Entomologia Experimentalis et Applicata **100:** 131–134, 2001.

Short communication

Potential for the evolution of resistance to pheromone-based mating disruption tested using two pheromone strains of the cabbage looper, *Trichoplusia ni*

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Accepted: April 10, 2001

Key words: mating disruption, pheromone, resistance, Lepidoptera, *Trichoplusia ni*

The reliance of most moth species on female-produced sex pheromones for mate attraction makes their communication system an ideal target for pest management. Mating disruption, by release of synthetic sex pheromone into the atmosphere, has been developed to control many lepidopteran pests and is available commercially for several species (Cardé & Minks, 1995). Selection pressure provided by continuous application of mating disruptants could result in the evolution of resistance to this tactic, and this possibility should be evaluated before it occurs.

The potential for resistance to mating disruption will depend on genetically-based variation in production of and response to pheromone within and amongst populations (Löfstedt, 1990, 1993; Phelan, 1992). The diverse phylogenetic pattern of lepidopteran pheromones suggests that natural selection has shaped the evolution of species-specific pheromones. Long-term use of mating disruptants that effectively control pest populations, could provide the necessary selection pressure for an alteration in the communication system and the evolution of resistance to mating disruption (Cardé & Minks, 1995).

In the cabbage looper, *Trichoplusia ni* (Hübner), the sex pheromone consists of a main component, *Z*7-dodecenyl acetate (*Z*7-12:Ac), and five minor components (Bjostad et al., 1984). Haynes & Hunt (1990) discovered an abnormal pheromone phenotype in a laboratory colony of *T. ni* that was the result of a single, recessive autosomal gene mutation. Mutant females release a pheromone that contains a twenty-fold increase of one minor component, *Z*9-tetradecenyl acetate (Z9-14:Ac) and a thirty-fold decrease of another minor component, *Z*5-dodecenyl acetate (*Z*5-12:Ac). Initially, male *T. ni* that carried the mutant gene responded like normal males, demonstrating a preference for the normal pheromone. However, after 49 generations within a pure mutant colony, males responded equally well to both mutant and normal pheromones (Liu & Haynes, 1994). Thus, there are genetically-based differences in both female pheromone and male response (Liu & Haynes, 1994).

The potential for mating disruption to result in evolutionary changes in the pheromone was tested on laboratory-derived populations of *T. ni* using a replicated selection experiment in large field cages. We manipulated the environment (pheromone-treated *vs.* clean-air control) that moths were exposed to and followed the evolution of the pheromone phenotype in five closed (no gene flow) populations across four offspring generations. The normal pheromone blend applied as a mating disruptant provided selection pressure that influenced the frequency of the mutant allele in the population.

Materials and methods

Insect colonies used are described in Haynes & Hunt (1990). Five parental populations were established by pairing individual 2–3-d old females with individual 3–4 d old males in 400 ml paper cartons (15 Normal (N) ♀ × N ♂, 15 Mutant (M) ♀ × M ♂, 30 N ♀ × M ♂ and 30 M ♀ × N ♂ pairs for each population). From each pair, ∼50 eggs were placed on pinto bean-based diet (25–30 eggs / ∼110 g diet) kept at ambient

conditions until pupation. Pupae were separated by sex and housed in different environmental chambers (L14:D10; night 21: day 25 °C). Individuals from all mating pairs were used to establish parental populations consisting of 90, 3–5-d old males and females for each line. The mutant allelic frequency in the parental populations was 0.5.

Four field cages (5 × 5 × 1.8 m, L × W × H) were constructed on Spindletop Farm, University of Kentucky Agricultural Experiment Station (38 °06′ N, 84 °29′ W). Two cages were positioned 85 m apart in each of two blocks separated by ∼200 m. One cage in each block was randomly chosen to be treated with normal *T. ni* pheromone released from 18 red rubber septa dispensers (Thomas Scientific, Swedesboro, NJ, USA 5 × 9 mm, red). Pheromone lures were loaded with 192 μg of 12:Ac (Aldrich Chemical Co., Inc., Milwaukee, WI, USA), 220 μg of *Z*5-12:Ac (Bedoukian Research Inc., Danbury, CT USA), 2000 μg of *Z*7-12:Ac, 64 μg of 11-12:Ac, 44 μg of *Z*7-14:Ac and 36 μg of *Z*9-14:Ac (Bedoukian) in 50 μl of HPLC grade hexane. Pheromone ratio composition was verified with gas chromatographic analysis (30 m DB Wax 20 M capillary column in a Hewlett-Packard 5890 gas chromatograph linked to a Hewlett-Packard 5970 mass selective detector). Treated rubber septa were attached at 30 and 90 cm above the ground on each of nine bamboo stakes erected in a 1 × 1 m grid within the cage. The control cage was similarly treated except that septa were loaded with 50 μl HPLC grade hexane alone. New septa were used for each replicate and generation. Between replicates and generations cage position was randomized.

