Pink Bollworm[1]: Response of Native Males to Ratios of *Z,Z*- and *Z,E*-Isomers of Gossyplure in Several Cotton Growing Areas of the World[2,3]

H. M. FLINT[4], M. BALASUBRAMANIAN[5], J. CAMPERO[6], G. R. STRICKLAND[7], Z. AHMAD[8], J. BARRAL[9], S. BARBOSA[10], AND A. F. KHAIL[11]

ABSTRACT

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A standard test consisting of 7 ratios of the *Z,Z*-, and *Z,E*- isomers of gossyplure was conducted in several major cotton producing areas of the world to determine the pheromone preference of *Pectinophora gossypiella* (Saunders). Catches in traps baited with 50 or 60% of the *Z,Z*- isomer in mixtures of *Z,Z*-, and *Z,E*- isomers were not significantly different in 5 of 7 areas (India, Australia, Pakistan, Argentina, and the U.S.). Catches by the 50% baits were significantly higher in Brazil, and catches with the 60% baits were significantly higher in Egypt. Therefore, baits with either 50 or 60% *Z,Z*- isomer will be useful in survey or control work throughout most of the area of distribution of pink bollworm.

When the test was conducted several times during the season in Australia and Pakistan, catches indicated no shift from a statistically equal preference for baits containing 50 or 60% of the *Z,Z*- isomer.

Pexicopia sp., a genus related to *Pectinophora*, was captured primarily in traps containing 20% *Z,Z*- isomer and 80% *Z,E*- isomer at Kununurra, N.W. Australia. The genera may be isolated from each other by pheromonal preferences in those areas where the distributions overlap.

The pink bollworm, *Pectinophora gossypiella* (Saunders), is an economic pest of cotton with world-wide distribution. The moth was originally described in 1843 from specimens collected in India. It was reported as a pest of cotton in Africa in 1904 (Noble 1969) and has been distributed to all major cotton-producing areas of the world. The pink bollworm entered Mexico in 1911 in seed imported from Egypt and entered the U.S. in 1917 in Texas.

Female pink bollworms produce a sex pheromone that is attractive to male moths (Ouye and Butt 1962). It was identified as a 1:1 mixture of the *Z* and *E* geometrical isomers of 7,11-hexadecadien-1-ol acetate by Hummel et al. (1973) who proposed the name gossyplure. Flint et al. (1977) reported that a mixture containing 60% of the *Z,Z*- isomer and 40% of the *Z,E*- isomer was more attractive early in the season than the 1:1 ratio, and this finding was confirmed in studies of the seasonal response of native and released laboratory-reared moths (Flint et al. 1978b).

Tests in Australia (Rothschild 1975) indicated that the pink bollworm responded better to the 1:1 ratio than to a 10:1 ratio; however, intermediate ratios were not tested. Marks (1976) reported that the response of the pink bollworm to a wide range of ratios of *Z,Z*-, and *Z,E*- isomers in Malawi showed preference for mixtures containing 55–60% of the *Z,Z*- isomer. Similar results were obtained by Boness (1975) in Spain. These are the only complete tests of ratios outside the United States of which we are aware.

Australian species of *Pectinophora*, including the pink bollworm, appear to be reproductively isolated by variations of the ratios of *Z,Z*-, to *Z,E*- isomers of gossyplure (Rothschild 1975). The *Z,E*- isomer of gossyplure was identified by Vick et al. (1974) as the primary pheromone of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). Widely distributed "pheromonal phenotypes" (Klun et al. 1975) or sibling species (Cardé et al. 1978) of the European corn borer use various blends of 2 component pheromone for communication. Among these tortricine moths attraction specificity appears to be a primary mechanism for species partitioning (Minks et al. 1973, Cardé et al. 1977). Thus, considerable divergence can occur in pheromone communication systems among closely related species, and there is divergence even within species that have been geographically or otherwise isolated. These studies indicate the necessity for considering pheromonal variability in programs in which pheromones are to be used for either survey or control of widely distributed pests.

In the case of the pink bollworm, recent development of control techniques such as confusion (Gaston et al. 1977) and trapping (Huber et al. 1979) plus the need to survey and monitor populations have made it necessary to determine pheromonal response of pink bollworm males in cotton producing areas outside of the U.S. We report here the results of such determinations in several areas of the world.

