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Identification of a second sex pheromone component of the Vine Mealybug

Abstract - The Vine Mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), is a serious pest of vineyards in the Mediterranean region, California and South Africa. In an attempt to develop a monitoring system for the mealybug, research teams in California and Israel have studied, independently, its pheromone system. The Californian team (Hinkens *et al.*, 2001) identified (S)-lavandulyl senecioate (I) as the female sex pheromone of the mealybug. Lately, we identified (S)-lavandulyl isovalerate (II) as an additional active component. The attraction of the vine mealybug males to both compounds was demonstrated by bioassays in petri dish arena and flight assays in the mealybug rearing room. Indoor, both compounds displayed a similar level of attractiveness to the mass-reared males. However, trials in a vineyard indicated that males were attracted only to compound I.

Key words: Vine Mealybug, *Planococcus ficus*, sex pheromone, chiral GC analysis, bioassay

INTRODUCTION

The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) occurs in many countries in the Mediterranean region, in Central Asia and South Africa and was introduced recently into the Americas. It causes damage to grapevine (*Vitis* spp., Vitaceae) and figs (*Ficus carica*, Moraceae) (Ben-Dov, 1988). Damage is mainly caused due to migration of the adult females and larvae to the grape bunches, soiling them with a mixture of dead mealybugs and honeydew on which sooty mold develops. The chemical control of the mealybug, using several treatments of nonselective insecticides, is difficult due to the cryptic behavior of the mealybug and its typical waxy body covering. Hence, monitoring the population densities of the vine mealybug by improved means, such as pheromone traps, is needed. The objective of this study has been the identification of the vine mealybug sex pheromones and the development of a convenient monitoring system based on pheromone traps. The research group of Jocelyn Millar (UCR) identified recently (*S*)-lavandulyl senecioate (I) as the sex pheromone of *P. ficus*. We report here the identification of a second component of the pheromone, as well as laboratory and field tests with both compounds.

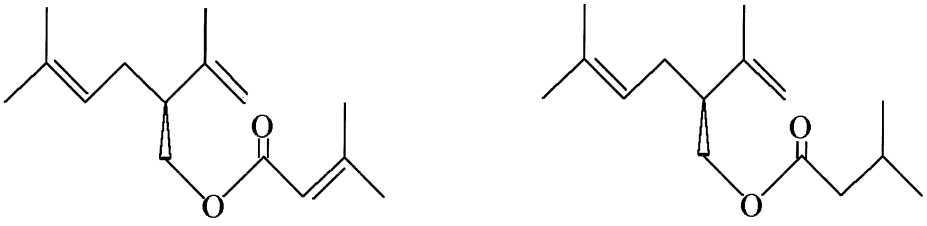
MATERIALS AND METHODS

Collection of vine mealybug for laboratory mass-rearing

Vine mealybug females were collected in a vineyard (var. superior) in Gshur, southern Golan. Microscopic slides of F_1 young females were used to verify the taxonomic identity of the collected individuals (Cox, 1989). The mass-rearing was carried out using potato sprouts in controlled chambers at a constant temperature of 23^0C, in the dark. Male pre-pupae and pupae were manually separated from the female. The males at the pre-pupa stage tend to leave the sprout and pupate inside folded paper strips. Attempts to eliminate the males from the mass-rearing using selective juvenoids failed. Male emergence coincides with the appearance of young adult females.

Collection, analysis and synthesis of the pheromone

The crude pheromone of *P. ficus* was trapped on Porapak Q (50-80 mesh) in an airborne collection device, similar to that used for the collection of the *Matsucoccus josephi* sex pheromone (Mendel *et al.*, 1990; Dunkelblum *et al.*, 1993). Each batch consisted of 10-20 sprouts carrying about 10,000 virgin females. The pheromone was collected for 3-4 days period, repeated three times. The Poropak Q columns were washed with hexane and the pheromone solutions were concentrated with a nitrogen flow. Combined samples of 20,000 – 30,000 female equivalents were separated by capillary GC on a Carlo Erba 5300 instrument. Samples were injected in the splitless mode with the FID shut down. A DB5 30m x 0.32mm ID, 0.50 mm df was kept at 60^0C for 2 min and then programmed to 160^0C at a rate of 20^0C/min. Ten fractions were collected every 2 min in precooled (acetone/dry-ice bath) capillary tubes connected to the FID outlet, starting 8 min after injection. The tubes were washed with predistilled ether and hexane. Fractions were bioassayed in a petri dish arena as described by Mendel *et al.* (1990). The 12-14 min and 16-18 min fractions consistently attracted the males. The two pheromone components were identified by GC-MS in the EI and CI mode and by comparison with standards. A AutoSpec VG GC-MS