Parental populations were introduced into cages treated with pheromone or control septa 24 h previously. At the time of moth introduction, 16, 5–6 week old broccoli plants were positioned in a 1 × 1 m grid in the cage. The plants, with the *T. ni* eggs, were removed from cages 3 nights later and transported to the laboratory. Broccoli leaves and associated *T. ni* eggs were placed in sealed, clear plastic containers (2–4 plants/container), and kept at ambient conditions. The resulting larvae (2^{nd}-3^{rd} instar) were transferred to artificial diet (25–30 larvae/∼110 g diet). At pupation, males and females were housed separately (L14:D10; night 21:day 25 °C) until eclosion. Ninety, 3–5-d old males and females were selected from each treatment line, in each population to be re-introduced back into field cages, as before. This procedure was repeated for both treatment lines of each of five populations over four generations.

Gland dissections of ∼100 females (from each treatment line in each of five populations over four offspring generations) were conducted to determine the frequency of the mutant phenotype. Glands were dissected from >2-d-old females and extracted individually in 50 μl of a 1 ng μl^{-1} solution of *E,Z*-4,7-tridecadienyl acetate (an internal standard) in hexane for 1 h. After extraction, glands were discarded and additional hexane was added to obtain ∼50 μl. Extracts were analyzed using an autosampler attached to either a Hewlett-Packard 5890 or 6890 gas chromatograph, equipped with a 30 m DB Wax capillary column and linked to either a Hewlett-Packard 5970 or 5973 mass selective detector, using the selective ion method of Haynes & Hunt (1990). When necessary, gland extracts were stored in sealed autosampler vials at −80 °C before analysis.

Logistic regression (SAS, 1996) tested for changes in the proportion of the mutant phenotype in populations over time (generations 1–4) and by treatment (control *vs.* disruptant), and the interaction of time and treatment (Stokes et al., 1995). At our end point of selection (4th offspring generation), a replicated χ^2-test was used to determine if there was a difference in the proportion of the mutant phenotype in control *vs.* treated lines (Sokal & Rohlf, 1981).

Results and discussion

Selection with the normal pheromone blend as a mating disruptant resulted in a divergence in the proportion of females exhibiting the mutant phenotype in the control and disruptant-treated populations (Figure 1). By the fourth generation, the proportion of females with the mutant phenotype was significantly greater in the treated populations (31.0%) than in the control populations (18.1%) (replicated Chi-square test; total $\chi^2 = 24.39$, df = 5, $P < 0.001$; pooled $\chi^2 = 11.06$, df=1, $P < 0.01$). There was variation amongst the replicates in the magnitude of difference between treated and control populations (heterogeneity $\chi^2 = 13.33$, df=4, $P < 0.01$), but the consistency of pooled and total Chi-square statistics indicates that the pattern of a higher proportion of the mutant phenotype in treated populations was robust.

The interaction between time and treatment had a significant impact on the proportion of females with the mutant pheromone phenotype, consistent with an incremental change across generations (Logistic regression; $\chi^2 = 5.99$, df=1, $P = 0.014$). Each gener-

