not use long-range sex or aggregation pheromones, and are not restrained by predators or parasitoids, trapping and classical biocontrol measures are not management options. Furthermore, coping with the beetle in avocado orchards alone may be inadequate, as the beetle affects many other such as castor bean and box elder. Thus, greater understandings of the relationship between avocado trees and the PSHB is required. The major objectives of this research were to study the host plant range of the beetle with an emphasis on avocado cultivars, and to reveal significant aspects in the relationship between the PSHB and avocado. This study was conducted between 2012 and 2016, mainly in different avocado production areas in the central coastal plain of Israel.

Material and methods

Host plant survey

Surveys were conducted in parks, botanical gardens, home backyards and ornamental landscapes of different Kibbutzim. Susceptible native trees, particularly maples and oaks, were also examined in their natural habitats. The cortex of suspect infested trees was removed to expose the beetle's gallery. Beetles were collected and identified, and in most examined trees a core sample (0.5 × 10 cm) was removed with a tree increment borer (Haglöf group, Långsele, Sweden) or knife for isolation of the common symbiotic fungus, *Fusarium euwallaceae*, as described by Freeman et al. (2016). The tree was considered suitable for reproduction when larvae, pupae or callow adults were detected in the exposed galleries. Tree species that were labeled as highly susceptible produced high beetle populations and significant numbers of these hosts were eventually killed.

Attack preference among avocado cultivars

The density of beetle attack was compared in different avocado cultivars in five orchards in the central coastal plain of Israel, two of which were 8 to 9 yr. old (Eyal and Ga'ash) and three of which were 20 to 25 yr. old (Ma'agan Michael, Nordia and Magshimim). The younger plantations consisted of Hass and Ettinger, whereas the older plantations consisted of multiple cultivars (Ardit, Fuerte, Galil, Nabal, Fino, Pinkerton, Reed and Horshim, besides cv. Hass and cv. Ettinger) in different combinations Ma'agan Michael and Nordia, and as a collection in Magshimim. In four of the plantations, 136 to 162 trees were examined, compared to 909 in Eyal. Each plantation was visited once during the summer of 2013, during which the number of lesions on stems and main branches up to 5 m above ground level were recorded. The size of the lesions were also determined with respect to branch diameter and whether or not they were colonized by the beetle.

Comparison of branch wilting between avocado cultivars Hass and Ettinger

Between 2013 and 2016, the intensity of branch wilting following colonization by PSHB was studied in four plantations, two in Eyal and one each in Nachsholim and Shfaim. A range of 20 to 112 Hass trees and 18 to 28 Ettinger trees were examined; all wilted branches on these trees were studied and only those that were successfully colonized by the beetle were recorded.

Comparison of attack and colonization of avocado and box elder by PSHB

The patterns of lesions and a gallery density index (GDI) was determined for PSHB in avocado cv. Hass and box elder, *Acer negundo*, and related to stem and branch diameter. Avocado trees were sampled in February 2014 in a mature plantation in Lehavot Haviva in the eastern Sharon plain that was clear-cut. Stem sections were divided into four groups according to diameter: group A - >20 cm (32 sections), group B - 10 to 20 cm (72), group C - 6 to 10 cm (52) and group D - 2 to 6 cm (45). The numbers of lesions in each section (Fig. 1c, e) were counted and their density per 1000 cm^2 bark surface was calculated. Branches were then sectioned and GDI was evaluated with the following scale: 0 = no galleries detected; 1 = 1 to 2 galleries; 2 = 3 to 5 galleries; 3 = 6 to 10; and 4 = > 10 galleries per 1000 cm^2 stem surface. Stem surface area was greater than 1000 cm^2 in groups A to C, and that for D ranged between 700 to 1100 cm^2. Lesion patterns and GDI in box elder (Fig. 2) was determined with stem sections taken from trees in the botanical garden of the Hebrew University in Givat Ram, Jerusalem, Ramot HaShavim in the Central Coastal Plain, and kibbutz Sde Nehemya in the Northern Hula Valley. The above avocado criteria were used for box elder, and a total of 31, 49, 29 and 45 stem sections were examined for groups A to D, respectively.

Infestation pattern and age distribution of PSHB development stages in avocado and castor bean plants

The infestation pattern and the age distribution of PSHB developmental stages was studied in a 'cv Pinkerton' avocado orchard in Rishpon (July 2012), a 'cv Hass' orchard in Eyal (October 2013), and a 'cv Hass' orchard in Nachsholim (March 2015). In addition, infested castor bean plants were sampled in Binyamina (March 2015). All studied avocado branches were those with typical dieback symptoms and most of them displayed beetle emergence holes. Spots on the branch where emergence holes were observed were identified with water-resistant paint, and each orchard sample consisted of 10 branches, 40 to 50 cm in length, 3 to 6 cm in diameter, and proximal to branch insertion points. Similar size stems of castor bean were removed from plants with typical beetle colonization symptoms.

Stem sections were carefully dissected in the lab and eggs, young larvae (the first and the second instars), the third instar larvae, pupae and adults of the beetle were enumerated. Larval instars were separated based on the width of the head capsule (Fig. 3). In April, 2014, beetle emergence was determined for week-old stem sections from seven locations in an avocado plantation at Givat Haim (three to five stems each, 20 to 40 cm in length). Stems were maintained at room temperature, and the numbers of adult beetles that emerged were collected every week until emergence terminated.

Location of reproductive galleries of PSHB on avocado branches

The location of PSHB galleries along the branches was examined in nine avocado orchards (in alphabetic order): Eyal (cv. Hass, in 2015), Kfar Masaryk (cv. Hass,

Fig. 2 **a** – large lesion developed on stem of box elder; **b** - typical penetration spot on stem of box elder **c** – section of a box elder branch with typical galleries of PSHB; **d** - high density of galleries of PSHB in the lower trunk of box elder

