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POTENTIAL FOR EVOLUTION OF RESISTANCE TO PHEROMONES

Worldwide and Local Variation in Chemical Communication System of Pink Bollworm Moth, *Pectinophora gossypiella*

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Abstract—Female *Pectinophora gossypiella* (Saunders) from most of the desert cotton-growing areas of southern California emitted significantly more pheromone in 1984 and 1985 than in preceding years (1982 and 1983). This increase amounted to almost 20% by 1985. It is unlikely that this small change would represent effective resistance to disruptant pheromones, but this increase could reflect the result of selection pressure imposed by the use of mating disruption for population control. A worldwide survey of emitted pheromone from this species found that there was much more variation in the emission rate than the blend ratio of the two pheromone components. The emitted blend ratio was remarkably consistent over time (in southern California) and throughout the worldwide range of the insect. Small differences in the blend ratio that were detected probably have no major biological significance because of the relatively broad response spectrum of males to changes in the blend of pheromonal components. Populations of males did not consist of several phenotypes, each with a different preference for specific blend ratios. Rather, the broad response spectrum to blend ratios in a population can be attributed to variation in the response of any individual. Therefore, selection for a response phenotype that is narrowly tuned to the blend emitted by females may be difficult.

Key Words—Resistance, mating disruption, sex pheromone, (Z,Z)-7,11-hexadecadienyl acetate (*Z,E*)-7,11-hexadecadienyl acetate, Lepidoptera, Gelechiidae, pink bollworm, *Pectinophora gossypiella*.

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INTRODUCTION

Selection imposed by the use of synthetic sex pheromones to disrupt mating of key agricultural insect pests could result in evolutionary changes in their chemical communication systems. The potential for rapid evolution of resistance to these mating disruptants will depend in part on heritable variation in the characteristics of chemical communication such as the emission rate, the emitted blend ratio of pheromone components, or phenotypic variation in the response of males to different blend ratios of pheromone components.

An ideal subject species for studying the potential for evolution of resistance to pheromones is the pink bollworm moth, *Pectinophora gossypiella* (Saunders), because its communication system has been examined extensively and certain populations have been exposed to mating disruptants for many generations. The sex pheromone of *P. gossypiella* consists of two components (*Z,Z*)- and (*Z,E*)-7,11-hexadecadienyl acetate (*ZZ*7,11–16:Ac and *ZE*7,11–16:Ac, respectively) (Hummel et al., 1973; Bierl et al., 1974). A blend of these components centered about a 1:1 *ZZ*/*ZE* ratio has been used by many cotton growers in the desert Southwest to disrupt mating of this species since the first commercially available mating disruptant was registered by the U.S. Environmental Protection Agency in 1978 (Doane and Brooks, 1981). Recently, Haynes et al. (1984) reported that these components were emitted from the sex pheromone gland in a blend ratio of 61:39 *ZZ*:*ZE*7,11–16:Ac, and Linn and Roelofs (1985) found that male *P. gossypiella* responded optimally to *ZZ*/*ZE* blend ratios between 60–65:40–35 at low emission rates of these components. Haynes et al. (1984) examined populations of *P. gossypiella* throughout southern California for evidence of resistance to sex pheromones used as mating disruptants. No differences in the emission rates or blend ratio of these pheromone components were documented in a comparison of females from fields with a long history of pheromone treatments to females from fields that received principally insecticide treatments. While these results were encouraging for continued effective use of mating disruption to control this species, it was important to expand this study in three critical ways. First, there was a need to continue this sampling for several years in case resistance to sex pheromones could not be detected in local populations because of migration among these populations. This procedure might establish a temporal record of changes in the communication system. Second, by sampling throughout the much broader geographical distribution of this species, in this case the worldwide distribution, one might detect differences between these populations that could correlate to the general pattern of use of mating disruptants in an area. In addition, this study of geographical variation in the emission rate and blend ratio might establish whether changes in the communication system of this species have occurred over the much longer period of time in which the species range has expanded. Third,

since heritable variation in the response of males to blend ratios could accelerate significantly the development of resistance, an analysis of phenotypic variation in the behavioral response to blend ratios was undertaken within a single field population.

