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On the physiological and behavioral mechanisms of pheromone-based mating disruption

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Abstract: Over the past several years my colleagues and I have been exploring ways of achieving moth mating disruption exceeding 95% efficacy even under high densities with limited insecticide inputs. The foundation for this work has been a series of studies examining the mechanisms underlying pheromone-based mating disruption in moth pests of pome fruit, stone fruit, walnuts, and citrus. Collectively, the results support competitive attraction or false-plume-following as an essential component of communicational disruption of moths in the family Tortricidae. Habituation of central nervous system (CNS) response appears to be an important supplementary mechanism for moths having oriented along plumes of high-dosage dispensers. Four main lines of evidence have led to these conclusions. Pheromone-based disruption of moths is density-dependent. Under high population densities, disruption increases as a function of increasing density of pheromone release sites. Effective mating disruption using high-dosage dispensers occurs in the field despite overall atmospheric concentrations not reaching levels high enough to desensitize moths by adaptation or habituation without close range (within cm) exposure to high dosage dispensers. Males are attracted to high-dosage dispensers in the field and such encounters desensitize the CNS but do not affect sensitivity of the peripheral nervous system. If competitive attraction followed by CNS habituation are the combined mechanisms achieving mating disruption, the following practical implications should be considered for developing high performance approaches and formulations: 1) distribution of dispensers should be uniform rather than clumped, 2) dispenser density should be high, and 3) release rate from synthetic dispensers should be within a physiologically attractive range but also sufficiently high to habituate male moth response following orientation.

Keywords: semiochemical, competitive attraction, CNS habituation, peripheral adaptation, camouflage

BACKGROUND

Several hypotheses have been proposed to explain how sexual communication in moths is disrupted by deploying formulations of synthetic pheromones to prevent mating. These "mechanisms of disruption" were originally defined in a review by R.J. Bartell [1] and re-analyzed in a recent series of articles by J.R. Miller and colleagues [2, 3]. Of these hypotheses, perhaps the most commonly-cited mechanisms are: false-plume following, camouflage, desensitization, and sensory imbalance. False-plume following, also called competitive attraction, is the decrease in visitation rate of calling females by available males due to preoccupation with false plumes sent out by competing dispensers of synthetic pheromone. For camouflage, it is believed that the boundaries of a calling female's plume are obscured by a background concentration of synthetic pheromone; this mechanism assumes that the male's sensitivity to pheromone is unaffected by continual exposure to high concentrations of background pheromone. Desensitization is defined as decreased sensitivity to pheromone due to continuous exposure to high background concentrations of pheromone. This mechanism is comprised of two possible sensory changes: 1) adaptation is defined as decreased sensitivity of the peripheral nervous system, while 2) habituation is defined as decreased sensitivity of the central nervous system. Finally, for sensory imbalance, it is believed that the natural pheromone component ratio released by females and required by males for normal orientation is adulterated by dispensing large amounts of one or more synthetic components into the crop atmosphere. Elevating the background concentration with a partial blend of synthetic pheromone component(s) may alter this required balanced ratio of sensory input perceived by males and thus disrupt oriented response.

CONTEMPORARY INVESTIGATIONS OF DESENSITIZATION

In a recent investigation of codling moth disruption, Judd et al. [4] quantified the airborne concentration of codlemone required for both adapting male antennal sensitivity and reducing subsequent behavioral response. Exposure to ca 35 μg of codlemone/l of air in static-air chambers for 10-30 min reduced electroantennogram (EAG) responses and nearly eliminated subsequent male orientation in a flight tunnel. The effect was reversible and behavioral responses were subnormal for a much longer interval (ca 4 hr) than antennal sensitivity (ca 1 hr) [4]. This result suggested that habituation rather than adaptation was the more important and longer-lasting component of desensitization mediating disruption following high-dosage exposure to pheromone. Stelinski et al. [5] confirmed that the duration of peripheral adaptation in male codling moth following prolonged exposure at μg/l dosages of airborne pheromone lasts ca. 1 h. The duration of peripheral adaptation in codling moth males is substantially longer than that recorded for several other tortricids [*Choristoneura rosaseana* (Harris), *Argyrotaenia velutinana* (Walker) *Grapholita molesta* (Busck), and *Pandemis pyrusana* Kearfott] after exposure to their pheromone components at the same dosages; these durations of reduced antennal sensitivity range between < 1 min – ca 15 min [5, 6]. Regarding the extraordinary duration of adaptation in codling moth males, Judd and colleagues [4] postulated that the codlemone diene alcohol may adsorb into the insect's waxy cuticle to a greater degree than the acetate and aldehyde pheromones of the other above-mentioned tortricids in which antennal adaptation has been investigated.