I Lavandulyl senecioate

II Lavandulyl isovalerate

Fig. 1 - *Planococcus ficus* pheromone components.

instrument, equipped with a DB1 25 m x 0.32 mm, 0.25 df column (J & W Scientific) was used for the analysis. The column was kept at 60^{0}C for 2min and then programmed to 200^{0}C at a rate of 15^{0}C/min. For the determination of the chirality of the pheromone components, a sample of the crude pheromone was hydrolyzed with KOH/Methanol for 2h, diluted with water and extracted with ether. The chiral analysis was performed on a Rt-bDEXsm, 30m x 0.25mmID, 0.25mm df column (Resteck), isothermally at 110^{0}C in the split mode (ratio of 100:1), using a HP 6898 instrument. Commercial racemic lavandulol (Fluka) and (*R*)-lavandulol obtained from hydrolyzed lavandin oil served as chiral standards. Chiral columns CDX-B, 30m x 0.25mmID, 0.25mm df (J & W Scientific) and Lipodex-G, 25m x 0.25mm, 0.25mm df (Macherey – Nagel) did not separate the lavandulol enantiomers. The terpenoid C5 esters, including the two pheromone components, were synthesized from the commercial terpenic alcohols (geraniol, nerol, (-)-linalool and (±)-lavandulol) and different C5 acylchlorides (Aldrich) in dry ether at 0^{0}C with pyridine as base. The esters were purified by column chromatography on SiO_2, using hexane + 2% ether as eluent.

Bioassays and field tests

The two pheromone components were bioassayed in the petri dish arenae, separately and concurrently, at dosages of 5-50 ng/paper-disk with untreated paper disks as control in each test. Flight assays were conducted in the mealybug rearing room using delta traps with sticky plates (10x22 cm). Each test included three treatments consisting traps baited with 50 mg or 200 mg of the two pheromone components or hexane for the control. Gray rubber septa (West Co Pennsylvania, USA) were used as lures. Traps were suspended from the ceiling, exposed for 4-9 days and their position was daily permutated. Field tests were conducted in an organic vineyard in Lachish, southern Judean foothills. Delta sticky traps, baited with 25 mg – 200 mg of pheromone components were exposed in the vineyard for one-week periods.

RESULTS AND DISCUSSION

Two active fractions were isolated from the airborne collections of the vine mealybug virgin females. One fraction contained lavandulyl senecioate (I), recently identified as the sex pheromone of *P. ficus* in California (Hinken *et al.*, 2001). In the second one, we identified lavandulyl isovalerate (II) as an additional component of the sex pheromone of *P. ficus* (Fig. 1). The structures were identified by GC comparison with synthetic standards and by GC-MS (Fig. 2). The two natural components are chiral. Attempts to separate racemic I and II on three different chiral cyclodextrin columns failed. Conversely, racemic lavandulol gave a base-line separation on the Rt-bDEXsm column. Therefore, a sample of the natural pheromone was hydrolyzed. The formed lavandulol was compared with *R*-lavandulol that was isolated from lavandin oil and racemic lavandulol. This GC analysis established the *S*-configuration of both