METHODS AND MATERIALS

Collection and Handling of Insects from California. Cotton bolls were collected from selected cotton fields in the three major cotton-producing valleys in southern California (Coachella, Imperial, and Palo Verde valleys) during August through October. In each valley an attempt was made to select fields with different histories of use of mating disruptants. When possible, fields with no exposure to disruptant pheromones (called insecticide-treated fields) and fields with three to five years of treatment with disruptant pheromones (called pheromone-treated fields) were selected. Because of prevailing agricultural practices in some valleys (e.g., crop rotation) and changing patterns of disruptant pheromone use, it was not always possible to group fields into these two categories. For example, no pheromone-treated fields were identified in Coachella Valley in 1985, and every cotton field in Imperial Valley was treated with disruptant pheromone in 1982. Between 1000 and 3000 cotton bolls were collected in each field, and the bolls were transported to Riverside, California, where they were stored in screened cages in a lath house. Handling of cotton bolls and emerging insects was identical for all four years of the experiment and followed the protocol detailed by Haynes et al. (1984).

Laboratory *P. gossypiella* were reared on shredded wheat-germ diet in half-gallon cartons (Haynes et al., 1984). The procedure for handling the laboratory-reared pupae and adults was identical to that followed for field-collected insects. The laboratory population originated from insects collected before 1976 from fields in Coachella Valley.

Collection and Handling of Insects from Around the World. Collaborators in Argentina, Brazil, China, Egypt, Mexico, and Pakistan shipped field-collected last-instar larvae or pupae by the quickest practical method to the quarantine facility in the Division of Biological Control, Department of Entomology. At this facility pupae were separated according to sex. Adult females that had emerged within a 24-hr period were taken out of the quarantine facility in individual cages that were then housed in an environmental chamber with laboratory-reared females.

Collection and Quantification of Emitted Pheromone. Pheromone was collected from individual female *P. gossypiella* during their peak period of calling during the last half of the scotophase, following the procedures described by Haynes et al. (1984). The female's wings were folded back over her head and

she was inserted abdomen-first into a 2.0-mm-ID glass tube with a 0.5-mm (diam.) hole at the distal end. This hole was large enough to allow only the ovipositor and associated pheromone gland to emerge when light pressure was applied to the female's head with a pipe cleaner. The glass tube was then inserted through a Teflon-coated GLC septum into the collector. Volatiles emitted from the gland's surface were collected for 10 min (at ca. 25°C). An internal standard [3.0 ng of (*Z*)-7-hexadecenyl acetate (*Z*7–16:Ac) in 5 μl of CS_2] was added to the glass wool before the inside of the collector was rinsed with ca. 200 μl of CS_2. This volume of CS_2 was reduced under a nitrogen stream to ca. 6 μl before it was pulled up into a 10-μl syringe for injection onto the GLC column.

It was critical that the internal standard remained at a constant concentration throughout the four years of the experiment. We ensured this consistency by maintaining several reference standards of *Z*7–16:Ac that were not used as internal standards, but were compared by GC analysis to the internal standard on a daily basis. In this way a change in the concentration of the internal standard of a few percent was easily detected. An increase in concentration occurred during repeated opening of the vial containing the internal standard. An internal standard was discarded as soon as the concentration change was detected. All standards were stored at −20°C. Before each year's collections, a set of new standards were prepared from >98% pure *Z*7–16:Ac and were compared to the previous years'. No changes in concentration of the standards were noticed during storage between years.