Materials and Methods

General Methods

A standardized trap test for the pink bollworm was conducted in several parts of the world. The standard test consisted of 20 traps of each of 7 mixtures of the *Z,Z*-, and *Z,E*- isomers of gossyplure (20, 30, 40, 50,

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[4] Western Cotton Research Laboratory, Agric. Res. SEA, Phoenix, AZ 85040.
[5] Dept. of Agricultural Entomology, Agricultural College and Research Institute, Coimbatore 641 003, India.
[6] Jefe Seccion de Entomologia, UNDP/FAO, Av. de Circunvalacion, Casilla 1281, Santa Cruz de la Sierra, Bolivia.
[7] Dept. of Agric., O.O. Box 19, Kununurra, Western Australia 6743.
[8] Cotton Research Institute, Ministry of Food, Agric., and Under Developed Areas, Multan, Pakistan.
[9] I.N.T.A. Estacion Experimental, Casilla de Correo No. 164, 3700 Peia. Roque Saenz Pena, Chaco, Argentina.
[10] Centro Nacional de Pesquisa do Algodao, Divisao de Improtacao da EMBRAPA, Super Center Venancia 2.000 - 8. ander, 70,000 - Brasilia - Df Brasil.
[11] Sakha Agric. Res. Station, Kafr El Sheikh, Egypt.

60, 70, and 80% of the *Z,Z-* isomer). All test materials except the stakes for hanging the traps were prepared in Phoenix and immediately air mailed to the cooperators for placement in the field. Test traps were usually exposed for 2 weeks or until a catch of ca. 50 moths/trap was obtained in the traps with the greatest catches. The arrangment of the traps in fields was determined by the cooperators, but specifications required a minimum of 30 m between traps and that a randomized block design be used in placement of the 7 treatments.

The traps used at all locations were Delta traps (Tetron Inc., Newark, NJ) originally designed for survey of the gypsy moth, *Lymantria dispar* (L.). Baits were made from rubber septa (no. 1F-66F red rubber septa, the West Co., Phoenixville, PA) treated with 1000 μg of lure dispensed in 50μliters of methylene chloride (dichloromethane) (Flint et al. 1974, Maitlen et al. 1976). The lure and solvent were placed in the up-turned cup of the septa and allowed to penetrate the rubber, usually within 5 min. Baits prepared in this manner are effective in the field for several months (Flint et al. 1978a). The *Z,Z-*, and *Z,E-* isomers of gossyplure used in the tests were obtained in a single lot in 1976 from Farchan Chemical Co., Willoughby, OH. These isomers were bioassayed in field tests with isomers of known purity (greater than 97% purity of isomers) obtained from R. T. Staten (Animal and Plant Health Inspection Services, Phoenix, AZ) and trap catches were not significantly different. These isomers also were used in other studies conducted during 1976–78 (Flint et al. 1977, 1978a, b, Smith et al. 1978).

Results were analyzed by analysis of variance (AOV) using a program that provided for missing data where necessary (missing data were infrequent). When a significant AOV was obtained, a Duncan's multiple range test was used to partition the means into significant ranges.

Samples of trapped moths from India and Bolivia were submitted to the Systematic Entomology Laboratory, Agric. Res., SEA, USDA, Beltsville, MD, for positive identification. Moths captured in Australia were identified by the cooperating author.

Specific Tests

In Argentina, the standard test was placed in a cotton field at Chaco on Feb. 2, 1978, and left in place for 1 week. The cotton, 'Toba II', a local short-staple variety, was planted Sept. 15, 1977, and was at the 1st picking stage by the test date (September–May production season). Populations of adult pink bollworms are abundant during February and March (before the initiation of diapause) at this location. The test was repeated at the same location 1 month later, Mar. 9–14.

In Bolivia, the standard trap tests were conducted during the period February–May 1976, at Abapo-Izozog, Santa Cruz, an area that is semiarid (10 cm precipitation/year) and is used for commercial production of cotton. During the test period, the mean temperatures ranged from 20° to 28°C, and maximums reached 40°C. The standard test was conducted at mid-season on 12-ha of commercial short staple cotton with Huber traps (Flint et al. 1976) since Delta traps were not available. The 2nd test was conducted with Delta traps over a 6-week period later in the season in the same area. Moths from traps containing 20, 50, or 80% *Z,Z-* isomer were returned to the U.S. for identification.