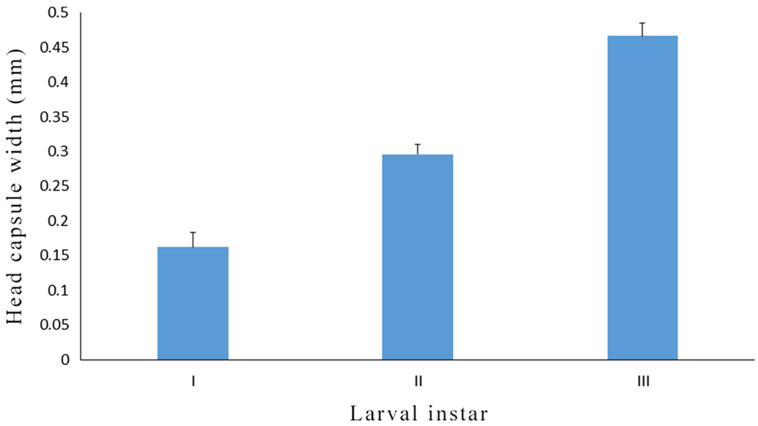


Fig. 3 Mean (+SD) head capsule widths (mm) of the three larval stage of *Euwallacea fornicatus* (*n* = 10)

in 2016), Lehavot Haviva (cv. Hass, in 2015), Maabarot (cv. Reed, in 2013), Ma'agan Michael (cv. Hass, in 2013), Mishmarot (cv. Hass, in 2016), Nachsholim (cv. Hass, in 2016), Nordia (cv. Hass, in 2013) and Rishpon (cv. Pinkerton, in 2012). Sampled branches included those displaying typical wilt symptoms or those that were already dry. Galleries exposed and beetle stages recorded for each penetration point and PSHB emergence hole (which are easily detected by their shape and presence of sugar). The location of the galleries was measured according to the distance, close or as far as 30 cm from the branching point, and those located further away along the branch.

Induced colonization of adult PSHB in avocado (cv. Hass) branches

Attempts to artificially infest avocado branches with the PSHB were conducted in three plots of cv. Hass trees in Palmachim (July 2012) and in Eyal (July and August, 2013). In each plot, four trees were selected, and to each tree, ten 2 ml Eppendorf tubes were attached (Fig. 1f, g). Two adult PSHB females, 4 to 24 h post-emergence, were placed in each tube, which was attached to a branch using elastic bands; with the opening facing the bark. The beetles were taken from transparent emergence boxes where naturally infested branches of castor bean and box elder were maintained under 24 °C under natural photo period conditions. The tubes were attached to 3 or 4 branches, 5 to 8 cm diameter each. A total of 10 tubes were secured per tree, with a total of 40 tubes containing 80 females per plot. The points of artificial colonization were examined after 4 to 5 weeks. Three levels of interaction were identified: (i) 'penetration' - indicated by a clear penetration hole and the presence of frass and sugar exudation, (ii) 'gallery initiation' – excavation of a clear gallery in the sapwood, and (iii) 'offspring production' – exposure of eggs or larvae in the galleries.

The effect of early colonization attempts in avocado branches on beetle penetration intensity and reproductive success

Comparisons were conducted on the effect of early colonization attempts by PSHB on further infestation by the beetle in an avocado plantation in Eyal (cv. Hass). In early March 2013, 220 branches (1 to 3 per tree), measuring 6 to 9 cm diameter were selected. The branches were numbered and a 70 to 100 cm section along each branch was marked with ribbons at both edges of the selected section. All the designated branches were healthy, viz., no lesions or penetration points of PSHB or any clear sign of other types of injury were observed along the marked section, and at least 20 cm away from the marked borders. Beetle attack was induced on these branches, as described earlier with five to six 2 ml Eppendorf tubes. The branches were divided into four groups. Group (i) consisted of branches that were artificially colonized between early March to late April (*n* = 33). Group (ii) consisted of 30 branches that were artificially colonized between early May and early August and 39 branches of similar sizes that were naturally attacked by the beetles in the same period interval (*n* = 69). Group (iii) consisted of 25 branches that were artificially colonized between early August and early October, and 33 of similar sizes that were naturally attacked by the beetles during this period (*n* = 58). Group (iv) served as a control, that included intact branches (n = 41). The naturally infested branches [groups (ii) and (iii)], displayed 2 to 4 typical lesions with sugar exudation. Among the 220 marked branches, few were lost due to uncoordinated pruning or breakage of a main branch. In early October, branches that were attacked during more than a single period were excluded but served to examine the performance of the beetles. The xylem below 27 penetration holes was exposed and occurrences of immature beetle stages were recorded. All branches included, a total of 201, were examined again during three sampling periods, in late October 2013, late April 2014 and mid July 2014. The new lesions were again recorded according to the period of beetle attack. In mid-July, 25 penetration holes were observed among branches of groups (i), (ii) and (iii) and again the occurrence of immature beetle stages was recorded for each examined point.

Seasonal activity of the beetle in avocado plantations

Seasonal flight activity of the beetles was determined by monitoring the occurrence of lesions on the stem and the main branches of 9-yr. old avocado plantations (cv. Hass) in Eyal and Ma'agan Michael, between April and December, 2013. A total of 910 trees in Eyal and 480 in Ma'agan Michael were examined. The number of additional lesions per tree was calculated for a given month by subtracting the number of accumulated lesions in the previous months to those appearing in the current one.

Statistical analyses

Statistical analyses of the data were performed using JMP 12.0.0 (SAS Institute Inc. 2015). Enumeration data were square-root transformed in order to normalize and to stabilize variances. Gallery density values were rank-transformed before analysis in order to perform non-parametric ANOVA. Means were compared by one-factor or two-factor ANOVA as relevant, and post-hoc pairwise comparisons were performed by the Tukey-Kramer HSD test, $\alpha = 0.05$. Incidence of wilting was compared for varieties and locations by nominal logistic regression. Nominal Logistic Regression followed by the Chi-square test was applied to test the significant difference between orchards with respect to the location of reproductive galleries along the branches.

Results

Host plants of PSHB in Israel

In the present study, 52 tree species from 26 families were attacked by the PSHB only six of which are native to Israel (Table 1). Beetle reproduction occurred in only 12 species, eight of which supported extensive reproduction and were considered highly susceptible. Among the native tree species *Platanus orientalis* was highly susceptible, in both ornamental and natural settings. *Fusarium euwallceae* was isolated from 33 of 41 botanical species that were assayed.

Attack preference among avocado cultivars and intensity of sugar exudation

All examined avocado cultivars were attacked by the beetle (Fig. 4). In general, Hass was much more susceptible than Ettinger to beetle attack although Reed, Nabal and Pinkerton were also markedly affected. Cultivar had a significant effect in Eyal ($F_{1,907} = 287.1$, $P < 0.0001$), Ma'agan Michael ($F_{4,131} = 9.2$, $P < 0.0001$) and Nordia ($F_{5,156} = 5.7$, $P < 0.0001$) but not in Ga'ash ($F_{1,160} = 2.6$, $P < 0.1091$) and Magshimim ($F_{1,907} = 2.4$, $P < 0.051$) plantations. Large lesions with excessive sugar exudation, with or without dark staining on the bark surface, occurred on stems and main branches. Less sugar exudation was observed on small diameter branches, and was minimal where successful colonization had already occurred (Fig. 1b, c and e).