Analyses were made on a Varian 3700 gas chromatograph equipped with a hydrogen flame detector, a Hewlett-Packard 3380A integrator, and a Silar 10C packed column (ca. 4 g of 10% Silar 10C on acid-washed 100–120 mesh Chromosorb W; glass column 3 m or 4 m; oven temperature ca. 175°C; N_2 flow rate of 30 ml/min). Over the four-year course of the experiment, several columns were used. Consistency in the ability of the columns to separate the pheromone isomers was checked by analyzing a standard that contained 2 ng *ZZ*7,11–16:Ac and 1.33 ng *ZE*7,11–16:Ac in 5 μl of CS_2 (60% *ZZ*7,11–16:Ac). The column was replaced or repacked if accuracy was lost in determining the blend ratio of this standard.

The amount of each isomer was calculated from a standard curve relating peak to mass. These values were corrected for recovery efficiency by standardizing the measurements relative to the internal standard. The lower analytical limit of our technique was ca. 0.1 ng (0.01 ng/min).

Variation in Males' Responses to Different Blend Ratios. Synthetic *ZZ*- and *ZE*7,11–16:Ac were purchased from Scentry Inc. (Buckeye, Arizona) and were determined to be greater than 93% free of other sex pheromone-like volatiles by GLC analysis. *ZZ*7,11–16:Ac contained as much as 4% *ZE*7,11–16:Ac. *ZE*7,11–16:Ac contained as much as 3% *ZZ*7,11–16:Ac. Hexane solutions of four blend ratios (38:62, 48:52, 58:42, and 68:32 *ZZ*:*ZE*) were

prepared taking into account the cross-contamination of the original materials. Using a pheromone collection device, it was determined that the emitted blend ratios from rubber septa treated with 5 mg of the two acetates were within 1% of the desired 40:60, 50:50, 60:40, and 70:30 blends of *ZZ*- and *ZE*7,11-16:Ac. Interestingly, the emitted blend ratio was always slightly biased towards the *ZZ* isomer relative to the blend loaded onto the rubber septum. The two pheromone components together were emitted at a rate of ca. 1.8 ng/min.

Sixty-four modified traps for marking males with fluorescent powders were deployed in a cotton field in Coachella Valley, California, during September 1984. These marking stations were evenly spaced in an 8 × 8 grid with 20 m between traps, and there was at least 40 m between every trap and the edge of the cotton field. The 64 marking stations consisted of 16 quartets arranged in a square. Each of the four blend ratios was used to bait a single marking station within each quartet.

The marking stations were cylindrical metal traps (height 13.5 cm, diam. 25 cm) with four 12.5-cm (width) by 3.5-cm (height) holes spaced evenly around the midline of the cylindrical surface. Each station contained a paper plate (25 cm diam.) that fit into the trap's bottom below the level of the holes. About 50 ml of fluorescent powder (Day-Glo Color Corp., Cleveland, Ohio) were spread evenly over the plate's surface. Four colors of fluorescent powder were used: Aurora Pink for the 40:60 blend, Horizon Blue for the 50:50 blend, Saturn Yellow for the 60:40 blend, and Blaze Orange for the 70:30 blend of the *ZZ* to *ZE* isomers. A pheromone-impregnated rubber septum was fixed with a color-coded straight pin to a cork that was in turn stapled to the paper plate.

Traps that were used to permanently capture males were similar to the marking stations, except paper plates coated with Stickem Special replaced the plates covered with fluorescent powder. Both marking stations and traps were placed at a height of about 1 m with three 1.2-m-long redwood stakes. Traps were placed at the center of each quartet of marking stations. Marking stations were only baited on the first, third, and sixth nights of the experiment, and traps were baited on the second, fourth, fifth, seventh, and eighth nights; thus marking stations and traps were not baited at the same time. Positions of traps were rerandomized before each trapping night of the experiment.

