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This issue mailed September 30, 1988

Fall Armyworm Symposium

PREFACE

NOTE: Reprints of each paper of the Fall Armyworm Symposium are available from the respective authors.

The 1988 Fall Armyworm Symposium is the fifth formal conference held in conjunction with the annual meeting of the Southeastern Branch of the Entomological Society of America. We gratefully acknowledge the assistance of Wayne Gardner, University of Georgia, Dept. of Entomology, Griffin, GA with reviewing and editing of papers. This symposium is dedicated to Ferdinand F. Dickie. Mr. Dickie's long and distinguished career has provided significant contributions to entomology in the areas of host plant resistance, biology and ecology, and control methodologies.

Topics included in this year's symposium dealt with host plant resistance, population dynamics, biology, management tactics, and genetics of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). The annual symposium reflects the varied aspects of research and the mutual sharing of data by scientists studying the fall armyworm. Allozyme loci and mtDNA restriction enzyme profiles were reported to differ between two fall armyworm strains. Strains also differed in physiological and developmental traits and resistance to insecticides. The impact of these findings on research related to the fall armyworm is far reaching and could significantly alter our thinking on the biology and ecology of this pest and screening plants for insect resistance.

The continued interest and efforts of researchers in the Americas and Caribbean Basin reflect the importance of the fall armyworm as a significant pest of agronomic crops throughout this region.

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THE CONTRIBUTIONS OF FERDINAND F. DICKE

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ABSTRACT

Ferdinand F. Dicke's contributions to entomology have spanned more than sixty years. Mr. Dicke has made significant contributions to entomology in the areas of host plant resistance, biology and ecology, disease transmission by insects, chemical and biological control, and methods for breeding for plant resistance to insects. Mr. Dicke's long and distinguished career in the field of entomology is truly outstanding and worthy of our recognition.

RESUMEN

Por más de sesenta años Ferdinand F. Dicke ha contribuido a la Entomología. El señor Dicke ha hecho contribuciones significantes en las áreas de resistencia de plantas hospederas, en la biología y ecología, en la transmisión de enfermedades por insectos, en el control químico y biológico, y en el fitomejoramiento de plantas resistentes a insectos. Mr. Dicke ha tenido una larga y distinguida carrera en el campo entomológico que ha sido sobresalitente y digna de nuesto reconocimiento.

Mr. Ferdinand F. Dicke was born at New Bremen, Ohio, August 25, 1899. He graduated from New Bremen High School in 1917. He received a B. Sc. degree from Ohio State University in 1927 with a major in entomology and botany. From 1938 through 1942, he attended graduate school (at night) at George Washington University, majoring in plant physiology and mycology.

Mr. Dicke's research experience with the United States Department of Agriculture began at Monroe, Michigan (1927-1929) where he worked on chemical control, cultural control, and varietal resistance of the European corn borer. From 1930-1933 he worked on biology and ecology and chemical control of the corn earworm at Charlottesville, Virginia.

He worked at Arlington, Virginia, and Beltsville, Maryland, on several research problems from 1933-1942. Research on the corn earworm included biology and ecology and varietal resistance of dent corn, disease transmission by insects, vectors and transmission of Stewart's bacterial wilt in corn, biology of corn flea beetles, potato leafhopper on alfalfa and peanuts, entomogenous fungi, sericulture, and collection of varieties of silkworms in the U.S.

Mr. Dicke worked on varietal resistance to the European corn borer at Toledo, Ohio from 1942-1950. He was project leader in the development of corn genotypes for resistance to the European corn borer at Ankeny, Iowa from 1950-1963. During this period he was also Professor, Department of Zoology and Entomology at Iowa State University and provided counsel and direction for many graduate students. On July 6, 1963 (after 37 years of research) he retired from USDA and started a 20-year career with Pioneer Hi-Bred International, Inc., Johnston, Iowa. He served as a consultant on insect and diseases in Pioneer's domestic and overseas research program to improve varieties of corn, sorghum, soybeans, alfalfa, cotton, wheat, and sunflowers. His major activities with Pioneer consisted of evaluating crop germplasm for insect and disease resistance.

From 1984 to the present, he has been Research Collaborator with USDA-ARS-Iowa State University at Ankeny, Iowa.

During Mr. Dicke's service with the U. S. Department of Agriculture he made significant research contributions in the areas of host plant resistance to insects, biology and ecology, disease transmission by insects, chemical control, biological control, and methods of breeding for plant resistance to insects. His primary research has been on cereal and forage crop insects. Some specific accomplishments are as follows: (1) Early contributions on the effectiveness and phytotoxicity of insecticides when used against the European corn borer, and reactions of the corn borer to a variety of host plants. (2) Biology of the wheat jointworm with studies on the development of gall tissue. (3) New interpretations on the seasonal populations of the corn earworm and factors affecting winter survival. (4) Identification and evaluation of relative resistance of inbred lines of corn against the corn earworm. Several of the lines found to be resistant are now used in hybrids and are also used in developing synthetic varieties. (5) Identification of new sources of resistance against the European corn borer through the development of new resistant inbred lines from single crosses that had susceptible lines in their parentage, demonstrating transfer and intensification of resistance; several of the lines have been released by the Iowa and Minnesota stations and are now used in commercial hybrids approved on a regional basis. He has made important contributions to an understanding of the genetics of resistance. (6) Important contributions to the large scale production of corn borer egg masses in the laboratory and their efficient use for artificially infesting corn in resistance and other experimental work. (7) Detailed studies on the biology of the corn borer on susceptible and resistant strains of corn leading to the development of important time-saving visual methods for identifying and evaluating resistance before pollination allowing effective selection in segregating plant populations and greatly reducing pollination work and progeny testing. These methods have significantly enhanced the studies on the genetics of resistance and breeding for resistance. (8) Important contributions in a biological and statistical study demonstrating a highly significant correlation between visual numerical leaf damage ratings, sheath and midrib lesions, stalk cavities, and surviving larvae and the use of these criteria in connection with corn breeding as well as with chemical, biological and other research. This work has brought about significant savings in field work and has permitted an increase in the scope of the program.

Mr. Dicke received the U. S. Department of Agriculture Superior Service Award on June 5, 1956, while employed at the European Corn Borer Research Laboratory, USDA-ARS, Ankeny, IA. This award was given for his outstanding contributions in the field of agriculture, especially in connection with his work on corn borer resistant varieties of corn. He was featured in 1957 in a Northrup-King and Co., Minneapolis, MN, publication because of his accomplishments in developing corns that are resistant to the European corn borer. He received the Alumni Member Award of Merit from Gamma Sigma Delta, Iowa Beta Chapter, April 1975. On March 24, 1982, he received the North Central Branch ESA Award of Merit. The CIMMYT Maize Program and the HPR Symposium participants dedicated the International Symposium on Methodologies for Developing Resistance to Maize Insects (March 16-20, 1987) to Mr. Dicke in recognition of his long and distinguished career in the field.

CURRENT STATUS OF FALL ARMYWORM HOST STRAINS

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ABSTRACT

Research on two host strains of the fall armyworm (FAW), *Spodoptera frugiperda* [J. E. Smith], is reviewed. They differ at allozyme loci and mtDNA restriction enzyme profiles, in their development on a variety of plants, and in their resistance to insecticides. Data on interstrain matings indicate unidirectional incompatibilities in the laboratory and strong preferences among males for females of their own strain in nature. Further study is required to clarify precise strain affinities for the long list of FAW host records. Information on phenologies and geographic distributions is presented but clearly in need of in-depth study. Those studying FAW are recommended to collect from a single host, to keep host records with laboratory colonies, to replenish colonies with individuals from the same host, and to establish a set of voucher specimens for each collection or study.

RESUMEN

Se revisa el estudio sobre dos tipos de hospederos del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). Ellos difieren en el loci de alozyme y en la restricción del mtDNA de las enzymas, en su desarrollo en varios tipos de plantas, y en su resistencia a insecticidas. Datos sobre cruce de razas indican incompatibdades no-direccionales en el laboratorio, y una gran preferencia entre los machos hacia hembras de su propia raza bajo condiciones naturales. Se requiere un estudio adicional para aclarar las afinidades de las razas hacia la larga lista de hospederos del gusano cogollero. Se presenta información sobre la fenología y distribución geográfica, pero está claramente necesitada de un estudio a fondo. Se recomienda a aquellos que estudian el gusano cogollero, que lo coleccionen de un solo hospedero, que mantengan un registro del hospedero de las colonias de laboratorio, que reemplacen las colonias con indivíduos del mismo hospedero, y que establescan un grupo de espécimenes justificante de cada colección o estudio.

The fall armyworm (FAW), *Spodoptera frugiperda* [J. E. Smith], has been subdivided recently into two strains on the basis of genetic differentiation associated with larval host plants (Pashley et al. 1985, Pashley 1986). One strain feeds primarily on corn (*Zea mays* L.) and the other on rice (*Oryza sativa* L.) and various forage and native grasses. The strains may represent one of three types of taxa. They may be biotypes in which genetic differences are due to a selectively-mediated polymorphism within a single randomly-mating species. They may be host races in the initial stages of speciation in which interbreeding is reduced due to host preference differences (Diehl & Bush 1984). Lastly, they may be sibling species that are either capable of hybridizing to a limited degree or completely reproductively isolated.

In this paper, I review research relevant to the status of these strains, elaborating on some of the more problematic areas. I also emphasize the impact that these taxa have on our current thinking about FAW biology and end with some recommendations for those studying FAW.

Relevant Background on FAW Biology

FAW has been reported on more than 80 plant species in 23 families (Table 1). It is primarily regarded, however, as a pest of grasses (Poaceae). The two host strains appear to utilize different sets of hosts (Fig. 1). The corn strain feeds primarily on corn, cotton (*Gossypium hirsutum* L.), and sorghum (*Sorghum vulgare* Pers.), but occasionally will feed on other plants either growing in proximity to primary hosts (such as signal grass, *Brachiaria platyphylla* (Nash), [LA 8/85 in Fig. 1]), or in isolation (LA(BH) 8/85 in Fig. 1). The rice strain has been reported on rice, signal, bermudagrass (*Cynodon dactylon* [Pers.]), and Johnson grass (*Sorghum halepense* [Pers.]). It has been suggested that early season FAW feed on corn and then shift to forage grasses after corn is harvested in the south (Morrill 1978). Genetic data do not support this hypothesis.

TABLE 1. Host plant records for FAW. Plant families arranged phylogenetically, species alphabetically. Species introduced to the Western Hemisphere indicated by an asterisk. All references from Luginbill (1928) unless footnoted.

FAMILY	SPECIES
1. Chenopodiaceae	*Chenopodium album* L.* (lambs quarters); *Spinacia oleracea* L.* (spinach); *Beta vulgaris* L.* (sugar beets)
2. Amaranthaceae	*Amaranthus* spp. including *spinosus* L.[a] (pigweed)
3. Portulacaceae	*Portulaca oleracea* L.* (purslane)
4. Polygonaceae	*Fagopyrum esculentum* Moench* (buckwheat)
5. Malvaceae	*Althaea rosea* Cav.* (hollyhock); *Gossypium hirsutum* L. (upland cotton)
6. Violaceae	*Viola* spp. (violets)
7. Caricaceae	*Carica papaya* L.[b] (papaya)
8. Cucurbitaceae	*Citrullus vulgaris* Schrad.* (watermelon); *Cucumis sativus* L.* (cucumber); *Cucurbita pepo* L.[c] (pumpkin)
9. Brassicaceae	*Brassica napobrassica* Mill.* (rutabaga); *B. oleracea* L.* (cabbage, kale); *B. rapa* L.* (turnip)
10. Rosaceae	*Fragaria chiloensis* Duchesue (strawberry); *Prunus persica* Batsch* (peach); *Malus pumila* Mill.* (apple)
11. Fabaceae	*Arachis hypogaea* L. (peanut); *Cajanus cajun* Millsp.* (pigeon pea); *Cicer arietinum* (L.)* (chick pea); *Glycine max* Merr.* (soybean); *Medicago sativa* L.* (alfalfa); *Pisum sativum* L.* (pea); *Pueraria lobata* Ohwi* (kudzu); *Stizolobium deeringianum* Bort.* (velvet bean); *Trifolium pratense* L.* (red clover); *T. repens* L.* (white clover); *Vigna unguiculata* Walpers* (cowpea)
12. Myrtaceae	*Eucalyptus camaldulensis* Dehuh.*[b]
13. Vitaceae	*Vitis* spp. (grape)
14. Rutaceae	*Citrus aurantium* L.* (seville orange)
15. Apocynaceae	*Plumeria rubra* L.[d]
16. Solanaceae	*Capsicum* spp.[b] (red peppers); *Lycopersicon esculentum* Mill. (tomato); *Nicotiana tabacum* L. (tobacco); *Solanum tuberosum* L. (potato)
17. Convolvulaceae	*Ipomoea* spp. (wild morning glory)
18. Asteraceae	*Lactuca sativa* L.*[e] (lettuce); *Xanthium italicum* Moretti (cocklebur)
19. Cyperaceae	*Cyperus rotundus* L.* (nut-grass)
20. Poaceae	*Agrostis hyemalis* (Walt.) (spring bentgrass); *A. stolonifera* L. (redtop bentgrass); *Andropogon virginicus* L. (broomsedge); *Avena sativa* L.* (oats); *Axonopus affinis* Chase (carpet grass);

TABLE 1. (Continued)

FAMILY	SPECIES
	Brachiaria platyphylla (Nash)[f] (signal grass); *Cenchrus tribuloides* L. (sandspur grass); *Chloris gayana* Kunth* (Rhodes grass); *Cynodon dactylon* (Pers.)* (bermudagrass); *Dactyloctenium aegyptium* (Beauv.)* (crowfoot grass); *Digitaria sanguinalis* (Scop.)* (crabgrass); *Hordeum vulgare* L.* (barley); *Oryza sativa* L.* (rice); *Panicum maximum* Jacq.* (guinea grass); *P. miliaceum* L.* (broomcorn millet); *P. purpurascens* Raddi[g] (paragrass); *P. texanum* Buckl. (Texas millet); *Pennisetum glaucum* (R. Br.)* (pearl millet); *P. purpureum* Schum.*[h] (Napier grass); *Phleum pratense* L.* (timothy grass); *Poa pratensis* L.* (Kentucky bluegrass); *Saccharum officinarum* L.* (sugarcane); *Secale cereale* L.* (rye); *Sorghum sudanense* Hitch.* (sudan grass); *S. vulgare* Pers.* (sorghum); *S. halepense* (Pers.)* (Johnson grass); *Triticum aestivum* L.* (wheat); *Zea mays* L. (corn); *Z. mexicana* Reeves & Mangelsdorf (teosinte)
21. Liliaceae	*Allium cepa* L.* (onion); *A. sativum* L.*[e] (garlic); *Asparagus officinalis* L.* (asparagus)
22. Iridaceae	*Gladiolus* spp.*[b]
23. Pinaceae	*Pinus caribaea* Morelet[i] (Caribbean or slash pine)

[a]Chereguino & Menendez 1975; [b]Bruner et al. 1975; [c]Pretto 1970; [d]Santiago-Blay 1983; [e]McGuire & Crandall 1967; [f]D. P. Pashley, pers. obs.; [g]Ashley et al. 1983; [h]Piedra 1974; [i]Howell 1979

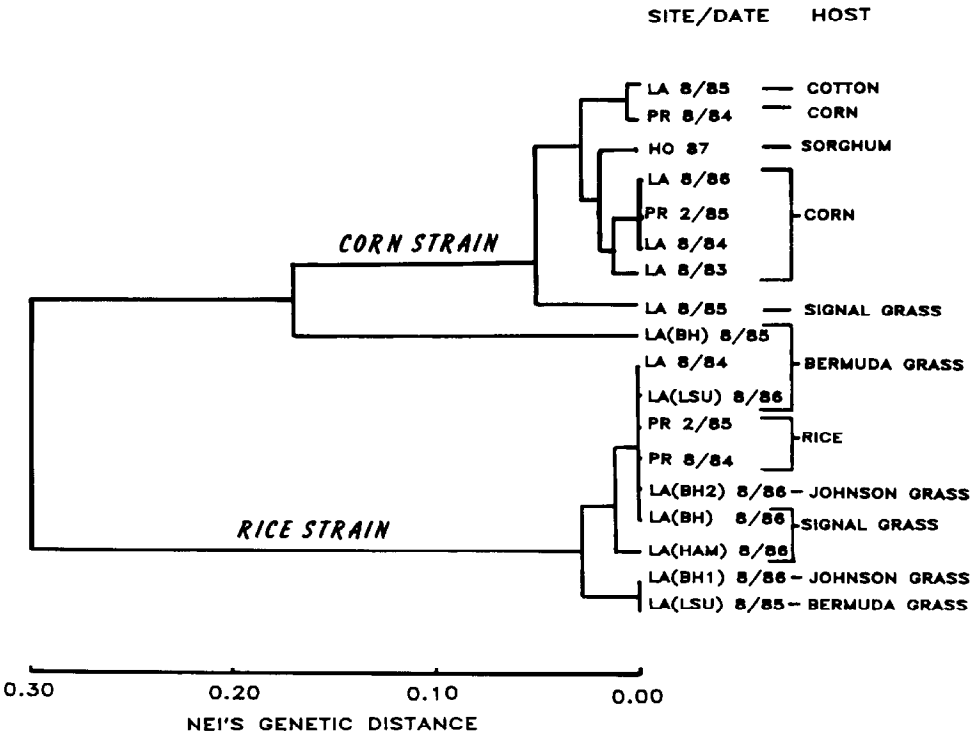

Fig. 1. Genetic relationships among wild FAW collected from different host plants. Genetic distances are based on three polymorphic loci used to separate strains (esterase, hydroxybutyrate dehydrogenase, and peptidase). PR = Puerto Rico, LA = Louisiana (sites in parentheses are different locations in LA), HO = Honduras.

Data on the phenology of strains in Louisiana indicate that the rice strain is present throughout much of the year (D. P. Pashley, T. N. Hardy & A. M. Hammond, unpublished data). Populations remain at low density on various grasses until a rapid buildup that generally occurs in late summer. Corn strain individuals are also present from at least late spring through December but densities peak in early to mid-summer prior to corn harvest. At present it is unclear where these individuals spend the rest of summer. They may migrate northward following developing corn, shift to some other locally available host, die because no hosts are available, or, although unlikely, enter some sort of summer aestivation.

The geographic distribution of the corn strain is well documented (Pashley et al. 1985, Pashley 1986). It occurs throughout much of the western hemisphere, generally in most places where corn is grown (Fig. 2). Less is known about the distribution of the rice strain because very little sampling has been done on hosts other than corn. It occurs sympatrically with the corn strain, often in neighboring fields, in Puerto Rico, southern Florida, Georgia, and Louisiana. Reported attacks on forage grasses throughout much of Latin America are more than likely due to the presence of the rice strain. Future collaboration with scientists from these localities will allow a more precise characterization of the rice strain's range.

Research on Strain Differences

Populations of FAW sampled from corn exhibit significant differences at five allozyme loci from populations collected from rice or bermudagrass (Pashley 1986). Cross-rearing experiments in which larvae were reared on their own and the other strain's host indicated that differences were not due to different selective regimes of the host environment (Pashley 1986).

Although it is possible to use genotypes from multiple allozymes to type individuals to strain with a high degree of accuracy, there are no diagnostic loci. Because of that, an independent genetic marker has recently been examined for use in strain identification. Mitochondrial DNA (mtDNA) has been isolated from individuals of each strain and restriction enzyme patterns at 35 enzymes have been examined (D. P. Pashley, unpublished data). Preliminary data indicate that major strain differences exist at two restriction enzymes (Table 2), and possibly at two others (MboI and MboII; D. P. Pashley, unpublished data). Like the allozyme data, genotypes are strongly associated with hosts; the small amount of overlap (e.g., 2 rice collected individuals with AA and 2 corn collected individuals with BB in Table 2) is probably due to a small number of individuals within each strain that use the other strain's host. Further analysis and geographic sampling are required to determine if differences in mtDNA are diagnostic. Host-associated differences at these two very independent markers (allozymes and mtDNA) are strong evidence for the existence of sibling species.

Many developmental differences have been reported between the strains (Pashley et al. 1987a, Pashley 1988, Whitford et al. 1988). Rates of development and pupal weights tend to differ consistently. The only adult character that differs significantly is preoviposition period (D. P. Pashley, T. N. Hardy & A. M. Hammond, unpublished data). In addition, the rice strain appears to be physiologically adapted to its hosts. It performs well on bermudagrass and rice but poorly on corn, whereas the corn strain is less influenced by larval host. The rice strain is probably the more specialized of the two strains.

Reproductive incompatibilities have been reported in interstrain matings performed in the laboratory (Pashley & Martin 1987). Rice strain females mated successfully with corn strain males but matings in the reciprocal cross (corn females with rice males) did not occur. In backcrosses using individuals from the successful cross, only F_1 hybrid

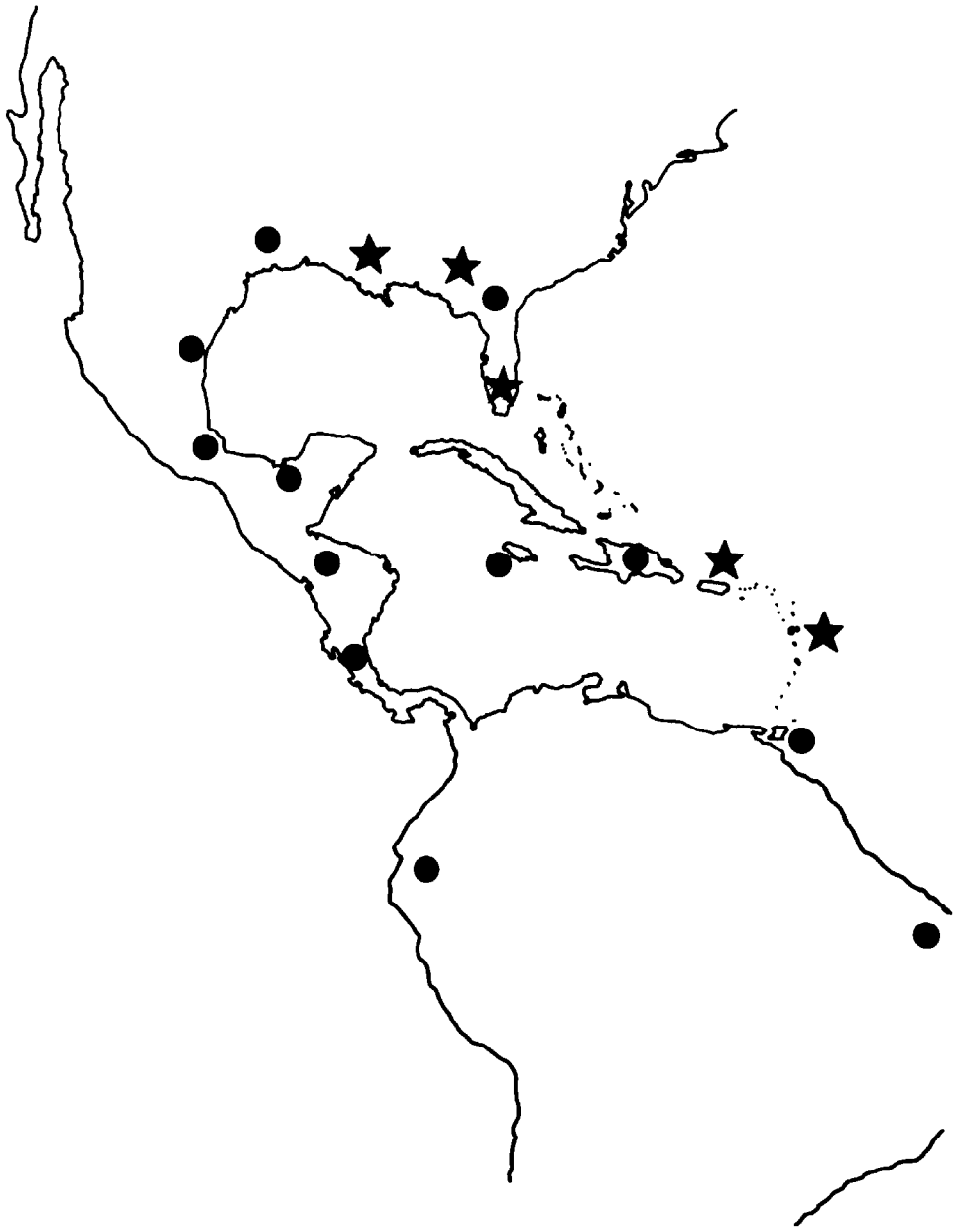

Fig. 2. Collection sites of FAW strains. Circles indicate locations where the corn strain has been collected. Stars indicate locations where both corn and rice strains coexist.

males mated with parentals while F_1 hybrid females would not. In a subsequent study (Whitford et al. 1988), strains successfully mated in both directions. In the two experments reported by Pashley & Martin (1987), material was relatively freshly collected (first and second lab generations in one case and fourth and fifth in the other). Whitford et al. (1988) used colonies at least three years old. Incompatibilities may be due to behavioral characteristics that are altered during the selection process that accompanies colonization. Future studies should focus on newly collected material to clarify this discrepancy.

TABLE 2. MTDNA GENOTYPES AT TWO RESTRICTION ENZYMES (*HINFI* AND *BSTNI*, INDIVIDUALLY, AND COMPOSITE TWO-ENZYME GENOTYPES) IN INDIVIDUAL FAW COLLECTED ON RICE (N = 15) AND CORN (N = 18) IN PUERTO RICO IN JAN. 1985. THE LOW OVERLAP (COMPOSITE GENOTYPE BB ON CORN AND AA ON RICE) IS PROBABLY DUE TO INDIVIDUALS OF ONE STRAIN FEEDING ON THE HOST OF THE OTHER STRAIN.

	HinfI		BstNI				HinfI/BstNI			
	A	B	A	B	C	D	AA	AC	AD	BB
Corn	16	2	14	2	1	1	14	1	1	2
Rice	2	13	2	13	0	0	2	0	0	13

The detection of incompatibilities in laboratory mating studies is informative because it probably reflects conditions in nature (particularly when intrastrain breeding is routinely successful under laboratory conditions). However, when taxa are interfertile in a laboratory environment, it cannot be assumed that mating occurs in nature (Mayr 1970, Diehl & Bush 1984). Therefore, definitive tests of interbreeding must involve studies of cross-attraction and mating in the field. Preliminary data from several ongoing studies suggest that although males are attracted preferentially to females of their own strain, some cross-attraction occurs. In Louisiana and Georgia, females of each strain have been placed in traps and captured males identified to strain using electrophoresis. In Louisiana, most rice strain males (119/166 = 72%) were attracted to rice strain females and most corn strain males (12/16 = 75%) were attracted to corn females (D. P. Pashley, A. M. Hammond & T. N. Hardy, unpublished data). Sample sizes are smaller in Georgia but roughly the same percentages, 75% fidelity for the rice strain and 86% for the corn strain, were observed (J. E. Carpenter & D. P. Pashley, unpublished data). Similarities between these separate studies suggest that either a pheromonal or calling time difference facilitates assortative mating within strains. Current efforts are being focused on the analysis of captured field-mated pairs and observations at mating tables to determine if the attraction of males to females of the opposite strain actually results in interstrain mating (J. E. Carpenter & D. P. Pashley, unpublished data).

Data have been gathered or studies initiated on several other features of the biology of the two strains. They have been reported to exhibit differences in resistance to several insecticides (Pashley et al. 1987b) and differences in survival and development on selections of bermudagrass (Pashley et al. 1987a). Morphological differences at traits in both larval and adult stages have been detected (A. M. Hammond, unpublished data; D. P. Pashley & E. R. Taylor, unpublished). So far, however, no differences have been found in pheromone chemistry (A. M. Hammond & H. Fescmeyer, unpublished data).

PROBABLE TAXONOMIC STATUS OF FAW STRAINS

Differences have been detected between strains at almost every character examined to date. It is unlikely that selective regimes on the two hosts could be maintaining differences at all of these independent characters. The strains are probably not interbreeding to a large degree or differences would quickly disappear. Therefore, these taxa are not biotypes and represent either host races or sibling species. Because the life history characteristics of FAW are not in any way similar to those proposed for host races (Bush 1975a, 1975b), they are probably sibling species. When studies of reproductive isolation in nature are complete, the correct taxonomic status of FAW host strains will be resolved.

RECOMMENDATIONS

It shoud be clear that much is yet to be learned about the biology of these two host-associated strains. Their taxonomic status is only a technical point. Differences exhibited at many different traits should cause those involved in research on these taxa to be extremely cautious. Material for study should be collected from a single host plant species and records of those collections should be kept with laboratory colonies. Colonies should not be replenished with collections from other hosts. Voucher specimens should be set aside for every collection made in the event that morphological differences can eventually be used for strain identification. If there is access to an ultra-cold (–70°C) freezer, a sample should be frozen for electrophoretic analysis, if necessary. If these suggestions are followed, research results will not be invalidated if the strains are concluded to be separate species.

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OVIPOSITION PREFERENCE, MATING COMPATIBILITY, AND DEVELOPMENT OF TWO FALL ARMYWORM STRAINS

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ABSTRACT

Host strains (corn and rice) of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), were evaluated for oviposition preference, mating compatibility, and development on artificial diet and four plant species. Both strains oviposited a greater percentage of egg masses on corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench.), and bermudagrass (*Cynodon dactylon* [L.] Pers.) than on centipedegrass (*Eremochola ophiuroides* [Munro] Hack). The corn strain oviposited preferentially on corn and sorghum, while the rice strain preferred bermudagrass. The number of fertile intrastrain and interstrain pairs ranged from 77 to 100%. Egg hatch was extremely high for all crosses (>80%). The F_1 (rice♀ x corn♂) interhybrid cross had fewer pairs mating and a decreased number of egg masses per female than the F_1 (corn♀ x rice♂) interhybrid cross. The corn and rice strain progeny had similar larval weights and survivorship, but the two strains differed significantly in pupal weights and rates of development on modified pinto bean diet. The corn strain developed equally well on corn, bermudagrass and sorghum, whereas the rice strain developed best on bermudagrass. On these hosts the corn strain had significantly heavier larvae and pupae than rice strain feeding on the same hosts. A discussion is provided that delineates problem areas associated with bermudagrass/FAW resistance studies.

RESUMEN

Se evaluaron razas hospederas (maíz y arroz) del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), sobre su preferencia oviposicional, compatibilidad de apareamiento, y su desarrollo en dieta artificial y en cuatro especies de plantas. Ambas

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RESUMEN

Se evaluaron razas hospederas (maíz y arroz) del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), sobre su preferencia oviposicional, compatibilidad de apareamiento, y su desarrollo en dieta artificial y en cuatro especies de plantas. Ambas

razas pusieron un mayor porcentage de las masas de huevo en el maíz (*Zea mays* L.), sorgo (*Sorghum bicolor* [L.] Moench.), y en la hierba-bermuda (*Cynodon dactylon* [L.] Pers.) que en la hierba-centipede (*Eremochola ophiuroides* [Munro] Hack). La raza del maíz preferentemente ovipositó en el maíz y el sorgo, mientras que la raza del arroz prefirió la hierba-bermuda. El número de parejas fértiles dentro de la raza y entre razas varió del 77 al 100%. La salida del cascarón fue extremadamente alta en todos los cruces (>80%). El F_1 (arroz♀ X maíz♂) del cruce entre híbridos tuvieron un menor número de apareamientos y una disminución en el número de masas de huevos por hembra que el F_1 (maíz♀ X arroz♂) del cruce entre dos razas. La prole de la raza del maíz y del arroz tuvieron larvas con peso y sobreviviencia similares, pero las dos razas fueron significativamente diferentes en el peso de las pupas y en el grado de desarroollo en la dieta modificada del frijol pinto. La raza del maíz se desarrolló igualmente en el maíz, la hierba-bermuda y en el sorgo, mientras que la raza del arroz se desarrolló mejor en la hierba-bermuda. En estos hospederos la raza del maíz tuvo significativamente larvas más pesadas que las razas del arroz comiendo del mismo hospedero. Se discuten problemas que delinean áreas problemáticas asociadas con estudios de resistencia sobre la hierba-bermuda y los gusanos cogolleros.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is an important agricultural pest on graminaceous crops in the southeastern United States (Luginbill 1928). The annual influx of FAW moths in early spring is generally assumed to originate from overwintering adults along the Gulf Coast or from long distance movements from Central and South America (Mitchell 1979, Sparks 1979, Johnson 1988).