Comparison of branch wilting between Hass and Ettinger varieties

Approximately 30% of the Hass trees in the four orchards displayed branch wilting compared to only 2% of the trees of Ettinger (Fig. 5). A significant difference was observed between the two cultivars ($\chi^2(1) = 49.8$, $P < 0.0001$), and also between locations ($\chi^2(3) = 14.98$, $P = 0.0018$).

Comparison of attack and colonization of avocado and box elder by PSHB

Significant, but contrasting differences were observed for the effects of stem diameters on lesion density and gallery density index (GDI); significant interactions existed between branch group diameter and the tested parameters

Table 1 Tree species attacked by *Euwallacea* nr. *fornicatus* in Israel

Family	Plant species[a]	Reproduction of PSHB[b]	Recovery of *Fusarium euwallaceae* from xylem sample[c]
Altingiaceae	*Liquidambar styraciflua*	u	n
Anacardiaceae	*Pistacia atlantica**	u	n
Arecaceae	*Roystonea regia*	u	?
Betulaceae	*Alnus cordata*	u	r
	Corylus colurna	u	r
Bignoniaceae	*Jacaranda mimosifolia*	u	?
Casuarinaceae	*Casuarina cunninghamiana*	u	n
Ebenaceae	*Diospyros kaki*	u	r
Euphorbiaceae	*Ricinus communis*	hs	r
	Sapium sebiferum	u	?
Fabaceae	*Bauhinia variegata*	u	n
	Dalbergia sissoo	u	n
	Gleditsia japonica	u	?
	Inga vera	u	?
	Tamarindus indica	u	?
Fagaceae	*Albizia julibrissin*	u	r
	*Quercus ithaburensis**	s	r
	Quercus calliprinos[a]	s	r
	Quercus hartwissiana	u	r
	Quercus infectoria	u	r
	Quercus pedunculiflora	hs	r
	Quercus pontica	u	r
	Quercus robur	hs	r
	Quercus suber	u	n
Juglandaceae	*Juglans regia*	u	?
Lauraceae	*Persea americana*	hs	r
Magnoliaceae	*Magnolia grandiflora*	u	r
Malvaceae	*Ceiba speciosa*	u	r
Moraceae	*Morus alba*	s	r
Moringaceae	*Moringa* sp.	u	r
Oleaceae	*Olea europaea**	u	n
Platanaceae	*Platanus occidentalis*	hs	r
	*Platanus orientalis**	hs	r
	Platanus racemosa	c	r
Proteaceae	*Macadamia integrifolia*	u	n
Rhamnaceae	*Hovenia dulcis*	u	?
	*Rhamnus alaternus**	u	r
Rosaceae	*Prunus domestica*	u	?
	Pyrus malus	u	r
Salicaceae	*Salix eastwoodiae*	u	r
	Acer negundo	hs	r
Sapindaceae	*Acer buergerianum*	hs	r
	Acer campestre	u	r
	Acer obtusifolium	s	r
	Acer pseudopltanus	s	r
	Koelreuteria bipinnata	u	?

[a] – * = native to Israel

[b] – u = unsuitable for reproduction, s = suitable for reproduction, hs = highly susceptible and suitable for reproduction, c = found suitable for beetle reproduction in California (Eskalen et al. 2013)

[c] – n = the fungus was not isolated, r = the fungus was isolated, ? = the fungus was examined

Table 1 (continued)

Family	Plant species[a]	Reproduction of PSHB[b]	Recovery of *Fusarium euwallaceae* from xylem sample[c]
Sterculiaceae	*Brachychiton acerifolius*	u	r
	Brachychiton populneus	u	r
	Brachychiton rupestris	u	r
Taxodiaceae	*Taxodium distichum*	u	r
Ulmaceae	*Zelcova carpinifolia*	u	r
	Ulmus glabra	u	?

($F_{1,3}$ = 75.3036, P < 0.0001, and $F_{1,3}$ = 78.2505, P < 0.0001, for lesion density and GDI, respectively). Lesion density in avocado increased as branch diameter decreased, from ca 0.8 lesions per 1000 cm^2 for the largest branches (> 20 cm in dia) to about 5 lesions for the smallest branches (2–6 and 6–10 cm in dia) (Fig. 6). In contrast, lesion density in box elder increased as branch diameter increased, from ca 0.3 lesions per 1000 cm^2 for the smallest branches to about 8 lesions for the largest branches (Fig. 6). Lesion densities were significantly different between the four diameter groups for avocado and box elder ($F_{3,197}$ = 15.5601, P < 0.0001 for avocado and $F_{3,135}$ = 191.7082, P < 0.0001 for box elder).

Significant differences in GDI were detected between avocado and box elder (F_1 = 87.03, P < 0.0001), and trends were similar to those noted for lesion densities. In avocado trees, GDI increased as branch diameter decreased, from approximately 0.03 in the largest to 1.4 galleries in the smallest stems. However, in box elder GDI increased as branch diameter increased, from ca 0.3 to 2.9 galleries per stem. GDIs were significantly different between the four diameter groups for avocado and box elder ($F_{3,197}$ = 30.1979, P < 0.0001 for avocado and $F_{3,135}$ = 52.7443, P < 0.0001 for box elder).

Induced colonization of adult PSHB in avocado (cv. Hass) branches

A total of 120 penetration points were examined, 4 to 5 weeks after beetle exposure. Significant differences were