RESULTS

Comparison of Populations from California Valleys and Laboratory over Four Years. The emission rate of *ZZ*7,11-16:Ac from field-collected females was significantly higher during the last two years of the experiment (1984 and 1985) than during the first two years (1982 and 1983) (Table 1). On average a field-collected female emitted 19.3% more *ZZ*7,11-16:Ac during 1985 (0.117

TABLE 1. EMISSION RATE OF ZZ7,11-16:Ac (ng/min) FROM THREE VALLEYS IN CALIFORNIA AND LABORATORY COLONY FOR 1982–1985

	$\overline{X}$ Emission rate (ng/min) of ZZ7,11-16:Ac + SD (N)			
	Coachella	Imperial	Palo Verde	Laboratory
1982	0.086 ± 0.049 (47)cde[a]	0.099 ± 0.054 (145)bc	0.100 ± 0.056 (216)bc	0.094 ± 0.050 (52)cd
1983	0.082 ± 0.033 (76)cde	0.074 ± 0.031 (54)e	0.092 ± 0.042 (227)cde	0.080 ± 0.046 (80)de
1984	0.115 ± 0.041 (90)ab	0.117 ± 0.037 (100)a	0.131 ± 0.046 (77)a	0.085 ± 0.049 (76)cde
1985	0.120 ± 0.034 (35)a	0.119 ± 0.042 (62)a	0.114 ± 0.049 (101)ab	0.090 ± 0.054 (83)cde

[a] Any means followed by the same letter are not significantly different ($P > 0.05$, analysis of variance and Duncan's new multiple-range test).

ng/min) than during 1982 (0.098 ng/min). The difference between field-collected females and the laboratory-reared population was greatest in 1984 when the former (0.120 ng/min) emitted 41.2% more ZZ7,11-16:Ac than the latter (0.085 ng/min). Unlike the results of analyses of volatiles from field-collected females, the emission rate of pheromone did not change from year to year in the laboratory population.

The blend ratio of the two pheromonal components showed no significant trend towards increasing or decreasing over the course of our sampling in either the field populations or the laboratory population (Table 2). No valley consistently had females that emitted a higher or lower blend ratio than those from the other valleys or the laboratory. The significant differences that were documented between valleys were small—the range of mean percent ZZ7,11-16:Ac was only 60.1–62.9.

For the subsample of fields that could clearly be separated according to their history of pheromone use, there was no relationship between this history and the emission rate or blend ratio of components (Table 3).

Worldwide Variation in Chemical Communication. The highest emission rate of ZZ7,11-16:Ac was found in females from Californian populations (0.119 ng/min), followed by a population from Egypt (0.103 ng/min) (Figure 1). The average emission rate from females from a Chinese population was 0.057 ng/min, and was significantly lower than the other populations with the exception of that from Brazil, which was intermediate (0.082 ng/min).

The Chinese population was also unusual in terms of the emitted blend ratio of pheromonal components (Figure 2). Females from this population emitted 57.5% ZZ7,11-16:Ac, significantly lower than all other populations sam-

TABLE 2. EMITTED BLEND RATIO OF TWO PHEROMONE COMPONENTS EXPRESSED AS PERCENT OF ZZ7,11-16:AC ISOMER IN TWO-COMPONENT BLEND OF FEMALES FROM THREE VALLEYS IN CALIFORNIA AND LABORATORY COLONY FOR 1982–1985

	$\bar{X}$ % ZZ7,11-16:Ac ± SD (*N*)			
	Coachella	Imperial	Palo Verde	Laboratory
1982	60.4 ± 4.2 (45)e[a]	62.4 ± 5.4 (118)a	62.1 ± 4.0 (192)ab	61.0 ± 3.8 (51)bcde
1983	61.0 ± 2.9 (75)bcde	60.6 ± 3.2 (54)de	60.8 ± 3.3 (217)de	60.6 ± 3.5 (77)de
1984	61.8 ± 1.8 (90)abcd	61.8 ± 2.0 (100)abcd	62.2 ± 2.1 (77)abc	61.0 ± 2.8 (72)bcde
1985	60.1 ± 1.7 (35)e	60.1 ± 2.5 (62)e	62.9 ± 2.6 (98)a	60.9 ± 2.9 (76)cde

[a] Any means followed by the same letter are not significantly different ($P = 0.05$, analysis of variance of arc sin $\sqrt{\%\ ZZ}$ transformed data and Duncan's new multiple-range test).