Entomologists have assumed that one interbreeding species of FAW exists throughout the Western Hemisphere; however, Pashley (1986) presented electrophoretic evidence that FAW populations were actually composed of two genetically differentiated host-associated strains. One strain feeds primarily on corn (corn strain), while the other feeds on rice or bermudagrass (rice strain). Pashley & Martin (1987) indicated that pre-reproductive isolating mechanisms exist between the female of the corn strain and the male of the rice strain. These reproductive isolating mechanisms suggest that the one recognized species of FAW is actually composed of one or more sibling species (Pashley & Martin 1987).

The biological criteria presently used to evaluate bermudagrass, *Cynodon dactylon* (L.) Pers., genotypes for resistance to FAW are insect development, larval consumption and utilization, and survivorship (Lynch et al. 1983, Quisenberry & Wilson 1985, Jamjanya & Quisenberry 1988, Quisenberry & Whitford 1988). The host strain used in the screening process can influence the selection of bermudagrass genotypes resistant to FAW (Pashley et al. 1987). Physiological differences between the corn and rice FAW strains have been identified and include differences in growth and development on artificial diets (Quisenberry & Whitford 1988), on each other's host plants (Pashley 1988), and on bermudagrass genotypes (Lynch et al. 1983, Pashley et al. 1987, Quisenberry & Whitford 1988). The inadvertent use of different FAW strains in screening trials makes biological data very difficult to assess. This further complicates our ability to compare research results between different laboratories studying bermudagrass genotype resistance to the FAW.

The purpose of this research was to investigate the oviposition preference, mating compatibility, and development of intrastrain, interstrain, and interhybrid crosses on artificial diet and four host plants of the two FAW strains (corn and rice).

Materials and Methods

Fall Armyworm Sources and Colony Maintenance. The two FAW colonies used in this study had previously been identified electrophoretically as the rice (Pashley et al.

1987) and corn (Mason et al. 1987, Pashley et al. 1987) strains. The rice strain was initially field collected as larvae on bermudagrass from East Feliciana Parish in Louisiana (August 1984). The corn strain was established from eggs obtained from corn by F. M. Davis, ARS-USDA, Crop Science Research Laboratory, Mississippi State, Mississippi, and in 1986 was crossed with feral males obtained by rearing egg masses collected on corn, *Zea mays* L. Both strains were maintained on a modified pinto bean diet according to procedures by Perkins (1979) for one generation to remove any confounding effects of the artificial diet (Quisenberry & Whitford 1988). Strains were maintained in a growth chamber at 26.7 ± 0.5° C, 14:10 (L:D) photoperiod, and > 50% RH.

Oviposition Preference Study. All plants were grown in plastic pots (1.89 liter) that contained potting soil (Fisons Western, Vancouver, B.C., Canada). Corn and sorghum, *Sorghum bicolor* [L.] Moench, plants were thinned after germination to 4 and 6 plants per pot, respectively. Plants were in the 5-leaf stage when both trials were conducted. Two wk prior to use, vegetatively propagated bermudagrass and centipedegrass, *Eremochola ophiuroides* (Muro) Hack, were clipped (7-cm) and fertilized with N-P-K.

One pot each of corn, sorghum, bermudagrass, and centipedegrass was placed equidistantly in a galvanized metal container (52 liter) with vermiculite used as a substrate around the pots. A plastic pipe (13 mm diam.) cage frame (48 by 48 by 92 cm) was inserted into the vermiculite, and a fine mesh screen (0.5 mm) placed over the frame. Four replicates (cages) were established per treatment (FAW strain) and repeated in two trials.

Pupae were placed in paper cartons (3.78 liters) and allowed to emerge. Each carton contained one hundred (trial 1) and 120 (trial 2) FAW (1♀:1♂ ratio) pupae from each strain. Two days after the emergence of the first adult, one carton of adults from one of the two strains was released into each cage. During the first trial, adult moths were released on 19 June and 21 June for the rice and corn strain, respectively, and on 18 July (rice strain) and 19 July (corn strain) in the second trial. Releases were made between 8 and 9 am. An adult diet (honey-beer: honey [150 ml] + distilled water [150 ml], + beer [335 ml] + ascorbic acid [12 g]) and water were provided to moths. After a 4-day oviposition period, each plant was examined and the number of egg masses per plant recorded.

A completely randomized design with four replicates per treatment (FAW strain) was used in each trial. Data were analyzed using analysis of variance, and significant means separated by orthogonal contrasts (SAS Institute 1985) and Duncan's multiple range test (Duncan 1955). Data were analyzed within each trial and strain because adult emergence differed between strains, and the number of adults used were different between trials.

Mating Compatibility Study. Male and female pupae from the rice and corn strains were placed individually in polystyrene cups (29.7 ml). The underside of the cup lid was fitted with moistened cellulose cotton wadding to help maintain humidity. At emergence, pairs (corn♀ x corn♂, corn♀ x rice♂, rice♀ x rice♂, rice♀ x corn♂) of virgin FAW adults were placed in oviposition chambers (946 ml) containing moistened vermiculite and an ovipositional substrate (cheesecloth top). An adult diet (honey-beer) and water were provided in wicked polystryrene cups (29.7 ml). Egg masses were collected daily from the cheesecloth and weighed. Egg mass weights were adjusted by subtracting the weight of the cloth material. The total number of eggs from three egg masses in each of five weight categories (5-10, 11-15, 16-20, 21-25, 26-30 mg/egg mass) was used to determine the relationship between the number of eggs per mass (y) and egg mass weight (x). Regression analyses ($y = a + bx$) were performed separately for each intrastrain and interstrain cross to estimate the number of eggs per egg mass. Egg masses laid on the sides of the ovipositional chambers were counted but not weighed. A completely randomized design was used with mating type as the treatments.

Treatments were replicated 10 to 18 and 13 to 15 times in trials 1 and 2, respectively. The parameters measured were the number of egg masses per female, egg mass weight, percent hatch, and the number of eggs per mass. Data were subjected to analysis of variance and significant means separated by least-squares means (SAS Institute 1985). Treatments were maintained in a growth chamber at 26.7 ± 0.5°C, 14:10 (L:D) photoperiod, and > 50% RH.

F_1 interhybrid larvae were placed individually in polystyrene cups (29.7 ml) containing a modified pinto bean diet. Pupae were sexed and held according to the procedures described above. A completely randomized design was used with the treatments consisting of two interhybrid crosses. Each treatment was replicated 11 and 18 (rice ♀ x corn ♂) and 5 and 26 (corn ♀ x rice ♂) times in trials 1 and 2, respectively. Data collected were egg mass number per female, percent hatch, egg mass weight, and number of eggs per mass. Data were analyzed using analysis of variance, and significant means separated using the F test (SAS Institute 1985).

Development on Artificial Diet. One hundred pupae (1♀:1♂ ratio) for each intrastrain and interstrain cross were placed in individual paper cartons (3.78 liter) that contained moistened vermiculite, an adult diet (honey-beer), and a cheesecloth top. Egg masses from the cheesecloth top were collected daily, placed in plastic bags, and allowed to hatch. Newly emerged neonate larvae of the four mating combinations were placed individually on a modified pinto bean diet in polystyrene cups (29.7 ml). Treatments were maintained in a growth chamber at 26.7 ± 0.5°C, 14:10 (L:D) photoperiod, and > 50% RH.

A completely randomized design with six replicates per cross was used in two trials. A replicate consisted of measurements averaged within a subsample of five larvae. Developmental parameters measured were 9-day larval weights, pupal weights, larval and pupal duration, and survivorship to adult eclosion. Data were subjected to analysis of variance and significant means separated by least-squares means (SAS Institute 1985).

Influence of Host Plant on Development. All grasses were grown in metal flats (50 x 35 x 9 cm) containing potting soil (Fisons Western, Vancouver, B.C., Canada). The bermudagrass ('Grazer') and centipedegrass (common) were cut (7 cm) 2 wk prior to feeding and fertilized with N-P-K. Hybrid field corn (Pioneer Brand 3055), and hybrid grain sorghum (Funk's 522DR) seeds were planted 10 days prior to feeding. Plants were maintained under greenhouse conditions at 29 ± 5°C, 16:8 (L:D) photoperiod, and > 50% RH.

Neonate larvae of the corn or rice strain were placed individually in a clear petri dish (10 x 2 cm) that contained a moistened filter cellulose wadding and excised leaves. Excised leaves of corn and sorghum (3-6 leaf stage) and bermudagrass and centipedegrass (regrowth at 2 wk) were initially replaced after 2 days and renewed every day thereafter. The experimental design was completely randomized with six replicates. Treatments consisted of a FAW strain (rice or corn) and host plant (corn, bermudagrass var 'Grazer', sorghum, or centipedegrass) combination. Each replicate consisted of five FAW larvae, and all parameters tested were averaged within each subsample. Biological measurements taken were 9-day larval weights, pupal weights, larval and pupal duration, and survivorship to adult eclosion. Data were subjected to analysis of variance, and significant means separated by least-squares means (SAS Institute 1985).

Results and Discussion

Oviposition Preference. Corn and rice strain female oviposition preference for corn, sorghum, bermudagrass, and centipedegrass is presented in Table 1. A greater percentage of egg masses for both strains was oviposited on corn, sorghum, and bermudagrass

TABLE 1. OVIPOSITION PREFERENCE (PERCENTAGE OF EGG MASSES OVIPOSITED ON EACH HOST PLANT) OF THE CORN AND RICE FAW STRAINS ON CORN, BERMUDAGRASS, CENTIPEDEGRASS, AND SORGHUM.

	Trial 1		Trial 2	
Grass Host	Corn strain	Rice strain	Corn strain	Rice strain
corn	41.7a[1]	24.0b	32.7a	29.2ab
bermudagrass	16.0c	40.5a	20.7b	20.5b
centipedegrass	16.5c	15.5b	14.7b	16.5b
sorghum	25.0b	19.7b	32.0a	34.0a
---------- Contrast[2] ----------				
Corn, sorghum bermudagrass versus centipedegrass	**	**	*	**
Corn, sorghum versus bermudagrass	**	**	*	*
Corn versus sorghum	**	**	ns	ns

[1]Means within a column (grass hosts within strain) followed by the same letter are not significantly different ($P > 0.05$, Duncan's [1955] multiple range test).
[2]NS = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$.

than on centipedegrass during both trials ($P < 0.05$; orthogonal contrasts). Centipedegrass had previously been identified as unsuitable for FAW development (Wiseman et al. 1982, Quisenberry & Whitford 1988). Corn strain females laid a greater percentage of their egg masses on corn and sorghum than on bermudagrass ($P < 0.05$; orthogonal contrasts). The corn strain females showed an ovipositional preference for corn over sorghum during the first trial ($P < 0.001$; orthogonal contrasts). FAW moths have been shown to prefer corn and sorghum rather than bermudagrass (Pitre et al. 1983). These authors used FAW moths obtained from the USDA, ARS, Southern Grain Insect Laboratory at Tifton, Georgia; a laboratory colony that was subsequently identified as a corn strain by Mason et al. (1987) and Pashley et al. (1987).

Rice strain egg-laying preferences were predominately bermudagrass (trial 1) and sorghum (trial 2) (Table 1). Pitre et al. (1983) indicated that as one preferred host becomes less attractive for oviposition, FAW moths will switch to a second host. We suggest that rice strain females in the second trial switched their oviposition preference to sorghum and corn because the bermudagrass was not as suitable for oviposition (i.e., the regrowth was not as dense and plant heights were shorter) as the grass used in the first trial. Our results indicate behavioral differences in oviposition between the two host-associated strains.

Mating Compatibility. The percentage of corn and rice females mated with intrastrain or interstrain males was 77 to 100% (Table 2). The corn intrastrain matings resulted in the highest number (12.2) of egg masses per female. In contrast, the rice intrastrain and rice♀ x corn♂ crosses tended to produce fewer egg masses in the first trial; however, during the second trial the rice intrastrain cross produced a higher number of egg masses than the rice♀ x corn♂ cross. Egg masses among the rice and corn crosses weighed 10 to 15 mg per mass. The number of eggs per mass was highly variable; however, the rice♀ x corn♂ cross produced a higher number of eggs per mass. Egg hatch was extremely high for the intrastrain and interstrain crosses (ca. 90%).

The F_1 (rice♀ x corn♂) interhybrid cross produced fewer matings, decreased number of egg masses per female ($P = 0.06$), and lower percent hatch than the F_1 (corn♀ x rice♂) interhybrid cross. Pashley & Martin (1987) observed fertility rates of

TABLE 2. THE EFFECTS ON REPRODUCTION OF INTRASTRAIN, INTERSTRAIN, AND INTERHYBRID CROSSES OF CORN AND RICE FAW STRAINS.

Trial	FAW strain		Reproduction parameters							
	female	male	Pairs (no.)	Pairs fertile (%)	Egg mass/ female (no.)	Egg mass weight (mg)	Regression equation: Total eggs/mass	R^2	Eggs/ mass (no.)	Hatch (%)
1	corn	corn	18	94	12.2a[1]	10.4a	y= 52.9 + 8.4x	.23	164.4c	91a
	corn	rice	10	80	8.0ab	14.3a	y= 16.0 + 8.1x	.87	112.8c	81a
	rice	rice	15	80	7.0b	12.8a	y= 79.3 + 10.5x	.38	269.6a	95a
	rice	corn	18	100	6.9b	14.9a	y=-36.5 + 14.9x	.65	222.1ab	81a
	F_1 (r♀ x c♂)	F_1 (r♀ x c♂)	11	73	6.8A[2]	14.7A	y=-12.4 + 8.4x	.40	129.2A	74A
	F_1 (c♀ x r♂)	F_1 (c♀ x r♂)	5	100	10.0A	10.8A	y=-20.6 + 15.1x	.86	143.2A	96A
2	corn	corn	15	100	10.2a	12.0a	y= 66.3 + 11.6x	.74	205.1b	97a
	corn	rice	15	100	8.7a	11.3a	y= 97.4 + 8.8x	.53	197.2b	99a
	rice	rice	15	93	9.6a	11.2a	y=-62.8 + 19.5x	.73	156.0b	87a
	rice	corn	13	77	6.5a	14.4a	y= 62.1 + 14.9x	.65	276.4a	88a
	F_1 (r♀ x c♂)	F_1 (r♀ x c♂)	18	45	7.6A	10.3A	y= 26.9 + 12.4x	.35	154.7A	98A
	F_1 (c♀ x r♂)	F_1 (c♀ x r♂)	26	100	10.2A	13.7A	y= 37.7 + 11.8x	.85	200.2A	90A

[1]Means within a column (intrastrain and interstrain matings) followed by the same lowercase letter are not significantly different ($P > 0.05$; least squares means [SAS Institute 1985]).
[2]Means within a column (interhybrid mating) followed by the same uppercase letter are not significantly different ($P > 0.05$; F test [SAS Institute 1985]).

25% when F_1 (rice♀ x corn♂) were crossed. Additionally, they were not able to make interhybrid F_1 (corn♀ x rice♂) crosses because the corn♀ and rice♂ were reproductively incompatible.

Development on Artificial Diet. The development of intrastrain and interstrain progeny on modified pinto bean diet is presented in Table 3. Larval weights at 9-days exceeded 300 mg, except for the the corn intrastrain cross in the second trial. Rice intrastrain pupae weighed significantly less than other crosses. Quisenberry & Whitford (1988) also reported that pupae of the corn strain reared on modified pinto bean diet were significantly heavier than those of the rice strain. The corn intrastrain cross took ca. 0.4 to 1.4 days longer to complete larval development than the other crosses. Survivorship of neonate larvae to adult eclosion was high (87 to 100%) for all crosses. Thus, the corn and rice strain progeny had similar larval weights and survivorship, but the two strains differed significantly in pupal weights and rates of development on modified pinto bean diet.

Influence of Host Plant on Development. Strain development was strongly influenced by larval host (Table 4). Larvae that were fed centipedegrass produced significantly smaller larvae and required a longer time to complete development than those on corn, bermudagrass, or sorghum. Wiseman et al. (1982), Chang et al. (1985), and Quisenberry & Whitford (1988) found centipedegrass significantly reduced larval and pupal weights and increased the time necessary to complete larval and pupal development. Development of the corn strain was comparable on corn, bermudagrass, and sorghum. The larvae and pupae of the corn strain on these hosts were heavier than those of the rice strain on the same hosts (orthogonal contrasts; $P < 0.0001$). However, rice strain larvae on bermudagrass had larval weights comparable to those of the corn strain on corn, bermudagrass and sorghum. The rice strain larvae completed larval development more rapidly on bermudagrass than on the other grass hosts. Pupal weights of the corn strain individuals were heavier on corn and bermudagrass. In contrast, rice strain pupal weights were significantly heavier on bermudagrass than on corn, centipedegrass, and sorghum. The survivorship of the corn strain was high on all hosts (> 80%); however, less than 80% of the rice strain survived to adult eclosion. These results, along with Pashley (1988), confirm differences in development between the corn and rice strains. In both studies, corn strain larvae and pupae were heavier, on average, than rice strain individuals. The corn strain also developed equally well on corn and rice (Pashley 1988) or corn and bermudagrass (Table 4). In contrast, the rice strain developed better on rice or bermudagrass than on corn.

TABLE 3. FALL ARMYWORM DEVELOPMENT OF INTRASTRAIN AND INTERSTRAIN PROGENY REARED ON MODIFIED PINTO BEAN DIET.

Trial no.	Strain cross		Weight (mg)		Duration of stage (days)		Percent survival
	female	male	Larval	Pupal	Larval	Pupal	
1	corn	corn	336.0b[1]	270.1a	13.1a	8.5a	97a
	corn	rice	339.7b	229.8b	12.7b	8.9a	93a
	rice	rice	322.5b	199.0c	12.5b	8.7a	90a
	rice	corn	381.2a	224.2b	12.7b	8.4a	100a
2	corn	corn	239.9b	264.4a	14.3a	9.1a	87a
	corn	rice	380.5a	245.6b	12.5bc	8.0c	83a
	rice	rice	380.9a	221.3c	12.4c	8.3b	83a
	rice	corn	362.2a	258.9ab	12.9b	8.6b	90a

[1]Means within a column by trial are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

TABLE 4. DEVELOPMENT OF THE RICE AND CORN FAW STRAINS ON FOUR GRASSES.

Strain/host	Larval weight at day 9 (mg)	Larval duration (days)	Pupal weight (mg)	Pupal duration (days)	Survival (%)
Corn/corn	275.5a[1]	14.2c	205.7a	8.6ab	90a
Corn/bermudagrass	274.6a	14.3c	192.3ab	8.3bcd	93a
Corn/centipedegrass	30.7d	27.3a	167.6c	8.5a	83ab
Corn/sorghum	290.8a	14.3c	191.3b	8.6ab	90a
Rice/corn	203.9bc	14.1c	147.1d	7.8d	77ab
Rice/bermudagrass	251.0ab	13.0d	166.3c	8.2bcd	77ab
Rice/centipedegrass	27.6d	24.7b	137.8d	8.9a	42c
Rice/sorghum	174.4c	14.4c	148.6d	8.0cd	60bc

[1]Means within a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

CONCLUSIONS

The bermudagrass variety 'Tifton 292' (Leuck et al. 1968, Lynch et al. 1983, Chang et al. 1985) has been identified as a resistant genotype to FAW; however, Jamjanya & Quisenberry (1988) reported 'Tifton 292' to be only intermediately resistant to FAW when compared with a susceptible variety ('Grazer'). It is apparent that factors other than the plant mechanisms (Quisenberry et al. 1988) are confounding our ability to accurately assess resistant bermudagrass genotypes to FAW. Researchers working with host plant resistance to the FAW need to be cognizant of three potential problem areas: 1) the influence of environment (field versus greenhouse-grown grass) on the acceptability of the bermudagrass to FAW, 2) the effects of chemical composition variation in bermudagrass genotypes from spring to fall on utilization of plant material by FAW, and 3) the host-associated FAW strain used in screening bermudagrass genotypes.

Jamjanya (1987) reported bermudagrasses grown in the field were lower in protein, higher in fiber content, and less acceptable to FAW than the same genotypes cultured in the greenhouse. Furthermore, field-grown bermudagrass genotypes were initially lower in protein content and total digestibility in June and declined more rapidly through September. For example, FAW fed greenhouse 'Tifton 292' in June had lower larval weights, increased larval duration, and lower survivorship than larvae fed the same field grown genotypes during the same time period (Jamjanya 1987). Conversely, larvae fed field-grown 'Tifton 292' in August had lower larvae weights and decreased developmental rates than larvae fed greenhouse-grown plants. Consequently, 'Tifton 292' would be classified as a susceptible genotype when grown in one environment (field) in the spring and resistant in the same environment later in the season.

Pashley (1986) suggested that two strains of FAW can be identified. The detection of corn and rice strains constitutes an important discovery and provides an essential refinement to the studies of host plant resistance to the FAW. The biological differences between the two strains are especially pronounced when 'Tifton 292' is evaluated for FAW resistance. Lynch et al. (1983) found 'Tifton 292' to be extremely resistant (100% larval mortality) to FAW. These authors used a laboratory colony that was subsequently identified as a corn strain (Pashley 1986). Pashley et al. (1987) also found 'Tifton 292' to be resistant when tested with the corn strain, but more importantly, it was susceptible when tested with the rice strain.

Physiological (i.e., development) and behavioral (i.e., ovipositional preference) differences were detected in this study between the corn and rice strains. Oviposition preference and greater larval development by the rice strain on bermudagrass indicate a degree of adaptation to bermudagrass. However, the corn strain appears to be more of a generalist feeder due to greater acceptance of corn, bermudagrass, and sorghum. These results confirm the conclusions by Pashley (1986, 1988) that two host-associated strains exist. We suggest that further research needs to be conducted to clarify the taxonomic status between the corn and rice strain and to determine if the concept of host-associated strains/sibling species can be applied to FAW populations over its entire geographic range.

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FEEDING RESPONSES OF FALL ARMYWORM LARVAE ON EXCISED GREEN AND YELLOW WHORL TISSUE OF RESISTANT AND SUSCEPTIBLE CORN

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ABSTRACT

Green and yellow whorl-stage foliage of resistant and susceptible corn, *Zea mays* L., were fed fresh as excised foliage or mixed in meridic diets to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), neonates. MpSWCB-4 manifested its resistance when fall armyworm larvae were fed fresh foliage (1985-87) but not when larvae were fed on tissue in a meridic diet. Larvae fed the green foliage were larger than those fed on yellow foliage tissue, irrespective of whether the foliage was resistant or susceptible. The resistance appears to be more behavioral than has been reported earlier.

RESUMEN

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neonatos del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). MpSWCB-4 manifestó su resistencia cuando el gusano cogollero se alimentó con follaje fresco (1985-87), pero no cuando las larvas se alimentaron con una dieta merídica. Las larvas alimentadas con follaje amarillo eran más grande que aquellas que se alimentaron con tejido foliar amarillo, aparte de que el follaje sea resistente o susceptible. La resistencia parece ser debida más al comportamiento que al que se ha reportado anteriormente.

The discovery of resistance to the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), in the Coastal Tropical Flint corn, *Zea mays* L., (Anonymous 1965, Wiseman et al. 1966), has encouraged numerous studies in plant resistance to FAW (Wiseman & Davis 1979). Mihm et al. (1978) developed a mechanical device to artificially infest whorl-stage corn with FAW neonates. Wiseman et al. (1980) modified this device and showed its usefulness in mass rearing programs, infestations in the greenhouse, and in small or large field tests. Wiseman et al. (1981, 1983) also demonstrated through novel techniques that two of the resistance mechanisms (antibiosis and nonpreference) are present in MpSWCB-4 and Antigua 2D-118. Wiseman & Widstrom (1986) and Wiseman et al. (1986) later studied silk resistance in corn (Zapalote Chico) and panicle resistance in sorghum to the FAW through the use of a laboratory bioassay.

Extensive attempts have been made to develop a laboratory bioassay to explain the biochemical basis for corn resistance to the FAW. We have experienced difficulty in developing a laboratory bioassay giving consistent positive results using MpSWCB-4, Pioneer X304C, and Antigua 2D-118 as known resistant plant material. Here, we report laboratory tests to demonstrate a relationship between FAW larvae feeding on whorl-stage tissue of resistant and susceptible corns.

Materials and Methods

Single-row bulk plantings (6.1 m long and 0.76 m apart) of Cacahuacintle X's (Susc.), Pioneer XZ304C (Resist.), and MpSWCB-4 (Resist.) were made during 1985-87 at the Insect Biology and Population Management Research farm in Tift County, Ga., using agronomic practices common to the area. Whorls of 10-12 leaf stage corn were excised, brought to the laboratory, and processed for use in the laboratory tests.

The whorl of each genotype was separated on the basis of green or yellow leaf tissue. Approximately 7.6 to 10.2 cm of tissue in the middle of each leaf was discarded and only green or yellow tissue was tested. The midrib of each leaf also was excised and discarded. Two separate tests were conducted each year. In one test, only fresh leaf tissue was fed to neonate FAW. In another test, the leaf tissue was oven-dried at 103°F (10 d to 2 wk), ground to a fine powder, and processed into meridic diets for laboratory bioassay as described by Isenhour et al. (1985), Wiseman & Widstrom (1986), and Wiseman et al. (1986).

Fresh Leaf Tests: The neonate FAW used in testing were maintained on bean diet as reported by Perkins (1979). Neonate FAW (100/large dish or 3/small dish) were fed fresh leaf tissue of each genotype for 3 d. After the 3 d of initial feeding, individual larvae were placed on excised leaf tissue (green or yellow) on pieces of moistened paper towel in Growth Chambers® (small petri dishes; Wiseman et al. 1981). Fresh leaf sections (ca. 5.08 cm^2) were replaced every other day or as needed. Tests were maintained in an environmentally controlled room at 80 ± 2% RH and 27 ± 2°C. Weights of larvae were recorded after 7 d in 1985, and after 7 and 10 d in 1986-87. The tests consisted of a split-plot design with 16 replications in 1985 and 24 replications in 1986-87, with two dishes or larvae/replicate. Whole plots were leaf tissue (green or yellow) and subplots were genotype entries.

TABLE 1. MEAN WEIGHTS (MG) OF FALL ARMYWORM LARVAE FED GREEN VS. YELLOW FRESH EXCISED 10-12 LEAF, WHORL-STAGE CORN.[1]

	1985			1986				1987			
	7 d wts			7 d wts		10 d wts		7 d wts		10 d wts	
Genotype	Green	Yellow		Green	Yellow	Green	Yellow	Green	Yellow	Green	Yellow
Cacahuacintle X's	160a	116a		61a	77a	336a	247ab	105b	64b	292b	206b
X304C	141b	82b		34b	59b	288a	280a	143a	77a	362a	254a
MpSWCB-4	127b	77b		15c	24c	165b	227b	87c	44c	220c	149c
Mean	142	* 92		37	* 54	263	ns 251	112	* 62	291	* 203

[1]Mean weights within a column not followed by the same letter and green-yellow means separated by * are significantly different (k ratio = 100; $P \leq 0.05$; Waller-Duncan t-test and least significant differences, respectively [Waller & Duncan 1969; SAS Institute 1982]).

Bioassay Tests: Fresh leaf tissue (60 g) blended in 300 ml of bean diet and 100 ml of distilled water was used in 1985. Oven-dried leaf tissue (20 g) blended in 300 ml of bean diet and 150 ml of distilled water was used in 1986-87 as outlined by Wiseman (1988). Tests were arranged as a split-plot design with 18 replications with two cups per replicate. The weight of larvae was recorded on the 9th day after the cups of diets were infested with neonates.

All data were analyzed by an analysis of variance and significant mean differences were separated with the use of Waller-Duncan k ratio t test and k ratio = 100 and $P \leq 0.05$ (Waller & Duncan 1969, SAS Institute 1982).

Results and Discussion

Table 1 presents the mean weight of 7-d-old (1985) and 7- and 10-d-old FAW (1986-87) larvae fed fresh excised whorl tissue. In four out of five instances, FAW larvae fed on the yellow whorl tissue were smaller than those fed green whorl tissue. But in three out of five instances, FAW larvae were significantly smaller. In each case, larvae that fed on fresh whorl tissue of MpSWCB-4 were signifcantly smaller than larvae fed on Cacahuacintle X's. Larvae that fed on Pioneer X304C were intermediate in size, in that for 50% of the cases, larvae were smaller than those fed on Cacahuacintle X's. The resistance of MpSWCB-4 manifested itself in all test years irrespective of whether larvae fed on green or yellow whorl tissue. The only significant whole plot-subplot interaction involved the 10-d weight for larvae that fed on fresh foliage in 1986. The interaction was due to the magnitude of weight differences for larvae fed on Cacahuacintle X's and those fed on Pioneer X304C.

The resistance of MpSWCB-4 to feeding by larvae was not manifested in the larvae that fed on plant materials in meridic diet mixtures (Table 2). No interactions between whole and sub-plots occurred, but in all 3 years the larvae that fed on yellow whorl tissue mixed in diet weighed significantly less than larvae fed on the green whorl tissue mixed in diet. The results for weights of larvae fed on green or yellow tissue in diet are consistent with results of larvae fed fresh whorl foliage. There were no significant separations of weight of larvae that fed on resistant versus the susceptible foliage diet mixtures. In fact, larvae that fed on the resistant foliage in diet media tended to be larger at 9 d than larvae that fed on susceptible foliage in diet media; this was especially true when compared to those fed the bean diet check.

TABLE 2. Mean weights (mg) of fall armyworm larvae that fed on fresh or oven-dried 10-12 leaf, whorl-stage corn in meridic diets.[1]

	1985		1986		1987	
	9 d wts		9 d wts		9 d wts	
Genotype	Green	Yellow	Green	Yellow	Green	Yellow
Cacahuacintle X's	191a	180a	332a	273a	510a	404a
X304C	177b	162b	355a	257a	420b	436b
MpSWCB-4	199a	187a	357a	289a	531c	456c
Bean check	181ab	177ab	250b	256a	354a	347a
Mean	187 *	177	323 *	269	454 *	411

[1]1985 diets used fresh foliage and 1986-87 used oven-dried foliage. Means within a column not followed by the same letter and gree-yellow means separated by * are significantly different (k ratio = 1.00; $P \leq 0.05$; Waller-Duncan t-test and least significant differences, respectively [Waller & Duncan 1969; SAS Institue 1982]).