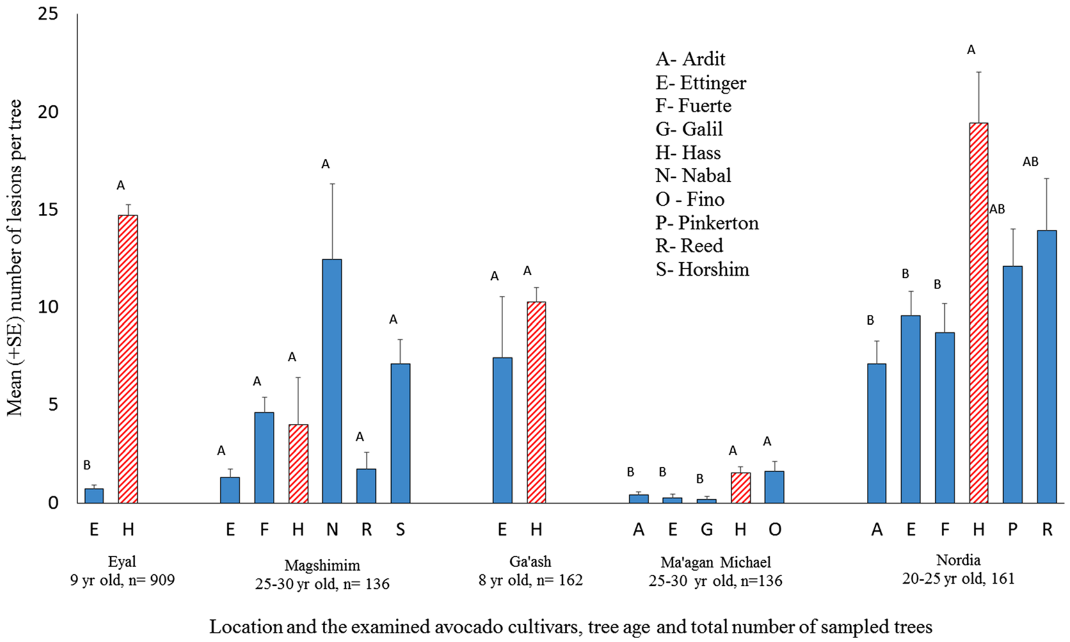
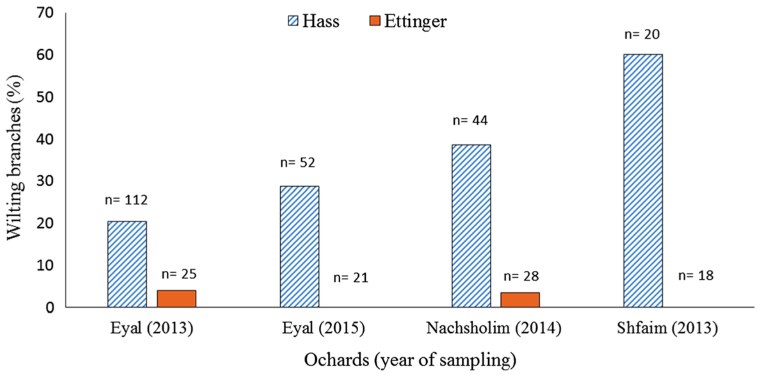


Fig. 4 Comparison of densities of beetle attack on various avocado cultivars, growing in five different orchards. Means with a *different letter* within orchards are significantly different. Hass cv. columns are emphasized by a *red dash* color. Comparisons for all pairs were performed using Tukey-Kramer HSD: α = 0.05

Fig. 5 Percent of branch wilting of two avocado cultivars planted in four different orchards. Ettinger cv. trees (served as pollinators) were planted within the row of Hass cv. trees, while in Shfaim some of the Ettinger trees were planted next to the Hass plot (n = number of examined trees)



detected between colonization stages ($F_{2,36}$ = 114.99, $P < 0.0001$) and orchard ($F_{2,36}$ = 11.03, P = 0.0002). Approximately 92.8% of the points were penetrated by at least one of the two beetles exposed at each point, and in 69% of the cases a clear gallery was excavated in the sapwood. However, reproduction was initiated in only ca 3.2% of the cases, as determined by the presence of eggs and/or beetle larvae (Fig. 7).

Effect of early colonization attempts in avocado branches on beetle penetration intensity and reproductive success

Between March and September 2013, new penetration points on avocado cv. Hass branches were monitored. The mean density of new penetration points per branch examined during three sampling periods, in late October 2013, late April 2014 and mid July 2014, as related to the four branch groups, is presented in Fig. 8. The density of new lesions did not differ much between the four branch groups in late October and ranged from 0.03 to 0.12 lesions per branch. The lesion density increased during the second sampling period reaching 0.81 to 0.94 lesions for groups i to iii, which was significantly higher than that of branch group iv, 0.02 (branches which were not previously attacked). A similar trend was observed in the third sampling period, where the lesion density increased significantly between the second and the third sampling, but did not differ significantly between branch groups i to iii, 1.75 to 2.07 lesions, which was significantly higher than that of branch group iv, 0.27 lesions, as shown in Fig. 8. Exposure of the xylem of the branches of groups i to iii to penetration points in mid-August 2013 and in July 2014 indicated that exposure of immature stages of the beetle were detected in only one case among 27 (3.7%), at the early examination period, and 18 out of 25 exposures (72%) at the late examination period. Significant differences existed between colonized and the non-colonized branches ($F_{3,591}$ = 14.90, $P < 0.0001$) and between the three attack periods

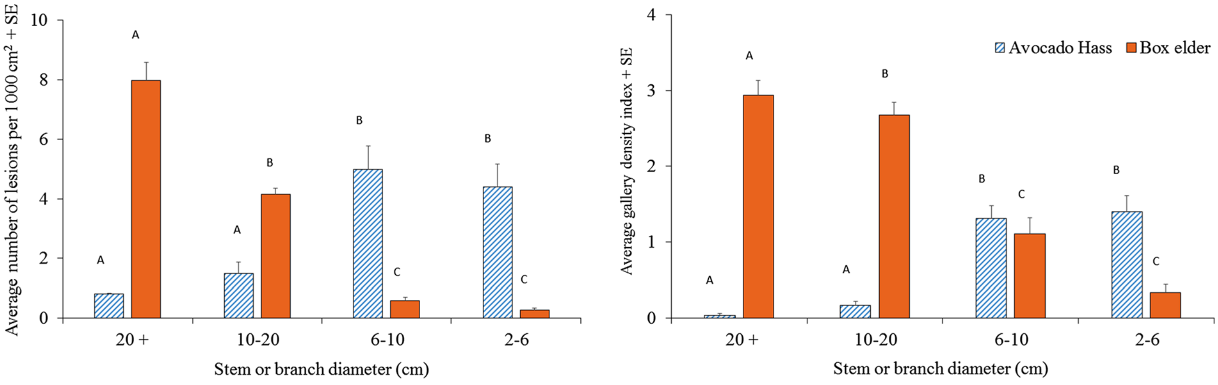


Fig. 6 Lesion occurrence patterns (*left*) and gallery density index (GPI) (*right*) per 1000 cm^2 bark surface of PSHB in avocado (Hass cv.) and box elder as related to stems and branches. Levels with a *different letter*, for each tree and tested parameter, are significantly different (comparisons for all pairs were performed using Tukey-Kramer HSD; α = 0.05)