In India, the standard test was conducted in commercial short staple cotton at Coimbatore on Nov. 21, 1976. The traps were checked weekly for a period of 4 weeks. The temperatures in the test area were very cool during December, and moth populations declined sharply as the growing season was terminated. Moths from traps containing 50% *Z,Z-* isomer were returned to the U.S. for identification.

In Brazil, the standard test was conducted July 12–19, 1978, mid-season in the test area. During this season temperatures never went below 20°C, and rains were frequent. The cotton was cultivated perennial variety *Gossypieum hirsutum L.* var. *marie-galante* (Watts) J. B. Hutch in its 2nd year of growth. The plants had squares, flowers, and bolls.

In Pakistan, during the 1977 season, 5 traps for each ratio were placed in a 2-ha field planted with *Gossypium hirsutum* var. 149F. The traps were checked weekly and replaced each month June–October 1977. When the traps were set out June 8, the cotton was the earliest available in an area where cotton farming is intensive, and application of insecticides had begun (for control of thrips). Five traps/ratio were replaced each month. The field was treated with insecticides at irregular intervals during the test period.

In 1978, the standard ratio test was conducted in fields of B. 557 variety. The test was begun July 13 and terminated Aug. 17. Traps were checked and cleaned weekly. The same traps and baits were used throughout the test period.

In Australia, the standard test was conducted at Kununurra in the Ord Irrigation District, Western Australia, during the 1977 season. At this time, commercial cotton production had come to an end in the area because of uncontrollable infestations of *Heliothis armigera* (Hübner). However, volunteer cotton was available in mixed stands with weeds. The test was conducted during the period Mar. 23–30 when the cotton had flowered and pink bollworms were available. The traps were then maintained weekly through May 25. The 10-week test period included the end of the monsoon season and the commencement of the dry season. During this period, 111 mm of precipitation were recorded, and mean temperatures ranged from 23°–31°C. Catches of *Pexicopia* sp., a genus containing some 20 species in Australia that is closely related in *Pectinophora* (Common 1958), also were recorded.

In Egypt, 2 ratio tests were conducted at Kafr El Sheikh during the 1978 season. A preliminary test with 5 traps/ratio was conducted June 20–July 3 during the earlier part of the blooming period. The standard test with 20 traps/ratio was conducted July–Aug. 11. Both tests were made in a 15-ha field planted with medium long staple cotton ('Giza 75') ca. Apr. 1. This field received no treatments of insecticide because boll infestations were low (1–3%) throughout the trial.

In the U.S., the standard ratio test was conducted Aug. 7, 1978, in short staple cotton ('Deltapine') at Phoenix, AZ. (Because the moth populations were so large, the traps were in the field for 1 night only). The

date was ca. mid-season in Arizona; the field received no insecticidal treatments.

Results

General Results

The summary of the results of the standard trap tests (Table 1) indicates that catches in traps baited with 50 or 60% of the *Z,Z-* isomer in mixutures of *Z,Z-*, and *Z,E-* isomers were not significantly different in 5 of 7 countries (India, Australia, Pakistan, Argentina, and the U.S.). The 50% baits caught significantly more of the moths in Brazil, and the 60% baits caught significantly more in Egypt. Generally, the preference for the 50 and 60% baits was clearly defined.

Specific Results

In Argentina, the results of the standard test indicated equal preference for baits containing 50 or 60% *Z,Z-* isomer (Table 1). Also, the results of the 2nd test late in the season (March) showed the same preference: average catches were 146.5 and 136.0 total moths/trap in traps with 50 or 60% baits, respectively (no seasonal differences between tests).

In Chaco, Bolivia, male pink bollworms were apparently more responsive to all ratios than they were elsewhere. The catches were 26a, 30ab, 32ab, 34bc, 40c, 32ab, and 33abc, for the 20-80% *Z,Z-* isomer baits, respectively, in the standard test. Thus, a majority of the test ratios was as preferred as the 50% baits. The explanation for the situation in Bolivia was found when traps baited with the 20, 50, or 80% *Z,Z-* isomer were used to collect moths for identification. All moths taken in traps with 50% baits were *P. gossypiella*. Moths taken in traps containing the 20 and 80% baits were not *P. gossypiella*. At the time of this writing, they remain unidentified, but several closely resemble and would easily be taken for *P. gossypiella*.