Therefore, the development of a laboratory bioassay such as described by Wiseman (1988), Wiseman et al. (1986), and Wiseman & Widstrom (1986) needs more study. One explanation for weight differences of FAW fed resistant and susceptible foliage may be that the whorls of Cacahuacintle X's are much deeper and tighter than those of MpSWCB-4 (Fig. 1). Whorls of MpSWCB-4 are short and more open, and thus, if larvae of FAW fed higher in the whorl of Cacahuacintle X's and only on green tissue, this

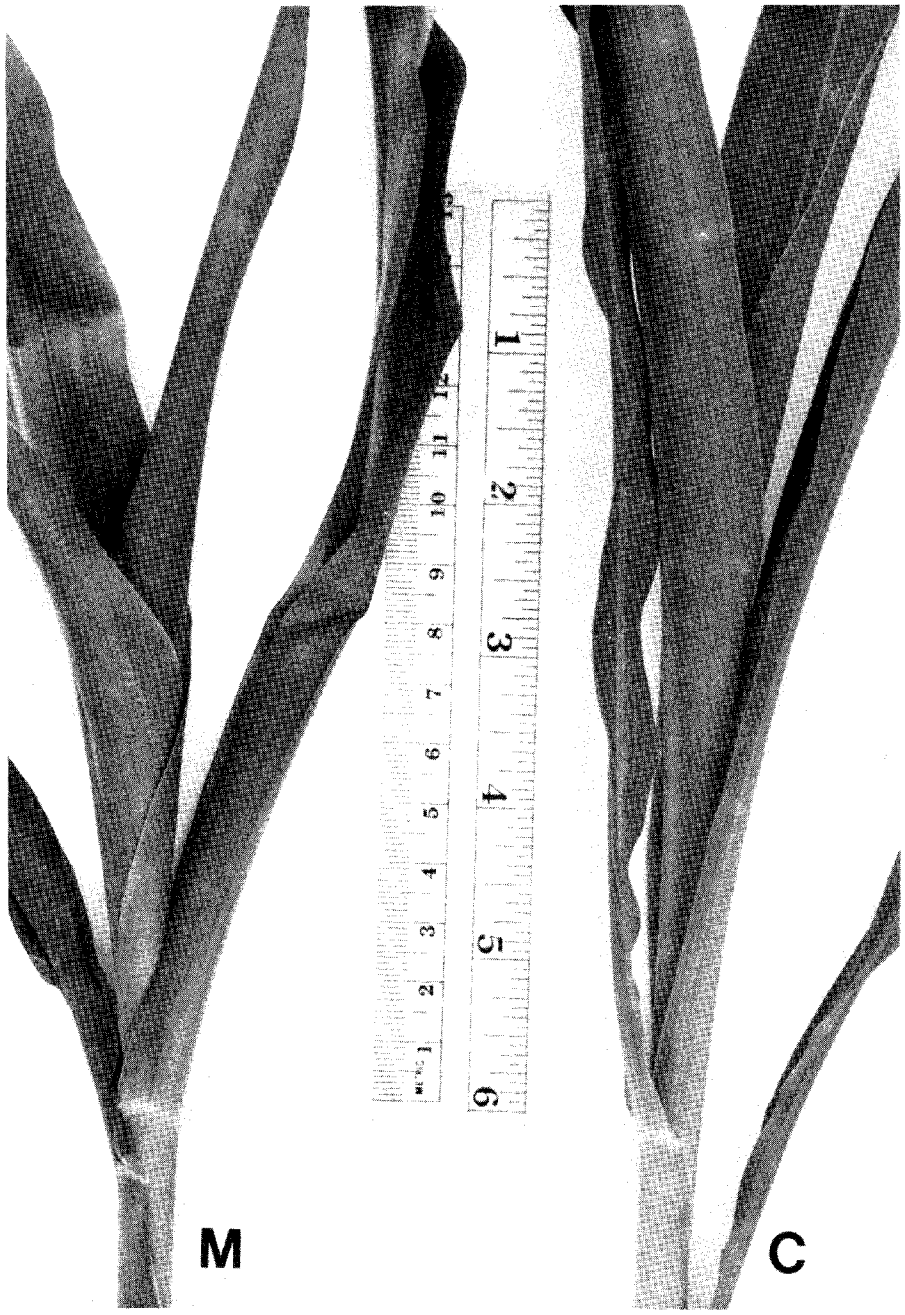

Fig. 1. Comparison of whorl lengths of MpSWCB-4 (M) and Cacahuacintle (C) susceptible.

feeding response would result in larger larvae. If FAW fed lower in the whorls of MpSWCB-4, this probably would be in the green-yellow to yellow whorl tissue, which would result in the production of small larvae. Then, the magnitude of differences between the weights of larvae fed the resistant and susceptible probably would be identified easily. However, if the resistance is due to nonpreference (Wiseman et al. 1983) such as occurs with Pioneer X304C and Antigua 2D, then forced-feeding tests as reported here probably would not identify the resistance as was the case with the Pioneer X304C (Table 1). In addition, the possible loss of leaf volatiles or surface waxes during the laboratory processing of meridic diets could account for the lack of resistance response. Also, enzymatic degradation may occur when the resistant plant material is mixed with the hot bean diet, possibly resulting in a loss of resistance activity.

In summary, the resistance of MpSWCB-4 to feeding of FAW larvae was manifested in the fresh whorl tissue test but not in laboratory diet bioassays. The resistance of whorl-stage corn may be more of an interaction of larval behavior and physical characteristics of the plant due to the feeding location of larvae within the whorls of resistant or susceptible corns than was expected earlier (Wiseman et al. 1981). The laboratory diet bioassay we use at present appears impractical for the FAW and stage of resistance. Therefore, more study is needed to develop a consistent bioassay for laboratory investigations.

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EFFECT OF PERIOD AND LEVEL OF INFESTATION OF THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA*, ON IRRIGATED MAIZE YIELD

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ABSTRACT

The effect of period and level of infestation by the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), on irrigated maize, *Zea mays* L., yield was determined. Two applications of the insecticide chlorpyriphos (336 g[ai]/ha) applied directly to the whorl produced yields equivalent to treatments with three applications. FAW infestation of 100% caused 45% yield reduction. A linear regression model (yield[g/plant] = 87.84 – 0.384 (% plants infested by FAW)) explained 46% of the yield variation. The economic injury level (EIL) was calculated as 2% of the plants infested. This low EIL is due to currently high subsidies provided pesticides in Nicaragua. The impact of pesticide subsidies on EIL's is discussed.

RESUMEN

Se determinó el efecto del período y nivel de infestación del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuide), sobre el rendimiento del maíz, *Zea mays* L., de riego. Dos aplicaciones del insecticida chlorpyrifos (336 g ia/ha) aplicados directamente al cogollo resultaron en rendimientos iguales que tratamientos con tres aplicaciones. Una infestación de 100% por el gusano cogollero causó una reducción de rendimiento de un 45%. Un modelo lineal de regresión (rendimiento [g/planta] = 87.84 – 0.384 [% plantas infestadas por *S. frugiperda*]) explicó el 46% de la variación en rendimiento. Se calculó el nivel de daño económico (NDE) como 2% de las plantas infestadas. Se debió este NDE bajo a los subsidios de insecticidas en Nicaragua. Se discute el efecto de subsidios de insecticidas sobre NDE's.

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In Central America, maize (*Zea mays* L.) is traditionally grown during the rainy season (May-December), and two crops can be harvested per year. In an effort to become self-sufficient in maize production, Nicaragua has added a third crop each year, during the dry season, through the introduction of irrigation on the Pacific Plain. Because this new production system is only 3 years old, an integrated pest management (IPM) program for the important insect pests of irrigated maize has not yet been developed. Because we already know the principle pests of irrigated maize, the first step in developing an IPM program is to determine the critical periods of protection and economic injury levels (EIL).

One of the principal pests of maize in Nicaragua is the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith). FAW is present during all three cropping cycles, but is much more abundant during the second natural rainfall season and in irrigated maize. Pest populations are very high in irrigated maize in the Pacific Plain, where 100% of untreated plants are infested, with up to three 6th-instar larvae per plant.

The current recommendations issued by the Ministry of Agriculture to control FAW in irrigated maize are to apply insecticides 5 times during the vegetative phase every 5 days, three of which are directed solely against FAW (MIDINRA, 1984). Some producers apply insecticides up to 12 times per cropping cycle (personal observation).

Given the current control recommendations and practices used against FAW in irrigated maize and as a first step in developing an IPM program, we carried out a study with 3 objectives: 1) to determine the critical periods of protection from FAW during the vegetative phase, 2) within the periods of infestation which cause yield reduction, to determine the damage function between infestation and yield, and 3) use the damage function to calculate the economic injury level for FAW in irrigated maize.

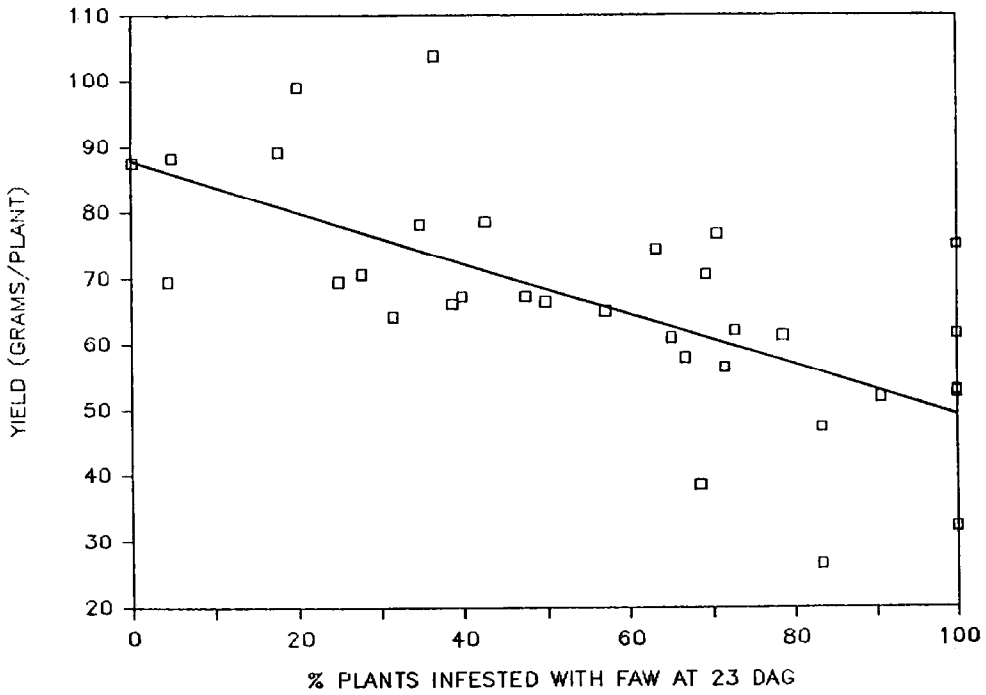

Fig. 1. Effect of FAW infestation at 23 DAG on Yield.

Materials and Methods

Open-pollinated maize variety NB-6 was planted at the National Center for Basic Grain Research in Managua, Nicaragua, in March, 1987, with 7 plants/m and 80 cm between rows. Due to the naturally occurring 100% infestation level of FAW in maize, we created the treatments by applying insecticide to individual plants. Chlorpyrifos (480 E) was mixed with sawdust and applied by hand directly into whorls (336 g[ai]/ha), a commonly used practice against FAW in Central America.

A randomized complete block design with 20 treatments and 8 blocks was used. Treatments were combinations of 3 periods and 4 levels of infestation. The three periods were: 5-17, 17-31, and 31-45 days after germination (DAG), corresponding to seedling and early whorl, mid-whorl, and late whorl to tassel. The four levels of infestation were 100%, 70%, 40%, and 0% of the plants infested with FAW larvae (Fig. 1). Infestation by FAW was determined at 10, 23, 30, and 39 DAG, using the center row of each plot, by visually examining the whorl for FAW larvae, feeding damage, or frass.

At 115 DAG the central row of each plot was harvested, and total number, number broken, and number of lodged plants were counted. Mean yield of commercial grain at 15% humidity per plant was calculated for each plot.

To determine the effects of periods of infestation on yield, the 7 treatments that had 100% infestation during the periods were analyzed with an analysis of variance (ANOVA). Differences among treatments were determined with Tukey's multiple comparison test (Steel & Torrie 1980). Regression models were used to determine the relation between FAW infestation and yield. Stepwise regression was used to determine which date of infestation was the best predictor of yield.

Results and Discussion

There was a significant effect of infestation period on yield (ANOVA, $F = 3.53$, d.f. = 6,38, $P < .01$). Treatments that were infested with FAW for 2 or 3 periods had a significantly lower yield than treatments that were not infested, or were infested during one of the periods (Table 1).

Using treatments that had infestation in two or three of the periods, a stepwise regression analysis indicated that, of the 4 infestation dates, 23 DAG was the best predictor of yield. Linear and quadratic regression models were compared, with the linear model explaining a greater percentage of the variance in yield. The estimated regression model is: $Y = 87.84 - 0.384(PI)$, with $r^2 = 0.458$, where Y = yield (g/plant) and PI = % plants infested (Table 2).

The results indicate that there is no one period that is more critical in causing yield reduction, although there is a non-significant tendency for infestation during later periods to cause greater yield loss (Table 2).

The results demonstrate that the entire vegetative phase of irrigated maize does not have to be protected from FAW to produce maximum yield. Two applications of chlorpyriphos produced yields that were not different from the treatment with three applications (Table 2). The effect of infestation by FAW on yield of irrigated maize appears cumulative during the vegetative phase, but maize plants are able to compensate for damage inflicted during any one period. Thus, the current insecticide recommendations could be reduced from current levels of 3-8 applications from seedling until tassel stage, to 2 during mid- to late-whorl stage, without reducing yield.

The shape of the damage function is different from the function obtained in similar experiments under natural rainfall where the regression model was curvilinear and yield did not decrease until 40% of the plants were infested (Hruska et al. 1987). Damage functions may be very specific to environmental conditions.

TABLE 1. EXPERIMENTAL TREATMENTS; PERIOD AND LEVEL OF INFESTATION BY *S. FRUGIPERDA*.

Treatment	Period and Level of Infestation[1] Days After Germination			
	5	17	31	45
1.	------100%------			
2.	------ 70%------			
3.	------ 40%------			
4.		------100%------		
5.		------ 70%------		
6.		------ 40%------		
7.			------100%------	
8.			------ 70%------	
9.			------ 40%------	
10.	------100%------	------100%------		
11.	------ 70%------	------ 70%------		
12.	------ 40%------	------ 40%------		
13.		------100%------	------100%------	
14.		------ 70%------	------ 70%------	
15.		------ 40%------	------ 40%------	
16.	------100%------	------100%------	------100%------	
17.	------ 70%------	------ 70%------	------ 70%------	
18.	------ 40%------	------ 40%------	------ 40%------	
19.				
20.	------100%------	------100%------	------100%------	

[1]Lines indicate infestation by FAW and numbers the percentage of plants infested.

TABLE 2. EFFECT OF PERIOD OF INFESTATION ON YIELD.

Treatment	Period of Infestation (Days After Germination)				Yield[1] (g/plant) (S.D.)	
	5	17	31	45		
1.	----------				69.76 (11.82)	A
2.		----------			64.95 (19.66)	A
3.					59.11 (27.83)	A
4.			----------		58.73 (13.84)	A
5.	----------	----------			51.72 (8.82)	B
6.		----------	----------		41.12 (10.51)	B
7.	----------	----------	----------	----------	38.67 (16.77)	B

[1]Means followed by the same letter do not differ significantly according to Tukey's test (P>.05).

Yield was reduced by 45% when 100% of the plants were infested. This yield reduction is more severe than studies conducted under natural rainfall, where 100% infestation caused between 15-30% yield reduction (Obando 1976, Van Huis 1981, Hruska et al. 1987).

The difference in the shape of the yield/infestation function and the greater reduction in yield in irrigated maize may be due to greater water loss in plants under irrigated maize than under natural rainfall. Irrigated maize grown in the hot dry season with typically strong winds would undergo high rates of evapotranspiration. Equal amounts of defoliation would be expected to produce greater water loss in the dry season than under natural rainfall.

Using the estimated damage function, the economic injury level (EIL) was calculated to be 2% of the plants infested. This is far lower than any other calculated EIL for FAW in maize, where published values vary between 20 and 50% (Sarmiento & Casanova 1975, Young & Gross 1975, Obando 1976, Van Huis 1981, Young & Gross, unpublished).

EIL's change any time there is a change in the damage function, value of the crop, cost of control, or efficacy of control changes (Hruska & Rosset 1987). An examination of the EIL calcualtion revealed that the low value was due to the relatively low cost of pesticides.

In Nicaragua, as in many developing countries pesticides are heavily subsidized by the government (Repetto 1985). Nicaraguan farmers pay between 3-5% of the world market cost for agricultural inputs. At the same time that pesticide prices are low, crop prices are relatively high, due to a shortage of basic grains in the country. The relation between the cost of insecticide and the value of maize is 0.52 (price of 3.78 liter of chlorpyrifos to the producer/value of 45.4 kg of maize), whereas in the United States the realtionship is 13.45 (Hruska & Gladstone 1987).

If we use world prices for insecticides and maize the EIL is 20% of the plants infested, similar to values reported in the literature (Sarmiento & Casanova 1975, Obando 1976, Van Huis 1981).

The *raison d'etre* of the EIL is to eliminate uneconomical pesticide applications. The decision criterion is set at maximizing profit for the individual farmer. What maximizes individual profit, however, may not maximize social profit.

Pesticide subsidies are a double-edged sword. On the one hand they make agricultural inputs readily available to farmers with limited resources, while on the other hand they may encourage unnecessary pesticide use. The classical EIL calculation may not provide a useful decision rule for farmers and countries with subsidized pesticides. A new decision rule is necessary, one that estimates and incorporates the values of "externalities" in the calculation. Hruska & Gladstone (1987) suggest a method to calculate a revised EIL and set pesticide subsidy levels to meet the goals of society.

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EVALUATION OF RICE CULTIVARS FOR ANTIBIOSIS AND TOLERANCE RESISTANCE TO FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The effects of antibiosis on the growth of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), larvae by rice, *Oryza sativa* L., seedlings at different plant stages and the tolerance of seedlings to artificial defoliation at the 7-leaf stage were evaluated in five plant introductions (PI) and the check cultivar 'Mars'. FAW larvae fed 5-leaf stage foliage had significantly lower larval, pupal, and adult weights than larvae fed 3-leaf stage foliage. The effects of antibiosis on the growth of FAW were evident in PIs 160842, 160827, 346833, and 346839, but not in PI 160823 or Mars. PIs 160842, 160827, and 160823 exhibited higher tolerance in plant regrowth after defoliation than PI 346833, 346839, or Mars. PIs 160842 and 160827 exhibited both the antibiosis and tolerance resistance.

RESUMEN

Se evaluó en cinco plantas introducidas (PI) y en la variedad testigo 'Mars', el efecto de antibiosis en el crecimiento de larvas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), en plantas de semillero de diferentes etapas de crecimiento del arroz, *Oriza sativa* L., y de la tolerancia de las plantas a la defoliación artificial en la etapa de 7-hojas. Larvas, pupas, y adultos del gusano cogollero alimentadas con follaje de la etapa de 5-hojas, tuvieron significativamente un peso menor que las larvas que se alimentaron con follaje de la etapa de 3-hojas. El efecto de antibiosis en el crecimiento

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The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a polyphagous insect which is an important pest on many crops (Luginbill 1928). However, cereals and grasses are the most preferred among its host plants (Crumbs 1927). On rice, *Oryza sativa* L., the FAW defoliates the leaf particularly at the seedling stage and is a sporadic pest in the southern U.S.A. (Bowling 1978, Smith et al. 1986). Severe damage by FAW to rice has been reported in Puerto Rico (Chandler et al. 1977), and several countries in South America (Navas 1966, Gallego 1967, Machado 1978).

Plant resistance to FAW has been studied on many crops as reviewed by Davis (1980). Pantoja et al. (1986) evaluated over 5,000 rice plant introductions for resistance to FAW defoliation. In their study, 11 plant introductions (PI) were found resistant in choice and no-choice experiments in the greenhouse and field.

The antixenosis, antibiosis, and tolerance resistance are all important to the implementation of resistant cultivars into an insect pest management program. Both rice stands and yield are reduced due to the damage caused by FAW feeding during the seedling stage (Bowling 1978). For this reason, the ability of rice to regrow after damage by FAW is an useful indicator for evaluating the tolerance resistance of rice germplasm. Similarly, the defoliation of rice by FAW or by artificial methods has differential effects on rice yield. Upland rice in Panama tolerates substantial artificial or FAW defoliation without incurring significant yield loss (Navas 1976). Artificial defoliation of the upper 2/3 of the plants of a leafy rice variety, N.P. 130, increased yields by 10-34% depending on the age of the plants at defoliation (Tripathi et al. 1973). Mukerji (1973) reported that rice plants pruned at the seedling stage have slight reductions in yield.

The objectives of this study were to evaluate the effects of rice plant growth stage on the growth of FAW larvae and to evaluate antibiosis and tolerance of five rice PIs found to have antixenosis resistance by Pantoja et al. (1986).

Materials and Methods

Four resistant PIs (160842, 346833, 346839, and 160823), one susceptible PI 160827, and check cultivar 'Mars' (PI 009945) were selected from the study of Pantoja et al. (1986). Seeds were pregerminated in an oven at 30°C for 3 d before being sown into pots in a greenhouse.

In the antibiosis test, pregerminated seeds were sown into the center of a plastic pot (5.1 by 5.1 by 5.1 cm) with ca. four to six seeds per pot. Baccto® potting soil was used, but no fertilizers were applied during the experiment. Pots were set in plastic-lined benches in the greenhouse, and the water level in benches was maintained at a height of 2-3 cm. Pots were moved to the labortory when most of the plants reached the 3-leaf stage (Yoshida 1981). All of the plants in each pot were confined in a transparent plastic tub (2.5 cm diam., 45.0 cm high). One FAW neonate larva per pot was introduced onto plants inside the tube with a camel's hair brush. A total of 20 pots of seedlings of each PI were used. Infested plants were placed inside an incubator at 28 ± 2°C, 14:10 L:D, and 70% RH. Larval weights were measured daily from the 8th day until larvae became prepupae. Pupal weights were recorded within 24 h after the pupation of each larva. The duration and mortality of the entire larval stage, the prepupal stage, and the pupal stage were also recorded. Individual larvae on severely consumed plants were transferred into cages with plants of the same stage to ensure that enough fresh plants were provided throughout the larval stage.

The same procedures were repeated using rice seedlings at the 5-leaf stage of growth. Effects of rice plant growth stage on growth of FAW larvae were compared by a t test (SAS Institute 1985). Means of measurements were subjected to Duncan's multiple range test (Duncan 1955) to compare antibiosis effects among PIs.

In the tolerance test, pregerminated seeds were sown into plastic pots (15 cm diam., 15 cm high) with six to tn seeds per pot. The soil was a mixture of rice field soil and Baccto® potting soil at a 4:1 ratio. No fertilizers wer applied to the soil during this test. Pots were set in plastic-lined benches in greenhouse, and the water level in the benches was maintained at ca. 10 cm. The number of seedlings was thinned to two plants per pot at the 4-leaf stage. Leaf blades were removed with scissors at rates of 0, 33, 66, and 99% dfoliation at the 7-leaf stage, and pots were flooded after defoliation. This experiment was conducted with a split-plot design with four replications. The six PIs were the whole plots and the different percent defoliation levels were the subplots. There were three pots for 0% defoliation (control) and two pots for each of the remaining treatments in each replication. Four weeks after defoliation, plants were removed from pots and soil was washed from the roots. The number of tillers, vegetation wet weight, root wet weight, and root volume were measured in each pot. Plant dry weights were measured after drying at 55°C for 4 d.

Analysis of variance (ANOVA) was performed to compare the effects of different defoliation levels on plant growth among and within rice PIs (SAS Institute 1985). The relationships between plant growth measurements and rice PIs were quantified by correlation analysis (SAS Institute 1985). The percent reduction in plant dry weight,

$$\frac{\text{(Dry Wt. of Uninfested Plant} - \text{Dry Wt. of Infested Plant)}}{\text{Dry Wt. of Uninfested Plant}} \times 100\%,$$

where plant dry weight = dry weight of vegetative parts + dry weight of roots, was calculated to compare tolerance among PIs (Panda & Heinrichs 1983). The relationship between antibiosis and tolerance among PIs was quantified by regrssing the last day larval weight (maximum larval weight) at the 5-leaf stage (antibiosis index) on the average percent plant loss in dry weight (tolerance index) (SAS Institute 1985). This relationship was used to measure the relative contributions of the antibiosis and tolerance components of resistance in each of the tested PIs (Ortega et al. 1980).

Results

Rice plant growth stage had a significant effect on the growth of FAW (Table 1). Larvae fed 5-leaf stage foliage weighed significantly less and had a longer duration of the larval stage than larvae fed 3-leaf stage foliage. The prepupal duration was longer on 5-leaf stage foliage than on 3-leaf stage foliage; however, the duration of the pupal stage was not different. Both pupal and adult weights of larvae fed on 5-leaf stage foliage were also lower than weights of larvae fed on 3-leaf stage foliage. The ovrall mortality of FAW was ca. 10% and showed no differences due to the rice plant growth stage. No larval mortality was observed, with the exception of one larva fed 5-leaf stage 'Mars' foliage. Most of the mortality was observed in the pupal stage. Adults emerging from larvae fed both plant growth stages appeared normal.

The effects of rice PI on larval and pupal weights and th duration of the larval and pupal stage were significant at both the 3-leaf and 5-leaf stages (Table 2). Larvae exhibited different trends in growth on different PIs tested. Therefore, the larval weight at the last larval day (maximum larval weight) was used to compare antibiosis effects. Maximum larval weights on PIs 346833 and 346839 were lower than those on all other PIs in the 3-leaf stage of growth. The same trend appeared among weights of larvae fed 5-leaf stage foliage, except that the maximum larval weight of FAW larvae fed PI 160827 was the same as that of larvae fed PI 346833. The duration of larval

TABLE 1. EFFECTS OF RICE PLANT GROWTH STAGE ON THE GROWTH AND DEVELOPMENT OF FALL ARMYWORM.

FAW Growth Index	Plant Growth Stage		t Test		
	3-leaf	5-leaf	t	df	P>F
8th day larval wt. (mg)	147.22	109.66	4.58	218	0.0001**
Last day larval wt. (mg)	424.17	388.92	4.62	233	0.0001**
Larval duration (day)	11.62	12.36	-3.91	196	0.0001**
Prepupal duration (day)	1.87	2.01	-3.10	226	0.0022**
Pupal duration (day)	7.30	7.49	-1.84	193	0.0665^{NS}
Pupal wt. (mg)	172.10	145.06	7.99	210	0.0001**
Adult wt. (mg)	81.93	67.30	6.63	207	0.0001**
Percent mortality	9.60	10.00	0.67	7	0.5188^{NS}

**highly significant at $\alpha = 0.05$; ^{NS}not significant at $\alpha = 0.05$.

development appeared to be correlated to the larval weight at the 8th day of development. The lower the larval weight on day 8, the longer the larval duration and vice versa ($r = -0.805$, $P = 0.0001$, $n = 235$). Pupal weights were not correlated to the duration of development among larvae fed rice at the 3-leaf stage ($r = 0.007$, $P = 0.940$, $n = 115$) or the 5-leaf stage ($r = -0.165$, $P = 0.080$, $n = 113$) of growth. However, pupal weights were significantly correlated to maximum larval weights (3-leaf stage; $r = 0.736$, $P = 0.001$, $n = 115$; 5-leaf stage; $r = 0.755$, $P = 0.001$, $n = 113$). FAW larvae had the highest pupal mortality when fed foliage of PI 160827 at both stages of rice growth.

In the tolerance test, the effects of PI were significant in relation to the number of tillers ($F = 15.04$; $df = 5,15$; $P = 0.0001$), vegetation wet weight ($F = 10.47$; $df = 5,15$;

TABLE 2. GROWTH AND MORTALITY OF FALL ARMYWORM LARVAE FED FIVE RICE PLANT INTRODUCTIONS (PI) AND THE CULTIVAR 'MARS' AT TWO PLANT GROWTH STAGES.

PI Number	Larval Weight (mg)		Duration (d)		Pupal Wt. (mg)	% Mortality
	8th Day	Last Day	Larva	Pupa		
	3-leaf stage					
160842	57.7 d[1]	458.6 a	13.2 a	7.6 a	179.4 ab	0
346833	105.0 c	400.0 bc	12.0 b	7.7 a	165.4 bc	5.0
346839	179.2 b	385.3 c	10.7 c	7.4 a	162.5 c	10.5
160823	202.6 ab	433.2 ab	10.9 c	6.6 b	173.9 abc	10.0
Mars	123.6 c	446.7 a	11.9 b	7.8 a	186.2 a	5.3
160827	218.8 a	438.0 a	10.9 c	6.4 b	164.9 c	31.6
	5-leaf stage					
160842	105.3 c	388.2 bc	12.2 c	7.4 a	138.2 c	0
346833	66.5 d	354.4 c	13.7 b	7.7 a	131.8 c	0
346839	185.8 a	381.8 bc	10.4 e	7.8 a	139.9 b	0
160823	145.6 b	448.0 a	11.3 d	7.0 b	181.3 a	11.8
Mars	42.7 e	406.0 b	14.7 a	7.8 a	154.2 b	5
160827	108.7 b	354.4 c	12.0 c	7.0 b	128.4 c	22.2

[1]Means followed by the same letters in each column for each plant growth stage are not significantly different (P = 0.05; Duncan's [1955] multiple range test).

$P=0.0002$), vegetation dry weight ($F=14.50$; $df=5,15$; $P=0.0001$), root wet weight ($F=9.18$; $df=5,15$; $P=0.0004$), root dry wight ($F=3.42$; $df=5,15$; $P=0.0294$), and root volume ($F=3.58$; df=5,15; $P=0.0248$). Defoliation also significantly affected the number of tillers ($F=12.62$; $df=3,54$; $P=0.0001$), vegetation wet weight ($F=27.50$; $df=3,54$; $P=0.0001$) and dry weight ($F=27.50$; $df=3,54$; $P=0.0001$), root wet weight ($F=8.69$; $df=3,54$; $P=0.0001$) and dry weight ($F=6.21$; $df=3,54$; $P=0.0011$), and root volume ($F=9.70$; $df=3,54$; $P=0.0001$). Interactions of PI and defoliation were not significant across any rice plant growth measurements. Among the PIs tested, 346833 and 160823 had higher growth measurement values than the remaining PIs (Table 3), indicating that these two PIs have a larger biomass than the other PIs tested.

The 33% defoliation rate did not induce a significant effect among PI on percent reduction in plant dry weight, but the 66% and 99% defoliation rates did ($\alpha=0.10$) (Table 4). In general, PIs 160842 and 160827 had the lowest reductions among all rice PIs tested. PI 160823 had a moderate reduction in plant dry weight, whereas the susceptible cultivar 'Mars' had the highest plant dry weight reduction across all three defoliation levels.

The linear relationship between the average percent reduction in plant dry weight and the maximum weight of larvae fed foliage of plants at the 5-leaf stage of growth (Fig. 1) had a very small slope. Among the five PIs tested, only the plotted values of PIs 160842 and 160827 fell within the region in which both antibiosis and tolerance were present. Values of PIs 346833 and 346839 fell in the region in which only antibiosis was present. PI 160823 had the highest larval weight among the PIs tested, but still plotted just within the tolerance quadrant. Values for the susceptible cultivar Mars fell within the region in which neither antibiosis nor tolerance was expressed.

Discussion

The use of whole rice seedling plants proved to be a useful bioassay technique to evaluate the antibiosis effects of rice on the growth of FAW. During the experiment, the first, second, and third instars fed exclusively on leaf blades while the fourth and fifth instars fed randomly on leaf blades or leaf sheaths. Pupal cells were built successfully under the base of plants by pooling plant fragments, frass, and soil. No prepupal mortality was observed during the test.

The significant differences in the growth of FAW fed rice plants of different stages indicated that plant growth stage affects the growth of FAW on rice. Older 5-leaf stage seedlings had more detrimental effects on the growth of FAW than younger 3-leaf

TABLE 3. Effects of artificial defoliation on the regrowth of five rice plant introductions and the cultivar 'Mars'[1].

PI Number	No. of Tillers	Vegetation Weight		Root Weight		Root Volume (ml)
		Wet (g)	Dry (g)	Wt (g)	Dry (g)	
160842	9.3 c[2]	65.5 b	8.6 c	96.7 b	17.6 bc	86.4 bc
346833	13.5 b	94.4 a	12.2 a	181.9 a	35.7 a	135.1 a
346839	10.6 c	62.0 b	7.5 c	71.1 b	14.0 c	66.1 c
160823	17.8 a	84.2 a	11.8 ab	166.6 a	33.9 ab	127.4 ab
Mars	10.1 c	67.8 b	8.9 c	106.8 b	20.0 bc	86.6 bc
160827	13.1 b	71.9 b	10.6 b	89.7 b	24.0 bc	84.8 bc

[1]Plants were defoliated at the rate of 0, 33, 66, and 99% at the 7-leaf stage of growth. Plant growth measurements were taken 4 weeks after defoliation.

[2]Means followed by the same letters are not significantly different (P = 0.05; Duncan's [1955] multiple range test).

TABLE 4. PERCENT REDUCTION IN PLANT DRY WEIGHT OF FIVE RICE PLANT INTRODUCTIONS AND THE CULTIVAR 'MARS' AFTER DIFFERENT RATES OF ARTIFICIAL DEFOLIATION.

