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EAG responses to pheromone as a tool in the control of population dynamics of the gypsy moth *Lymantria dispar*

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Introduction

Sex pheromones play a major role in regulating behaviour of insects, as well as other organisms. The reproductive programme of the gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae), the most important defoliator of Sardinian *Quercus suber* L. forests, is based on production and release of its sex pheromone (+)disparlure by calling female moths and its perception by conspecific males. The acquisition of information about any factors influencing male sensitivity to this sex attractant may help improve field techniques against this defoliator or possibly provide a basis for predicting where favourable conditions for control of population dynamics will occur. In this respect, the pheromone might be used as a specific tool for olfaction research and to monitor changes in male sensitivity, thus leading to a better control of population.
For example, by means of the electroantennogram technique (EAG), pheromone stimulations were used to verify if olfactory activity could vary in adult males healthy and treated with *B. thuringiensis* during different larval instars, with the aim of ascertaining if delayed sublethal effects of this microbial agent could be reflected in a diminished sensitivity to pheromone and used in the population control other than the well known effect on mortality rate. Moreover, stimulations with pheromone were also used to verify if males from two localities at different microclimatic conditions could show different sensitivity levels. Traditionally these parameters were sorted out on historical data and basically on oviposition density and distribution, or on the map of defoliated area in previous years, instead we have taken into account, as principle variable in population dynamics, the ability of males to recognize pheromone signalling from females.

Materials & methods

All EAGs were performed on adult males collected in two different localities from central and northern Sardinia, Abbasanta (40°12'N 8°47'E; alt.: 360 m a.s.l.), and Chiaramonti (40°45'N 8°49'E; alt.: 350 m a.s.l); larvae were reared on artificial diet in environmentally controlled chambers (25±5°C; 60±5% RH and 16 hrs daylight regime) until pupation. As regards the infection protocols, newly molted 3rd, 4th and 5th instar larvae were treated by means of a 24-hours exposition to a contamined diet with *B. thuringiensis* aqueous suspension (Foray 48B; 10,600 UI/mg) at different concentrations. Insects surviving infection and control ones were individually placed in petri dishes with untreated diet and monitored daily. Larvae from Abbasanta were infected during the 4th instar, while those from Chiaramonti were infected during the 3rd, 4th and the 5th. Each antenna (one per moth) was stimulated by means of an odour delivery system with (+)disparlure solutions within the 10^{-1} - 10^{-5} dilution range in paraffin from pure pheromone and presented in increasing concentration sequence. Each stimulation lasted 2 s and a recovery interval of 3 min was allowed between any two successive stimulations, with the intent of minimizing adaptation effects. Differences in EAG values were calculated by means of the Student's "*t*" test (Statistical software package) with a 95% confidence level, while Pearson's *"r"* (product-moment correlation) was used to correlate electrophysiological data and climate variability.

Results & discussion

All tested concentrations of (+)disparlure evoked antennal responses of a negative sign, i.e. a depolarization, interpreted as a net excitatory response of the chemosensory neurons in the antennae; in all specimens a weak sensitivity was observed at the lowest pheromone concentrations (10^{-5} - 10^{-4}) while increasing responses and a dose-response relationship were clearly present at higher concentrations. EAG

mean amplitude values from antennal receptors of healthy (control) and infected males collected in Abbasanta, showed a sensitivity level from 0.56 ± 0.1 to 3.04 ± 0.29 mV and from 0.7 ± 0.12 to 3.4 ± 0.5 mV for healthy and treated samples respectively, in the range $10^{-3} \div 10^{-1}$ of pheromone concentration; no statistical difference was found. As regards specimens from Chiaramonti, EAG values obtained from the control population ranged from 0.37 ± 0.04 to 2.67 ± 0.28 mV for (+)disparlure concentrations of 10^{-3} and 10^{-1}, whereas from 0.37 ± 0.06 to 2.87 ± 0.28 mV, from 0.23 ± 0,03 mV to 2.08 ± 0.16 mV and 0.23 ± 0.03 to 2.08 ± 0.16 mV respectively for 3rd, 4th-low mortality and 4th-high mortality infected larvae in the same interval of (+)disparlure concentrations. The comparative analysis between infected specimens and the control shows that a significant difference exists only between the 4th instar infected sample at low rate of mortality and its control at 10^{-3} and 10^{-2} pheromone concentrations; high variabilities observed within each specimen, probably explain undetected differences. EAGs recorded from the sample from the same locality but infected during the 5th instar larvae showed responses ranging from 0.33 ± 0.07 to 1.37 ± 0.13 mV and from 0.25 ± 0.04 and 1.47 ± 0.15 mV for healthy and treated samples respectively, in the same range of stimuli.

As regards the modalities by which adult male sensitivity to pheromone can be influenced by microclimatic variations during larval instars, our results suggest no correlation between EAGs and the temperature of the two different localities over a four-years period (1995-1998); conversely, a negative correlation between the same parameter and precipitation was found. Our data are in good agreement with those reported in the literature, according to which a negative correlation between defoliation and precipitation was found. In fact, if male capability to find females decreases inside a population, the possibility of mating should also decrease as well as the number of egg masses and defoliation risk attached to next generation.

Conclusions

Our electrophysiological results following stimulation with pheromone show no statistical differences in adult male sensitivity to pheromone between healthy and treated samples with *B. thuringiensis*, regardless of age of larval infection; surviving males retain a complete sensitivity towards pheromone so that, at least in that case, this kind of infection could be considered irrelevant as an alternative method for population control. On the other hand, the negative correlation between male responses to pheromone and precipitation during their larval cycle, may help understand how population expansion occurs in different areas with different microclimatic conditions.

One more employment of this sex attractant will be addressed to obtain analogues of the pheromone molecule showing higher activity and/or more stable and with a longer decay time than the natural one; once identified and synthesized, these compounds can be used to bait traps that provide simple, specific tools for monitoring insect pests and can also be used to control insect population by mating disruption. They may have the advantage of being non-toxic and specific for the target pest.

References

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