Only data for the 1st week of trapping at Coimbatore in India are presented in Table 1. Catches in the same traps during the next 3 weeks were greatly reduced (avg of 2 moths/trap for 60% baits during the 4th week). There was no significant difference between 50 or 60% baits in any of the weekly totals. Over the 4 weeks, 25 and 31% of the total males were taken in traps with the 50 or 60% baits, respectively. Samples of captured males were determined to be *P. gossypiella* by the Systematic Entomology Laboratory. (During the 1st 2 weeks of trapping, large numbers of *Melipona* bees were found in some traps but catches were not correlated in any particular test bait.)

In Pakistan, the results of the standard test indicated a distinct preference for 50 and 60% baits (Table 1). Also, the monthly tests conducted June–October 1977, showed no significant differences between these baits with the exception of July when the 50% baits were significantly preferred. We therefore saw no consistent preference for either the 50 or 60% baits in the tests in Pakistan.

Data for the standard test for 1 week in Australia indicated an equal preference for 50 and 60% baits and a strong preference for these baits over baits containing other proportions of the 2 isomers (Table 1). This pattern persisted during additional tests during the months of March, April, and May. Table 2 reports numbers of *Pexicopia sp.* captured in traps baited with 20 or 30% *Z,Z-* isomer. *Pexicopia* feed in the seed capsules of Malvaceae and are common in *Sida* and other malvaceous weeds, so the weediness of the test site undoubtedly accounts for their abundance in the traps. Populations of both *Pexicopia* and the pink bollworm declined similarly during the test period. A preference of *Pexicopia* for baits containing maximum amounts of the *Z,E-* isomer (80% *Z,E-*) was apparent, so greater catches might have been achieved with greater proportions of this isomer.

In Egypt, the preliminary test resulted in average catches per trap of 1.6c, 2.0c, 3.6c, 9.7b, 14.6a, 3.6c, and 1.2c for the 7 baits, respectively. These results were substantiated later in the season when the standard test was conducted (Table 1). In both of these tests, blank traps were included in numbers equal to the number used with each bait. The average catch in these blank traps was 0.2 moths/trap for both tests (significantly below the catch in traps containing any bait).

Discussion

The pink bollworm is thought to be indigenous to Australia (Holdaway 1929) though specimens have been identified from most cotton-producing areas of the world (Common 1958). Holdaway (1929) provided an interesting discussion of the possibility that the moth is, in fact, native to Africa, Asia, and Australia because these continents were at one time contiguous. Certainly, the pink bollworm was found in north and north-western Australia in 1911, well before commercial cotton was

Table 1.—Catches of male pink bollworm moths in Delta traps with bait containing 20–80% *Z,Z* isomers in a mixture of *Z,Z-*, and *Z,Z* isomers of gossyplure (1 mg of lure/bait, 20 traps/treatment, 2-week test period).[a]

% Z,Z- isomer in mixture	Avg Catch per trap for indicated country						
	India	Australia	Pakistan[b]	Argentina	U.S.	Brazil	Egypt[d]
20	2.2 a	4.1 a	0.0 a	14.3 a	1.8 a	0.9 a	1.0 b
30	4.6 a	3.0 a	0.5 a	31.1 a	1.4 a	1.3 a	0.8 ab
40	10.2 a	14.9 b	11.5 a	70.8 b	5.8 a	6.3 b	6.3 c
50	32.7 bc	94.8 c	47.4 b	126.2 c	22.7 b	13.4 c	6.6 c
60	44.5 c	84.3 c	61.5 b	119.0 c	22.6 b	8.3 b	7.8 d
70	18.9 ab	12.5 b	9.0 a	69.2 b	2.5 a	2.5 a	1.3 b
80	7.0 a	1.0 a	0.0 a	27.1 a	0.9 a	0.9 a	0.9 ab

[a] Means within vertical columns not followed by a common letter are significantly different at the 0.05% level.
[b] 4-week avg.
[c] 1-night catch.
[d] Avg. catch/day during 2-week period

Table 2.—Catch of *Pexicopia* sp. in pink bollworm traps during the 1977 season at Kununurra, Western Australia.