PI Number	Defoliation Rate			
	33%	66%	99%	Average
Mars	33.28	51.35 a[1]	83.16 a	56.02 a
346839	30.33	44.17 ab	56.07 b	43.47 a
346833	2.01	50.42 a	62.13 ab	36.00 ab
160823	27.75	5.62 b	54.95 b	31.61 b
160827	–0.95	22.17 ab	48.44 b	23.78 b
160842	–14.23	18.82 ab	50.41 b	19.70 b
$F=$	0.82	2.28	2.55	3.64
$df=$	5, 14	5, 15	5, 15	5, 80
$P=$	0.5544	0.0987	0.0733	0.0485

[1]Means followed by the same letters are not significantly different (P = 0.10; Duncan's [1955] multiple range test).

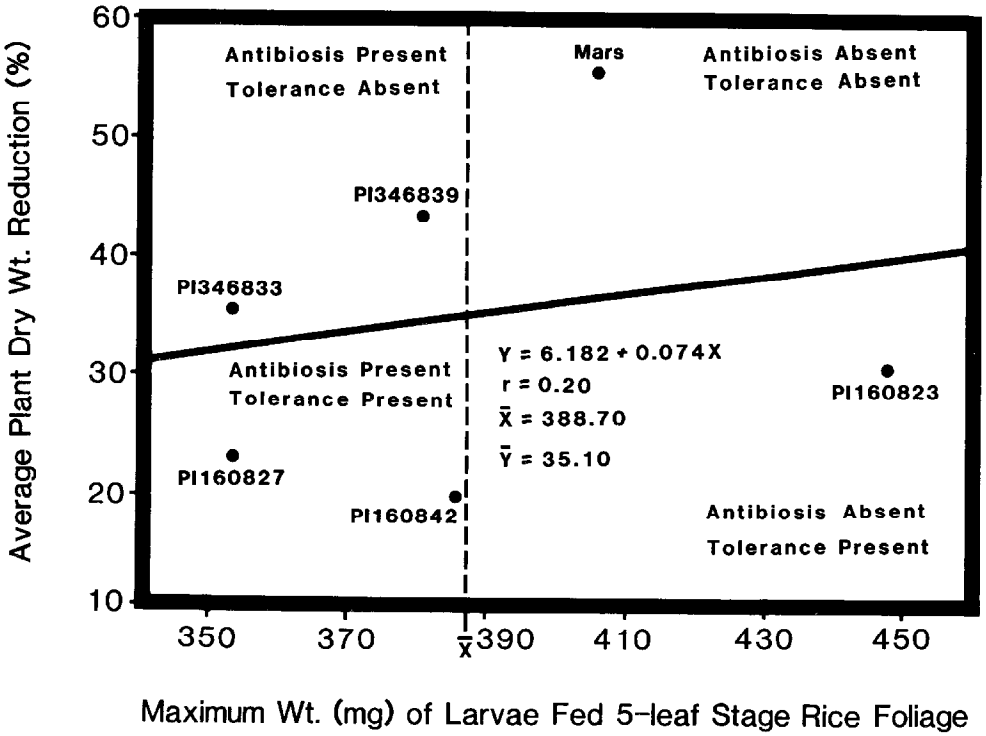

Fig. 1. Relationship between percent rice plant dry weight reduction (tolerance) and maximum larval weight of fall armyworms (antibiosis) fed foliage of five rice plant introductions and the cultivar 'Mars' at the 5-leaf stage of development.

stage seedlings. Hardy et al. (1986) reported that both neonate and fourth instar FAW larvae feed more on new growth than on older growth of tall fescue grass. FAW larvae also prefer young corn leaves over old leaves as a food source (Garner & Lynch 1981). Similarly, the resistance level of rice plants may vary as the plants mature. In our study, the maximum larval weight of FAW larvae on PI160827 was among the highest when fed 3-leaf stage foliage, but became the lowest when larvae were fed 5-leaf stage foliage.

Larval weight has been used previously as an indicator of rice plant resistance to insects. Oliver & Gifford (1975) studied the effects of seven rice lines on the larval weight of the rice stalk borr, *Chilo plejadellus* Zincken, and identified two cultivars as potential sources of stalk borer resistanc. In our study, the maximum larval weights of FAW fed 5-leaf stage foliage of PIs 346833 and 160827 were the lowest among the five PIs tested, indicating that these two PIs are more resistant to FAW than the other PIs. In general, PIs 160842, 346833, 346839, and 160827 had more antibiotic effects on FAW growth than Mars and PI160823, based on the maximum larval weight of FAW fed 5-leaf stage foliage.

The percent reductions in plant dry weight of the five rice PIs tested were lower than that of the check cultivar 'Mars'. PI 160827, which did not exhibit antixenosis (Pantoja et al. 1986), had an antibiotic effect on FAW growth and was tolerant to defoliation as indicated by its low percent reduction in plant dry weight. These results indicate that the resistance by one cultivar cannot be justified by a single category of resistance. The relationships between antibiosis and tolerance (Fig. 1) demonstrate the relative contributions of each type of FAW resistance in PIs tested. PIs 346833 and 346839 have antibiosis but no tolrance to FAW damage. PIs 160842 and 160827 appear to be sources of antibiosis and tolerance resistance to FAW; however, PI160842 appears to be the most promising PI to use for development of lasting resistance to FAW, because it also has the antixenosis resistance reported by Pantoja et al. (1986).

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DEVELOPMENT OF OPEN-POLLINATED VARIETIES, NON-CONVENTIONAL HYBRIDS AND INBRED LINES OF TROPICAL MAIZE WITH RESISTANCE TO FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA:NOCTUIDAE), AT CIMMYT

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ABSTRACT

As part of the overall improvement of maize, *Zea mays* L., the International Maize and Wheat Improvement Center (CIMMYT) has been working to develop tropical maize materials with improved levels of resistance to fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), and good agronomic qualities. Efficient mass rearing and infestation techniques have been developed, many of which have been adopted or adapted by other FAW host plant resistance workers. Our initial efforts to develop FAW resistance consisted of a population improvement approach. Recently, CIMMYT's maize insect resistance improvement program has been amplified to include screening and development of new sources of resistance, international testing of these sources, and inbred line and 'non-conventional' hybrid development, in addition to the continuing development of open-pollinated varieties.

RESUMEN

Como parte del programa de mejoramiento de maíz, el Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) ha trabajado para desarollar el germoplasma de maíz con niveles de resistencia de la planta huésped contra al "cogollero", *Spodoptera frugiperda* (J. E. Smith). Se han desarollado técnicas eficientes de crianza masiva e infestación artificial al nivel de campo. Muchas de esas técnicas han sido adoptadas por otros trabajadores en resistencia de la planta huésped para facilitar sus trabajos y estudios. Nuestros trabajos iniciales fueron sobre el mejoramiento de resistencia al nivel de poblaciones de maíz. Recientemente, el programa de entomología sobre la resistencia de las plantas huéspedes ha sido ampliado para incluir la evaluación y desarollo de fuentes de resistencia nuevas, ensayos internacionales para probar dicha resistencia, y la formación de variedades de polinización libre, lineas endocriadas e híbridos 'no-convencionales' con resistencia al cogollero.

For more than a decade, the maize improvement program of the International Maize and Wheat Improvement Center (CIMMYT) has included improvement of host plant resistance (HPR) to the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), as an integral part of its selection and improvement process in maize materials of tropical lowland and subtropical adaptation (Mihm 1985). The FAW is a major destructive field pest of maize in tropical and subtropical areas of the Americas, and in favorable years even damages fields in temperate production areas. Enhanced levels of resistance to

TABLE 1. INTERMEDIATE GERMPLASM PRODUCTS OF THE CIMMYT MAIZE IMPROVEMENT PROGRAM.

Category	Composition	Number
Pools	± 500 Half-sibs	± 50
Populations	± 250 Full-sibs	± 40
Experimental varieties	± 10 Full-sibs	± 600
Inbred lines	S_3-S_5 level	±5000
'Non-conventional' hybrids	Variety & Topcross	± 500

FAW in maize cultivars is highly desirable and would have great utility to CIMMYT's clientele, the National Agricultural Research Systems (NARS) and their farmers. In many developing countries, where low input agriculture predominates, HPR is the only practical means of managing pest populations and minimizing production losses resulting from pest attack.

Because of its facilities for insect mass rearing, its personnel (breeders, entomologists, and pathologists), its resource commitment to the improvement of maize germplasm, and its well established ties to NARS for testing and production research, CIMMYT is uniquely qualified to conduct FAW HPR research (Mihm 1983).

Before 1984, HPR improvement to FAW was carried out in selected CIMMYT germplasm 'pools' and 'populations' (Table 1) of lowland tropical adaptation (Ortega et al. 1980). The goal was gradual improvement of resistance on a population level, and extract superior progenies to form open-pollinated experimental varieties with improved resistance (Mihm 1985). When the work was begun in the late 1970s, such varieties were the most appropriate intermediate product for CIMMYT to produce because of the ease of maintenance, seed production, and distribution for most client countries.

In 1984, it was apparent that the progress made was slow because of low gene frequency and competitive selection concerns. Also, several NARS were developing capabilities of viable hybrid seed production systems, especially for utilizing varietal or topcross hybrids (termed 'non-conventional' by the CIMMYT maize program) (Table 2). Nearly all the maize grown in developed countries is produced from single-, three-way, or double-cross hybrids, which are formed from crosses of highly inbred maize lines, and growers purchase new seed for each planting. In developing countries, it is difficult to maintain inbred line purity, to produce good quality seed of hybrids, to get high enough seed yields, and to get seed distributed on time to farmers at a price they can afford. Many of these problems are minimized in the production of 'non-conventional' hybrids. Although these are less uniform for many characteristics than conventional

TABLE 2. CONVENTIONAL HYBRIDS, MADE BY CROSSING HIGHLY INBRED LINES, CONTRASTED TO 'NON-CONVENTIONAL' HYBRIDS, WHERE AT LEAST ONE PARENT IS NON-INBRED.

Conventional	Components	'Non-conventional'	Components
Single cross	A x B	Topcross:	Commercial hybrid x Line
Three-way	(A x B) x C		Family (half-, full-sib) x Line
Double cross	(A x B) x (C x D)		Variety x Line
		Varietal:	Family (half-, full-sib) x Family
			Variety x Family
			Variety x Variety

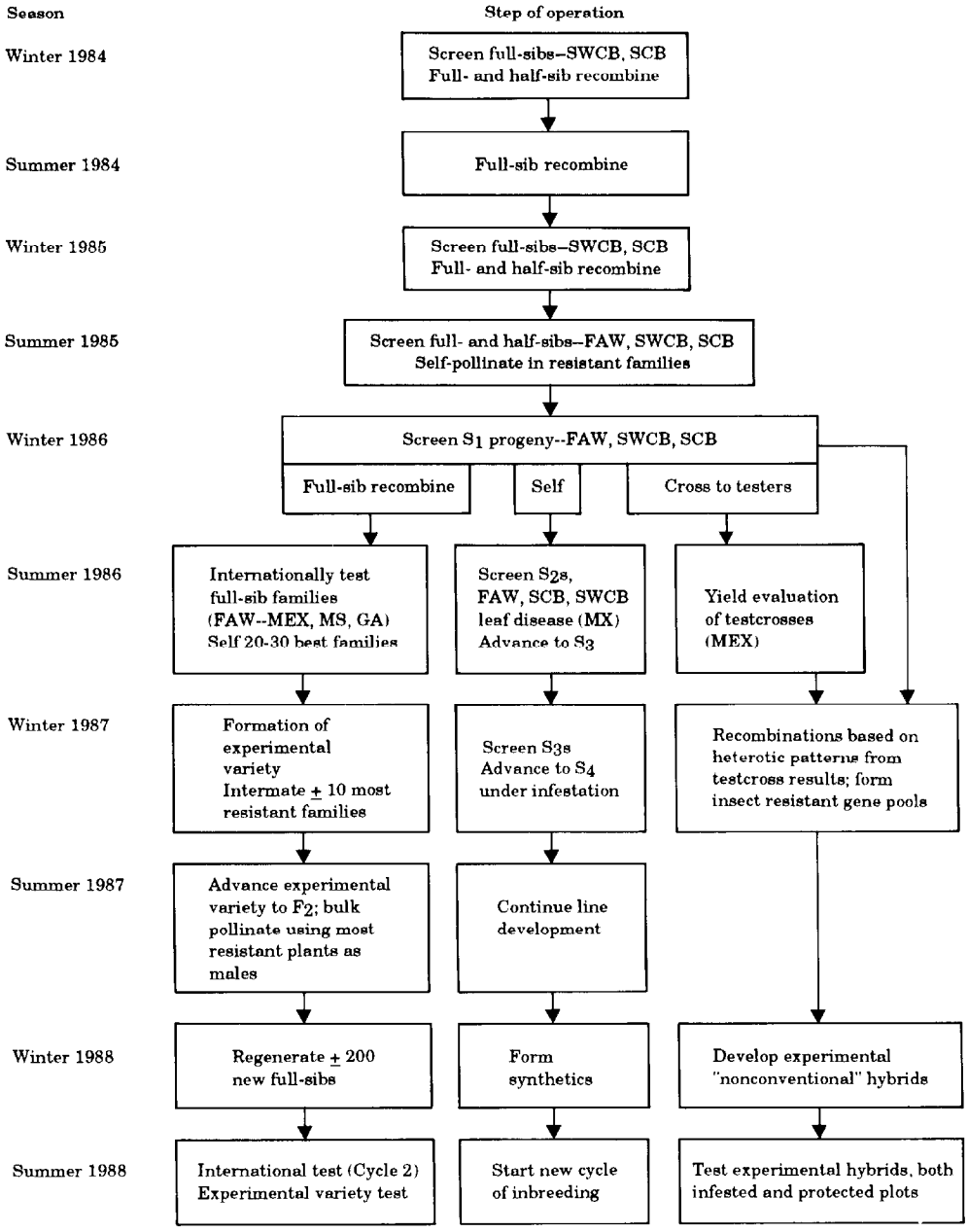

Fig. 1. Flow-chart showing operations and breeding methodology used in developing the Multiple Borer Resistance (MBR) population, inbred line extraction, and the formation of experimental varieties and 'non-conventional' hybrids.

hybrids, this usually does not detract from their acceptability. Many developing country farmers actually benefit from some heterogeneity in their cultivars, as their production fields are often not uniform, and harvest is done by hand labor, not machines. Suitable cultivars for most subsistence farmers need only be fairly uniform in grain color and texture, maturity, yield, and postharvest storability (Deutsch 1988).

In order to make greater and faster progress in developing FAW resistance, and to produce an array of germplasm products with greater utility to a wider range of maize

improvement programs in both developing and developed countries, CIMMYT formed a new maize population and began improving it for resistance to several maize field pests. This population was named the Multiple Borer Resistance (MBR) population (Fig. 1). It was formed from predominantly subtropical and temperate materials that were reported to have high or intermediate levels of resistance to FAW and the following subtropical or temperate region maize stem borers: *Ostrinia nubilalis* (Hubner), *Diatraea grandiosella* (Dyar), *D. saccharalis* Fabricius, and *Chilo partellus* (Swinhoe) (Benson 1986, Mihm 1986, Smith et al. 1988).

Progenies from this population were evaluated internationally for resistance in 1986. Two hundred full sib families were sent to interested cooperators who had the capability to artificially infest at least 10 plants per progeny row with FAW or one of the maize stem borers to assess resistance to first and/or second generation damage by that pest (Smith et al. 1988). Each cooperator infested 10-15 plants per row with 30-40 newly hatched FAW larvae (Mihm 1983). Leaf feeding ratings were taken 14-21 days after infestation using a 1 to 9 scale (1 = highly resistant, 9 = highly susceptible. These, and other data of interest were reported to CIMMYT. The number of families in each resistance category from the Mississippi, Georgia, and Mexico FAW evaluations are presented in Fig. 2. The results indicated fairly high frequencies of FAW resistance, with most of the families showing high (1-3 ratings) or intermediate (4-6 ratings) levels of resistance. Only a few families were classified in the susceptible category. These results indicate this population is an excellent source of FAW resistance; most breeding materials and commercial cultivars are found to be susceptible when artificially infested with FAW.

In addition to taking resistance ratings, each cooperator selected the 20 to 30 most resistant families (equal or superior to the best resistant check entry) with good agronomic characters and self pollinated the best plants in these families. The majority of the seed harvested from these plants remained with the cooperators for use in their HPR program. Most of the cooperators sent 50-100 seeds of each selected ear to CIMMYT, where they are being used to regenerate a new set of progenies for testing in 1988, and for line and variety development. For each test site that successfully evaluated the population, there is the potential to develop an experimental variety and/or extract inbred lines with potentially superior resistance. If the population was tested for the same species at more than one site, there is the potential to develop an across site experimental variety, using the 8-10 most resistant families across sites as progenitors. Based on the results from the 1986 MBR international test, CIMMYT decided to develop only Across 86 MBR-FAW for the following reasons: (1) the cooperators from Mississippi and Georgia have the production of resistant inbred lines as their goal; (2) both sites are similar in latitude and general adaptation to the Mexican test site; and (3) most of the selected families were common across sites, therefore site specific experimental varieties would not differ greatly.

Seed of selected S1's returned to CIMMYT from these and other sites which evaluated for stem borer resistance was used to continue inbred line development and to regenerate a new set of full sib progeny for international testing in 1988 (Fig. 1). Some of the initial S1 lines used in developing the full sib families for the 1986 international progeny testing trial (IPTT) were also testcrossed to a set of testers to determine combining ability groups. These testcrosses were grown in a subsequent season under both infested and protected conditions to identify their resistance reaction and yield performance. The results were used to help form two insect resistant pools with good potential combining ability. Information from the testcross performance also will be used to identify lines for forming synthetics and experimental 'non-conventional' hybrids with high yield potential and good resistance, primarily for developing country NARS, but of possible value in developed country programs as well.

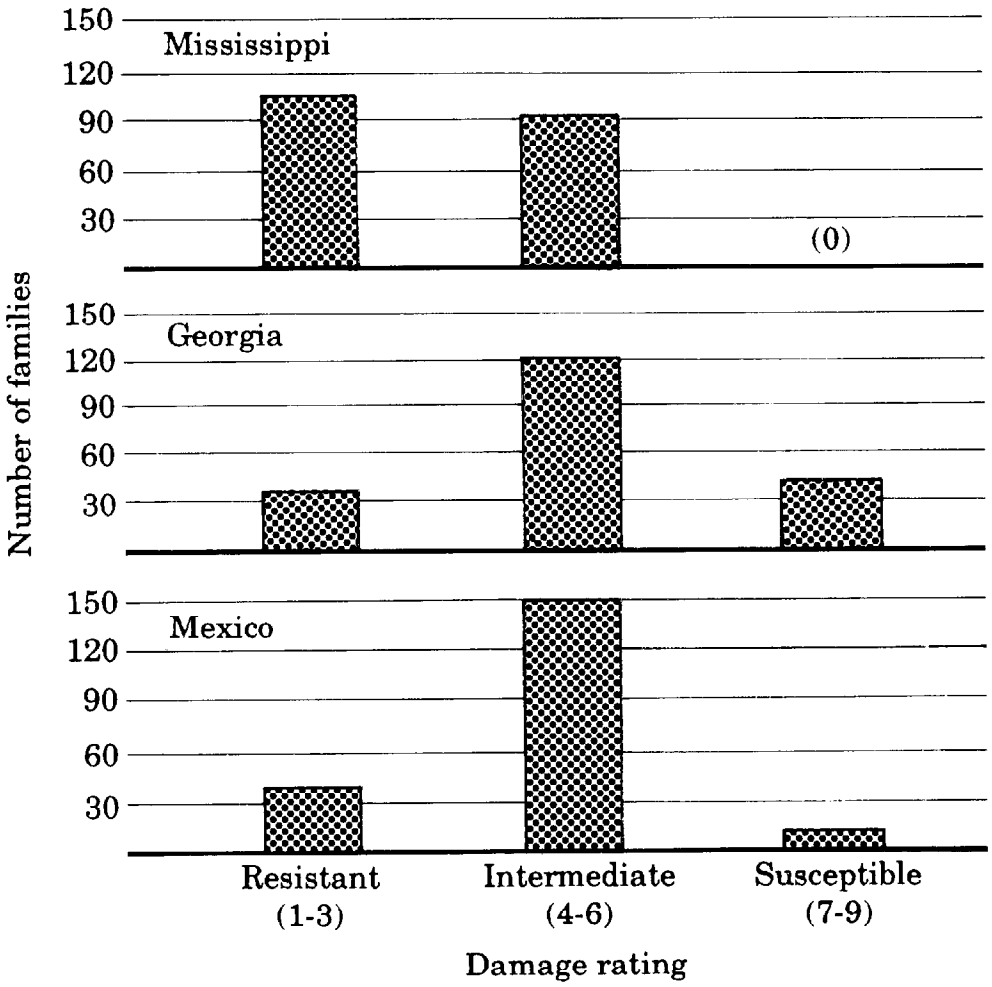

Fig. 2. Fall armyworm leaf-feeding ratings of 200 full-sib families of MBR population at three locations.

In developing the MBR population and monitoring the sites where it was internationally tested, two things were observed. First, although there was notable expression of daylength sensitivity in this germplasm, cooperators were able to obtain viable seed from testing sites more than 40° North latitude to 30° South latitude, indicating broad general adaptation of the population. Second, there was variability for resistance to common foliar diseases within the population. As we are aware of the importance of resistance to plant pathogens as well as insect pests, and know that varieties will not be acceptable to farmers unless they are resistant to both, we also screen and select for resistance to leaf diseases, and ear and stalk rots.

Lines from the MBR population that are selected for insect resistance are being screened for reaction to common rust, *Puccinia sorghii* Schw. and *Helminthosporium* sp. leaf blights. The most resistant disease selections are then re-screened for insect reaction and re-incorporated into the population.

Testing of the MBR indicated a more complex approach was needed for developing resistant germplasm for lowland humid tropical areas, especially where the downy mildew disease complex or maize streak virus diseases occur (although there were apparent differences for streak reaction at the Kenya testing site). In order to develop insect

TABLE 3. EXPERIMENTAL VARIETIES, FAMILIES, AND INBRED LINES SELECTED FOR RESISTANCE TO FALL ARMYWORM AT CIMMYT.

Material	Selections available	Adaptation
Tuxpeno	PR 8321, Exp'l. variety	Tropical/subtropical
Antig.-Ver. 181	PR 8424, Exp'l. variety	Tropical/subtropical
Pools 24, 26	Half- & full-sib families, S_1 lines	Tropical/subtropical
MBR	Across 86-FAW, Exp'l. var.	Subtropical/temperate
Varios x Mp lines	Full-sibs, S_1 & S_2 lines	Tropical/subtropical
MIRT	Population development	Tropical/subtropical

resistant germplasm to serve in such areas, another population is being developed. It is named the Multiple Insect Resistant, Tropical (MIRT) population (Mihm 1986, Smith et al. 1988). The germplasm incorporated into this population is predominantly of lowland tropical adaptation. Crosses with sources of downy mildew resistance from Thailand and streak resistance from the CIMMYT/IITA program in Nigeria were included. Selections of FAW and stem borer resistance from CIMMYT pools and populations, from 'Antigua' collections from the CIMMYT maize germplasm bank, and Mississippi resistant lines and hybrids were added.

The MIRT population is still being formed, but we expect to have a set of full-sib progenies ready for international testing in 1989. We hope to send it for insect resistance screening to cooperators in countries with lowland humid tropical climates, where FAW and other armyworms and borer pest complexes occur. Additional evaluation for disease reaction will be useful though not essential at this time.

The best sources of FAW and stem-borer resistance available to the present, the Mississippi-released inbred lines, are deficient in many agronomic characteristics (Williams & Davis 1988). However, Overman (1988) was able to use them to develop new hybrids with multiple resistance to leaf-feeding and stalk-boring lepidopterous pests by crossing them with susceptible, agronomically superior U.S. Corn Belt lines, then extracting new resistant lines. These lines, in hybrid combinations, are competitive in yield with current commercial hybrids in the absence of pest attack, yet superior in yield under insect infestation (Overman 1988). Some of the recently extracted CIMMYT 'Antigua' lines are equal to the Mississippi lines in FAW and stem borer resistance, and superior to them in standability and leaf blight, stalk and ear rot resistance under Mexican growing conditions. Their combining ability and yield performance are yet to be tested.

Table 3 lists various materials that have been selected for FAW resistance at CIMMYT that are presently available in small quantities for testing. In the near future, we expect to develop and have available new materials, in the form of open-pollinated varieties, synthetics, inbred lines, and experimental non-conventional hybrids with potential resistance to FAW and other insect and disease pests of maize from temperate to tropical growing areas.

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FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) INFESTATIONS IN NO-TILLAGE CROPPING SYSTEMS

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ABSTRACT

Field experiments comparing no-tillage and plow-tillage practices demonstrated that infestations by the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), ultimately become similar in either cropping system. However, in certain no-tillage situations where high mulch concentrations were present on the soil surface, oviposition and damage were reduced. Significantly fewer egg masses and damage were sampled on corn, *Zea mays* L., (3-leaf stage) while seedlings remained within no-tillage mulch. Oviposition quickly became similar to that observed in plow-tillage systems when the plants grew above the mulch canopy of no-tillage. The number of egg masses on corn older than 4 leaves was similar in either cropping system, and leaf injury at plant silking was the same. In a comparison of corn, sorghum (*Sorghum bicolor* [L.] Moench.), and soybeans (*Glycine max* L.), the latter crop had no damage in either tillage system while corn and sorghum were heavily infested. Efficacy of chlorpyrifos (0.56 kg [AI]/ha) for controlling fall armyworm leaf damage was similar in corn and sorghum in either cropping system.

RESUMEN

Experimentos donde se comparó el no labrar y el labrar con arado demostró que las infestaciones por el gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), al final, son igual en cualquiera de los dos sistemas. Sin embargo, en ciertas situaciones donde no

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se ara y está presente una concentración alta de capa vegetal en la superficie del suelo, se redujo la oviposición y el daño. Significativamente menos masas de huevos y daño se encontró en muestras de maíz, *Zea mays* L., (etapa de 3-hojas), mientras las plantas de semilleros permanecieron dentro de la capa vegetal no arada. La oviposición se igualó rapidamente a aquella observada en el sistema labrado con arado cuando las plantas crecieron por arriba de la capa vegetal cuando no se aró. El número de masas de huevos en maíz más viejo de 4-hojas, fue similar en cualquiera de los dos sistemas de labranza, y el daño a la hoja cuando la mazorca está produciendo seda también fue igual. En una comparación entre el maíz, el sorgo (*Sorghum bicolor* [L.] Moench.), y la soya (*Glycine max* L.), el último cultivo no tuvo daño en ninguno de los sistemas de labranza, mientras que el maíz y el sorgo fueron muy infestados. La eficacia de clorpirifos (0.56 kg [AI]/ha) en controlar el daño a las hojas por el gusano cogollero fue igual en el maíz y el sorgo en cualquiera de los sistemas de labranza.

Entomologists recognize that the crop environments created by no-tillage practices are often greatly different from those of plow-tillage systems and are justifiably concerned about enhanced biological potential of pests like the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), in no-tillage (All & Musick 1986). Increased damage by other noctuids such as the armyworm, *Pseudaletia unipuncta* (Haworth), and the black cutworm, *Agrotis ipsilon* (Hufnagel), in no-tillage cropping systems has been reported (Musick & Petty 1973, Wrenn 1975), and the fall armyworm infests many of the same crops. Therefore, this paper reviews data from experiments designed to determine if fall armyworm hazard is influenced by lack of tillage.

Materials and Methods

Field experiments were conducted during 3 years (1985-1987) near Athens and Griffin, GA. The experiments had either a split plot or split-split plot design. The main plots were preplanting land treatments consisting of some form of no-tillage compared with plow-tillage. The designation 'no-tillage' consisted of treatments in which no plowing operations were used immediately prior to planting of seed. This included planting into fallow land with weed cover and/or debris from former crops. Also, no-tillage treatments were used in double cropping systems where a field crop was planted into standing small grains (including rye, *Secale cereale* L.; wheat, *Triticum vulgare;* and barley, *Hordeum sativum*, or into stubble following harvest of the grain.

Plow-tillage treatments consisted of tilling the soil with either a moldboard plow, disk harrow, or rotary tiller. Soil was tilled up to 5 times in some treatments to insure a smooth seed bed for planting. The size of no-tillage and plow-tillage plots varied from 0.03-0.1 ha with 3-5 replications in different experiments.

The subplots in the experiments were selected crops (corn, *Zea mays* L.; sorghum, *Sorghum bicolor* [L.] Moench.; and soybeans, *Glycine max* L.) and insecticide treated or untreated. In the crop comparison test, 16 rows (20 m long) of corn, sorghum, or soybeans were each planted on the same day in June plantings of replicated plow-tillage or no-tillage plots. Chlorpyrifos was applied at a rate of 0.56 kg [AI]/ha at 10 day intervals for 3 applications, beginning in the 3-leaf stage of development for corn and sorghum, and for 1 application in the V6 stage of soybeans. The chlorpyrifos treatments were 4 row plots x 8 m long and were paired with untreated plots in each of the corn, sorghum, and soybean treatments.

Sampling of fall armyworm infestations was done by examining 5 consecutive plants for egg masses at 3.3 m intervals, covering a field or plot uniformly. At least 500 plants were examined in each experiment. Damage was assessed on the plants when they were examined for egg masses by making a visual estimate of the degree of injury based on

a scale of 0-7. Plants received a 0 rating when no injury was present; 1 was slight (< 10%) leaf damage with no feeding in the whorl as evidenced by the lack of gummy excrement; 2 was moderate (10-20%) leaf damage and no whorl feeding; 3 was heavy (> 20%) leaf damage and no whorl feeding; 4 was < 20% leaf damage and light whorl damage with a slight amount of excrement; 5 was 20-40% leaf damage with a moderate amount of excrement in the whorl; 6 was severe leaf feeding (> 40%) with a large amount of excrement in the whorl; and 7 was plants that had leaves with only midribs remaining and buds destroyed (plant was virtually destroyed).

One or more pheromone traps baited with (Z)-9-dodecen-1-ol acetate were placed in the test fields to monitor fall armyworm adult seasonality patterns. Information on adult flights into fields was used in timing planting operations and insecticide applications in the tests.

The data were analyzed using analysis of variance (SAS Institute 1985) with procedures appropriate for a specified experimental design. Duncan's new multiple range test (Duncan 1955) was used to separate means.

Results and Discussion

Table 1 shows results from 3 tests with no significant difference between plow-tillage and no-tillage in % egg masses (except test 3) or foliar damage by the fall armyworm. These results are representative of general observations in many experiments in corn over several years (1980-1988) of fall armyworm infestations in fields with plants in the 6-leaf stage (25-30 days after planting) or older in comparisons of plow-tillage and no-tillage systems.

On several occasions, it was observed that damage to 2- and 3-leaf stage plants (10-15 days after planting) was greatly reduced in no-tillage as compared with plow-tillage, especially when the no-tillage fields had a heavy mulch from previous crops. Figure 1 shows results from a test using corn planted on July 29 following harvest of rye. A heavy rye mulch was present on the soil surface in the no-tillage plots. Corn germination and development in the no-tillage plots was similar to that observed in plow-tillage. The corn seedlings were shorter than the mulch height up to the early 3-leaf stage. Oviposition and damage were significantly less ($P < 0.05$) in no-tillage plots as compared with plow-tillage. Leaves were first observed above the mulch in the late 3-leaf stage 16 days after planting. This coincided with greater oviposition and, thus, at 20 days the number of egg masses on plants was similar in the 2 tillage systems. Damage remained significantly higher in the plow-tillage plots than in no-tillage. This trend also was evident after 25 days, but the differences were not significant. The number of egg masses that were sampled at 32 days dropped substantially and may reflect sampling error due to diffi-

TABLE 1. Comparison of fall armyworm infestations in no-tillage and plow-tillage corn.

	Till		No-Till	
Test[1]	% Egg Masses	Damage Rating	% Egg Masses	Damage Rating
1 (20 da)	0.8	3.8	0.3	3.7
2 (30 da)	5.7	4.9	6.8	4.0
3 (40 da)	3.0[2]	3.7	0.5	4.0

[1]Tests 1 and 3 were conducted near Griffin and test 2 near Athens GA. A minimum of 100 plants were examined in each plot at a specified number of (days) after planting.

[2]Significantly different from plow tillage in analysis of variance ($P < 0.05$).

