Use of pheromones and other semiochemicals in integrated production
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Females sex pheromone of oleander scale: quantitative aspects of its production and release

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Abstract: Sex pheromone of the Oleander Scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) was investigated to define its chemistry. Quantification of sex pheromone emitted by female of Oleander Scale was performed using headspace solid phase microextraction (SPME) subsequently analyzed by gas chromatography/mass spectrometry (GC/MS), adopting a cyclobutane derivative standard of Oleander Scale sex pheromone chemically synthesized as a standard. The headspace SPME of the volatile emission from about 30 Oleander Scales virgin females of a Sicilian population monitored for several days allowed individuating the initial point of sex pheromone production in females twenty-seven d-old. The amount of pheromone production has been determined in 10 pg for single scale per day.

Key words: Sex pheromone, oleander scale, SPME, lemon

Introduction

In Sicily, commercial citrus orchards are infested by Diaspid pests, commonly known as scales (Liotta, 1970; Liotta *et al*., 1977). These pests cause a general weakening of the tree, and infested fruits are graded down in the packinghouse making them unmarketable. Control of Scales consists mainly of insecticide applications which use has been rationalized due to the discovery and the synthesis of sex pheromones. The pheromones, in the scales Integrated Pest Management programs, are used to monitor scale male so that combining male capture data and degree day model upcoming events in the scale lifecycle the best time to make pesticide applications could be forecasted. So far, the sex pheromones of four species of Diaspididae have been chemically identified and adopted in IPM: the California red scale, *Aonidiella aurantii* (Maskell) (Roelofs *et al*., 1977), the yellow scale *A. citrina* (Coquillett) (Gieselmann *et al*.1979a), the San Jose scale *Quadraspidiotus perniciosus* (Comstock) (Gieselmann *et al*.1979b; Anderson *et al*., 1981) and the white peach scale *Pseudolacaspis pentagona*.(Heath *et al*.,1979). The sex pheromone of the Oleander scale *Aspidiotus nerii* Bouché, was recently chemically identified (Einhorn *et al*., 1998; Boyer & Ducrot, 1998). In all identification processes the methodology

used consisted in collecting in volatile substances emitted by a large number of females over a long time period. We used a different approach by means solid phase microextraction (SPME) followed by gas chromatography/mass spectrometry (GC/MS) that is an excellent concentration and preparation technique for the analysis of volatile and semi-volatiles compounds that can be found in the headspace above the sample. This solvent free technique is reliable because it has great sensitivity, it is fast, does not damage insects and can be applied over several consecutive days on the same sample. In this study SPME analysis was adopted for detection of *A. nerii* pheromone on lemons and for tentatively quantifies its production over several days.

Materials and methods

Insect cultures. The Oleander Scale colony was established from materials collected in Sicily from lemon trees. Insects were reared on lemon fruits previously coated with paraffin over about the 2/3 of their surface to avoid desiccation. Daily lemon fruit infestations were manually performed transferring on fresh fruit crawlers from gravid females and infested fruits were kept isolated in a plastic bottle cap at 24 ±1 °C, 70 ± 5% RH with a 16L:8D photoperiod. Twenty-four days after the infestation second instar males were manually removed, then two days before the second molt, lemons, infested with about 30 virgin females, were used for SPME collection.

Sample collection. Volatile collection was performed by SPME from the headspace over two infested lemons with Oleander Scale virgin females, held in a 300 ml glass vial sealed with a silicon septum, and left under laboratory conditions at 25 ±1 °C. Volatile was collected by a 70μm SPME (Supelco) CarboWax divinylbenzene (CW/DVB) coated fiber, for 18 hours starting at 2.00 p.m. on each day of the sampling period. Virgin females of Oleander Scale were sampled from twenty-five d-old to thirty-seven d-old. Between two volatile samplings, the infested lemon fruits were held sealed in the same glass vial.

Chemical analysis. All the samples were analyzed by GC on a fused capillary column HP5-MS (30m length, 0.25 mm I.D., 0.25 μm film thickness, Agilent) in a Agilent mod. 6890 chromatograph equipped with the mass selective detector Agilent 5973 in the following condition: the injector temperature was held at 250 °C; He as carrier gas at 10^{-3} l/min.; oven temperature program: 5 min. isotherm at 45°C followed by linear temperature increase of 4º C/ min. up to 300° C held for 10 min.

A cyclobutane derivative, standard of *A. nerii* pheromone, was tested in order to identify the response factor, the retention time on the chromatogram and the most characteristic fragments in the mass spectrum. Capacity of SPME to detect a pheromone concentration of 1.5 μg, in our experimental system, was tested to investigate the presence of some lemon peaks from the examined area (Fig.1). To improve the detection limit, the SIM mode was set on mass spectrometer.