TABLE 3. EMISSION RATE AND BLEND RATIO OF PHEROMONE COMPONENTS FROM FEMALES FROM PHEROMONE-TREATED AND INSECTICIDE-TREATED FIELDS

	Emission rate[a] ± SD (*N*)		Blend ratio[b] ± SD (*N*)	
	Pheromone-treated	Insecticide-treated	Pheromone-treated	Insecticide-treated
1982	0.095 ± 0.056 (252)	0.102 ± 0.053 (156)	61.7 ± 4.2 (218)	61.9 ± 5.0 (137)
1983	0.087 ± 0.040 (183)	0.087 ± 0.038 (174)	61.0 ± 3.2 (183)[c]	60.3 ± 3.2 (174)[c]
1984	0.127 ± 0.044 (109)	0.117 ± 0.036 (68)	61.6 ± 2.2 (109)	61.9 ± 2.0 (68)
1985	0.114 ± 0.044 (77)	0.118 ± 0.045 (121)	61.2 ± 3.2 (77)	61.7 ± 2.5 (118)

[a]Emission rate is ng of *ZZ*7,11-16 : Ac emitted per minute.
[b]Blend ratio is % *ZZ*7,11-16 : Ac in two-component blend.
[c]Significantly different pair of means ($P < 0.05$, Analysis of variance).

pled. The overall range in mean percent *ZZ*7,11-16 : Ac emitted was small (57.5-63.1).

Variation in Males' Response to Different Blend Ratios. Over the course of this experiment we captured 13,303 male *P. gossypiella*, of which 331 (2.5%) were marked at least once. The 70 : 30 blend of *ZZ*- to *ZE*7,11-16 : Ac captured significantly fewer moths than the 40 : 60 blend, and there were no other significant difference between blends. Because these traps were approaching their numerical capacity to capture and retain males, the absolute numbers captured are not useful. However, the proportion of males marked with fluorescent powders and subsequently captured should provide an accurate estimate of the proportion of males that actually visited the marking station.

Fluorescent marks were detected in the following proportions: 100 pink, 94 blue, 101 yellow, and 82 orange (pink = 40 : 60, blue = 50 : 50, yellow = 60 : 40, and orange = 70 : 30 *ZZ* : *ZE*7,11-16 : Ac). A 4 × 4 chi-square analysis revealed that there was no significant relationship between marking and recapturing at specific blend ratios ($\chi^2 = 4.2$, 9 *df*). Figure 3 illustrates that some individuals that had responded to specific marking blends were subsequently recaptured in every capture blend. Fourty-two males (0.3% of total number of males captured) were marked with two or more colors. They were captured in the following numbers 10 P/B, 5 P/Y, 5 P/O, 6 B/Y, 7 B/O, 9 Y/O. Of these males, 20 were captured at a blend that was different from any to which they were marked, indicating that they had responded to at least three blends. Two males were marked at three different blend ratios, and one of these had visited every blend ratio that was available when the capture blend was considered.