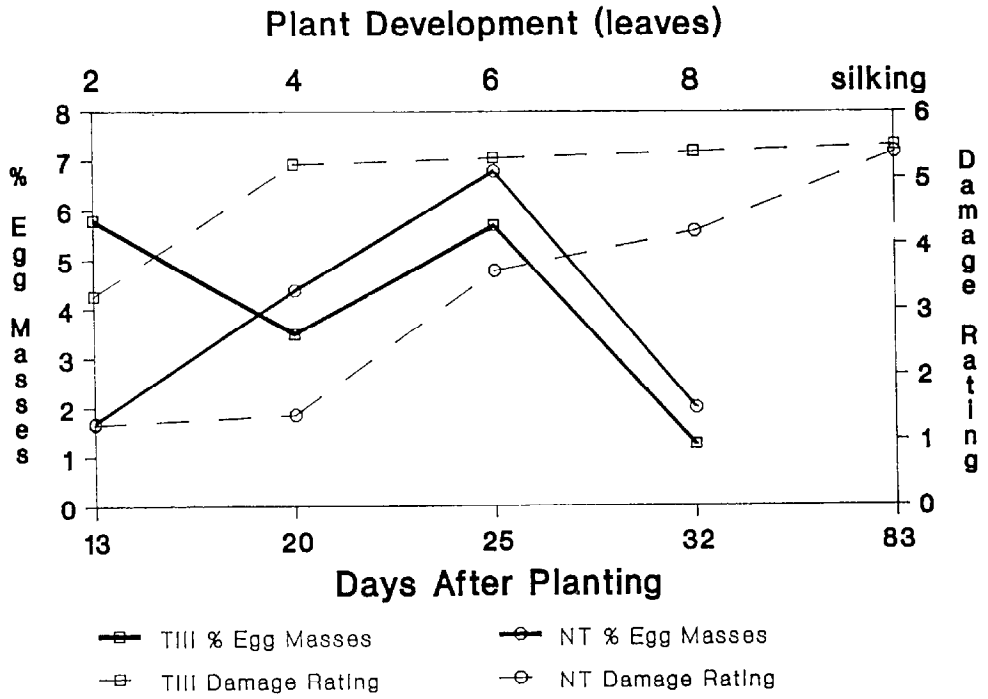

Fig. 1. Seasonal comparison of fall armyworm infestations in no-tillage and plow-tillage corn as expressed by sampling of unhatched egg masses on leaves and by visual estimates of leaf and whorl damage on a scale of increasing severity of 0-7. Significant ($P < 0.05$) differences occurred between tillage systems in egg masses and damage on day 13 and in damage on day 20 after planting.

culty in finding egg masses on the heavily damaged leaves of the late 8-leaf stage plants. Injury was not significantly different in the 2 tillage systems at 32 days, or when the plants initiated silking 83 days after planting. In this test, the yield was significantly ($P < 0.05$) higher in the no-tillage (5033.0 kg grain/ha) treatment as compared with plow-tillage (2597.7 hg/ha).

In another test in which corn, sorghum, and soybeans were compared, there was no significant difference in either fall armyworm damage or yield between the 2 tillage systems in corn and sorghum (Table 2). No infestation occurred in any of the soybean treatments. Significantly ($P < 0.05$) less damage occurred in the noninsecticide-treated sorghum as compared with corn. Chlorpyrifos sprays provided significant ($P < 0.05$) control of infestations in only the plow-tillage treatments of corn, but significant differences in yield occurred between insecticide and nontreated plots in both tillage systems of corn and sorghum.

In conclusion, these data show that infestations of the fall armyworm may be reduced in no-tillage systems under certain circumstances. Infestations on recently germinated plants are reduced during the short period of 5-10 days that seedlings remain below the mulch canopy of certain no-tillage systems. Less oviposition occurs on plants in these situations and may be due to an inability of moths to locate the crop within the mulch. When plants grow above the mulch, oviposition quickly becomes similar to that observed in plow-tillage systems. The ultimate defoliation damage in later crop developmental stages is similar in no-tillage and plow-tillage systems. The lag in the development of fall armyworm infestations in no-tillage may have pest management value by reducing the need for 1 or more insecticide applications to protect young seedlings during the critical post-germination stages of plant establishment and growth.

TABLE 2. FALL ARMYWORM DAMAGE IN CORN, SORGHUM, AND SOYBEANS PLANTED IN NO-TILLAGE AND PLOW-TILLAGE SYSTEMS WITH OR WITHOUT CHLORPYRIFOS (0.56 KG [AI]/HA) TREATMENT.

	Corn			
	Till		No-Till	
	Treated[1]	Untreated	Treated	Untreated
Damage Rating	1.3a	4.2b	2.0a	3.9a
Yield kg/ha x 100	73.2a	22.6b	68.0a	34.8c
	Sorghum			
	Till		No-Till	
	Treated	Untreated	Treated	Untreated
Damage Rating	1.3a	2.1a	1.6a	2.1a
Yield kg/ha x 100	22.6a	11.5b	19.2a	13.4b
	Soybean			
	Till		No-Till	
	Treated	Untreated	Treated	Untreated
Damage Rating	0a	0a	0a	0a
Yield kg/ha x 100	0.6a	0.6a	0.7a	0.5a

[1]Damage rating 60 days after planting was significantly different between crops, but not between tillage. Duncan's multiple range analysis ($P < 0.05$) values are for tillage and insecticide treatments within a crop. Damage and yield analyses are separate.

In comparisons of corn, sorghum, and soybeans, it is apparent that soybeans are least susceptible. Soybeans would be preferred over the other crops in high hazard situations for fall armyworm infestations, such as certain double cropping systems that have later than normal planting dates. In situations where insecticides are required to suppress fall armyworm infestations, data indicate that chemical control operations utilized in plow-tillage systems are applicable to no-tillage.

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POTENTIAL FOR USING MAIZE AS A TRAP CROP FOR THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE), WHERE SORGHUM AND MAIZE ARE INTERCROPPED ON SUBSISTENCE FARMS

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ABSTRACT

The effectiveness of a maize trap crop in reducing fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), larval infestations on sorghum, *Sorghum bicolor* (L.) Moench. in areas where sorghum and maize, *Zea mays* L., are grown together in the same field was investigated in small plots at Choluteca, Honduras in 1984 and at Starkville, Mississippi in 1984 and 1985. Infestations of FAW larvae were similar on sorghum treatments with and without the maize trap crop in Honduras and in one test in Mississippi, 1984. Apparently, the area ratio of 2:1 for sorghum : maize plantings in close proximity did not differentially restrict FAW activity on the preferred maize plants. However, significantly higher FAW larval infestations and plant damage were observed on maize than on sorghum in pure stand or when grown together with maize as the trap crop in a second test conducted in 1985 in Mississippi. The results of these studies support reported observations in greenhouse and field cages showing higher oviposition by FAW moths on maize than on sorghum. The higher infestation of FAW on maize further suggests the potential for use of maize as a trap crop for monitoring insect populations and its possible use as a trap crop control tactic for FAW in areas of low rainfall where sorghum is grown as a main crop and this insect is a serious pest.

RESUMEN

Se investigó la efectividad del maíz usado como trampa para reducir infestaciones por larvas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), en sorgo, *Sorghum bicolor* (L.) Moench., en áreas donde el sorgo y el maíz, *Zea mays* L., se cultivan juntos en el mismo campo, en pequeñas parcelas en Choluteca, Honduras, en 1984, y en Starkville, Mississippi, en 1984 y 1985. Infestaciones de larvas del gusano cogollero fueron similares en sorgo tratados con y sin trampas de maíz en Honduras, y una prueba en Mississippi en 1984. Aparentemente, la proporción de 2:1 de sorgo:maíz sembrados en corta proximidad, significativamente no restringió la actividad del gusano cogollero en las preferidas plantas de maíz. Sin embargo se observaron significativamente más altas infestaciones larvales y daño a las plantas de maís que en las de sorgo, o cuando cultivado junto con maíz como el cultivo de trampa en una sequnda prueba hecha en 1985 en Mississippi. El resultado de estos estudios apoyan las observaciones que se han reportado en invernaderos y jaulas en el campo que indicaron una mayor oviposición en maíz que en sorgo. La alta infestación de gusano cogolleros en el maíz sugiere además el potencial uso del maíz como un cultivo de trampa para chequear las poblaciones de insectos y su posible uso como táctica de control contra el gusano cogollero en áreas de pocas lluvias donde el sorgo es cultivado comc un cultivo principal y este insecto es una plaga seria.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a polyphagous insect that attacks 50 non-economically and 30 economically important plants (Ashley 1979). The FAW acts as a cutworm on young plants, cutting plants at soil level, and on more developed plants the larvae feed on foliage. When severe infestations occur on maize or sorghum, all the foliage may be consumed except the midrib (Andrews 1984). Yield reduction in maize by FAW feeding damage ranges from 20 to 87 percent (Henderson et al. 1966, Andrews 1980, respectively).

Fall armyworm infestations can be high on hosts in the grass family (Poaceae), wherein more oviposition occurs on maize than on sorghum, even when the two crops are grown together (Sifuentes 1967, Van Huis 1981). Resource management has received little attention in the past but is perhaps the most feasible overall approach to FAW control (Lewis & Nordlund 1980). Some research has been conducted using trap crops to attract a pest species or to provide a more favorable habitat to increase natural enemies. The interplanting of alfalfa strips in cotton fields is an example (Huffaker & Messenger 1976) since *Lygus hesperus* Knight prefers alfalfa over cotton as long as the alfalfa remains in a lush growing condition.

Because FAW prefer to oviposit on maize, we investigated the hypothesis that a maize trap crop would concentrate FAW larval populations in sorghum production fields. Thereby, insecticide applications for control of this pest could be limited to the area occupied by the trap crop. This pest management tactic has application in high technology agricultural production areas and would be of value to subsistence farmers in developing countries, such as Honduras, where 93% of the sorghum is intercropped with maize (Donaire 1982).

Materials and Methods

1984 Study I. In 1984 FAW larval infestations in sorghum grown with a maize trap crop were compared to infestations in pure stand sorghum (adjacent plots) (Fig. 1A) at Choluteca, Honduras. Sorghum and maize were planted on June 14 and June 21, respectively. Maize, planted in a block in the middle of the sorghum, comprised 20% of the total area in the trap crop treatment. Each treatment plot was 40 x 40 m arranged in a randomized complete block design with four replications. Infestation by FAW larvae and damage ratings (on a scale of 0-9; 0 = no damage, 9 = plant dead (Wiseman et al. 1966, Wiseman & Davis 1979) were recorded weekly, for six weeks, on 20 plants selected at random in each treatment plot. To find larvae feeding in the whorl, plants were pulled from the ground and the whorl leaves were separated. Using this destructive sampling technique, the whole plant was searched for larvae. The data were analyzed by analysis

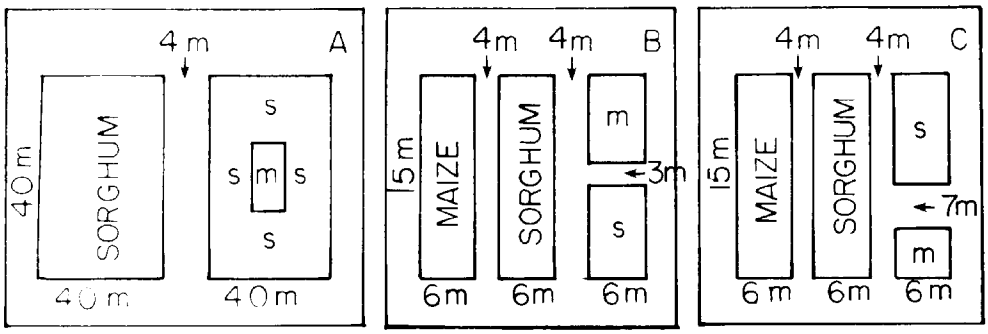

Fig. 1. Sorghum (S) and sorghum-maize (M) trap crop planting designs. A. 1984 Honduras; B. 1984 Mississippi, USA; C. 1985 Mississippi.

of variance and treatment means were separated by Duncan's multiple range test (Duncan 1955).

1984 Study II. Treatments included pure stand maize and sorghum and a trap crop plot with one-half of the area of each crop separated by 3 m of uncultivated land (Fig. 1B). Each treatment plot was 6 x 15 m separated by a 4 m uncultivated alley. Sorghum was planted on May 28 and maize on June 3 at Starkville, Oktibbeha County, Mississippi. The delay in maize planting was not intended. The experimental design, sampling of plants for FAW larvae, estimates of FAW larval feeding damage, and analysis of the data were as described for the 1984 study I.

1985 Study. In this study, sorghum and maize in pure stands were compared with a sorghum-maize trap crop where maize and sorghum occured in an area ratio of 1 maize : 2 sorghum (Fig. 1C). Sorghum was planted on May 28 and maize on June 3 to duplicate the 1984 Study II in the same field. Maize and sorghum in the trap crop treatment plots were separated by a 7 m uncultivated alley. The test design, sampling procedures, and analysis of data were as described above.

Results

Numbers of FAW larvae per plant or plant damage ratings did not differ significantly ($P>0.05$) on any sample date in the 1984 study in Honduras and the 1984 study in Mississippi. There was a trend for more larvae on maize than on sorghum in the Mississippi study in 1984. However, numbers of larvae per plant (Fig. 2) and plant damage ratings (Table 1) differed significantly on sorghum and maize in the different planting systems on all sample dates in the 1985 Mississippi study. More larvae infested maize than sorghum in pure stand or when grown together with maize as the trap crop. Fall armyworm larval feeding damage was consistently greater on maize than sorghum in these systems.

Discussion

The effective size and location of a maize trap crop in small production areas was not determined in these studies. Due to adjacent stands of maize and sorghum in the 1984 Study I, the FAW larvae and damage to both crops were distributed uniformly within and among treatment plots. With a 1:1 ratio of area planted to each crop separated by 3 m of uncultivated land (1984 Study II), the crops obviously attracted moths into the test area, but due to the relatively large area planted to maize and the close proximity of the crops, the FAW larval population and plant damage to maize and sorghum were uniformly distributed and similar among treatments. Where sorghum and maize were planted in a 2:1 ratio (1985 Study), there were significantly ($P>0.05$) fewer larvae on sorghum than maize in the trap crop system. The larger numbers of larvae on maize than sorghum in the pure stand and trap crop planting systems resulted in significantly ($P>0.05$) more damage to the maize than the sorghum. Fall armyworm larval infestations and damage on sorghum grown in pure stand did not differ significantly ($P>0.05$) from sorghum grown with a maize trap crop. In the relatively small test area, the FAW infestations on sorghum in adjacent treatments were uniform reflecting the equal attractiveness of the sorghum treatments in the test, whereas maize apparently attracted a higher population of FAW. The maize plantings in this study appeared to serve as the trap crop for all sorghum plantings regardless of treatment design. The close spatial arrangement of treatment plots, allowing for uniform dispersal of larvae, negated the possible separation of treatment effects on FAW infestations on sorghum in pure stand and with a maize trap crop.

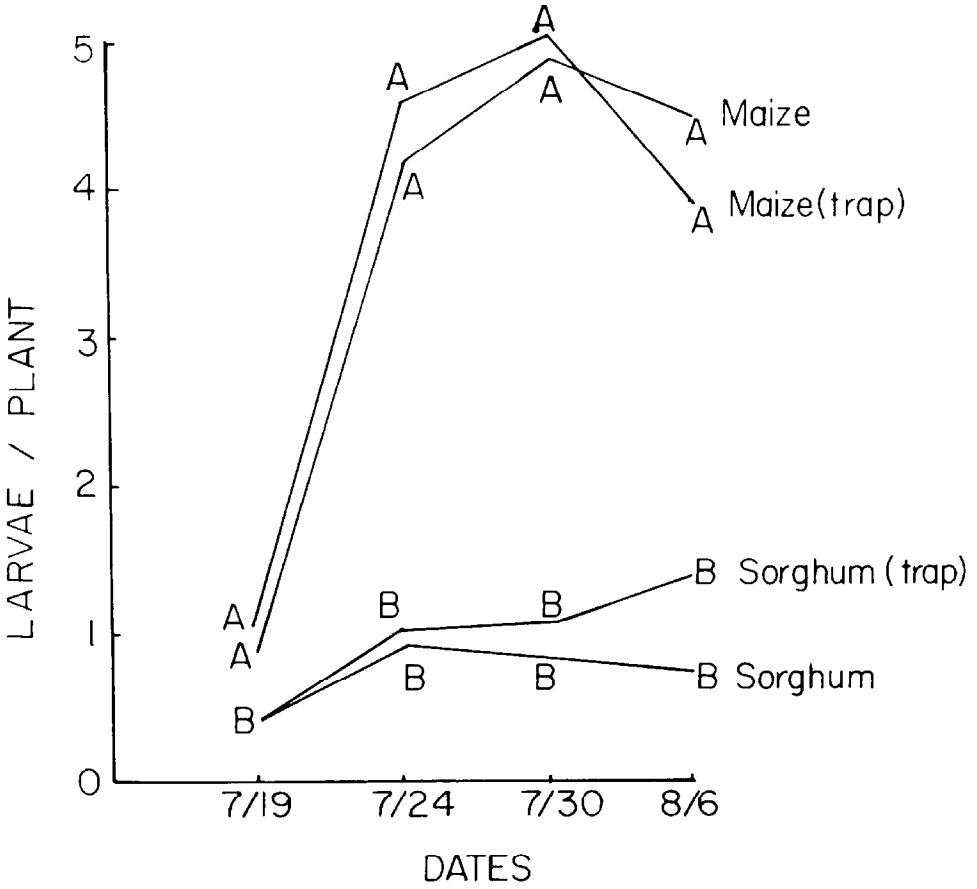

Fig. 2. Fall armyworm larvae on sorghum and maize in different treatment plots [trap indicates that sorghum and maize (the trap crop) in 2:1 crop area ratio (See Fig. 1C)]. Starkville, Mississippi. 1985 Study. Means on each date followed by the same letter are not significantly different [P>0.05; Duncan's multiple range test Duncan 1955)].

TABLE 1. FALL ARMYWORM LARVAL FEEDING DAMAGE TO SORGHUM AND CORN PLANTS IN DIFFERENT CROPPING SYSTEMS, STARKVILLE, MS, 1985.

System	Mean damage rating[1] per plant on dates				
	7/11	7/19	7/24	7/30	8/6
Trap Crop					
Corn	1.15 b[2]	2.70 a	2.40 a	3.70 a	3.40 a
Sorghum	0.72 bc	1.70 b	1.80 b	2.30 b	2.40 b
Pure Stand					
Corn	2.08 a	2.70 a	2.60 a	3.60 a	3.90 a
Sorghum	0.68 c	1.70 b	1.90 b	2.10 b	2.20 b

[1]Damage rating: 0 = no damage, 9 = dead plant.

[2]Means in a column followed by the same letter are not significantly different at [P>0.05; Duncan's multiple range test Duncan 1955)].

Where sorghum is intercropped with maize in many developing countries, the infestations of FAW on both the sorghum and maize plants would be expected to be high and similar due to the closeness of the plants in the system, often in the same hill. Due to general dispersal characteristics (Green & Morrill 1970, Morrill & Green 1973) of FAW larvae and competition for space and food resources, the larvae can readily disperse from the preferred maize plants to adjacent sorghum plants. Therefore, both maize and sorghum in intercropped plantings can be damaged severely by high FAW infestations.

These data support observations by others (Van Huis 1981) of higher FAW larval infestations on maize than sorghum as a result of the greater attractiveness of maize for oviposition. A reduction in area planted to maize compared to that planted to sorghum (less area planted to maize than the 2:1 sorghum : maize ratio used in the present study) and a greater separation of maize from sorghum in a trap crop planting system may improve the effectiveness of the trap crop management tactic by limiting FAW damage to the sorghum. This would be especially important in Honduras and surrounding areas, for example, during the early part of the crop growing season when the plants are attacked by a complex of lepidopterous defoliators, but this needs to be investigated. Additionally, the initial concentration of eggs and larvae on the maize in a relatively restricted cropping area, the maize trap crop, would provide pest survey information required for recommending the application of some pest control tactics. The early detection of FAW egg masses and small larvae should be made easier on the maize in the area of the concentrated trap crop. The dispersal of larvae and infestation on the sorghum crop can be reduced significantly by timely application and concentration of insecticide on the limited area of the maize trap crop. This will result in restricting the use of insecticides to a relatively small area compared with the total area on which the crop is produced and will represent a reduction in production cost to the farmer. The reduction in insecticide use will be less disruptive to the environment and particularly the associated beneficial organisms in the total cropping system.

These benefits would be desired in crop production systems experiencing pest problems regardless of the level of technology involved in producing the crop. In developing countries, where the subsistence farmer can ill afford the use of expensive production practices, the planting of a preferred host plant, in a small area relative to the total area of the main crop with little cost to the farmer to achieve an easy, deliberate method of detection and control of lepidopterous defoliators on crops in early growth stages, can be a valuable tool for the farmer. However, the use of this planting strategy to achieve insect pest control with minimal economic sacrifice must be evaluated in the field in large plots for effectiveness and acceptance.

Endnote

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SYMPOSIUM: NEW TECHNOLOGIES FOR THE TAXONOMIC IDENTIFICATION OF ARTHROPODS

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PREFACE

According to classical alpha-taxonomy a species is defined as a group of populations with similarity in appearance. All the individuals of a species are considered to be relatlvely uniform in anatomical features and clearly separable from other species. Type specimens are used with the presumption that they possess all the essential features of the species. Since the advent during the mid-l970s of electrophoretic methods of uncovering hidden genetic variability, studies have established that natural populations are highly variable. There is no single individual or a given population that represents the entire gene pool of a species. Genetic variability among populations reveals geographical, temporal, clinal and other ecologically related patterns. Unidirectional or bidirectional hybrid sterility has been demonstrated within natural populations that were almost morphologically inseparable. These observations led to the genetic concept of a species. Genetical species can be defined as groups of actual or potentially interbreeding populations reproductively isolated from other such groups. Thus populations of the same species are actually or potentially capable of exhanging genes; i.e., they share a common gene pool. By inference, populations of different species do not share a common gene pool and by definition are said to be reproductively isolated. Under this concept, morphologically indistinguishable, but genetically distinct species are called sibling species. Laboratory hybridization tests and comparative studies of karyotypes have traditionally been the methods for recognition of sibling species. Recently, the entire area of taxonomy is undergoing revolution in conepts and methodologies. Electrophoretic, chemical, and recombinant DNA techniques aided by computer programs,. have greatly facilitated the recognition of new genetical species. This symposium endeavors to address these new technologies for taxonomic identification of arthropods.

COMPUTER ASSISTED IDENTIFICATION OF HYBRID STRAINS OF WESTERN HONEY BEES

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ABSTRACT

Standard, generally-agreed-upon principles of taxomony guide the naming of groups as species. Chief among these principles is that species-specific characteristics can exist because members of different species do not freely produce fertile hybrids.

The history of the nomencalture of subspecies of Western honey bees exemplifies 3 additional taxonomic principles. First, subspecies-specific characteristics have not been found in honey bees. Second, subspecies are distinguishable as groups by the multivariate techniques of principal component analysis or discriminant analysis. Third, subspecies identifications are statistically based and hence probabilistic. These characteristics of subspecies identification are ideally suited to computer applications. Computer systems can be used to collect data and then used to apply complex statistical procedures to provide identifications.

Such techniques have been used to analyze the honey-bee populations of the Americas. The honey bees in the Americas constitute populations resulting from subspecies hybridization. Populations of Africanized bees from South America are morphometrically quite distinct from populations of *A. m. scutellata* in South Africa. In every respect, the Africanized bees clearly show the genetical influence of their European parents. Additionally, F_1 progeny of Africanized and European honey bees provide further evidence of the hybrid nature of Africanized bees. It is clear that Africanized bees in the Americas constitute a "hybrid swarm". That is, they are "a continuous series of morphometrically distinct hybrids resulting from hybridization".

RESUMEN

Las normas, de acuerdo común, de los principios de taxonomía son la guía en el nombramiento de grupos como especies. Primero dentro de estos principios es que características específicas a la especie pueden existir porque miembros de diferentes especies no producen híbridos fértiles libremente.

La historia de la nomenclatura de sub-especies de la abeja melífera Occidental hace ejemplo de 3 principios taxonómicos diferentes. Primero, características específicas a sub-especies no se han encontrado en la abeja melífera. Segundo, sub-especies son distinguidas como grupos por las técnicas multivariantes de análisis de componente principal o análisis discriminante. Tercero, identificaciones de sub-especies son basadas estadisticamente y así probabilísticas. Estas características de identificación de sub-especies se prestan idealmente para la aplicación a computadoras. Systemas de computación pueden ser usados para recolectar datos y entonces aplicarles procedimientos de estadistica complejos para identificarlos.

Tales técnicas se han utilizado para analisar la población de la abeja melífera en las Américas. Las abejas melíferas en las Américas se constituyen de poblaciones resultantes de hibridización de sub-especies. Poblaciones de abejas Africanizadas de America del Sur son morfométricamente muy distintas a la población de *A. m. scutellata* en Sur Africa. En todo respecto las abejas Africanizadas muestran claramente la influencia genética de sus padres Europeos. Adicionalmente, cria F_1 de abejas Africanizadas y Europeas proporcionan más evidencia de la índole híbrida de las abejas Africanizadas. Una comparación de abejas Africanizadas de Argentina y de Venezuela demuestra que

estos dos grupos son diferentes morfométricamente y proporciona más evidencia de hibridización. Queda claro que abejas Africanizadas en las Américas constituyen un "enjambre híbrido." O sea, son "una serie contínua de híbridos morfométricamente distintos resultando de la hibridización."

This discussion is first intended to describe the special taxonomic problems attending the subspecies identification of Western honey bees. Secondly, this paper will describe a developed system of identification that is reliant upon computer-assisted measurements of body parts and computer comparisons of measured data to baseline data through the application of multivariate discriminant analysis technologies.

SYSTEMATICS

A brief review of classical taxonomy reveals some rather clear concepts. First, the "species is the basic unit of taxonomy and evolution." (Mayr 1970). In order to name a group as a species, certain generally accepted questions are asked: (1) do similar groups have allopatric or sympatric occurrances; (2) do similar groups freely hybridize or not; and (3) do groups have species-specific characters?

Certainly a group that has species-specific characters which does not hybridize with similar groups and has an allopatric distribution with other groups is deserving of species designation. The more clearly the information at hand provides these answers the more certain it is that a species designation is correct. The difficult problems of species-level taxonomy, for example, undescribed ecoclines and sibling species, are problems of incomplete information. As more information becomes available, clearer and more correct applications of the principles of taxonomy can be made.

Different taxonomic problems arise with the Western honey bee (*Apis mellifera*). In contrast to other Apoidea where congeneric sympatry and limited distributions are typical, the Western honey bee occupies an amazingly large and varied area. Naturally, it ranges from near the Arctic circle in Europe to the Cape of Good Hope to the south and from the North and South Atlantic coasts of Europe and Africa to the Ural Mountains in the north and the Arabian peninsula's coast of Oman in the south. Populations of Western honey bees freely interbreed with opportunity and produce fertile offspring. Indeed, honey bees may have only occupied this area since the last ice age of the late Pliocene (Ruttner 1987). In additon to this natural distribution, man in the last 300 years has spread honey bees throughout the New World areas of North and South America, Australia and New Zealand. Although clearly one species, *A. mellifera* shows substantial variation in morphology, behavior and physiology (Cornuet & Louveaux 1981). This subspecific variation has been the primary focus of honey-bee taxonomists within this century. Historically, rules governing the description and naming of varing populations of honey bees at the subspecies level have been non-existent. Guides to such nomenclatural issues have frequently included political and sociological elements as well as biological ones. As recent as 1953, Maa presented a list of 146 subspecific names of honey bees which he considered acceptable. This list comprised a revision of 600 named groups of *Apis mellifera* used previously. The difficulties of subspecies honey-bee taxonomy are obviously entangling. If it were not important for achieving various social and economic goals having to do with the public health and commercial characteristics of honey bees, subspecies taxonomy of honey bees would probably have collapsed.

Nonetheless, biological differences among honey bees that reflect adaptations to ecological differences do exist. A long tradition of biometry (Buttel-Reepen 1906, Alpatov 1929, 1948, Goetze 1930, 1940, 1964, Dupraw 1965a, 1956, Daly & Balling 1978, Ruttner 1986) has attempted to describe these differences. Biometrical applications of

modern statistical methods have lead to certain suggestions for taxonomic decisions at the subspecific level with *A. mellifera*. Ruttner (1986) suggests the "operational taxonomic units" called for by Sneath & Sokal (1973) can be the means of standard individual measures. The application of standard multivariate methods which are capable of describing the magnitudes of differences between populations, such as discriminant analysis or principal component analysis provides a basis for the acceptance of groups as subspecies. Applying these principles Ruttner (1986) described 23 subspecies from the Old World range of the species. In doing so, he observed that variation among groups was quantitative not qualitative and frequently occurred within narrow limits.

The application of modern computer technology to the collection and analysis of morphometric honey-bee data was begun by DuPraw (1965a, 1965b) and refined by Daly & Balling 1978 and Daly et al. 1982.

Methods

Data collection was according to procedures adapted to the morphometric analysis of honey bees by Daly et al. (1982). Body-part images from wings, legs and sternites mounted on slides were projected with a standardized magnification from a projecting microscope to a digitizing tablet connected to a micro-computer (Fig 1, A and B). Various points on the projected images were identified by the operator with the aid of an electronic "mouse" or "cursor". The positions of the electronically marked points were transmitted to the micro-computer where they were converted to values representing actual lengths angles and counts of the 25 characters measured (Daly & Balling 1978).

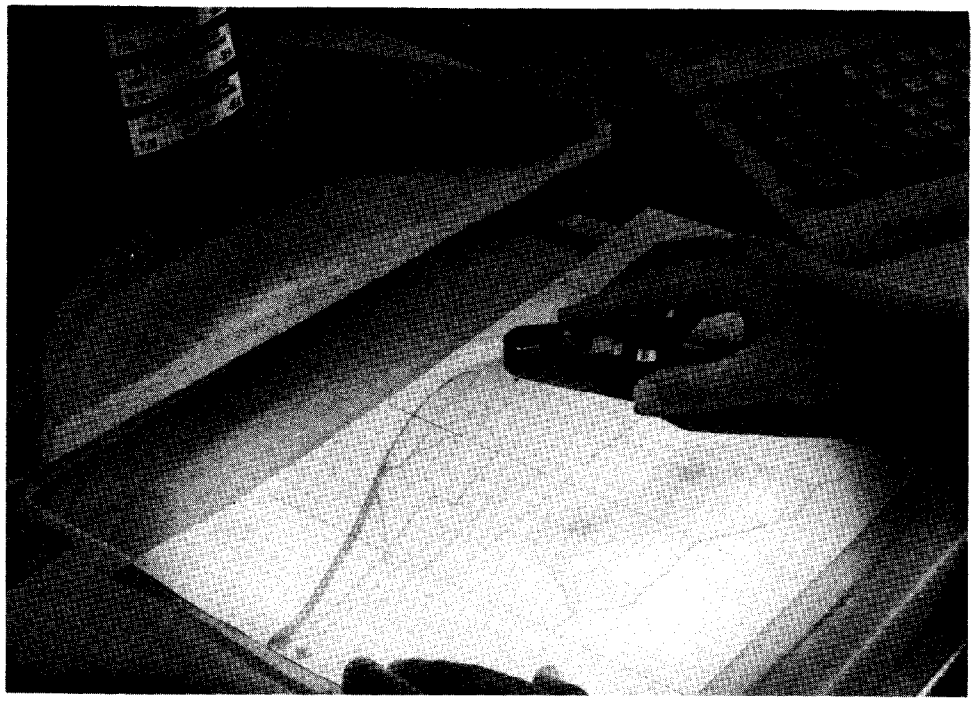
In the numerical analysis of a sample, reductionist statistical procedures as used to calculate and then compare descriptive values of the sample to similar values for baseline groups. The analysis provides probabilities of a sample belonging to groups, based on these comparisons. At least two and probably more of the major suppliers of statistical software provide discriminant analysis packages that are appropriate for use in honey bee taxonomy.