% Z,Z-isomer in mixture	Avg *Pexicopia* per trap during week ending on indicated date								
	3/23	3/30	4/6	4/13	4/20	4/27	5/4	5/11	5/18
20	4.0	11.4	9.2	6.7	1.1	1.0	0.5	0.7	0.7
30	1.0	2.2	0.3	1.7	0.5	0.2	0.1	0.3	0.0

grown there (Common 1958). It is this fact plus the wide range of native plants that are infested by the pink bollworm that have led to the theory that the pink bollworm is endemic to Australia. In addition, Australia has yielded the greatest number of related species and genera (of the areas studied). However, Wilson (1972) pointed out that trial shipments of cotton could have introduced the pest into Australia very early. Also, 1st reported finding of the pink bollworm in India (in 1849), casts some doubt on Australia as the original home of the moth because the insect is easily disseminated as diapausing larvae in cotton seed and was apparently spread by this method from India to Egypt and Mexico shortly after the turn of the century. In any case, it seems, reasonable to assume that Australia has harbored the pink bollworm and its relatives for a long time.

Our data from tests conducted in many cotton-producing areas of the world indicate a uniform response to blends of the Z,Z-, and Z,E- isomers of glossyplure. This finding might be anticipated since the pink bollworm is a comparatively recent introduction in most of these localities so pheromonal phenotypes have probably not had time to evolve.

There is also the matter of the attraction to gossyplure of related species. Common (1958) conducted taxonomic studies of *Pectinophora* and 3 related genera of Gelechiid moths in Australia. One of these, *Pexicopia*, was found in our traps (primarily in those containing 20% Z,Z- isomer) at Kununurra, N.W., Australia, where *P. gossypiella* also occurs. *Pexicopia* is recorded from the Northern Territory and northwestern Australia only, so in northwestern Australia where the genera overlap, they may be isolated by differences in their pheromonal response.

A still more closely related species, *P. scutigera* (Holdaway), infests wild malvaceous hosts throughout its distribution in the western Pacific (New Guinea, Micronesia, Hawaii) (Common 1958) but also infests cotton in central Queensland (Rothschild 1975). The adult and developmental stages of *P. scutigera* closely resemble *P. gossypiella*, and both attack the fruiting forms of cotton (Passlow 1963). However *P. scutigera* is attracted to a 10:1 ratio (Z,Z-:Z,E-) of the isomers of gossyplure and is thus isolated from *P. gossypiella* in areas where the species overlap (Rothschild 1975). Roelofs and Comeau (1969) reported that 2 morphologically similar co-occuring gelechiid moths (*Bryotropha* sp.) were likewise reproductively isolated through attraction to different geometrical isomers of 9-tetradecen-1-ol acetate. The relationship between *P. gossypiella* and *P. scutigera* appears to be comparable. Roelofs and Cardé (1974) suggest that unique pheromonal systems are of increased importance to those sympatric species for which other isolating mechanisms are not very important.

Our present data indicate that as many as 3 species of moths may have been attracted to our test traps in Bolivia. We know only that the moths captured in traps containing the 50% bait were the pink bollworm and those in traps containing 20 or 80% baits were not. We do not even know if these other moths are related to the pink bollworm. However, all moths were captured in cotton by the same pheromone components, and all, at least superficially, resemble each other, so we have to suspect some taxonomic relationship. We have not been able to pursue this study further.

On the other hand, Marks (1976) tested numerous ratios of the 2 isomers of gossyplure in Malawi, in east-central Africa. He observed significantly greater catches of pink bollwrom in traps containing baits with 55 or 60% of the Z,Z- isomer than in traps containing 50% of this isomer. However other species of moths were caught there in gossyplure baited traps, i.e., *S. cerealella* (Gelichiidae) which normally use the Z,E- isomer of gossyplure for pheromonal communication and *Catelaphris sp.* (Gelechiidae) a little known moth thought not to infest cotton. Boness (1975) conducted a ratio test in southern Spain during the 1974 season. His results indicated that baits containing 50% of the Z,Z- isomer, in mixtures of Z,Z and Z,E- isomers were comparable in attractiveness to mixtures containing 60 or 40% of the Z,Z- isomer. It was his observation that catches declined faster when the amount of Z,E- isomer was increased. Our results are in agreement with these findings.

In summary, with the standard test, there was no consistent preference for 50 or 60% baits. Also, in countries where tests were conducted over several months, there was no detectable seasonal variation. In the U.S. the pink bollworm has preferred 60% baits early and late in the season (Flint et al. 1978b). However, 50% baits have been as attractive or more attractive than 60% baits during most of the season, especially during the periods when survey trapping is most often practiced. Our present results indicate that the 50:50 blend of Z,Z- and Z,E- isomers of gossyplure could be used successfully in major cotton-producing areas of the world.

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