Despite the presence of a 60-75 min long duration of peripheral adaptation in male codling moth following exposure to pheromone, Stelinski and colleagues [5] questioned its potential importance as a contributor to mating disruption. Caging male codling moths for 30-34 hr in an orchard treated with a 1,000 Isomate C dispensers / ha did not impact the males' capability of subsequently orienting to pheromone sources in a flight tunnel [4]. Thus, male sensitivity to pheromone was not affected under the standard Isomate treatment, which is known to disrupt male orientation to traps and virgin females and reduce crop damage [7]. The findings with codling moth [4] were similar to those for the European grape moth, *Lobesia botrana* (Denis and Schiffermüller) [8] and the Oriental fruit moth, *G. molesta* [9]. For *L. botrana*, males were captured in attractive sticky traps in the field directly after 8 h of exposure in vineyards treated with polyethylene-tube dispensers (1 dispenser/5 m^2; each dispenser contained 500 mg of *E*7,*Z*9-dodecadienyl acetate) [8]. Reduction in male moth response to traps in the field occurred only after males were exposed in the laboratory at an airborne pheromone concentration of 4 μg / l of air [8]. For *G. molesta*, reduction of male captures in attractive sticky traps occurred only after one h of laboratory exposure to pheromone at 65 $\mu g/m^3$ (3200 female equivalents) [9]. Collectively, desensitization of tortricids, including codling moth, is not induced after field exposures at rates of synthetic pheromone dispensers per area of crop known to result in effective disruption.

In addition, the results of studies quantifying the airborne concentrations of pheromone achieved in the field by mating disruption dispensers suggest that laboratory experiments investigating desensitization have exposed moths to dosages of pheromone far greater than which is actually achieved in the field. Specifically, the average airborne concentration of pheromone achieved per cubic m of crop treated with mating disruption dispensers has been quantified as ca. 1-2 ng [10. Therefore, the airborne concentrations of pheromone that have been shown to desensitize moths in most laboratory investigations to date have far exceed (by ca. 1,000 X's) the actual concentration of pheromone achieved in the field by application of commercially-available dispensers such as Isomate C Plus.

IMPORTANCE OF COMPETITIVE ATTRACTION

Field observations have revealed that tortricid male moths orient to and approach polyethylene tube reservoir dispensers of pheromone characterized by release rates 1,000 fold higher than female moths as well as "female equivalent" dispensers which release pheromone at rates approximating calling females [11]. Far more males may actually orient to these dispensers than what has been actually observed given that oriented progress is likely terminated downwind at a certain distance at which the pheromone concentration is above the upper threshold for response [12]. These results suggest that competitive attraction between calling females and synthetic point sources of pheromone may be an important contributing mechanism to mating disruption.

Outcomes of mating disruption studies in which dispenser density per area of crop was varied corroborate the hypothesis that competitive attraction is an important mechanism contributing to mating disruption. Specifically, there is mounting evidence that disruption of various moth species is superior via higher rather than lower densities of pheromone release sites at common overall release rates of pheromone per ha [13, 14]. In addition, recently proposed mathematical models developed to differentiate between competitive versus non-competitive mechanisms of disruption [2] suggest that competitive disruption was the primary mechanism of disruption among 13 available studies of disruption [3]. Under the scenario of competitive attraction, plotting 1 / male visitation rate to a given attractant source on the y-axis against dispenser density on the x-axis yields a straight line with positive slope. Furthermore, plotting male visitation rate to a given attractant source on the y-axis against (dispenser density x visitation rate) on the x-axis yields a straight line with negative slope; disruption by a non-competitive mechanism was found not to share this set of properties [2]. The resultant analyses were consistent with the hypothesis that competitive attraction mediated disruption of several tortricid species with high densities of pheromone dispensers [3]. Collectively, these results suggest that false-plume following to pheromone dispensers contributes to disruption and that point source density and distribution may be critically important factors to achieving effective disruption; particularly under high moth densities. If competitive attraction is an important contributor to mating disruption, then efficacy should be highly dependent on moth population density and the density of synthetic point sources that are deployed [2, 3].

COMBINED EFFECT OF PLUME-FOLLOWING AND HABITUATION

Currently, hand-applied, Isomate-style dispensers deployed at ca. 1-4 per tree are the dominant method of dispensing pheromone for mating disruption of moth pests in orchards [15]. Although the average airborne concentration of pheromone achieved in orchards treated with these pheromone dispensers is unlikely to desensitize males flying or resting meters away from the source of emanating pheromone, anemotactic orientation of attracted male moths to within close proximity of dispensers likely does induce habituation [16]. Moths may be capable of making these close (within 1 m) approaches to high-dosage dispensers by orienting along the edge of the pheromone plume, modulating their exposure dosage [11, 17]. Thus, the combination of initial orientation by tortricid moth males along plumes of synthetic pheromone compounded by habituation of subsequent response due to over-exposure likely explains mating disruption by Isomate dispensers and related technologies. This potential explanation for mating disruption of moths, in general, was proposed almost a decade ago [18] and current evidence with tortricid moths is consistent with this hypothesis.

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