Many interesting problems in honey bee classification are found among the honey bees brought to the New World. These bees have come from a variety of sources and the populations of bees found in the Americas cannot be said to be members of identifiable sub-specific groups. Nonetheless, differences do occur among American populations of bees. Those populations derived in part from parents from the Highland population of East Africa (*A. m. scutellata*) are considered economically undesirable because of excess stinging (Collins et al. 1982) and inferior honey production (Rinderer et al. 1984, 1985). This parental stock was introduced to apiaries near Piracicaba Brazil from Africa in 1957 (Kerr 1967). Because of economic concerns, the identification of Africanized bees has become important.

As examples of the value of the discriminant analysis of morphometric measures, the results of two separate analyses are presented. The first compares a sample of a population of Africanized bees to a sample of the parental population in Africa. The second compares Africanized and European honey bees and F_1 progeny of crosses between these two.

Results and Discussion

One use of multivariate discriminant function analysis is to compare two or more groups on the basis of a variety of quantitative measures. Such an analysis was done in a description of morphometric differences between South American Africanized and South African (*Apis mellifera scutellata*) honey bees (Buco et al. 1987). This study compared the two groups for each of the univariate measures with the use of t-tests.



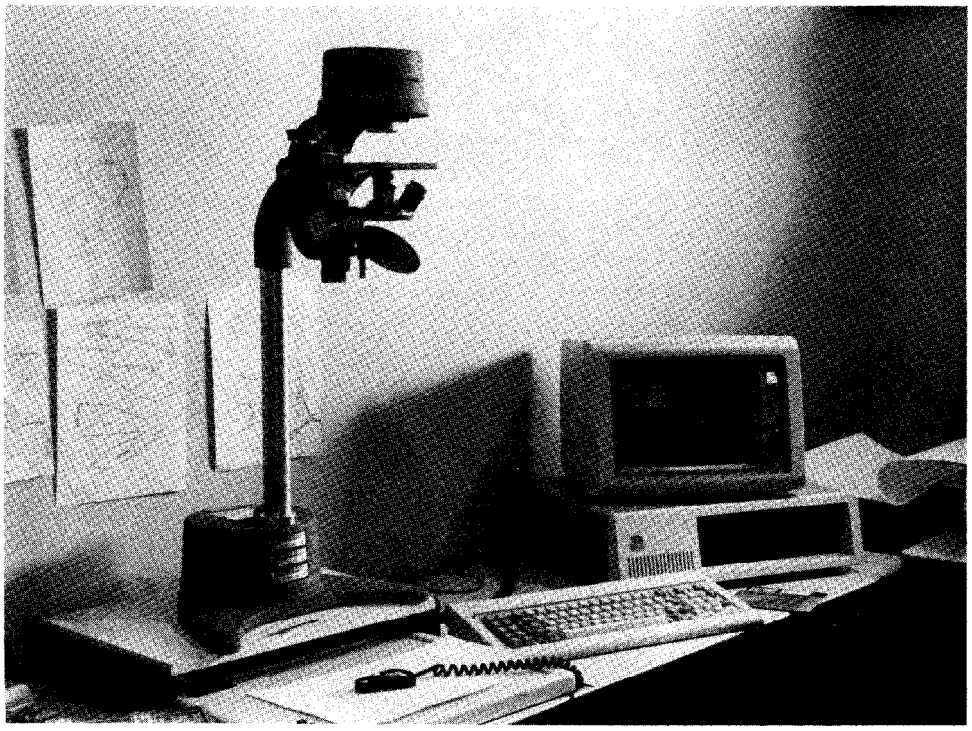


Fig. 1. Photographs of the data collection hardware. A. The projection of body part images onto the digitizing tablet. B. The data collecting assembly: projecting microscope, digitizing tablet and micro-computer.

Nineteen of the 24 characteristics differed between the groups (P = 0.05 or less). However, a full appreciation of the overall difference between the groups depended upon the multivariate discriminant further analysis.

The differences found in comparisons of individual characteristics were reflected in the multivariate discriminant function analysis. The two populations were completely separated by the discriminant function (Fig 2). The posterior probability of group membership for all cases indicated a correct classification of P = 0.9984 or greater using the derived discriminant function.

The feral bees of South America constitute a population which is morphometrically quite different from the parental population of *A. m. scutellata* in Southern Africa. Comparisons of individual characteristics, as well as the composite analysis represented by the discriminant function, indicated that Africanized bees are generally larger than their African parental stock.

The most likely explanation for these differences is that Africanized bees in the Americas are a subspecific hybrid swarm (King 1968) which has resulted from matings with European bees and then subsequent crossing and back-crossing. The generally intermediate morphometric character of Africanized bees suggests that a large number of loci are involved in the determination of the honey bee's morphometric make up. Predictable Mendelian events associated with crossing and then backcrossing between African and European bees would produce the Africanzed bees that were measured. Such bees clearly show the influence of both their European and their African parentage.

A different use for multivariate discriminant function analysis is to describe populations in terms of functions and use the functions to classify unknown samples. This was originally lone for Africanized and European bees for the Americas by Daly & Balling (1978). However, the experimental production of F_1 progeny of European and Af-

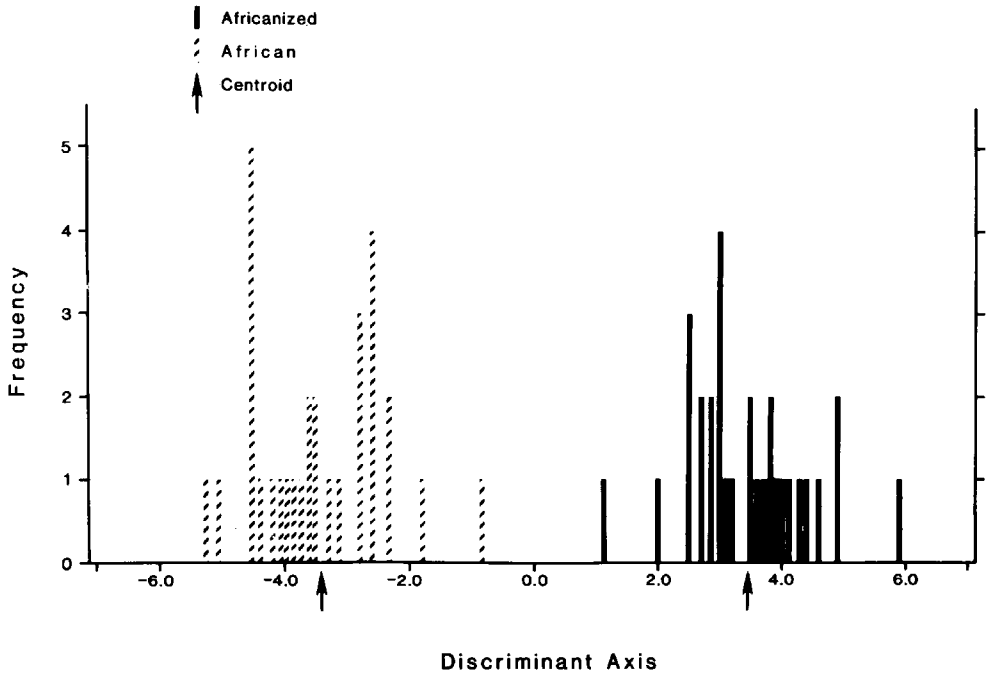


Fig. 2. Histogram of the results of the multivariate discriminant function analysis of African and Africanized bees.

ricanized parents made possible the development of discriminant functions for the three groups (Rinderer et al., unpublished).

The multivariate discriminant analysis correctly identified all samples as Africanized, European or F_1 hybrids (Fig. 3) Most classifications were at $P = 1.00$ and all but one were at $P \leq 0.090$. The remaining sample, an F_1 colony, was correctly classified at $P = 0.55$. As expected, the discriminant analysis procedure selected the F_1 category as the second most likely classification for all Africanized and European colonies. Interestingly, the second most likely category for the F_1 samples was European for all but 3 F_1 samples. Thus, the procedure was conservative in its classification of F_1 colonies in ways appropriate to regulatory needs. Only clearly Africanized or F_1 colonies would be so classified.

The identification of unknown samples by comparison to this baseline can be expected to correctly classify samples of Africanized and European worker bees and samples of worker bees that are completely or mostly F_1 hybrids. According to established practice, such identifications would be made with posterior probabilities of group membership of $P \geq 0.90$. Samples which are a mixture of bees would likely either be classified with a low probability of group membership ($P<0.90$) or the range for the majority of measurements would span 2 or 3 of the 3 group ranges.

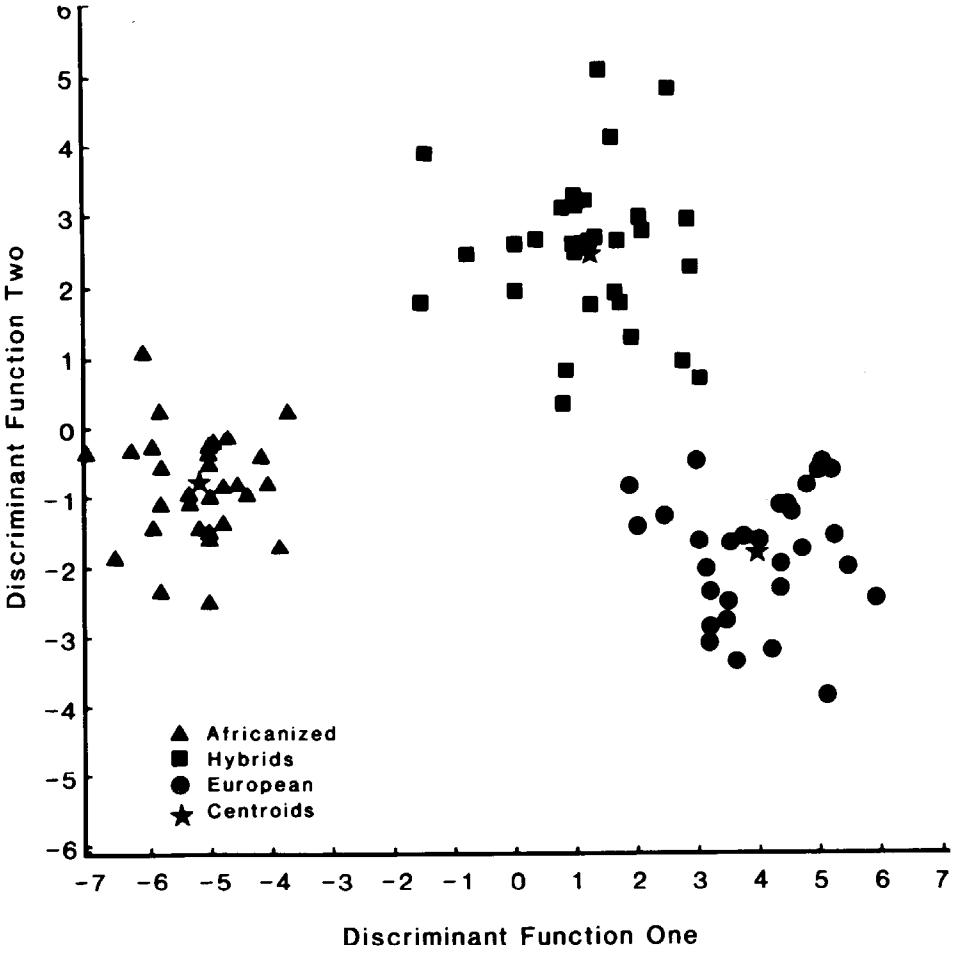


Fig. 3. Scatterplot of the results of the multivariate discriminant function analysis of Africanized, European and their F_1 hybrid bees.

Such samples would warrant close inspection. The classification of individual bees may provide evidence of mixed samples. Data supporting the classification of individual bees have been analyzed and discriminant functions of identifying individual bees have been developed.

Scientific and commercial issues regarding identifying Africanized bees and their hybrids have been well served by the computer-assisted discriminant function analysis of morphometric data. The process has permitted rather difficult taxonomic problems to be resolved clearly with numerical procedures. The success of the techniques with honey bee groups even at the subspecific level, suggests that other identification problems are within the capabilities of this tool to solve.

ACKNOWLEDGEMENT

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RIBOSOMAL DNA PROBES FOR IDENTIFICATION OF MEMBER SPECIES OF THE *ANOPHELES GAMBIAE* COMPLEX

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ABSTRACT

Many insects belong to species complexes wherein member species are morphologically indistinguishable. Yet, in numerous cases, both economic and medical exigencies require that the species of such individuals be reliably determined. The specific problem addressed here concerns the *Anopheles gambiae* mosquito complex which includes *An. gambiae* and *An. arabiensis*, currently the two major African malaria vectors. The present work uses a ribosomal DNA gene probe to differentiate member species of this complex. The method is shown to be extremely useful and sensitive because it can easily test just portions of a single dried adult. The rationale for using ribosomal DNA clones to provide such diagnostic probes to distinguish among other morphologically identical insect species is also discussed.

RESUMEN

Muchos insectos pertenecen a un complejo de especies donde los miembros son morfológicamente indistinguibles. Sin embargo, en muchos casos, exigencias económicas y médicas requieren que la especie sea identificada con seguridad. El problema específico tratado aquí le concierne al complejo del mosquito *Anopheles gambiae*, que incluye a *An. gambiae* y *An. arabiensis*, que son actualmente los dos vectores principales de la malaria Africana. El presente trabajo usa un gene ribosomal de DNA como sonda para diferenciar especies de este complejo. Se demostró que el método es extremadamente útil y sensitivo porque facilmente puede probar solo porciones de un solo adulto secado. Se discute tamabién la racional del uso de clones ribosomal de DNA para proveer sondas diagnósticas para distinguir entre otras especies de insectos morfológicamente idénticas.

Malaria is the most debilitating disease in the world today; among the 92 million new cases each year, there are close to 1 million deaths, nearly all of which are young children (Service 1985). Although malaria is found world-wide, the problem is most acute in subsaharan Africa where the disease presents an enormous obstacle to social and economic development. Although malaria was eradicated from most of its original

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RIBOSOMAL DNA PROBES FOR IDENTIFICATION OF MEMBER SPECIES OF THE *ANOPHELES GAMBIAE* COMPLEX

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ABSTRACT

Many insects belong to species complexes wherein member species are morphologically indistinguishable. Yet, in numerous cases, both economic and medical exigencies require that the species of such individuals be reliably determined. The specific problem addressed here concerns the *Anopheles gambiae* mosquito complex which includes *An. gambiae* and *An. arabiensis*, currently the two major African malaria vectors. The present work uses a ribosomal DNA gene probe to differentiate member species of this complex. The method is shown to be extremely useful and sensitive because it can easily test just portions of a single dried adult. The rationale for using ribosomal DNA clones to provide such diagnostic probes to distinguish among other morphologically identical insect species is also discussed.

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temperate range in the 1960s, its incidence in the tropics continues to increase, due in part to insecticide resistance in the mosquito vector and chemoprophylactic drug resistance in the malaria parasite (Service 1985).

Two of the principal African malaria vectors, *An. gambiae* and *An. arabiensis*, belong to the *Anopheles gambiae* species complex, which contains six member species. All of the species are morphologically indistinguishable, and two (and in some areas as many as four) of the species are often sympatric in many parts of Africa (White 1974). The species' two principal vectors, *An. gambiae* and *An. arabiensis*, differ in behavior and preferred habitat. Moreover, there is abundant evidence suggesting that the two major vector species may not be equally involved in malaria transmission, depending upon the season and location (Coluzzi et al. 1977). Therefore, one of the problems for epidemiological studies of these insect vectors is to determine whether an individual female mosquito is infected with the malaria parasite, and also, to what species does she belong. The latter consideration is most pressing for ecological studies of habitat and reproductive behavior, which then provide information essential for the design of various control strategies.

We have focused our work upon two areas: One involves development of a rapid, accurate and easy means of distinguishing the species of single mosquitoes. The other involves what will ultimately become a fairly detailed analysis of the ribosomal RNA genes from the perspective of population structure and evolution of the species complex.

In developing a rapid means of distinguishing species we chose to use some feature of DNA because of its stability in dried tissue. Dessication is the easiest way todeal with field specimens, and further, a rapid, highly sensitive assay was already in use for detecting the presence of the malaria sporozoite antigen in single dry mosquitoes (Collins et al. 1984). We chose therefore to focus on the ribosomal DNA gene family because they are most likely to be present in hundreds of copies per genome, thus increasing the sensitivity of a probe derived from this region. More important, the ribosomal genes contain a highly conserved coding region as well as a spacer region, and although the latter is transcribed, its sequences are processed out, and it is apparently subject to fairly rapid divergence (Beckingham 1982). Thus, when used as probes, sequences from portions of the coding region flanked by spacer sequence are expected to reveal species-specific restriction fragment length polymorphisms (RFLP). Such characteristic RFLP have been used in numerous instances to distinguish among closely related species (Jeffreys 1981, Avise et al. 1979, Langley et al. 1982).

Methods

A genomic library from *An. gambiae* was constructed in EMBL-3 as described in Collins et al. (1987). A variety of clones containing ribosomal coding sequences were isolated from this library as previously described (Collins et al. 1987), then restricted with EcoRI and SalI or whith EcoRI and BamHI and subjected to Southern analysis. The blots were probed with a ribosomal DNA clone isolated from *Sciara coprophila* (Renkawitz et al. 1979). Since the coding regions tend to be highly conserved they were expected to hybridize with the *Sciara* clone. However, the non-vector non-hybridizing fragments were expected to derive from spacer or intron regions, and we therefore chose these fragments for further study.

Southern analysis and probe preparation were carried out as described in Collins et al. (1987). An rDNA (pBC2) clone from *S. coprophila* was provided by S. Gerbi. The 18S and 28S coding region clones, derived from *Calliphora erythrocephala*, were provided by K. Beckingham (Beckingham 1980). Mosquitoes were supplied by the Malaria Branch, Centers for Disease Control, Atlanta. DNA extraction is a modification of a method reported by Livak (1984). All of the techniques used for Southern analysis are

fully described by Maniatis (1982); certain modifications are described in Collins et al. (1987).

RESULTS

Preliminary characterization of clone λAGr12. In order to be certain of the nature of the fragments we would ultimately use as probes to reveal a species-characteristic RFLP, we chose one representative clone for further study. A preliminary restriction map of this clone is shown in Figure 1. The coding regions were roughly delineated by Southern analysis, again using separate *Calliphora* 18S and 28S clones as probes. The arrangement left to right of 28S-18S-non-coding-28S is as expected for insect ribosomal genes, and this clone evidently contains one entire repeat unit. The fragments of interest were those which include both coding and adjacent spacer sequence.

Identification of species-diagnostic RFLP. The interesting fragments (identified according to the procedure outlined in Methods) were those which did not appear to hybridize to the heterologous rDNA probe, pBC2. Figure 1 shows the position of two fragments, pAGr12A and pAGr12B, within the rDNA colone. Such fragments were either subcloned or eluted from a gel and then used individually to probe blots containing genomic DNA from different members of the *An. gambiae* ccomplex. One such blot is shown in Figure 2A. This fragment (pAgr12A), when used as a probe, reveals a characteristic RFLP which, as demonstrated in the figure, can distinguish all five of the member species. Another fragment (pAGr12B) was sued to probe a similar blot, revealing a different RFLP which is shown in Figure 2B. The RFLP revealed in Figure 2A most likely reflects the changed position of the underlined EcoRI site (Figure 1). The RFLP shown in Figure 2B may reflect the changed position of the underlined HindIII site shown in Figure 1, although variation in the HindIII site to its left is also possible.

Ribosomal DNA probes are particularly useful because of their great sensitivity. Since the rDNA genes of many insects are present in 200-500 copies per nucleus, they are readily visualized with an overnight exposure of Southern blots hybridized with a moderately hot (10^7 cpm/μg) probe. The blot shown in figure 2 contains an amount of DNA equivalent to one fourth of a mosquito per lane.

DISCUSSION

Thus far, species identification in the *An. gambiae* complex relied almost exclusively on cytogenetic criteria (Coluzzi et al. 1979). This method requires a great deal of tech-

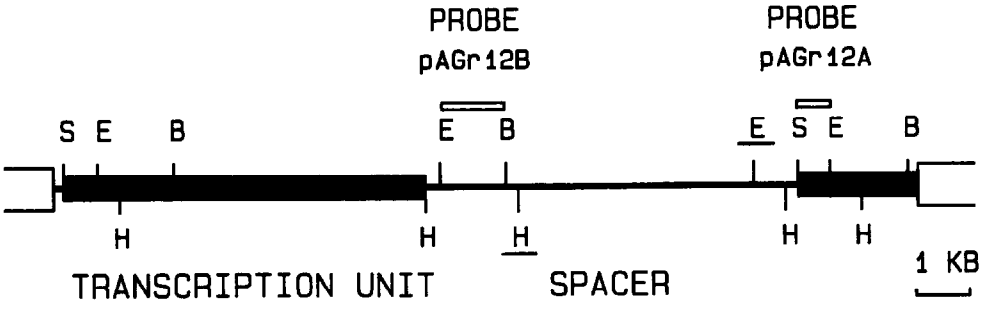

Fig. 1. Simplified restriction map of the *An. gambiae* rDNA clone, λAGr12. The dark bars represent transcribed regions, not all of which is coding region. The colone contains one complete intronless repeat unit. S = SalI, E = EcoRI, B = BamHI, H = HindIII.

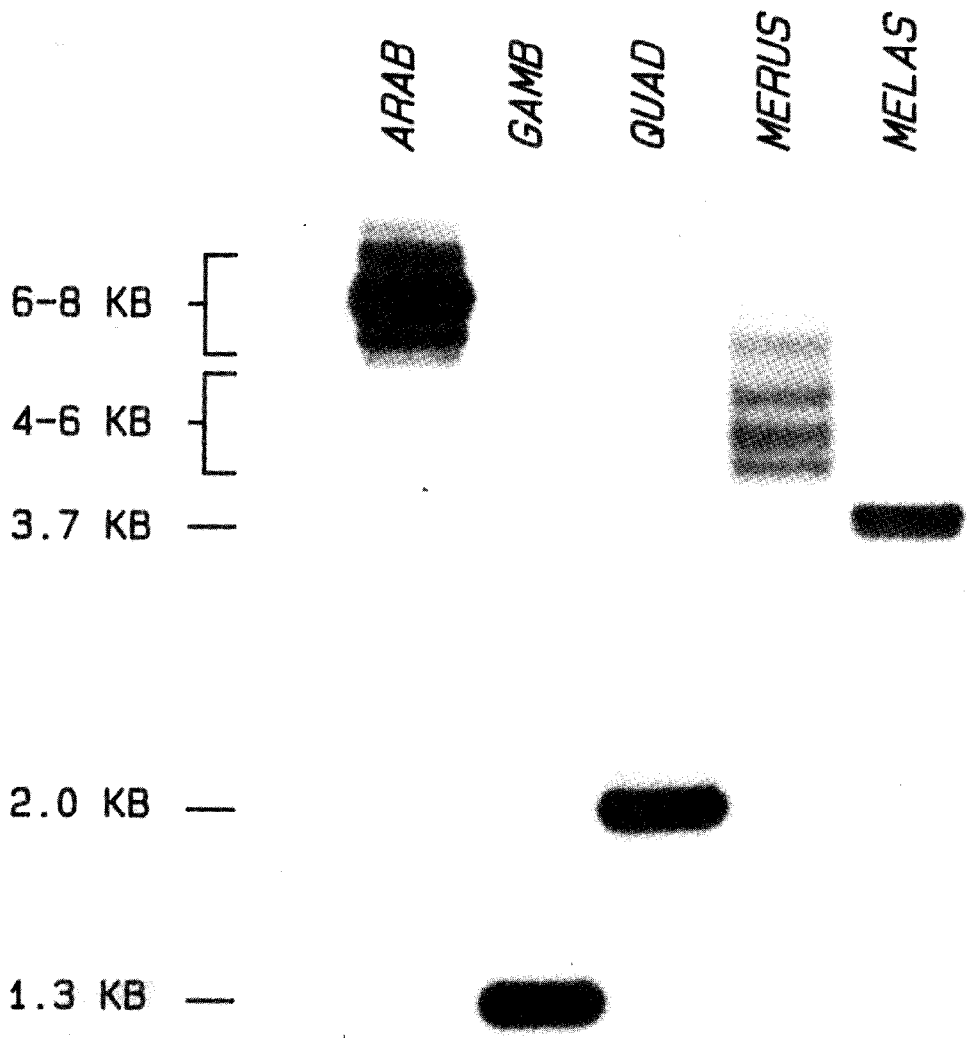

Fig. 2A. Autoradiograph of a Southern blot from an overnite exposure containing an EcoRI digest of the indicated mosquito DNAs probed with pAGr12A (10^7 cpm/μg). Shown from left to right are *An. arabiensis*, *An. gambiae*, *An. guadriannulatus*, *An. merus*, and *An. melas*. One quarter of a single mosquito DNA extract was used per lane.

nical expertise, as well as freshly blood fed mosquitoes. Alternative methods based upon differences in several dehydrogenase isozyme patterns have other disadvantages, among which is the requirement for fresh or frozen specimens (Miles 1978, Marchand & Mnzava 1985). Cuticular hydrocarbon profiles can be used to reliably distinguish dried specimens, but the method is not practical for large numbers of specimens or for field use (Carlson & Service 1980, Hamilton & Service 1983). Recently, we demonstrated that the RFLP revealed by rDNA probes were as reliable as either cytogenetic or isozyme methods by direct comparison of results from the same individuals or siblings from an isofemale line (Collins et al. 1988, Collins et al., unpublished). The DNA method has several clear advantages, one being that dried individuals of virtually all life stages can be used. Further, only part of the specimen is required for DNA analysis, so that

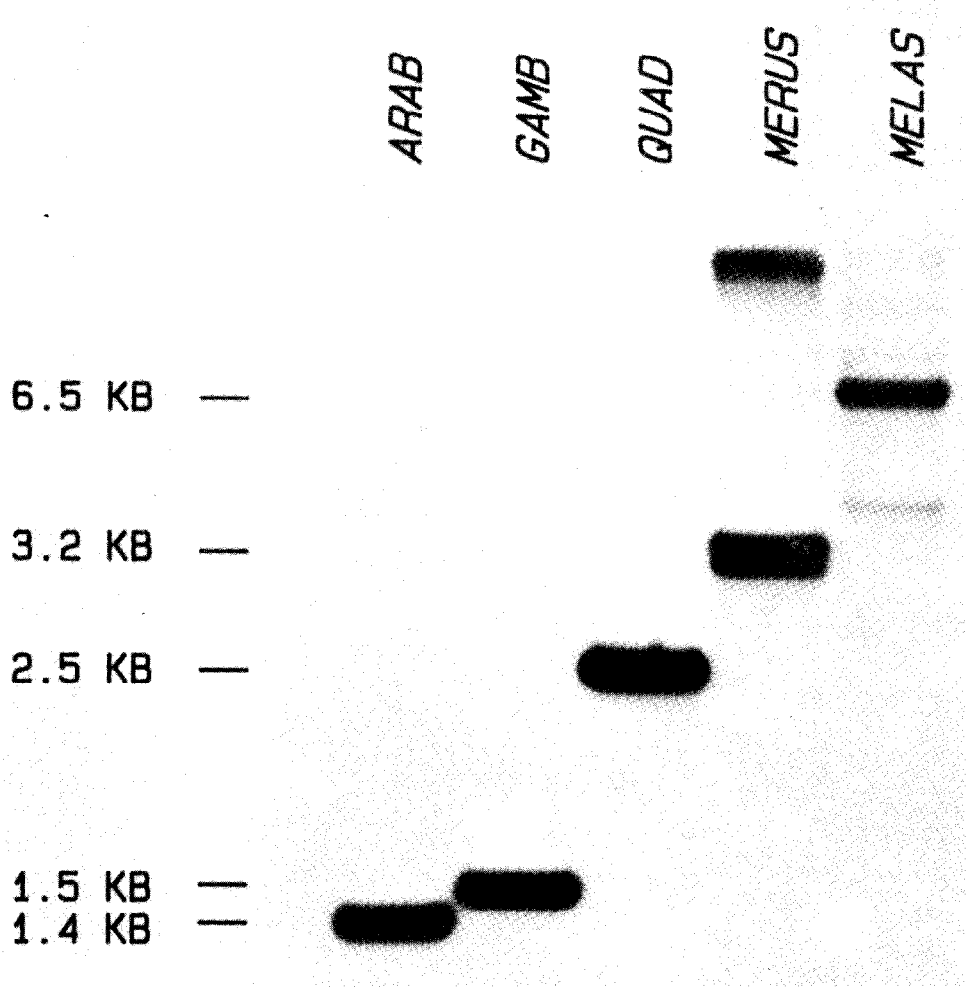

Fig. 2B. Autoradiograph of a Southern blot containing a HindIII digest of the indicated mosquito DNAs probed with pAGr12B. All other conditions are as described for figure 2A.

assessment of the blood meal source as well as the presence of sporozoite antigen can readily be done on the remainder of the specimen. However, it must be emphasized that the DNA method currently requires DNA extraction and Southern blotting. A more useful version of the test would emerge if the necessity of running and blotting a gel could be eliminated in favor of a "dot blot." Such a dot blot assay would require a series of species-specific probes. We have therefore begun to determine whether there are sequences other than the spacer within the ribosomal DNA genes which may have diverged sufficiently between species to the extent that they no longer behave as if they are homologous under the stringent conditions we use for Southern analysis. One obvious candidate is an intron region, and many Diptera contain a mixture of 28S gene types, some with a single intron, and others not having the intron, such as λAGr12, shown in Figure 1. Since mosquito rDNA genes may or may not bear such introns, the intron itself might be even more likely (than the transcribed spacer) to contain sequences which differ significantly between species (Gerbi 1985). Therefore we have examined our collection of *An. gambiae* rDNA clones in order to focus upon those which

might contain an intron in the 28S region. One such "species-specific" sequence has been subcloned from an *An. gambiae* intron. Preliminary analysis of this fragment indicates that it is perfectly useful as an *An. gambiae*-specific probe. Currently we are searching for analogous fragments in rDNA clones from the other members of the complex which could prove to be useful as species-specific probes. In contrast to the highly conserved 18S and 28S coding regions, insect rDNA is generally found to contain both spacer and intron sequences which appear to diverge more rapidly between even closely related species. The rDNA gene family is therefore an excellent candidate for providing (as it has in the present case) a number of fragments, some of which may be used to reveal a species-specific RFLP, as well as other fragments which could behave as species-specific probes.