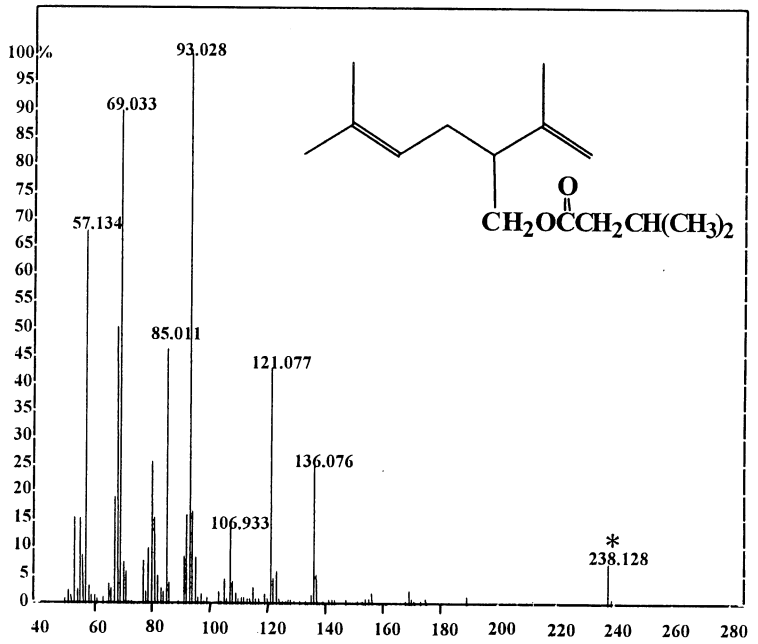


Fig. 2 - MS of lavandulyl isovalerate.

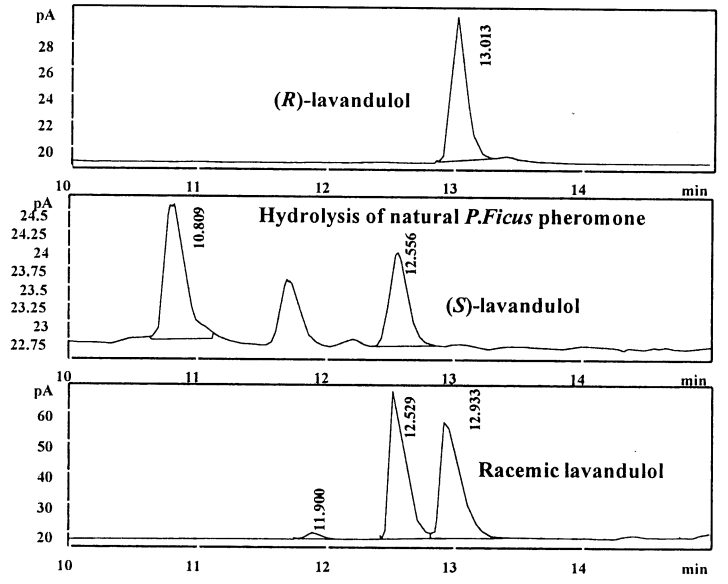


Fig. 3 - Chiral GC profile of lavandulol from hydrolysis of *P. ficus* pheromone *vs.* racemic lavandulol and (*R*)- lavandulol.

lavandulyl isovalerate (II) and lavandulyl senecioate (I) (Fig. 3). Data obtained by the petri dish assays and the flight assays in the rearing room (Fig. 4), proved that both components are similarly attractive to males from the mass-rearing. However, in the vineyard (Lachish) males were captured only by traps baited with lavandulyl senecioate (Fig. 5).

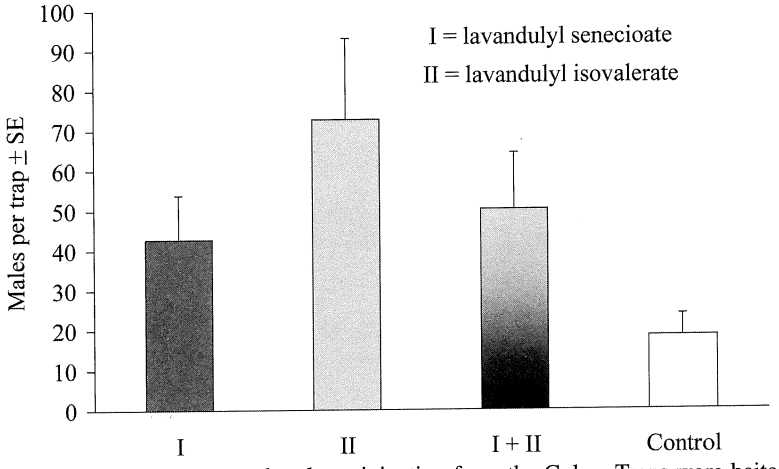


Fig. 4 - Flight assay of mass-reared males originating from the Golan. Traps were baited with 50mg of the pheromone components.