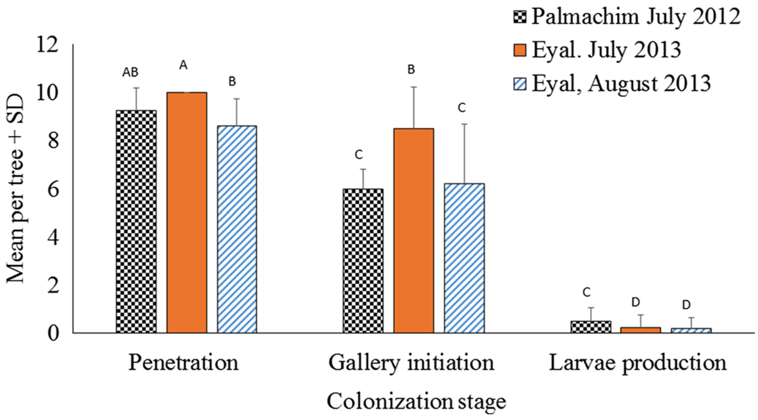


Fig. 7 Induced colonization of adult PSHB in different orchards (Palmachin and Eyal) at different periods in avocado (cv. Hass) branches as related to three early colonization stages 4–5 weeks, after beetle exposure. Values at each colonization stage with a *different letter* per column, are significantly different (comparison by LS Means Differences Student's t, $t = 2.02809$; $\alpha = 0.05$)

($F_{2,591} = 75.15$, $P < 0.0001$). There were also significant differences in lesion densities between the three attack periods for branches that were colonized between March to September 2013 ($F_{2,591}$ = 23.42, 53.13 and 31.59, both $P < 0.0001$), and between the three attack periods between October 2013 and July 2014, but not for non-colonized branches between March to September 2013 ($F_{2,591} = 0.83$, $P = 0.4355$).

Location of reproductive galleries of PSHB along the avocado branches

Frequency occurrence of galleries as related to their position along the branch, examined in different orchards, is displayed in Fig. 9. In all examined orchards reproductive galleries were largely found at the base of the branches, close to the branching point (Fig. 1a-b). Among a total of 121 branches examined, the calculated mean of reproductive galleries located up to 30 cm from the branching point was 94.2 ± 5.8%. There was no significant difference between orchard locations ($\chi^2(6) = 5.89$, $P = 0.4355$) with respect to the location of reproductive galleries along the branches.

Infestation pattern and age distribution of PSHB developmental stages in avocado and castor bean plants

Beetle emergence holes usually occurred approximately 20 to 40 cm away from the branching point in examined avocado branch sections. The tissue beneath the bark surface with emergence holes was always dry, and adult beetles were always present, although in distinct numbers, with the teneral ones rarely observed. The larvae and pupae and both mature and teneral adults were frequently found close to the branching points where the tissue was still moist. It may be important to note that

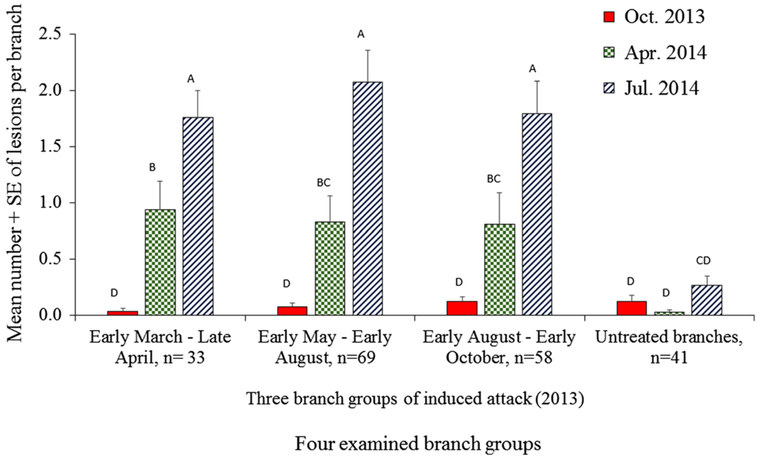


Fig. 8 New penetration points of PSHB, as related to induced colonization conducted during three periods in 2013 and a fourth group of untreated branches, as shown by new lesions, counted during three attack periods between October 2013 to July 2014. Values at each date with a different letter per column, are significantly different (comparison by LS Means Differences Student's t, $t = 1.96399$; $\alpha = 0.05$)

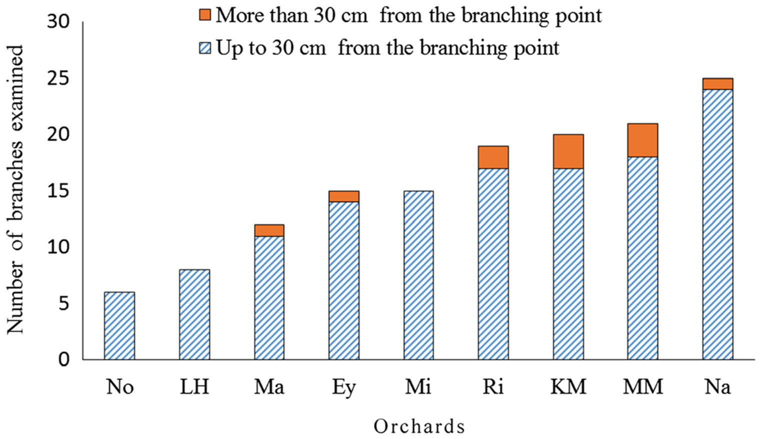


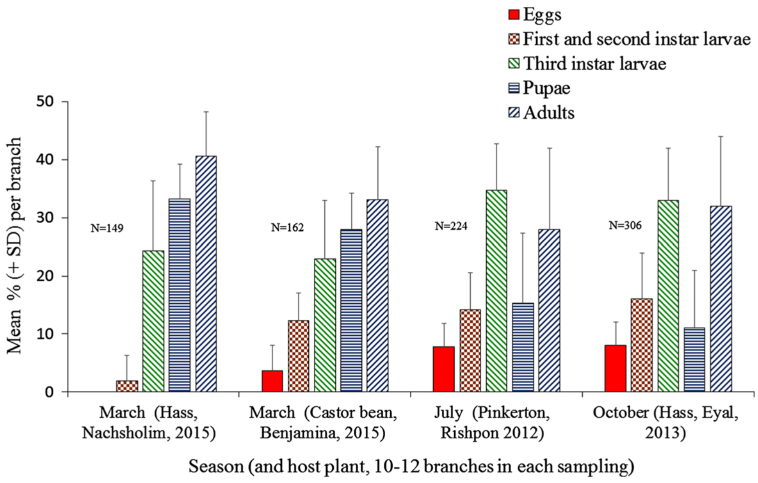
Fig. 9 Frequency occurrence of galleries as related to their position along the branch examined in different orchards, as denoted in parenthesis (avocado cultivar and year of sampling). No-Nordia (cv. Hass, 2013), LH-Lehavot Haviva (cv. Hass, 2015), Ma-Maabarot (cv. Reed, 2013), Ey-Eyal (cv. Hass, 2015), Mi-Mishmarot (cv. Hass, 2016), Ri-Rishpon (Pinkerton 2012), KM-Kfar Masaryk (cv. Hass, 2016), MM-Maagan Michael (cv. Hass, 2013(, Na-Nachsholim (cv. Hass, 2016)

the main branch tissue (the stem of the branch from which the examined infested branch originated close to the most proximate galleries) was unaffected, although a typical staining of the xylem was observed. At the time of beetle collecting, we did not separate between specimens retrieved from different parts (close and a bit further, under bark displaying typical emergence holes, of the branching point) of the examined sections. The beetle age distributions are displayed in Fig. 10. All developmental stages were present during the different sampling periods. The advanced developmental stages were more common than the younger ones. Eggs were not found in March in the avocado orchards but few were detected during the same period in castor bean plants. Emergence of adult females from branches collected from the orchard ground lasted for approximately one month (Fig. 11).