Acknowledgments

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DISTINGUISHING AFRICAN AND EUROPEAN HONEYBEES USING NUCLEAR DNA RESTRICTION FRAGMENT POLYMORPHISMS

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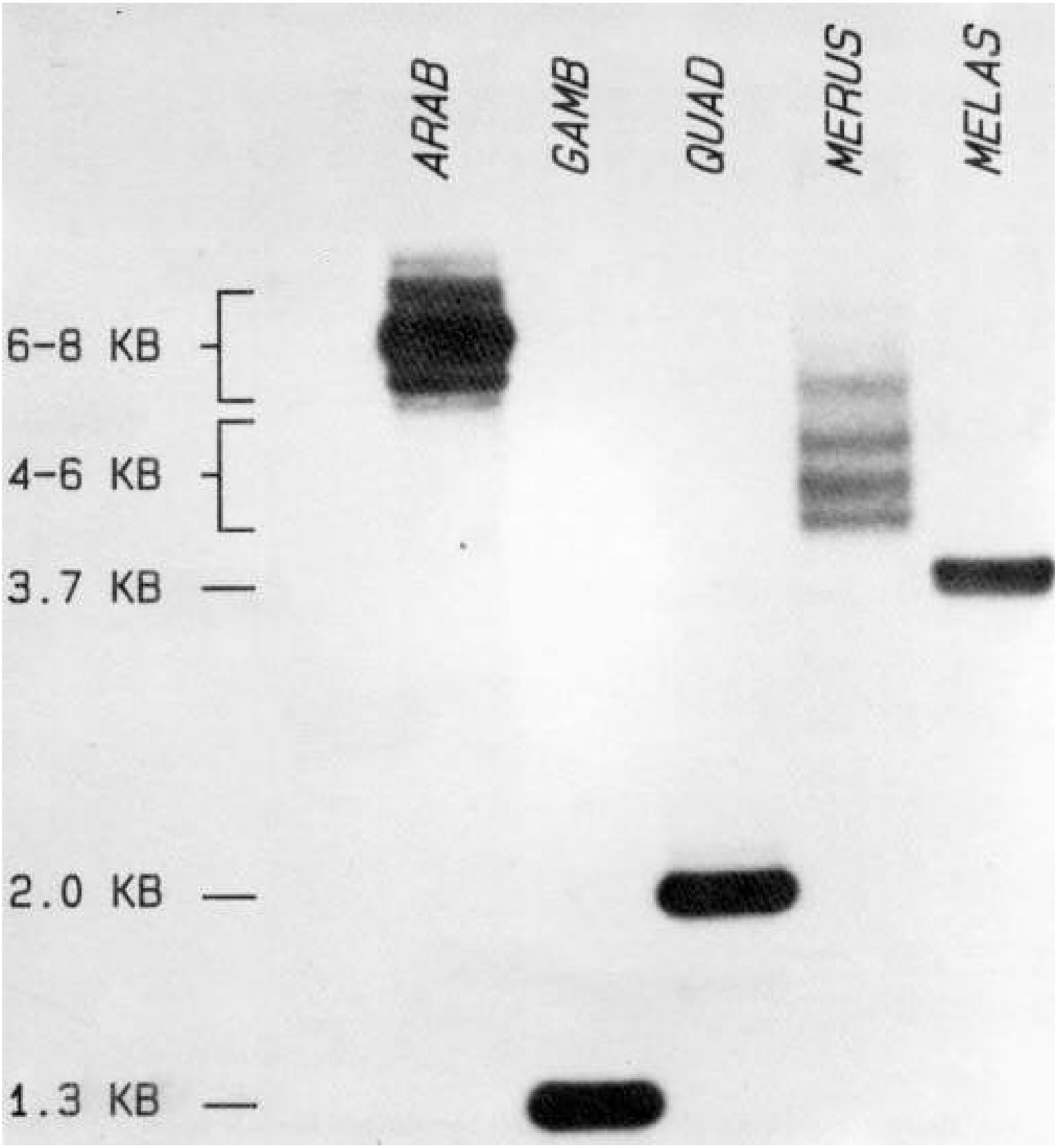
ABSTRACT

African-derived honeybees, expected to enter the United States within two years, will adversely affect the honey and pollination industries and will be an environmental hazard to the public. Control measures are dependent upon a reliable identification method, heretofore unavailable. Nuclear DNA restriction fragment polymorphisms are being found that successfully distinguish African and European bees as well as hybrids between the two. Beyond identification for regulatory purposes, DNA markers will enhance studies on African-European bee genetic interactions and population dynamics.

RESUMEN

Se espera que abejas africanizadas entren los Estados Unidos dentro de dos años y que adversamente afectarán las industria de la miel y de la polinización, y también serán un peligro al público. Las medidas de control dependen de un método de identificación de confianza, hasta ahora no disponible. El polimorfismo de la restricción de fragmentos de DNA nuclear se ha encontrado que distinguen con éxito entre abejas Africanas y abejas Europeas, así como entre híbridos de las dos. Después de la identificación por propósitos regulatorios, marcadores de DNA mejoraran los estudios sobre la interacción genética y el dinamismo de población de las abejas Africanas-Europeas.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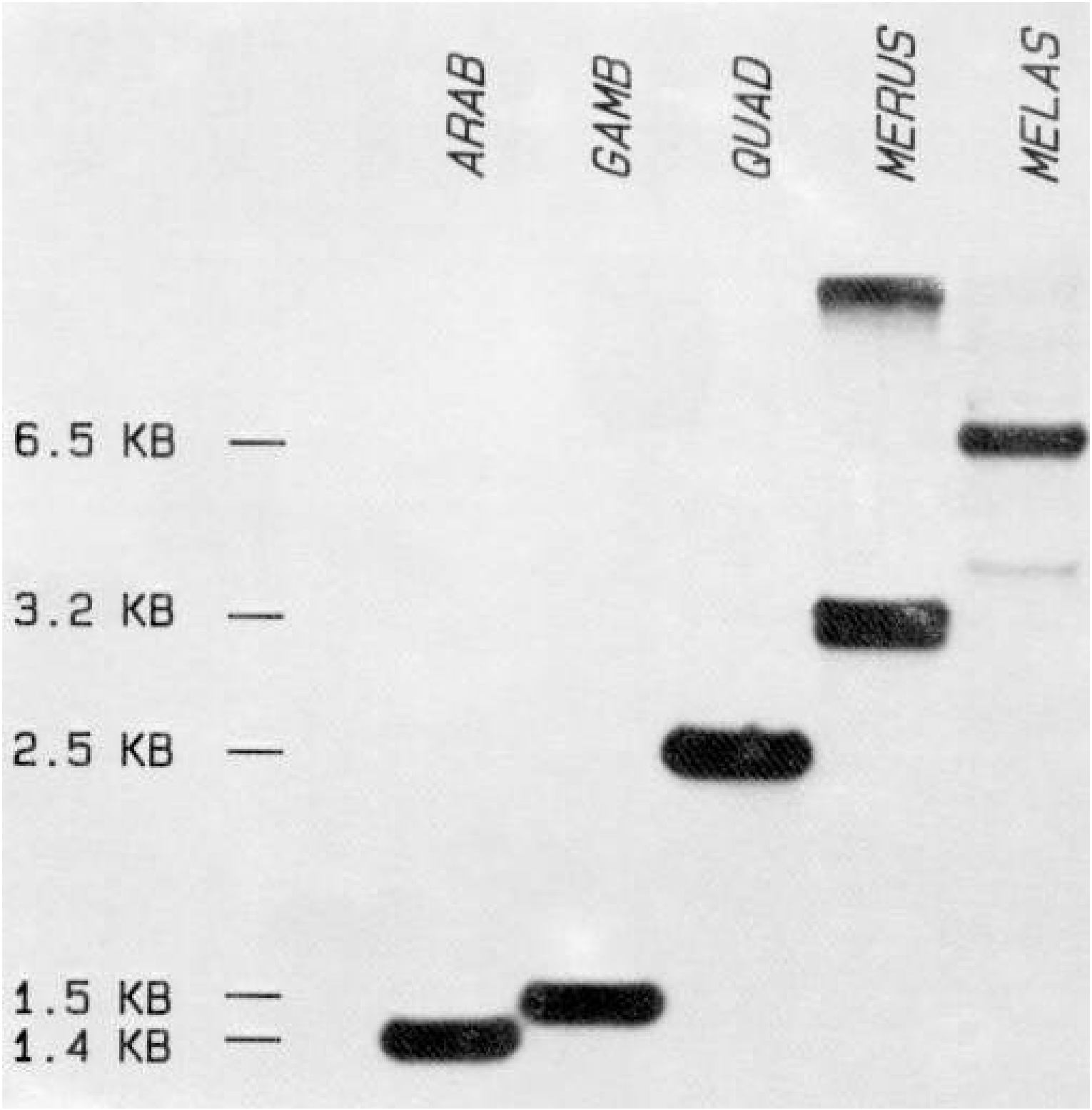
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European honeybees were imported to the American continents at the time of colonization. They are now vital to the pollination of many agricultural crops at an estimated US annual value of $19 billion (McDowell 1981). African honeybees (*Apis mellifera scutellata;* Ruttner 1976), accidently released in 1957 from experimental hives in Brazil (Kerr 1967, Woyke 1969, Michener et al. 1972, Michener 1975), have undergone a phenomenal migration with the replacement of the extant European bees throughout much of South and Central America (Taylor 1977). The bees are now in Mexico and are expected to enter Texas *en masse* within two years and the panhandle of Florida within six years (Taylor 1985). In Floridia's tropical climate, African bees could reach saturating densities (Taylor & Spivak 1984). Excessive stinging, excitability and swarming make management of African bees difficult. If they cannot be kept from apiaries, beekeepers will discontinue their business. Competitive European bee populations will be eliminated, and numbers of commercial colonies available for pollination will be reduced. Large populations of African bees will increase stinging incidents, and resulting publicity will likely impact tourism in Florida. The threat to human and animal populations has been sensationalized but is, nevertheless, real (Taylor 1986). Proposed methods of control include quarantine, extermination, and certification of breeding stock. These are dependent upon diagnostic identification of African honeybees (Page & Erickson 1985). However, current methods are inadequate. Morphometric statistical analysis, now most commonly used to identify African bees (Daly & Balling 1978, Daly et al. 1982), is subject to error due to environmental influences and overlapping distributions. The composition of cuticular hydrocarbons (Carlson & Bolten 1985, Smith 1988) has considerable promise for identification, but the inheritance of these compounds is not understood. Honeybees show limited allozyme variation which is generally characteristic of hymenopteran insects. Alleles of only four proteins are known to have significant frequency differences between African and European bee populations, but none are diagnostic. Therefore, allozyme analysis cannot make certain identifications, especially of hybrids after several generations (Rinderer & Sylvester 1981).

Honeybee subspecies are distinguished here through DNA fragments generated by restriction endonucleases. A number of studies have demonstrated the value of this approach in establishing the genetic relatedness of organisms (Brown et al. 1982, Cann et al. 1984, Ferris et al. 1982). DNA restriction fragment polymorphisms do not necessarily result in, nor their detection depend upon, functional changes subject to selection. Thus, they can provide allele distinction at many loci within natural populations. In the initial study with honeybees, greater DNA polymorphism was found than all the protein differences reported so far (Hall 1986).

Restriction enzymes recognize and cut DNA at specific short sequences, most commonly consisting of four to six nucleotides. Alterations in the sequences, due to genetic diverence, are manifested in the resulting DNA fragments, separated according to size by electrophoresis. When the total genome is digested, thousands of different-size fragments are generated. To visualize only a small portion at a time and to compare the homologous regions between individuals, use is made of DNA probes (Southern 1980, Maniatis et al. 1982). These short pieces of cloned radioactive DNA recognize homologous sequences, binding through complementary base pairing, i.e. hybridization. Thus genetic distinction is based on a small percentage of nucleotides comprising the restriction sites within the probe region (Jeffreys 1979).

Materials and Methods

As a source of probes, random fragments of honeybee nuclear DNA were cloned in *E. coli* bacteria, by insertion into plasmid pBR322 (Hall 1986). To have probes sufficiently large to overlap a number of sites, clones carrying inserts of honeybee DNA

greater than four kilobases were also selected. Clones with low copy number sequences were distinguished from those with repetitive sequences.

DNA was isolated from uncapped honeybee larvae. Each sample came from a single colony, thus represented a combination of progeny from a single queen mated with one to a dozen drones. European bees were provided by N. Gary (Dept. of Entomology, Univ. of Calif., Davis) established by R. Page (Dept. of Entomology, Ohio State Univ.) and E. Erickson (USDA-ARS, Tucson) as members of a closed breeding population from stocks across the United States. African samples from Costa Rica and Venezuela were provided by O. Taylor, M. Spivak (Dept. of Entomology, Univ. of Kansas) R. Hellmich, A. Collins and T. Rinderer (USDA-ARS, Baton Rouge). DNA can be isolated from any stage, but the soft larval tissue facilitates DNA isolation. Also, the use of larvae ensures the colony origin of samples, since adults can drift among hives. Each DNA sample was digested separately with different restriction enzymes that recognize short nucleotide sequences (four). Short sequence enzymes generate more fragments, increasing the chances of finding differences. Each enzyme digest was placed in a separate lane in an agarose gel, and the fragments were separated by electrophoresis and blotted onto membranes of derivatized nylon. Probes were radioactively-labelled with 32P deoxycytidine by nick translation. The blots were hybridized with the denatured labelled probes, one at a time, washed and exposed to X-ray film (Southern 1980, Maniatis et al. 1982).

Results and Discussion

DNA Differences:

DNA from a few European and African samples was digested with nine separate restriction enzymes. Of sixteen probes hybridized to these samples, three fourths revealed polymorphisms. Pairwise comparison of the fragments clearly showed that European samples from the U.S. were more distantly related to an African sample from Costa Rica than they were to each other (Hall 1986). Some of the first polymorphisms have promise as diagnostic markers. Enzyme-probe combinations that revealed differences among a few samples were tested against multiple samples of European bees from different U.S. locations and African bees from Costa Rica and Venezuela. So far three of the probes reveal restriction fragments present in all members of one group and absent in all members of the other group (Hall 1988 a,b). Other fragments are totally present or absent in one group but variable in the other. Although many more samples need to be tested, these results strongly indicate that numerous diagnostic differences will be found. By a rough estimation, the probes tested so far together represent only about 1/2000th of the genome.

Since the probes were derived from random fragments of DNA, presumably they represent loci scattered throughout the genome. Although clones to low copy number sequences were selected, some polymorphisms seen in very intense bands may, nevertheless, be of repetitive sequences. These could be advantageous, since each probe would represent many loci. To form the discrete bands needed for analysis, fragments would have to be derived entirely from within the repetitive sequence. Those spanning the repetitive sequence and different flanking DNA would be of many different sizes, forming an unresolvable smear.

Restriction site variations in nuclear DNA sequences show Mendelian inheritance as codominant alleles (Cavenee et al. 1982, Gusella et al. 1984) which enables the identification of heterozygous loci. A single fragment characteristic of a subspecies will be present in the homozygotes and heterozyotes but will be absent only in homozygotes of the other subspecies. Differences detected by two thirds of the probes in the samples

from South and Central America are represented by the lack of fragments, indicating that the loci are homozygous for African alleles (or at least not heterozygous with any of the alleles in the European samples). Interestingly, among Venezuelan bees, greater homozygosity is found in feral swarms. Samples from managed apiaries show varying, although minor, amounts of European-characteristic fragments, which probably represent a minority of colony members sired by European drones.

Use of DNA Markers to Study Honeybee Population Dynamics

DNA restriction fragment polymorphisms will enable studies that have been limited due to the lack of protein variation in natural bee populations. The term "Africanized bees" is a presumption that the honeybees in South and Central America are hybrids but further implies that they are mostly European with introgression of African genes. This was a logical assumption at the time the bees were released when, as it has been reported, there were only twenty-six African queens compared to the entire extant European population (Kerr 1967). However, the extent to which African and European bees freely interbreed and the degree of hybridization is not well documented and has become a controversial subject (Taylor 1985, Rinderer 1986).

Finding loci homozygous for African DNA alleles is consistent with the predominance of African morphological and behavioral characteristics. This may be solely a consequence of tropical environmental selection against hybrids of European bees. However, other mechanisms may exist that allow retention of the African genotype (Taylor 1985). The extent to which the South and Central American bees are hybrids can be clearly resolved with further DNA restriction fragment analysis. Possible genotype retention mechanisms can be followed in natural populations without need to introduce genetic markers that would disrupt the bees' genetic integrity.

Use of DNA Markers for Regulatory Control of African Bees

African bee identification through morphometrics is rapid, simple and can be used to test many colonies but with limited certainty. DNA and morphometric analyses together would make an effective combination for regulatory purposes. Restriction fragment analysis would be used for a smaller number of colonies when a high degree of reliability and hybrid identification are necessary. With diagnostic DNA alleles at many loci and codominant expression of restriction fragments, hybrids can be recognized after many generations and multiple segregational events. This will increase the efficacy of control measures (Stibick 1984) and will reduce the chances of misidentification leading to unnecessary regulatory steps, costly to the government and beekeepers. A quarantine operation to eliminate an isolated introduction in California (Cobey and Lawrence 1985) cost over $1 million. Regular requeening of apiary colonies with certified stocks would likely be a central control tactic. Presently, DNA analysis is the only promising approach for genetic certification. Simplification of the restriction fragment analysis, likely to be forthcoming, will make it more practical for routine use.

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USE OF DNA PROBES TO DISTINGUISH SIBLING SPECIES OF THE *ANOPHELES QUADRIMACULATUS* COMPLEX

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ABSTRACT

Isozyme electrophoresis has traditionally been the method of choice for analyzing the genetic structure of populations. It can also be useful for distinguishing morphologically identical sibling species. More recently mitochondrial DNA restriction analysis has become popular as an alternative for studying population structure; and the isolation of species-specific DNA probes has become the method of choice for distinguishing closely related species. We have evaluated the usefulness of these DNA based techniques in mosquitoes and discuss their advantages and disadvantages.

RESUMEN

La electroforesis de isoenzyma tradicionalmente ha sido el método preferido para analizar la estructura genética de poblaciones. También puede ser utilizada para distinguir entre especies hermanas morfológicamente idénticas. Más recientemente, análisis de la restricción de la mitocondria del DNA se ha hecho más popular como una alternativa para estudiar la estructura de la población; y el aislamiento de sondas de DNA específicas a especie ha sido el método preferido para distinguir especies cercamente relacionadas. Nosostros hemos evaluado la utilida de estas técnicas basadas en el DNA de mosquitos y se discuten sus ventajas y desventajas.

The *A. quadrimaculatus* species complex consists of at least four sibling species: species A, species B (Lanzaro 1986), species C and species D (unpublished data). We have used species A, B, and C of this complex to investigate the usefulness of DNA techniques in mosquito evolution research. Our first aim was an estimate of the degree of diversity between different populations (in this case the three sibling species) and the second was a rapid identification tool for wild-caught mosquitoes. These are quite different questions and have involved different approaches.

DNA restriction analysis has been widely used in vertebrate population and evolution studies (Brown 1985), and is becoming more popular in entomology (DeSalle et al. 1986, DeSalle & Giddings 1986, Hale & Beckenbach 1985, Hale & Singh 1986, Harrison et al. 1987, Hall 1986, LaTorre et al. 1986, Powell 1983, Shah & Langley 1979, Solignac

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et al. 1986a, 1986b, Solignac & Monnerot 1986). The basic approach is to isolate DNA from individual insects, cut it at specific sites using a restriction endonuclease, separate the fragments by size by agarose gel electrophoresis, and then probe to identify specific fragments of interest. Except for the restriction endonuclease step, this is analogous to the technique of isozyme electrophoresis. The resulting data is even very similar, consisting of a series of bands in lanes on a gel.

DNA probes have been used to identify species or strains of microorganisms when no reliable phenotypic key was available. The approach is to identify a DNA sequence that is present in only one species or strain, clone that sequence in bacteria, and then use the cloned DNA as a probe against DNA of an unknown specimen. This is similar to the use of antibodies for serotyping. Techniques such as this are not in general use for identifying higher eucaryotes, though several groups have used them to identify individual insects infected with parasites (e.g. Kirkpatrick et al. 1987).

RESULTS

Restriction analysis

The most widely used DNA for restriction analysis is mitochondrial DNA (mDNA). There are several reasons why this molecule is preferred: it is relatively small (about 16,000 base pairs in mosquitoes), it is present in thousands of copies per cell, and it can be isolated away from the bulk of the DNA in the nucleus (Brown 1985). Mammals are large enough that it is possible to isolate enough mDNA from a single individual to analyze it by staining. That is not possible in mosquitoes because of their small size, so we were forced to take a different approach to identifying the mDNA.

DNA fractionated on a gel can be transferred to a nitrocellulose membrane and hybridized to a radioactive probe sequence to detect specific fragments. We investigated various DNAs to use to detect the mDNA fragments. *Heliothus zea* mDNA (courtesy of S. Miller) hybridized to most of the *A. quadrimaculatus* mDNA fragments, but about 20% of the molecule did not hybridize. In addition, the sensitivity of this probe was rather low and it would have been difficult to detect the mDNA of a single individual.

We devised a procedure to isolate *A. quadrimaculatus* mDNA and used this as a probe. All of the fragments were easily detectable. Two problems were that contaminating nuclear DNA gave minor background bands and in some cases the patterns were too complex to easily analyze. As an alternative we used three cloned fragments of *Aedes albipictus* mDNA (Dubin et al. 1986, HsuChen et al. 1984) as probes. These hybridized as intensely as the *A. quadrimaculatus* probe, gave no background, and lit up only a subset of the bands (which made interpretation easier).

The sensitivity of the technique was studied by serial dilutions of the DNA from a single mosquito. The mDNA fragments could be scored in the 1/64 dilution. Therefore theoretically 64 different enzymes could be scored for each mosquito.

Different methods of preservation of material for DNA preparation were tried. Live mosquitoes gave the highest yields, but mosquitoes stored for several days in ethanol also produced DNA, and the banding patterns were unchanged (though less intense). This method of preservation could be of great utility when collecting field material in areas where dry ice or liquid nitrogen is unavailable.

Analysis of the three *A. quadrimaculatus* sibling species with thirteen different restriction endonucleases showed ten differences among the three mitochondrial genomes. Species C was most divergent but species A and B also differed. This work was done on pooled samples, so only the major forms would have been detected. Further work on individual samples will be necessary to determine if these variants are fixed in the different species.

The ribosomal RNA genes (rDNA) (Gerbi 1985) could also useful for restriction analysis. They are also repeated hundreds of times per cell and cloned probes are available. We probed the same blots used to analyze the mDNA with an *A. gambiae* rDNA probe (courtesy of V. Finnerty) and found that the sensitivity was about the same. The results when the rDNA patterns of the *A. quadrimaculatus* species complex were compared were similar to the mDNA results. With the thirteen restriction endonucleases tested, six differences were seen among the three species. This is slightly less variability than in the mDNA, but the *A. gambiae* probe only hybridized to the coding sequences, not the non-transcribed spacer region. If this region had been included the differences probably would have been about the same. This is similar to the situation in *Drosophila* (Powell et al. 1986) but different from mammals (Brown 1985) where the mitochondrial DNA evolves at a much faster rate than the nuclear genes. Reprobing the filters was little additional effort and generated a great deal of additional information, so it is highly recommended that both probes be used rather than just one.

Restriction enzyme analysis has to compared to isozyme analysis to determine its usefulness.

Disadvantages could be:

1. More time consuming.
2. Slightly more expensive.
3. Requires new expertise.

Advantages could be:

1. DNA is invariant in all cells (no artifacts based on comparing different life stages).
2. DNA is much more stable than enzymes.
3. Only one type of gel and DNA isolation are used for all insects, therefore results can be directly compared between different workers.
4. Restriction endonucleases are commercially available.
5. Polymorphisms usually result in changed numbers of bands, not slight changes in mobility, making analysis much simpler.

Species specific probes

In applied entomology it is often important to be able to identify field specimens rapidly and cheaply. For example, one member of a species complex might be a serious disease vector while another is not. Chromosome analysis, isozyme analysis, and other techniques that have been useful in research situations are slow and require expert interpretation.

We have developed a simple means of isolating cloned DNA sequences that are species-specific and used it to obtain aclone (Arpl) that is specific to *A. quadrimaculatus* species A. Arpl can be used as a probe to identify DNA from individual mosquitoes of the species in question. Since the assay is for presence or absence of the Arpl sequence, it is not necessary to digest the DNA or run gels. Hundreds of "bug blots" can be analyzed by a single person in a day.

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ELECTROPHORETIC METHOD FOR RECOGNITION OF SIBLING SPECIES OF ANOPHELINE MOSQUITOES A PRACTICAL APPROACH

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ABSTRACT

Laboratory hybridization tests, comparative study of the banding patterns of polytene chromosomes, and electrophroetic techniques for isozyme analysis are the methods that are usually employed for the recognition of sibling species of anopheline and some other mosquitoes. Hybridization tests under laboratory conditions are sufficient for measuring postmating reproductive isolation, but premating mechanisms are generally undetectable. Difficulty in obtaining good quality preparations tends to limit the usefulness of the chromosomal approach for the identification of sibling species. Generally, isozyme analysis is more accurate and reliable in uncovering cryptic variation, providing meaningful information on genetic relatedness, and in the identification of sibling species. We have presented a step-by-step analysis of electrophoretic data for the identification of both allopatric and sympatric sibling species.

RESUMEN

Pruebas de hibridación en el laboratorio, estudio comparativo de los patrones de las bandas de los cromosomas de polytene, y técnicas electroforéticas para el análisis de isoenzyma son los métodos que usualmente se emplean para reconocer especies hermanas de anopheline y algunos otros mosquitos. Pruebas de hibridación bajo condiciones de laboratorio son suficientes para medir la reproducción en aislamiento después del acoplamiento, pero mecanismos antes del acoplamiento no son generalmente detectables. Dificultad en obtener preparaciones de buena calidad tienden a limitar la utilidad del método usando los cromosomas para la identificación de especies hermanas. Generalmente el análisis de isoenzyma es más exacto y confiable en descubrir variaciones crípticas, en proveer información sobre la afinidad genética, y en la identificación de especies hermanas. Nosotros hemos presentado paso a paso un análisis de datos electroforéticos para la identificación de especies hermanas alopátricas y simpátricas.

Sibling species are genetically distinct, but these closely related types are quite often indistinguishable morphologically. Since speciation is a gradual process, sibling species are thought to represent an incipient level of evolutionary divergence in comparison to morphologically distinguishable species. The lack of clear morphological differences creates a serious problem for taxonomists and other scientists, especially control specialists who are attempting to exploit biological and genetic strategies for insect control. Before the development of electrophoretic methods that were suitable for analysis of isozymes (Hubby & Throckmorton 1965, 1968), sibling species were identified by laboratory hybridization tests and comparative study of karyotypes (especially the banding patterns of polytene chromosomes in species of Diptera). Both these approaches have serious limitations. For the most part, only postmating reproductive isolation mechanisms are detected in hybridization tests under laboratory conditions, because the abnormal situation of the laboratory can obscure premating isolation.For some species of anopheline mosquitoes, the polytene chromosomes are of good quality and

can be used for the purpose of correct identification of sibling forms, but there are problems in obtaining good preparations for some species, even to the extent that this technique can not be used on a routine basis. Such limitations do not exist in the electrophoretic analysis of gene-enzyme systems. A comprehensive discussion on the applications of hybridization, karyotypes, and electrophoretic studies for evolutionary and taxonomic inferences in the study of sibling species in mosquitoes can be found in review articles by Bullini & Coluzzi (1982) and Narang & Seawright (1988). Throughout the text, we have used three terms to designate a given sibling species in order to highlight the taxonomic tool used for its identification. A sibling species identified on the basis of diagnostic allozymes will be referred to as "allospecies", in contrast to "cytospecies" based on chromosomal identification, and "biological species" based on hybridization tests.

During the last few years, we have used electrophoretic methods for analysis of gene-enzyme systems to identify sibling species in natural populations of the *Anopheles quadrimaculatus* complex (Narang et al. 1988). A dichotomous electrophortic key was prepared (Narang et al. 1988) and can be used accurately for the identification of field-collected adults of the *A. quadrimaculatus* complex. In the early stages of this work, studies on ovarian nurse cell polytene chromosomes and laboratory hybridization tests were used to complement and correlate the identification of the members of the complex (Kaiser et al. 1988). In this paper, we present a step-by-step approach to the analysis of electrophoretic data for recognition of sibling species, both in sympatry and allopatry. Electrophoretic data of Narang et al. (1988) will be used to illustrate these steps.

Materials and Methods

The following text describes the materials and methods used for identification of sibling species of *A. quadrimaculatus* complex. This general approach should be useful for recognizing sibling species in other species complexes of mosquitoes.

Adult mosquitoes of *A. quadrimaculatus* were collected from about 30 localities throughout the distribution range of this species in the eastern half of the U.S. Wild-caught adults were stored frozen at –70° C until used for electrophoresis. Horizontal starch gel electrophoresis was performed according to Steiner & Joslyn with a few modifications (Narang et al. 1988). Gel trays measuring 13 X 20 cm were used. Individual mosquitoes were homogenized in 30ul of grinding buffer (10mM Tris, 1mM EDTA, 1mM 2-mercaptoethanol, pH 7.0) in a multiple sample grinding block. Homogenate of each adult was absorbed with three 3 X 10 mm wicks (Whatman 3 mm paper) and analyzed on three 10 mm thick starch gels (one wick from each mosquito per gel). Homogenates of thirty-two adults including equal numbers of mosquitoes from two localities and three samples of a standard reference strain were run on each gel as described by Narang et al (1988). After electrophoresis, each gel was cut into six 1.5 mm thick slices. These 18 slices from three gels were stained for different enzymes. Samples of 30-50 adults from each locality were analyzed for preliminary electrophoretic test for recognition of genetic substructuring. Electromorphs (enzyme bands) at thirty four presumptive loci of 16 enzyme systems were scored using bands of the reference strain as controls as described earlier (Narang et al 1988).

Statistical analysis of electrophoretic variability data was performed by using the computer program, Biosys 1 (Swofford & Selander 1981). The tests included: (1) chi-square test for conformance of observed electromorph frequencies to those expected under Hardy-Weinberg equilibrium; (2) Selander's (1970) coefficient for deficiency or excess of heterozygotes for each polymorphic locus; (3) genetic identity and genetic distance (Nei 1978); and (4) the diagnostic value of a locus (Ayala & Powell 1972).

Results and Discussion

The following text, divided into 5 steps, describes a sequential approach to the analysis of electrophoretic data for recognition of sibling species in natural populations of *A. quadrimacultus. We have used some data from previously reported studies on A. quadrimaculatus* (Narang et al. 1988) to serve as an example for this paper. Electrophoretic data on populations from four localities, Shell Mound, Levy Co. FL (LEV), Bear Bay Swamp, Dixie Co. FL (BBS), Noxubee, Noxubee Co. MS (NOX) and Panasoffkee, Sumter Co. FL (PAN), will be used for illustrations.

Step I. Recognition of genotypes with allelic clusters:

The first level of evidence for the occurrence of presumed sympatric sibling species (allospecies) can be obtained from visual observation of zymograms of polymorphic loci as shown in Figures 1-5. For example, in the LEV samples (lanes 5-8 in each figure), adults can be grouped into two classes. In the first (lanes 5 & 6 in each figure), there is a cluster of alleles such as: Had-1 (92), Had-3 (45), Got-2 (38), Pep-2 (110), Pgi (95), Idh-2 (162), Idh-1 (100). The second, (lanes 7 & 8 in each figure) lacks these alleles at the respective loci. These results give the erroneous indication of strong linkage among these loci. In addition, without formal statistical analysis, there are obvious deficiencies of heterozygotes for alleles at these loci. These results suggest that the two classes of adults are reproductively isolated from each other. Similarly, clustering of Idh-1 (86) and Idh-2 (162) electromorphs to certain individuals is indicative of sympatric sibling species in NOX. On the contrary, no allelic clustering is apparent in samples from PAN and BBS; therefore, presumably at this point it appears that pure populations were collected at those locations.

Step II. Chi-square test for Hardy-Weinberg equilibrium and Selander's D coefficient:

The chi-square test helps to define statistically the presence of sympatric sibling species. This test is based on the assumption that a population is in Hardy-Weinberg equilibrium, i.e., the observed frequencies of alleles can be used to calculate expected genotypic frequencies that are not significantly different from the observed genotypic frequencies. A significant chi-square value is indicative of genetic substructuring, i.e., sibling species. As shown in Table 1, there is a lack or significant deficiency of heterozygotes for alleles at certain loci in LEV (Had-1, Had-3, Got-2, Pep-2, Pgi, and Idh-2), and in NOX (Idh-1, Idh-2 and Had-1). Therefore, these results indicate that at both localities, there are sympatric allospecies. Furthermore, on the basis of the number and types of loci for which there a deficiency of heterozygotes, one of the species in LEV is different from the two species in NOX. The chi-square values for the BBS and PAN populations were not significant. Selander's D coefficient also indicates whether a population is in equilibrium, but this statistic also provides information on the proportion and direction (either positive or negative) of an unusual frequency of heterozygotes at each locus. For the data in Table 1, there was general agreement, as expected, between the values for Selander's D coefficient and chi-square. By Selander's D coefficient, there was no evidence for the occurrence of sympatric allospecies in the populations from BBS and PAN.