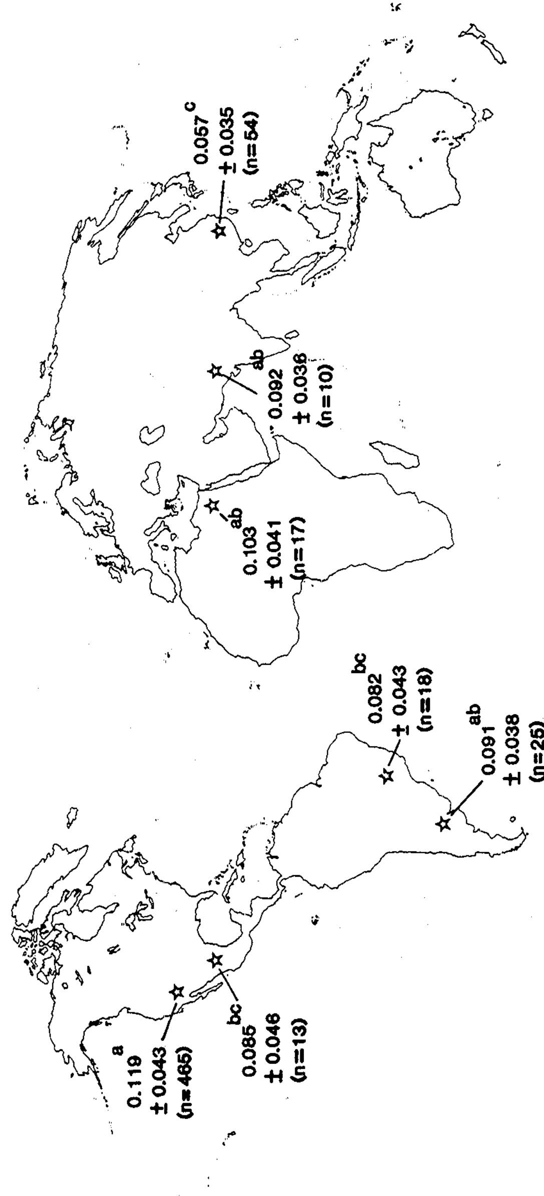

FIG. 1. Worldwide variation in the emission rate of *ZZ*7,11-16:Ac from the sex pheromone glands of female *P. gossypiella*. Means followed by the same letter are not significantly different ($P > 0.05$, analysis of variance and Duncan's new multiple-range test).

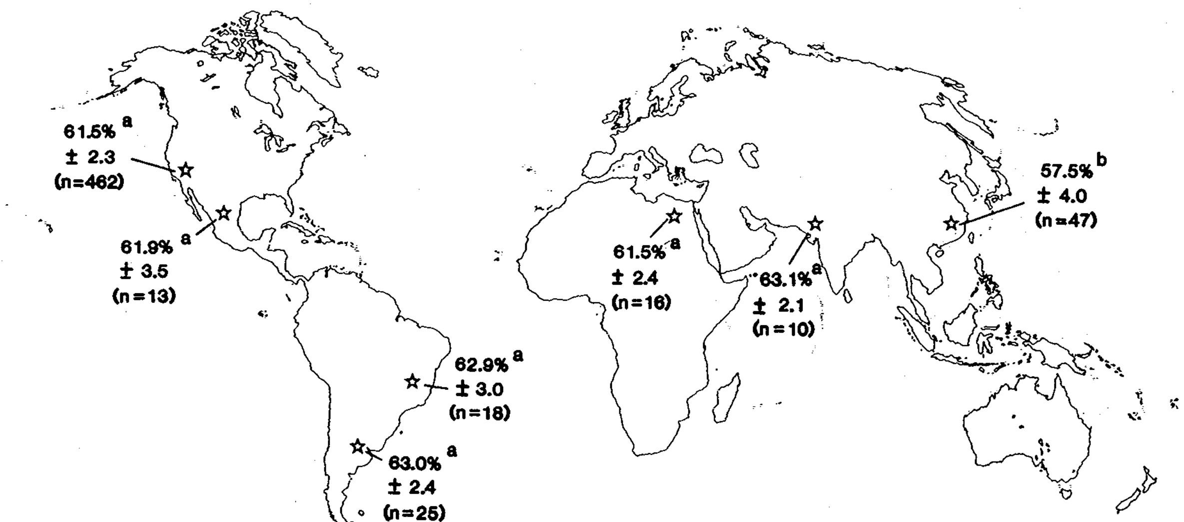

FIG. 2. Worldwide variation in the emitted blend ratio of *ZZ*7,11-16:Ac and *ZE*7,11-16:Ac expressed as % *ZZ*7,11-16:Ac, from female *P. gossypiella*. Means followed by the same letter are not significantly different ($P > 0.05$, analysis of variance of arc sin $\sqrt{\%\ ZZ}$ and Duncan's new multiple-range test).