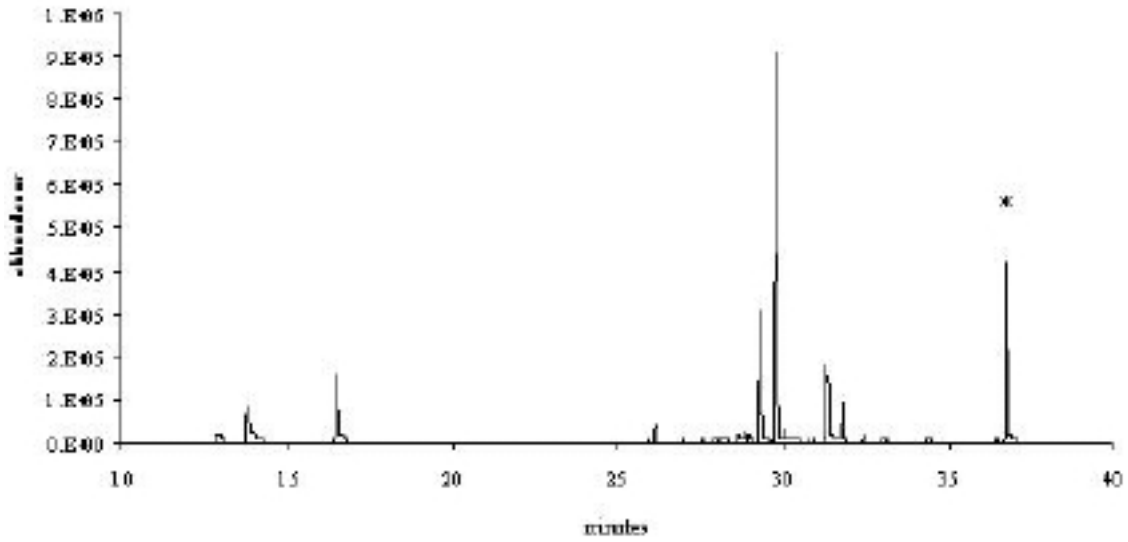

Figure 1. SPME (CW-DVB) chromatogram of synthetic pheromone of oleander scale on lemon substrate for 18 h (asterisk = pheromone peak)

Results and Discussion

The retention time of standard pheromone was found to be 36,8 ± 0.1 min. and the mass spectrum showed the following, more intense, ions 68, 79, 93, 107, 121, 133, 161, 189, 204 amu. In Figure 2 were reported the results of these experimentation. The y axis represents the total amount of pheromone collected by fiber in the glass vial closed with a silicon. The amount has been calculated from integrate area under the peak at ~36.8 min. of R.T. In the first two day no peak was detected over the instrument detection limit, for this reason we consider the first part of the curve as the "zero level" of pheromone presence. On the twenty-seventh day, one peak begun to grow up at ~36.8 min. of R.T. and on the twenty-eightieth day we had the maximal integrated area. After the twenty-eightieth day, the peak slowly decreased down to a constant value.

Assuming constant daily pheromone production for each individual, the shape of the growing part of experimental curve yields from different pheromone production start time among all the individuals. According to this supposition on the twenty-eightieth day all the scales were probably on production. The supposed amount of pheromone produced by all virgin scales in one day was determined by subtracting the amount of pheromone collected on the twenty-seventh day to the amount collected on the twenty-eightieth day. The result was 10 pg per single scale per day.

This experiment, under established conditions, gave us three important results agreeing with morphological aspects of scales: first, we were able to identify the beginning of pheromone production; second, it the last day of pheromone production

was determined; third, the pheromone production of a single female scale has been measured.

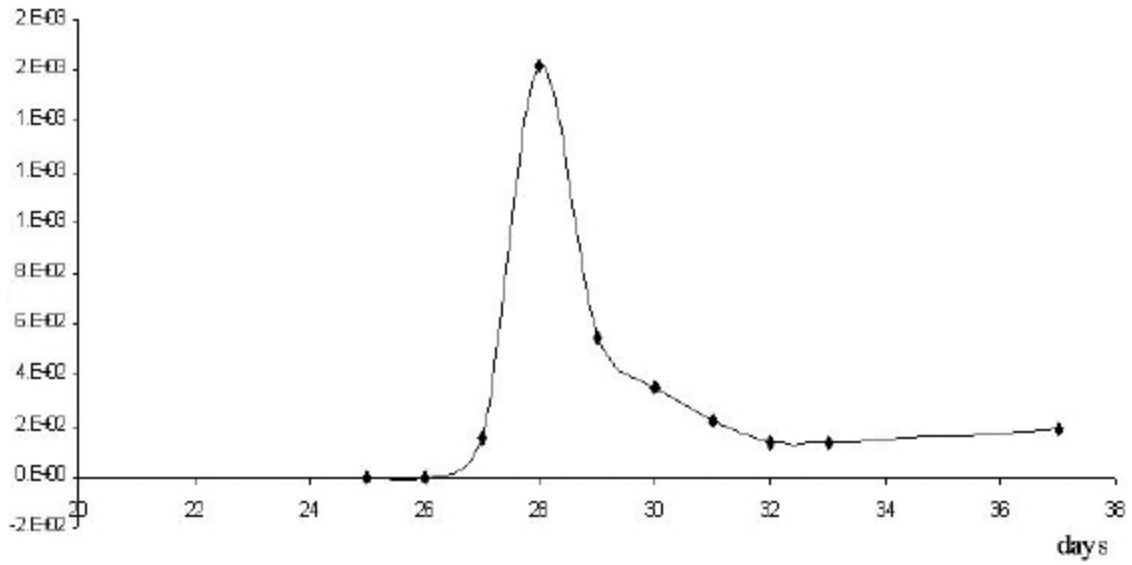

Figure 2. Sex pheromone of oleander scale - timing of production

In order to assess the importance of semiochemicals as sympatric speciation factors (Tremblay e Rotundo, 1978), as showed in some species by the presence of so-called "pheromone strains" (Aldrich *et al.*,1987), further research developments could be aimed at verifying whether the sex pheromone could be produced also by the *A. nerii* partenogenetic population and whether colonies of *A. nerii* collected from sicilian citrus orchards release only a cyclobutane derivate as attractive substance or other substances are involved instead, at least in determining pheromone specificity.

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