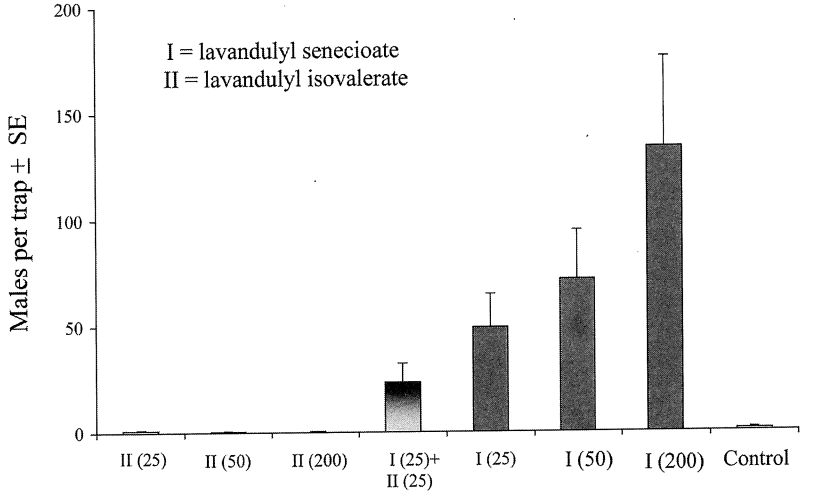


Fig. 5 - Capture of *P. ficus* males in Lachish vineyard. Traps were baited with different dosages of the pheromone components (µg).

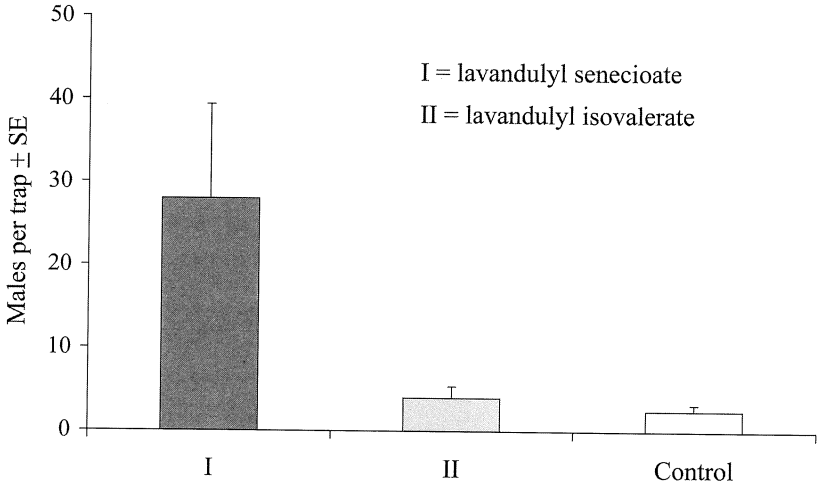


Fig. 6 - Flight assay of *P. ficus* males originating from Lachish. Traps were baited with 50mgr of the pheromone components.

Due to the different response of mass-reared males and feral males in the Lachish vineyard to lavandulyl isovalerate, we tested the attraction of F_1 males of females collected in Lachish to both components in the laboratory. These males responded both in the petri dish bioassay and in the flight assay (Fig. 6) only to lavandulyl senecioate, as in the vineyard.

The laboratory and field assay results indicate that feral males respond differently from the laboratory mass-reared males to the tested components. No similar phenomenon of this kind has been reported with scale insects. However, the different pattern of attraction of mass-reared males (originating from the Golan) and F_1 males from Lachish (about 300 km south of the Golan) may be related to the mass-rearing process or to genetic differences between the studied populations.

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