Seasonal activity of the beetle in avocado plantations

Figure 12 presents the seasonal flight activities of the beetles as represented by the occurrences of new lesions on stems and main branches of avocado trees (cv. Hass) in Nachsholim and Eyal orchards in 2013. The seasonal pattern was similar for both plantations. The first lesions were observed in April (late spring) and gradually increased until September, decreasing in the autumn from October to early December, when the last new lesions

Fig. 10 Age distribution of PSHB developmental stages in avocado cultivars and castor bean plants



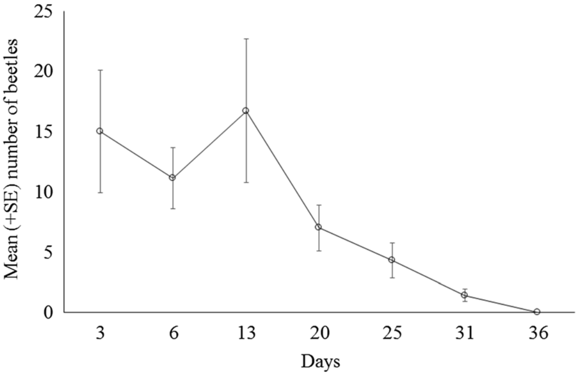


Fig. 11 Emergence (mean ± SE) of adult female *Euwallacea* nr. *fornicatus* beetles from avocado (cv. Hass) branches collected from the orchard ground at Givat Haim and maintained in emergence boxes at room temperature

were recorded. Significant differences existed in the mean lesions induced between the eight examined months ($F_{7,11,103} = 215.50$, $P < 0.0001$) and also between locations ($F_{1,11,103} = 20.79$, $P < 0.0001$).

Discussion

An intriguing ecological question is why few ambrosia beetle species successfully colonize and cause mortality in apparently healthy trees. Several members of this group have become pests of trees around the Mediterranean Basin. For example, *Platypus cylindrus* (Platypodinae; Platypodini) native in the west Mediterranean has become a primary pest (Bellahirech et al. 2016). In other cases invasive species have caused problems, such as *Megaplatypus mutatus* (Platypodini), a South American species (Alfaro et al. 2007); *Xylosandrus crassiusculus* (Scolytinae; Xyleborini) of East Asian origin (Pennacchio et al. 2003), and most recently, *Xylosandrus compactus* and its associated fungi in Italy (Vannini et al. 2017). Since 2010, severe damage to avocado plantations and mortality of several shade tree species has been associated with the polyphagus shot hole borer (PSHB) *Euwallacea* nr. *fornicatus* in Israel.

Similar to the situation in California (Eskalen et al. 2012; Umeda et al. 2016), the PSHB develops successfully in Israel on trees in different botanical families. The closely related TSHB was also recorded from trees belonging to various botanical families (Danthanarayana 1968). However, PSHB (6% of 103 in California (Eskalen et al. 2012) and 23% of 52 in Israel (reported herein) and TSHB manage to reproduce in relatively few tree species that they attack (16% of 49 in Sri Lanka (Danthanarayana 1968). *Fusarium euwallaceae* was isolated from many of the attacked species in California (35%, Eskalen et al. 2012) and in Israel (56%). Although the success

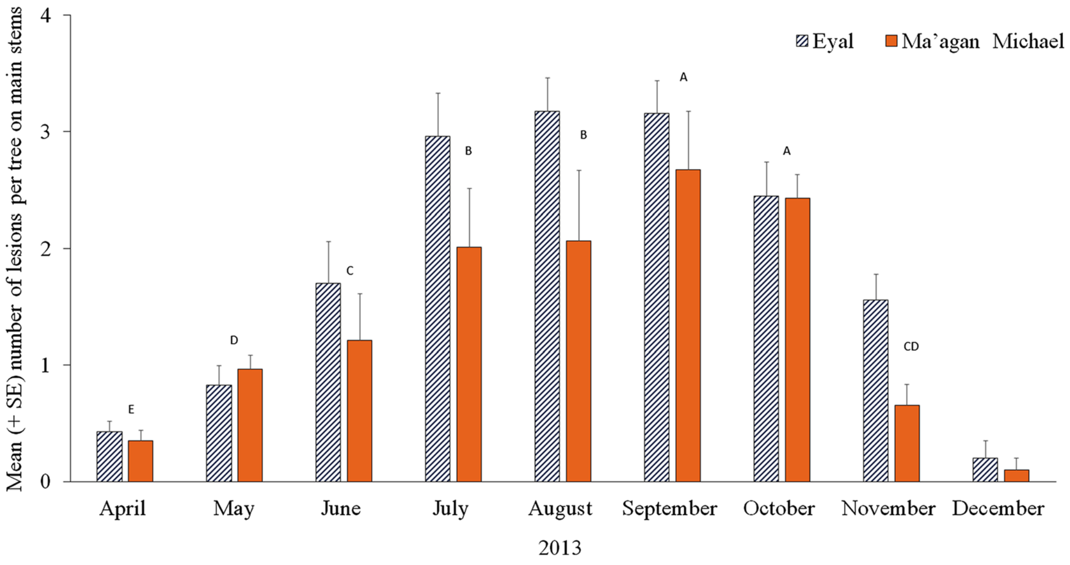


Fig. 12 Seasonal occurrences of new lesions on stems and main branches of avocado trees (cv. Hass) in two orchards in 2013. Values for each month with a *different letter* are significantly different (Tukey-Kramer HSD; α = 0.05). The comparison was performed on data pooled for each of the two sites. The data collected in December was not analyzed due to too small a sample number of trees

of PSHB establishment is probably associated with the ability of their symbiotic fungus to colonize a tree's xylem, it is not clear why the beetle does not reproduce in some trees in which *F. euwallaceae* does establish (e.g. persimmon, *Diospyros caci*, and *Brachychiton* spp.) (Table 1). Furthermore, even among the reproductive tree species for PSHB there are conspicuous differences. Whereas box elder has been destroyed in the low lands in Israel, another maple species that is native in Israel and grows as a garden tree, *Acer syriacus*, exhibited beetle reproduction in only one of the 20 locations that were examined (data not shown).