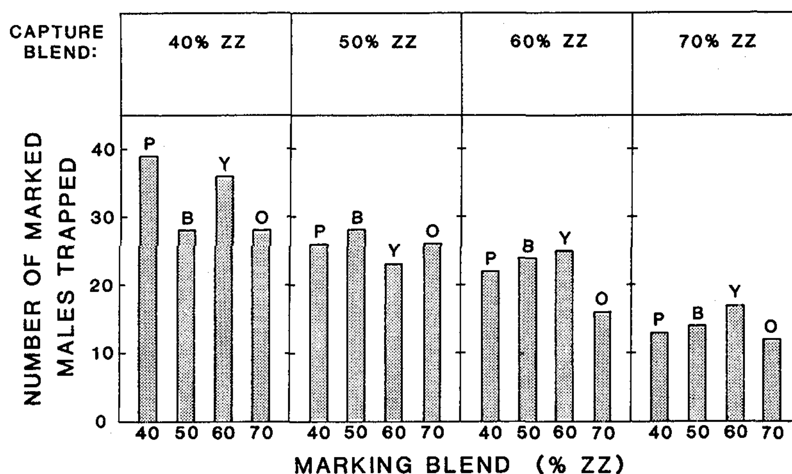

FIG. 3. The number of male *P. gossypiella* captured at indicated blend after being marked with fluorescent powders. Fluorescent marks established record of prior responses to specific blend ratios of pheromone components (P = pink = 40:60; B = blue = 50:50; Y = yellow = 60:40; O = orange = 70:30 *ZZ*- to *ZE*7,11-16:Ac).

DISCUSSION

The present study documents biological (phenotypic) variation in pink bollworm populations from southern California that has occurred over the four-year course of this experiment. Our data are not sufficient to unequivocally support the hypothesis that selection imposed by the use of mating disruption caused these changes. Rather this hypothesis should be considered as but one of a number of viable explanations, including undocumented changes in the environment with no underlying genetic change, selection imposed by some unknown factors that have resulted in the documented phenotypic change, or genetic drift with no underlying selection pressure. Regardless of the cause(s), however, the populations in 1984 and 1985 contained females emitting higher average amounts of pheromone and should have been, at least hypothetically, slightly less susceptible to the effects of synthetic disruptants than populations in previous years. We believe that any such advantage gained by females in these populations would have been too slight to result in significant loss of efficacy of the disruptant pheromone, and thus should not have had any major effect on overall population growth.

There is some indirect support for the idea that the increase in emission rate of pheromone in southern California in 1984 and 1985 could be related to the use of synthetic sex pheromone to disrupt mating. First, pheromones have been used there on a commercial basis for control of the pink bollworm moth

for over eight years. Second, field documentation of a possible selective advantage for a higher emission rate from point sources in disruptant-treated field was supplied by Doane and Brooks (1981), who showed that increasing emission rates of synthetic pheromone (*ZZ*- and *ZE*7,11–16:Ac) from traps lead to increasing captures of male moths in pheromone-treated fields. Third, in the laboratory, Collins and Cardé (1985) showed that the heritability of pheromone quantity was slightly greater than for blend quality, and they have been able to select for a line of *P. gossypiella* in which the females contain almost twice as much *ZZ*- and *ZE*7,11–16:Ac in their sex pheromone glands (personal communication). Thus the potential for a response to selection has been demonstrated in the laboratory.