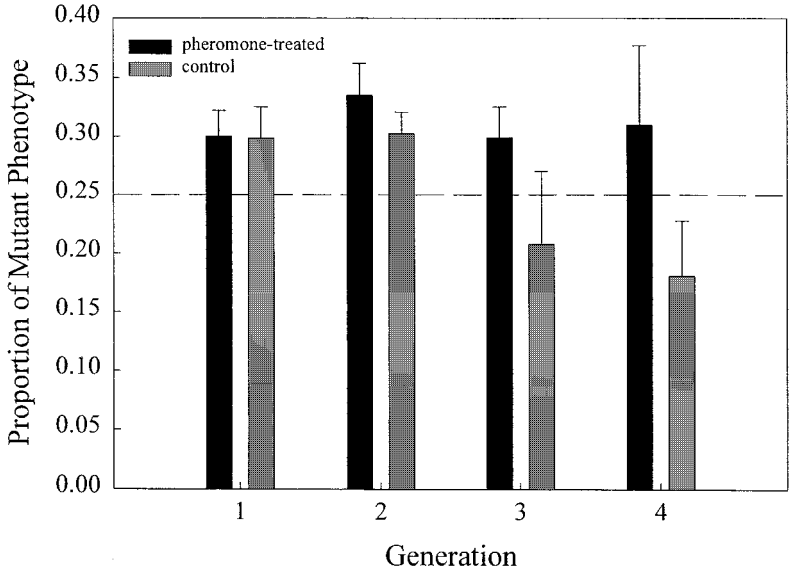

Figure 1. Mean proportion (+ s.e.) of females with mutant phenotype in the pheromone-treated and control lines ($n = 5$) over four offspring generations. The dashed line refers to the expected proportion of the mutant-phenotype females (0.25) if the assumptions of the Hardy–Weinberg equilibrium were met. By the fourth generation, there was a significant difference between the proportions of the mutant-phenotype females in treatment and control lines (replicated χ^2 test, $P < 0.05$). The number of glands dissected at each generation were: Generation 1, pheromone-treated: 505; control: 508; Generation 2, pheromone-treated: 498; control: 499; Generation 3, pheromone-treated: 498; control: 420; Generation 4, pheromone-treated: 443; control: 336.

ation the difference between treated and control cages increased (Figure 1). Alone, generation and treatment did not explain the observed pattern (Logistic regression; $\chi^2 = 0.41$, df=1, $P = 0.523$, $\chi^2 = 1.02$, df=1, $P = 0.313$, respectively).

We predicted that the frequency of the mutant phenotype would head toward extinction in our control cages because normal males mate preferentially with normal females, while mutant males do not discriminate between the two pheromone types (Liu & Haynes, 1994; Zhu et al., 1997). Hybrid males also respond preferentially to the normal pheromone phenotype (Hemmann, 2000), reinforcing the expected mating advantage of normal females. The initial frequency of the mutant alleles was 0.5, which would lead to a stable Hardy–Weinberg equilibrium of mutant phenotypes of 0.25 because the mutant allele is recessive. By the fourth generation, the frequency of the mutant phenotype was 0.31 and 0.18 in the treated and control lines, respectively. Selection imposed by the normal mating disruptant appears to counter the disadvantage of the mutant females, and preserve that genotype in the population. One mechanism for this response to selection could be that normal females become less apparent in disruptant-treated cages to both normal and mutant males, and mutant females may become relatively more apparent. Because mutant males respond equally well to the normal and mutant pheromone, they would be at an advantage to obtain matings from the more apparent mutant females in pheromone-treated cages.

An understanding of how selection influences the evolution of pheromone communication systems is essential if we are to understand how these signals originated, and how to maintain and expand the use of pheromones in pest management. Our results indicate that mating disruption with the normal pheromone blend under these experimental conditions did impose selection pressure that influenced the frequency of the pheromone phenotypes within the *T. ni* populations tested. This suggests that mating disruption using the most commonly produced pheromone blend in a pest population may select for uncommon pheromone phenotypes and gradually alter the chemical communication system of the population and contribute to the potential for the development of resistance to mating disruption. Further study, using field populations in which gene flow into the treatment area can occur by moth dispersal, would determine if the potential for resistance to mating disruption can be realized in actual pheromone-based pest management systems.

Acknowledgements

We thank B. Chastain, J. Vires , R. Hopper, J. Clements, B. Spohn and D. Hemmann for technical assistance. This work was supported by the Cooperative State Research Service, US Department of Agriculture under Agreement No. 97-35302-4324. The GC-MS system was purchased, in part, by funds from the University of Kentucky major equipment program (MRES94-01-Haynes). We thank Drs C. Fox and B. Webb for helpful suggestions on an earlier draft of this manuscript. This investigation (Paper no. 00-08-155) was conducted in connection with a project of the Kentucky Agricultural Experimental Station.

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