Typical sugar exudation (Mendel et al. 2012; Eskalen et al. 2012) was observed at the beetle penetration points on all ten examined avocado cultivars. However, in the present study there was a primary focus on 'Hass' and 'Ettinger', as they predominate in the Israeli avocado industry (Noy and Gafni 2015). Although a significant cultivar effect was observed only in three of five examined orchards, in all cases 'Hass' was more prone to attack than 'Ettinger' and other cultivars. Sugar exudation was intense from healthy tissues of large and medium diameter branches, > 5 to 6 cm, in which beetle attack rarely progressed to reproduction (see below). Only minimal sugar exudation was observed in thin branches >5 cm, particularly in branches that were weakened by previous successful beetle attacks. In the latter cases, successful colonization of the fungus was followed by wilting and death of the colonized branches (see below, and Fig. 1a). Hence, lesions with large sugar deposits usually indicated that the tissue was able to prevent further development of the beetle. In 'Ettinger', lesions rarely indicated successful colonization of the tree by the beetle. In the same plots, branch wilting was much more significant in 'Hass' than 'Ettinger' (Fig. 3). However, isolations of *F. euwallaceae*, either from beetle attack spots or from artificially inoculated branches, was similar between the two cultivars (Freeman et al., in preparation). These results suggest that the avocado cultivar 'Ettinger' displays a partial resistance to the beetle compared to that of 'Hass'.

The mode of PSHB attraction to different host species or between avocado varieties has not yet been studied. *Xyleborus glabratus* attacks avocado trees in Florida (Brar et al. 2013). It was found that captures of this beetle were positively correlated with emissions of several sesquiterpenes (Niogret et al. 2013). These researchers also discussed how short and long range distribution of sesquiterpenes may function as host-location cues in that ambrosia beetle.

While box elder is killed within a year of beetle attack (data not shown), mortality of avocado trees is quite rare in Israel (Mendel et al. 2012a, b). A similar distinction of susceptibility between these hosts was also evident in California (Eskalen et al. 2012, 2013). Hence, we compared the PSHB colonization pattern in box elder and 'Hass'. Lesion and gallery densities in Hass increased as branch diameter decreased, and reproductive galleries were quite rare on large diameter branches. In contrast, opposite trends in the attributes were noted in box elder (Fig. 2a-d). These distinctly different patterns in avocado and box elder may explain their relative susceptibilities to PSHB assault. These observations are similar to those on other susceptible trees, such as *Quercus pedunculifora*, *Q. robur* or *Platanus orientalis* (data not shown). Reproduction in these trees occurred mainly on large branches.

F. euwallaceae does not spread systemically (far from galleries) in infected xylem (Mendel et al. 2016; Freeman et al. in preparation). An absence of wilting and dieback in large branches of avocado probably reflects the sporadic and localized nature of galleries in these areas. On the other hand successful colonization of PSHB leads to the dieback of thin branches in avocado trees. A contrasting situation occurs in box elder, where high gallery densities in the main branches and trunks (Fig. 2d) lead to impeded water flow in the xylem and eventual mortality.

The distinct colonization pattern in avocado and box elder has implications for the numbers of beetles that would develop in these trees. Whereas reproduction in thin avocado branches usually lasts one or perhaps two generations, three to four generations develop in the main branches of box elder and the population densities are much more pronounced permitting a substantial increase in infestation levels from this host (see below).

The resistance of large avocado branches to attack by PSHB was also evident in 'forced' colonization experiments, as immature stages of the beetle developed in only 3% of the assays. Subsequent studies indicated that attacked branches were subjected to additional colonization attempts more frequently than neighboring healthy ones (Fig. 8). Based on these observations we raise the question whether colonization of small branches is more successful following repeated attacks. It appeared that these 're-attacked' branches were more preferable for colonization since earlier unsuccessful attacks were also involved in development of lesions, which in turn reduced branch tolerance/resistance to beetle attack. Moreover, fungal inoculation and subsequent development of *F. euwallaceae* at the attack points may result in emission of volatiles that attract the beetles. Many ambrosia beetles of the Xyleborini are known to rely on stress-related volatiles such as ethanol in search of a host tree (Ranger et al. 2010). Nevertheless, Hulcr et al. (2011) suggested that symbiont specificity and odor-perception specificity may be correlated traits, as they demonstrated that three Xyleborini species, *Xylosandrus crassiusculus* and *Xyleborus ferrugineus*, both highly polyphagous species, and *Xyleborus glabratus* adapted to members of the Lauraceae, are attracted to volatiles produced by their fungal symbionts. In the present study we showed that PSHB prefers to attack branches that had been previously colonized by its conspecifics and reproduction success in these branches seemed to be much higher than after the first attacks. We found that percent added lesions between late October 2013 and mid July 2014 (second phase of attack) was significantly higher in branches that were previously attacked by the beetle during March and September 2013 (first phase of attack) than that of branches that were not attacked by the beetles during the latter period. In addition, there was a clear indication that during the first phase of beetle attack (in artificially or naturally attacked branches) the number of branches where immature reproductive beetle stages were observed was rather low (3.7%), while this number rapidly increased (72%) during the second wave of attack. Ambrosia beetles may accelerate the deterioration of trees that are already weakened by other agents such as fungi (McPherson et al. 2005; Worrall et al. 2008). In the present study, the preference and the reproduction success of previously attacked branches may be related to their attractiveness to the beetles as mentioned above, and/ or their reduced resistance to the beetle attack, as compared with apparently healthy branches.

The increasing number of beetle attacks during the different seasons coincided with an increase in flight activity as determined by monitoring the occurrence of lesions on the stems and main branches of hundreds of trees in 2013 (Fig. 12). We found that occurrence of lesion frequencies increased from late spring (April) with a maximum being recorded during late summer (September). Thus, we suggest that the beetle flight increased in parallel to the elevation in mean temperatures recorded during the warm season. Desiccation of the typically thin dying branches colonized by the beetle may also accelerate the emergence of new generation adults.