One observation is not necessarily consistent with the hypothesis of selection for increased emission rate in field populations as a response to use of disruptant pheromone. In 1982 an areawide program was in effect in Imperial Valley in which all cotton growers utilized pheromone applications for control of *P. gossypiella* for part of the growing season. This program represented one of the most extensive uses of disruptant pheromones in agriculture to date, but it did not result in an immediate increase in pheromone emission rate from field-collected females. In fact, the emission rate from females collected in Imperial Valley in 1983 was 0.074 ng/min, the lowest average that was recorded.

Unlike the phenotypic variation in emission rate that was documented in field populations, the emission rate of *ZZ*7,11–16:Ac from laboratory females remained relatively constant throughout the four-year period. This consistency in the laboratory population was expected because of the constancy of environmental conditions and the lack of selection pressure for increased emission rate under laboratory conditions.

Based on our first year of sampling (Haynes et al., 1984), we anticipated that if any change occurred in the blend ratio, it would be an increase in the *ZZ* to *ZE* ratio, because a blend centered about a 50:50 ratio has been used as the standard commercial blend to disrupt mating, whereas females emit ca. a 60:40 blend. This could mean that directional selection might be imposed on the pheromone blend. However, in the present study the blend ratio of *ZZ*- to *ZE*7,11–16:Ac was relatively constant throughout the period of the study (Table 2). Selection on the emitted blend ratio may be more difficult because it would involve changes in both males and females, unless there was close genetic linkage between emission and response.

Our analyses of pheromone components emitted by females from around the world was consistent with greater interpopulational variation in the emission rate than in the blend ratio of components. While the blend ratios emitted by females from China were significantly lower that those from any other locality, the overall difference was small. In contrast, females from China emitted about half as much pheromone as those from southern California. It would be inter-

esting to obtain pink bollworm moths from Australia because *P. gossypiella* and *P. scutigera*, the pink-spotted bollworm, are sympatric in certain areas of this country. The two species respond to different blend ratios of *ZZ*- and *ZE*7,11-16:Ac (Rothschild, 1975), and thus interspecific selection pressure may have led to divergence of their blend ratios or to a narrowing of the broad response spectrum of males to blend ratios of the pheromone isomers. Our initial attempts to obtain sufficient numbers of moths from Australia were not successful because cotton production no longer occurs in areas where *P. gossypiella* occurs on wild plants (S.E. Learmonth and G.H.L. Rothschild, personal communication).

If selection is resulting in the changes in the pheromone communication system of *P. gossypiella*, then why are we unable to detect these differences between pheromone- and insecticide-treated fields in southern California? One possibility is that local differences between populations may be swamped by migration between fields within a valley and possibly between valleys. While the potential impact of migration on gene flow has not been evaluated in any quantitative way, the occurrence of such migration is well documented (Bariola et al., 1973; Flint and Merkle, 1981; Stern, 1979).

Since our marking–capture analysis of variation in the response of males to blend ratios from 40:60 to 70:30 indicated that there was no evidence of phenotypic variation between individuals, the potential for rapid selection for a preadapted phenotype that responds specifically to the blend released by the female and not to the blend used to disrupt mating seems unlikely. The major source of variation in the response of males to blends comes from variation within individuals rather than between individuals. It would seem that in order for resistance involving blend ratios to evolve rapidly, the population would need to begin with males having narrower blend ratio response spectrums than we measured here. Cardé et al. (1976), who first applied this marking–capture technique to the Oriental fruit moth, *Grapholita molesta*, also found no evidence of phenotypic variation in the response of males to different blends of the pheromone components of that species.

Considering the results of our experiments, it is possible that the observed increase in the females' emission rate of pheromone may be an early response to selection imposed by the use of disruptant pheromones, but other undocumented factors may play a role in this biological variation that has been followed for four years. Evolutionary changes in the blend ratio may be inherently more difficult because males have a broad response spectrum to variation in blend ratio (Flint et al., 1979; Linn and Roelofs, 1985) with no detectable phenotypic variation between individuals. Even so, shifts in the emitted blend ratio and the responses of males to blend ratios should continue to be considered as a possibility that may be expressed in individuals in field populations over evolutionary time.

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