Hulcr et al. (2007) reported that in the rain forest of New Guinea only a minority of the studied ambrosia species were associated with a particular tree part, but several species displayed clear preference to trunks, roots or twigs of the colonized trees. Reed et al. (2015) observed that four Scolytine ambrosia: *Xylosandrus crassiusculus, X. germanus, Xyleborinus saxeseni,* and *Xyleborus affinis*, were collected from black walnut trees of almost all branch and trunk dimensions, and that there was no preference for specific diameter sizes; however, most of these species were obtained from the stem sections. Artificially stressed trees combined with a reduction in host plant defenses may explain the lack of a diameter preference for attack in these situations by ambrosia beetles (Ranger et al. 2013). Kendra et al. (2013) found that female beetles of *X. glabratus* typically initiate boring in the trunk and large diameter branches of host trees, and that there was a strong, positive correlation between diameter of host tree and density of beetle populations. PSHB attacks box elder and avocado trees that were considered healthy and did not suffer any physiological stress or visible damage prior to the beetle attack. It is not known why in most tree species suitable reproduction of offspring occurs mainly on the stem and main branches, while in avocado small diameter braches serve for reproduction. However, in other studies it was suggested that differences in moisture content in different tree parts may determine the distribution of colonizing beetles (Fisher et al. 1953; Francke-Grossman 1967). Conspicuous internal xylem staining caused by the beetle attack also occurs in branches where beetle colonization attempts usually fail (Freeman et al. in preparation, but see Fig. 1c-d). However, the staining (Fig. 1d) may detrimentally affect xylem water conductivity, further affecting branch health leading to deterioration of upper branches which may facilitate colonization attempts by PSHB.

PSHB reproduction galleries may be found at different locations along avocado cv. Hass branches. However, successful reproduction of the beetle in approximately 94% of the branches in the present study, ended in brood emergence and branch dieback that occurred at a distance of 30 cm from the branching point. It was also observed that colonization of branches commenced from thicker branch sections. The syndrome of the branches damaged by PSHB is illustrated in a scheme presented in Fig. 13. The beetles colonize the xylem close to the base of a

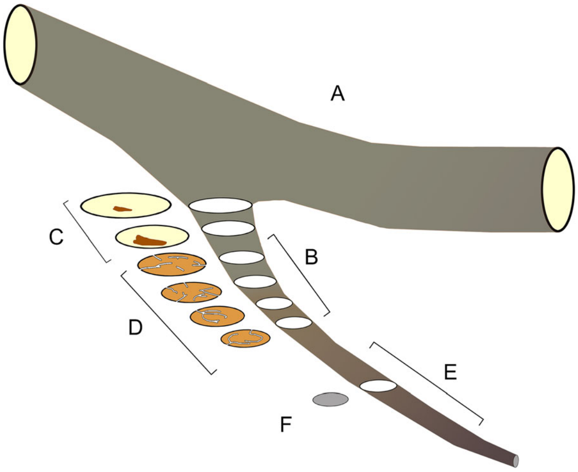


Fig. 13 Schematic colonization of PSHB in sections along an avocado branch. *A* – live healthy branch, *B* – infested section, *C* – slices of a live section with typical staining, this section is usually colonized at a later stage, *D* – slices of colonized sections of the branch with active galleries and beetle brood, *E* – dried section not colonized by the beetles, *F* – representative slice from *E*

branch near a point that was apparently subjected to unsuccessful attacks several months earlier, that displayed typical symptoms of sugar exudation. The branch sections further away from the branching point, were desiccated due to the severe disruption of water flow. It appears that the proximity of the colonization point to a functioning thick branch indicated the necessity of moisture for development of the fungal symbionts in the beetle galleries. Our observations of infested branches that were dissected in the laboratory imply that colonization of the branch section is a continuous process, with the first penetration points being observed at a distance of approximately 20 to 40 cm from the branching point. At the time of sampling, all developmental stages of the beetle were observed in the drying and wilted branches. It could not be determined whether the galleries close to the branching point were the result of activity of beetles that had already emerged from the neighboring galleries. However, it appears that the emergence process from colonized branches may last for an extended period (for at least one month). The overall period of emergence is extensive. Brar et al. (2013) reported that in the case of *X. glabratus* that developed in the lab on intact logs, adult emergence was first observed 60 days after colonization and maximal emergence lasted between 120 to 150 days. Although Brar et al. (2013) suggested that this extended period of emergence is probably related to an overlap of generations of beetles developing within the studied logs. In the present study, emergence of PSHB from trunk sections of box elder trees (displaying a significant number of emergence holes when brought to the laboratory) that served as a source of beetles for lab experiments, continued to emerge during at least 6 to 7 weeks following branch removal (data not shown). However, in the case of intact avocado cv. Hass branches, examined in the present study, emergence lasted for approximately only one month. However, our findings indicate that branch colonization is a rather long process and while immature beetle developmental stages were found near the branching point of the colonized branch sections, emergence took place at a distance of 20 to 40 cm further away along the colonized branch.

Management consideration

Control measures to protect ornamental trees against the damage caused by PSHB and its fungal symbionts focuses on the beetle alone since field application of fungicides against the fungal agents currently appears unfeasible due to the limited movement of systemic fungicides in woody tissues. Insecticide treatments are a realistic option when ornamental trees are considered. Sycamore and oak trees were protected at various degrees of success by cover sprays of pyrethroid compounds, by stem injections or soil application of systemic compounds (Mendel et al. unpublished data). However, to the best of our knowledge, effective IPM friendly and biocontrol compatible compounds with moderate toxicity to natural enemies (which play an important role in restraining several hemipteran pests in the avocado orchards) or those with no problematic residues, are not yet available. Lowering the pressure of PSHB in affected avocado orchards may be achieved by strict phytosanitation and or by means of mass trapping of flying females; the latter method is still under evaluation (Mendel et al. 2016; Maoz et al. unpublished). Our present findings indicate that the beetle may originate from two sources in the avocado orchards. In infested orchards, the main source are beetles that develop in the small diameter branches; and to some extent, beetles may also migrate from nearby infested vegetation, such as castor bean, a common element within and adjacent to plantations located along the coastal plain in Israel.

Preventive healthcare is likely to be the main approach for reducing damage caused by the PSHB. This may be accomplished by removal of colonized branches and intact infested pruned slash. Identification of branches subjected to early successful beetle colonization may be facilitated by observations of several points of sugar exudation at the base of a thin branch and not always by the wilting phenomenon of the foliage. Removal of dry branches at the branching section may not be adequate as the material may already be colonized and inhabited by different developmental stages of the beetle. In these cases, the severed point should be treated with an appropriate insecticide. In early spring (March), before the onset of emergence, most of the mature and teneral adults are found at the base of dead branches previously colonized by the PSHB, which indicates the importance of sanitation and removal of infested avocado plant tissue.

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