Step III. Significant differences in allelic frequencies:

Comparison of allelic frequencies at various loci will help recognize presumed allospecies in allopatry. As shown in Table 2, samples from PAN and BBS are either fixed

FIGURE 1

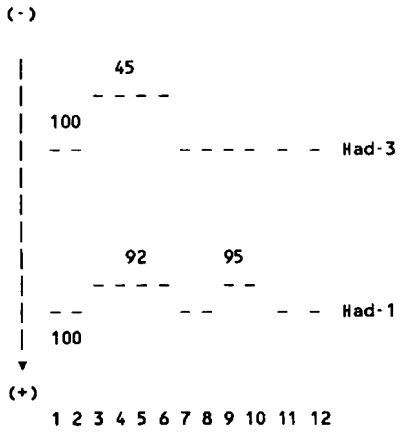

FIGURE 2

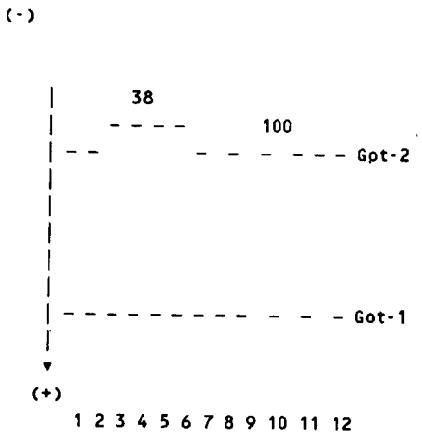

FIGURE 3

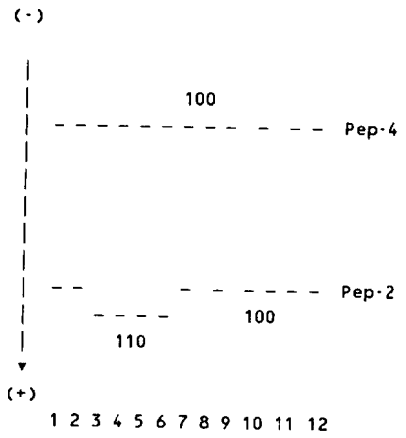

FIGURE 4

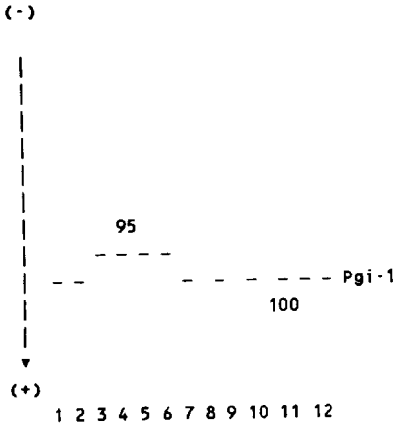

FIGURE 5

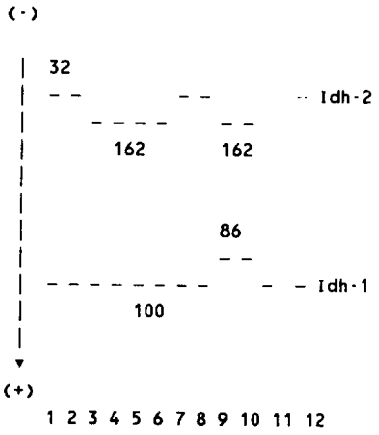

TABLE 1. COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY AT CERTAIN LOCI IN SAMPLES OF *ANOPHELES QUADRIMACULATUS* FROM FOUR LOCALITIES (PAN, BBS, NOX AND LEV).

Locus	Locality	N	Observed heterozygotes	Exptected heterozygotes[1]	X^2	Selander's D
Had-1	PAN	50	6	6	0	0
	BBS	42	0	0	—	0
	NOX	52	7	26	13.88*	–0.73
	LEV	78	1	18	16.06*	–0.94
Had-3	PAN	50	6	7	0.14	–0.14
	BBS	50	3	3	0	0
	NOX	260	40	41	0.02	–0.02
	LEV	243	25	73	31.56*	–0.66
Got-2	PAN	48	18	21	0.43	–0.14
	BBS	50	5	5	0	0
	NOX	154	26	26	0	0
	LEV	171	28	49	9*	–0.43
Pep-2	PAN	41	6	6	0	0
	BBS	44	8	9	0.11	–0.11
	NOX	63	0	0	—	0
	LEV	136	37	58	7.60*	–0.36
Pgi-1	PAN	50	3	3	0	0
	BBS	50	5	6	0.17	0
	NOX	119	5	5	0	0
	LEV	241	42	75	14.52*	–0.44
Idh-1	PAN	50	12	14	0.28	–0.14
	BBS	50	5	6	0.17	–0.16
	NOX	323	37	120	57.41*	–0.69
	LEV	171	22	25	0.36	–0.12
Idh-2	PAN	47	24	27	0.33	–0.11
	BBS	42	1	1	0	0
	NOX	227	33	80	27.61*	–0.59
	LEV	143	28	32	0.5	–0.13

*Significant chi-square values ($p < 0.05$).
[1]Based on Hardy-Weinberg equilibrium.

for different alleles or show significant differences in allelic frequencies (most common allele in PAN:BBS shown in parenthesis) at loci : Had-1 (100:92), Had-3 (100:45), Got-2 (100:38), Pep-2 (100:110), Pgi-2 (100:95) and Idh-2 loci (132:162). It is also clear that alleles at these loci in BBS are similar to alleles in some of the adults of LEV, indicating

Figs. 1-5. Schematic representations of zymograms of adult samples from PAN, BBS, LEV and NOX. Individual adults were run on one gel and gel slices stained for various enzymes. The sample order is same in all zymograms. From left to right samples no. 1-2 (PAN), 3-4 (BBS), 5-8 (LEV), 9-12 (NOX). Notice that samples no. 3, 4, 5 and 6 have different bands for most loci (species C specific cluster of alleles). Sample 9, 10 (species B) differs from others at Had-1, Idh-1 and Idh-2.

TABLE 2. FREQUENCIES OF ELECTROMORPHS AT SEVEN LOCI IN 3 SIBLING SPECIES OF THE *ANOPHELES QUADRIMACULATUS* COMPLEX. DIAGNOSTIC ELECTROMORPHS (RELATIVE TO SPECIES A) ARE UNDERLINED

Locus	A (NOX)	A (LEV)	A (PAN)	B (NOX)	C (LEV)	C (BBS)
Had-1						
(N)	17	10	50	35	68	42
104	0	0	0.05	0	0	0
100	1.00	1.00	0.93	0.12	0	0
95	0	0	0	0.88	0.02	0
92	0	0	0.02	0	0.98	1.00
Had-3						
(N)	80	39	50	180	204	50
156	0.07	0.05	0.09	0.05	0	0
100	0.91	0.87	0.89	0.93	0.03	0.01
45	0.02	0.08	0.02	0.02	0.96	0.99
25	0	0	0	0	0.01	0
Got-2						
(N)	57	28	48	97	143	50
170	0.02	0	0.03	0.01	0	0
138	0	0.04	0	0	0	0
115	0.02	0	0	0.02	0	0
100	0.86	0.67	0.69	0.92	0.06	0.05
80	0	0	0	0.01	0	0
38	0.10	0.29	0.28	0.04	0.94	0.95
Pep-2						
(N)	29	26	41	34	110	44
115	0	0.06	0	0	0.04	0.03
110	0	0.17	0.07	0	0.85	0.88
100	1.00	0.77	0.92	1.00	0.11	0.09
89	0	0	0.01	0	0	0
Pgi-1						
(N)	42	41	50	77	206	50
105	0.02	0	0	0	0	0
103	0	0	0	0	0	0
100	0.95	0.79	0.97	0.98	0.08	0.06
95	0	0.21	0.02	0.02	0.91	0.94
80	0.03	0	0.01	0	0.01	0
Idh-1						
(N)	100	23	50	223	148	50
110	0.03	0.02	0.02	0	0.01	0.05
100	0.75	0.88	0.83	0	0.97	0.94
86	0.17	0.10	0.15	1.00	0.02	0.01
83	0.05	0	0	0	0	0
Idh-2						
(N)	74	19	47	153	124	42
209	0.02	0.02	0.05	0	0.02	0.01
181	0.02	0	0	0	0	0
162	0.19	0.54	0.32	1.00	0.97	0.99
132	0.66	0.42	0.56	0	0.01	0
100	0.11	0.02	0.07	0	0	0

that the species in BBS is most likely the same that occurs in sympatry in LEV. Table 3 shows the diagnostic values (Ayala & Powell 1972) for these loci, which can be used for taxonomic identification of sibling species.

Step IV. Partitioning of genotypic frequency data of samples containing allospecies in sympatry into data corresponding to respective presumed allospecies (using information from step I and II). Hardy-Weinberg equilibrium test on partitioned data set:

The results in Table 1 and 2 (steps I and II) indicated that the NOX population was composed of two presumed allospecies which differ at the loci for Idh-1, Idh-2, and Had-1. Adults with the allelic cluster at these three loci, Idh-1 (86), Idh-2 (162) and Had-1 (95) can be tentatively assigned to one allospecies (species B). Adults of NOX lacking this cluster can be assigned to a distinct allospecies A (see alleles of A at three loci in Table 3). At this point the chi-square test for conformance of each data subset (of allospecies A and B) to Hardy-Weinberg equilibrium should be done. If the partitioned genotypic frequency data of each subset is in equilibrium (i.e.. the chi-square value is not significant), this can be considered adequate to identify and characterize sibling species A and B. When partitioned, the LEV population was found to be composed of two presumed species, (1) allospecies A similar in genotypes at Idh-1, Idh-2 and Had-1 loci to that of A in NOX and allospecies C characterized by a cluster of alleles such as: Had-1 (92), Had-3 (45), Got-2 (38), Pep-2 (110) Pgi (95) and a characteristic two-locus genotype, Idh-2:Idh-1 (162/100).

Step V. Genetic Identity (I) and genetic distance (D).

The coefficients of genetic relationships (Nei 1978). i.e. genetic identity (I) and genetic distance (D, –log I), can be very useful indicators for recognition of allopatric sibling species. As shown in Table 4 the intra-specific I is 0.95 to 0.97 in species A, 0.98 in species B and 0.98 in species C. The interspecific I ranged from 0.80 to 0.85 between A and B, 0.54 to 0.64 between A and C, and 0.54 between B and C. Allopatric populations of PAN and BBS, which were both in Hardy-Weinberg equilibrium for genotypes at all loci showed I value of 0.61 (N in each locality was 50) confirming the two populations were composed of two distinct allopatric allospecies.

TABLE 3. DIAGNOSTIC VALUES[1,2] OF SOME ENZYME LOCI, WHICH CAN BE USED TO IDENTIFY SYMPATRIC SIBLING SPECIES OF *ANOPHELES QUADRIMACULATUS* COMPLEX.

Locus	Species		
	A:B	A:C	B:C
Idh-1	98.82	—	99.97
Idh-2	95.03*	95.01*	—
Had-1	97.66*	99.98	99.97
Had-3	—	99.92	99.92
Got-2	—	99.19	99.75
Pep-2	—	99.07	99.36
Pgi-1	—	99.99	99.65

[1]Diagnostic values were calculated by the method of Ayala and Powell (1972). Frequencies of electromorphs of species A from pooled samples of PAN and NOX. B was from NOX and C from Levy Co. and BBS were used.

[2]A locus is considered diagnostic, if the probability of correct diagnosis of an individual to a given taxon is 99% or higher. Loci marked with asterisk (*) can be used only as two-loci diagnostics.

TABLE 4. ESTIMATES OF NEI'S (1978) UNBIASED GENETIC IDENTITY (ABOVE DIAGONAL) AND GENETIC DISTANCE (BELOW DIAGONAL) FOR NATURAL POPULATIONS OF SIBLING SPECIES OF *ANOPHELES QUADRIMACULATUS* COMPLEX.

Populations	1	2	3	4	5	6
1. PAN species A	—	0.973	0.948	0.825	0.578	0.539
2. NOX species A	0.027	—	0.924	0.818	0.541	0.563
3. LEV species A	0.053	0.079	—	0.799	0.642	0.610
4. NOX species B	0.193	0.201	0.442	—	0.544	0.587
5. LEV species C	0.548	0.614	0.442	0.610	—	0.964
6. BBS species C	0.618	0.574	0.494	0.533	0.037	—

An alternative approach to identify sympatric sibling species directly from step I consists of examining chromosomal banding patterns in adults showing allelic clusters. Ovaries from individual wild gravid females are removed for study of ovarian nurse cell polytene chromosome banding pattern and the remaining part of each mosquito is used for electrophoretic analysis of selected gene.enzyme systems (steps I). This simultaneous chromosome-electrophoretic analysis is helpful to correlate the identity of allospecies with that of cytospecies. Our results (Narang et al. 1988, Kaiser et al. 1988) showed that each of the three allospecies, A, B and C had indeed diagnostic chromosomal complements. Chromosomes of gravid females of allospecies A from NOX and LEV were similar to those of the pure population of allospecies A from PAN. Similarly, females of allospecies C from LEV were similar to those of pure population of species from BBS. In order to designate these species as true biological species (more so for species in allopatry), hybrid breakdown among these taxa must be established by hybridization crosses in laboratory condition. Crosses among A, B and C showed hybrid sterility to different degrees (Kaiser 1988).

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CYTOTAXONOMY AS A TOOL FOR IDENTIFICATION OF SIBLINGS OF THE *ANOPHELES QUADRIMACULATUS* COMPLEX

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ABSTRACT

Wild populations of *Anopheles quadrimaculatus* were collected in the southeastern United States for cytogenetic studies. Two approaches, i.e., polytene chromosome comparisons and hybridization studies were used. Observations on the ovarian nurse cell polytene chromosomes indicated two distinct X chromosomes, one with a standard banding arrangement and the other with a fixed inversion. Three polymorphic inversions on the right arm of chromosome 3 were associated with the normal X (cytotype A). Individuals with the inverted X contained a polymorphic and a fixed inversion on both 3R and the left arm of chromosome 2 (cytotype B). The two types shared a small inversion on 2L and a complex arrangement on the left arm of chromosome 3 that included two distinct homokarotypes. The ovarian polytene chromosomes of the F_1 hybrids (A x B and reciprocal) demonstrated complete asynapsis in the X chromosome and 3L, and extensive asynapsis in the other arms. Differential mortality was expressed in F_1 hybrids depending on the cross; some crosses produced only females, some produced only males, and others resulted in normal sex ratios. The reproductive organs of F_1 adults were always abnormal, ranging from atrophied to complete absence. These aberrations were expressed in backcrosses where very high embryonic and larval mortality were observed. These studies proved that the chromosomally differentiated types A and B are distinct sibling species of the *Anopheles quadrimaculatus* complex.

RESUMEN

Se colectaron poblaciones salvajes de *Anopheles quadrimaculatus* en el sudeste de los Estados Unidos para estudios citogenéticos. Se usaron dos aproches, comparaciones de cromosomas de politeno, y estudios de hibridación. Observaciones de los cromosomas de politeno de la célula nodriza del ovario indicó dos cromosomas X distintos, uno con unas bandas patrones, y otro con una inversión fija. Tres inversiones polimórficas en el brazo derecho del cromosoma 3 fueron asociadas con la X normal (citotipo A). Individuos con la X invertida contenían una inversión polimórfica y fija en ambos 3R y en el brazo

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izqierdo del cromosoma 2 (citotipo B). Los dos tipos comparten una pequeña inversión en el 2L y un complejo arreglo en el brazo izquierdo del cromosoma 3, que incluye dos distintos homokarotipos. Los cromosomas de politeno del ovario de híbridos F_1 (A X B y el recíproco) demostraron una asinapsis completa en el cromosoma X y 3L, y una extensiva asinapsis en en los otros brazos. Mortalidad diferencial se expresó en híbridos F_1 dependiente del cruce; algunos cruces producieron solo hembras, algunos producieron solo machos, y los otros resultaron en una proporción normal de los sexos. Los órganos reproductivos de adultos F_1 siempre fueron anormales, variando de atrofiados a completamente ausentes. Estas observaciones se expresaron en el cruce de la progene del F_1 de vuelta a los padres, donde se observó una alta mortalidad embriónica y larval. Estos estudios probaron que los tipos A y B de cromosomas diferenciados son especies hermanas pero distintas del complejo de *Anopheles quadrimaculatus*.

The use of cytology in conjunction with classical taxonomy is not a new idea, as it has been 40 years since Frizzi used salivary gland polytene chromosomes to distinguish members of the European Maculipennis sibling species complex (Frizzi 1947). Twenty years later Coluzzi & Sabatini (1967, 1968, 1969) published a series of papers that differentiated the members of the *Anopheles gambiae* complex according to differences in their salivary gland polytene chromosomes. Polytene chromosome maps have been prepared for most of the medically important anophelines, and these maps have been used to uncover sibling species complexes in *Anopheles stephensi*, *Anopheles culicifacies*, *Anopheles balabacensis* and *Anopheles farauti*.

Kitzmiller et al. (1967) reviewed the cytological relationships of the important North American anophelines and determined that many similarities of ethnological significance existed. Although the adults of the different species were morphologically distinguishable, many homologies in the DNA banding patterns were revealed. They used these homologies and the results of hybrid crosses to determine that *Anopheles freeborni* Aitken, *Anopheles aztecus* Hoffman, *Anopheles occidentalis* Dyar and *Anopheles earlei* Vargas were more related within the nearctic Maculipennis complex. *Anopheles punctipennis* Say and *Anopheles quadrimaculatus* Say were also related to this complex, but to a lesser degree. Kaiser et al. (1988) indicated that inversion sequences in *A. earlei*, *A. punctipennis* and *A. quadrimaculatus* demonstrated relatedness between these three eastern U.S.A. species. This, perhaps, should not be surprising because they occupy overlapping ranges. Therefore, it is clear that cytotaxonomy can be utilized to distinguish between morphologically similar species (siblings), and to demonstrate similarities among related species. This paper shows how cytology was used in a research program on *A. quadrimaculatus*, the goal of which was to determine if a sibling species complex existed in this species.

Background on *A. quadrimaculatus*

Anopheles quadrimaculatus was of medical importance because it was the primary vector of malaria in the eastern United States when it was endemic in this country. Today it is a serious pest in rice-growing areas and in the Tennessee Valley Authority (TVA) reservoir system, where pest management programs are monetarily and environmentally expensive. Permanent bodies of water with large amounts of aquatic vegetation provide excellent breeding habitats for the immatures, so the establishment and proliferation of exotic aquatic weeds in the eastern United States has enhanced the breeding potential of this mosquito. The broad distribution of *A. quadrimaculatus* extends from southern Florida and Texas to southern Canada; the northwestern range extends through southern Minnesota. The fact that the distribution of this mosquito covers such a large geographic area precludes the notion that environmental

homogeneity could exist throughout its breeding range. Therefore, we postulated that evolutionary processes may have occurred that resulted in geographic and, eventually, reproductive isolation (this is not meant to discount the possibility of sympatric speciation). Morphological changes may not have occurred or may have been minor because the speciation process was relatively new. Or, perhaps there was no advantage in morphological change. At any rate, the result of this speciation process would be a sibling species complex in *A. quadrimaculatus*.

To test this hypothesis we collected adults from throughout the eastern United States and returned them to our laboratory for study. Standard taxonomic keys from Carpenter & LaCasse (1955) and Darsie & Ward (1981) were used to verify that the adults were *A. quadrimaculatus;* females were blooded and incubated at 27°C for 24 hr and then transferred to a refrigerator (5°C) pending dissection. The ovaries were removed from these females and the ovarian nurse cell polytene chromosomes were prepared by the method of Kaiser et al. (1988a). Chromosomes were observed under phase-contrast and compared to the standard ovarian polytene map for *A. quadrimaculatus* prepared by Kaiser & Seawright (1987).

Males and females from some of the populations were mated to adults representing other populations; the wild adults would not mate freely in the laboratory so a forced copulation technique similar to that of Baker (1964) was employed. We used these matings to observe F1 hybrid mortality, polytene chromosomes and sterility.

Cytotaxonomic Methods

Cytological techniques for differentiating sibling species can be divided into two main categories.

Poytene Chromosome Comparisons

In anophelines, polytene chromosomes usually occur in the salivary glands of 4th instar larvae and in adult ovaries. The quality of the chromosome preparations varies from tissue to tissue and from species to species, so the various options should be considered. Polytene chromosome maps have been prepared for most of the important North American anophelines (Kitzmiller et al. 1967), but if not available they must be prepared. Although there was a salivary gland chromosome map available for *A. quadrimaculatus* (Klassen et al. 1965), the polytene chromosome preparations from the salivary glands were usually of very poor quality. Therefore, it was necessary to draw a map from the ovarian polytene chromosomes (Kaiser & Seawright 1987), the quality and consistently of which were good, even in wild females. The map was prepared from permanent slides, photographs of standard enlargement, and camera lucida drawings.

When suitable polytene maps are available, field populations can be analyzed by observing the chromosomes of random samples of larvae and/or adults for a) polymorphic and b) fixed inversions. Polymorphic (floating) inversions are important in regard to frequency and kind. Polymorphic inversions may be shared by sibling species, in which case variations in frequency may indicate separate mating populations (Bedo 979, Bryan et al. 1982). Or, more typically, they may be unique for a particular species. Inversion breakpoints are usually easy to identify and inversion loops are distinct features in polytene chromosome preparations that can be used to distinguish species with a high level of competency. Inversion freqencies of conspecific polymorphic inversions may vary, geographically and temporally, between populations, but this does not change their status as a diagnostic tool (Stalker 1964, Dobzhansky & Powell 1975, Coluzzi et al. 1979). In North American Anophelines, the right arm of chromosome 3 contains the most inversion polymorphisms; the X chromosome and the left arm of chromosome 2 may also contain inversion polymorphisms (Kitzmiller et al. 1967).

Fixed inversions, which are inversion homozygotes that are fixed in a mating population, i.e., no inversion polymorphism occurs, are often used to separate sibling species (Green & Hunt 1980, Rothfels 1981). Diagnosis may initially be difficult, but if camera lucida drawings of sections of the chromosome arms are compared to the respective sections in photographs, they can usually be detected. Then, prominent groups of bands within the inversion can be used as a diagnostic feature, by noting the order of the bands, to easily discern the presence of the homozygote. Fixed inversions may occur on the X chromosome or on either of the autosomes.

Hybridization Studies

Adults from different populations can be mated to each other and their progeny observed for a) aberrant polytene chromosomes, b) sterility and c) mortality. Polytene chromosomes of hybrids from interspecific crosses commonly have regions that are asynaptic, i.e., unpaired zones of a diploid chromosome (Kitzmiller et al. 1967). These asynaptic regions, which may have homosequential banding patterns, presumably have undergone changes at the molecular level that affect their normal affinity for each other. A general rule of thumb is that the degree of asynapsis is directly proportional to the degree of relatedness of the two parents. Another important reason for observing hybrid polytene chromosomes is that fixed inversions, which may be difficult to distinguish in normal chromosome complements, produce the standard inversion loop in their effort to pair with their homologous partner. An example of this can be seen in Figure 1b, where a very small region of a chromosome has been inverted and inserted at a new location. Although obvious here, this rearrangement would have been difficult to detect in a normal complement.

Hybrid sterility is the second method of assaying the compatibility of parents, and is usually a more accurate measurement of relatedness than asynapsis. Hybrid sterility, which in effect is reproductive isolation, is expressed in three ways. First, the testes and ovaries of hybrids may undergo structural changes; these changes can vary from a slight reduction in size, to atrophy, to total absence (Davidson 1964, Kreutzer & Kitzmiller 1971). The size reduction usually results in a decrease in fecundity for the females. The amount of sperm in testes is reduced and many of the sperm and spermatozoa are deformed. The last two expressions of hybrid sterility are pre- and post-zygotic mortality. When hybrid females and males are outcrossed to either of the parental types, fertility is usually reduced and mortality may be as high as 100%. The survival rate of the progeny that do hatch is less than normal (Curtis 1982, Kaiser et al. 1988).

Mortality of F1 hybrids may occur in one or both sexes, and the mortality may be unidirectional or in both directions (Kreutzer & Kitzmiller 1972, Miles 1981). Unidirectional mortality occurs when species P x species Q produces only females (or males) but sp. Q x sp. P results in a normal sex ratio. The mortality usually occurs in the larval or pupal stage.

Polytene Chromosome Comparisons in *A. quadrimaculatus*

The results of observing the ovarian polytene chromosomes of adults from several different populations and comparing them to the standard map (Kaiser & Seawright 1987) are described below.

X Chromosome

A small fixed inversion, In(X)a, occurred in a portion of the adults from several of the populations. The normal arrangement was also present, however, we failed to detect

Fig. 1. The ovarian nurse cell polytene chromosomes of hybrids of an interspecific cross between species A and species B of the *Anopheles quadrimaculatus* complex. A) Left arm of chromosome 2. B) Left arm of chromosome 2 that shows the fixed inversion, In(2L)c. C) Right arm of chromosome 3 that shows the fixed inversion, In(3R)e. D) Left arm of chromosome 3; this homokaryotype ($3L_1$) is one of two that have been described. E) The homokaryotype $3L_2$ (unpublished data).

any individuals that were heterozygous for this inversion. This was an indication that two separate mating populations, reproductively isolated, existed sympatrically at some of the collection sites. We provisionally called the new sibling species B. The two X chromosomes can be seen in Figure 2; the fact that the puff was included in the inversion made detection fairly easy.

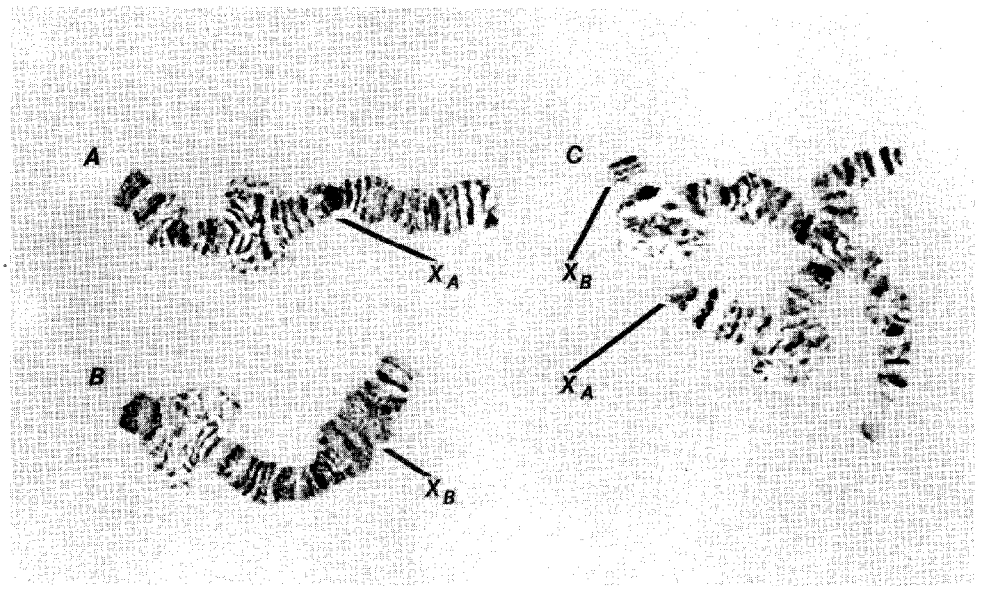

Fig. 2. The X chromosomes of *Anopheles quadrimaculatus*. A) Species A. B) Species B. C) Asynaptic X chromosomes of species A x species B hybrid (unpublished data).

Chromosome 2

Three inversions were observed on the left arm of chromosome 2. By using the X chromosome for species determination (A or B), we were able to associate the autosomal inversions with their respective species. A small inversion, In(2L)a, was located near the centromere and was seen in both species A and B. The two remaining inversions were found only in species B. The polymorphic inversion, In(2L)b, and the fixed inversion, In(2L)c, were located in the middle of the arm where they shared a common breakpoint; In(2L)c was also inserted into a new position on the distal side of In(2L)b (Fig. 1b). No inversions were found on the right arm of chromosome 2.

Chromosome 3

The left arm of chromosome 3 is very unusual in that two karyotypes, $3L_1$ and $3L_2$, exist in both species A and B. The heterokaryotype was observed much more often than either of the homokaryotypes (the ratios did not fit Hardy-Weinberg in any of the populations); most of the heterokaryotype is asynaptic which gives it a distinctive appearance. Two small fixed inversions have taken place on $3L_2$, and these are the only regions that pair in the heterokaryotype.

The right arm is typically the most polymorphic arm in North American anophelines. It is also the longest of the arms which makes identification easy. We have found six inversions in this arm, 4 in species A and 2 in species B (Figure 3). The polymorphic species A inversions are: In(3R)a, which is a small inversion near the distal end of the chromosome.; In(3R)b is a somewhat larger inversion occurring in the middle of the arm. This inversion sometimes includes a smaller inversion, i.e., included inversion.; In(3R)c is a small inversion that is adjacent to the centromere.; In(3R)f is the small inversion included in In(3R)b that occurs infrequently by itself. The two species B inversions are In(3R)d, which is polymorphic, and In(3R)e, which is fixed. They occur in the middle regions of 3R (Fig. 3d,e).

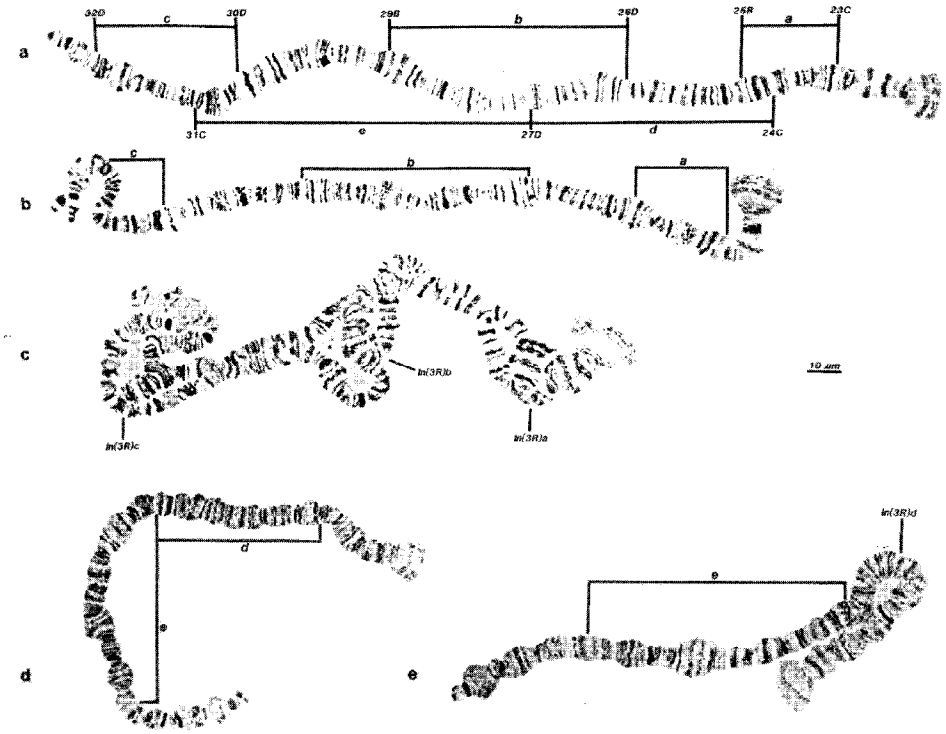

Fig. 3. The right arm of chromosome 3 of *Anopheles quadrimaculatus*. A) A standard 3R of species A that shows the inversion breakpoints for species A (above) and species B (below). B) 3R of species A with 3 homozygous inversions. C) 3R of species A with 3 inversion heterozygotes. D) 3R of species B with homozygous inversion (d) and fixed inversion (e). E) 3R of species B with heterozygous inversion (d) and fixed inversion (e) (Kaiser et al. 1988a).

The frequencies of the conspecific floating inversions varied between populations, i.e., geographic variation, and they were not polymorphic in all populations (Table 1) (Kaiser et al. 1988a). Species A inversions In(3R)a, b and c were never observed in adults collected from central and south Florida. However, populations in north Florida contained a few individuals heterozygous for these inversions. Populations of species A that were north and/or west of Florida were very polymorphic, and in some cases the homozygous inversion frequency was greater than that of the standard banding arrangement. The frequencies of species B floating inversions also varied between populations. However, since the species B populations were all above latitude 30°N no conclusions regarding north vs. south geographic variation in inversion polymorphism could be drawn.

Hybridization Studies in *A. quadrimaculatus*

Adult females from field populations were returned to the laboratory and used to establish isofemale lines. Cytology was used to determine species A and B lines, and forced copulation was used to make hybrid crosses (sp. A x sp. B and reciprocal). Cytology on F1 hybrids demonstrated that some portions of the polytene chromosomes were asynaptic (Fig. 1), and that others contained inversion loops indicative of fixed inversions (Fig. 1b,c). Complete asynapsis was noted in the X chromosome (Fig. 2c)

TABLE 1. VARIATIONS IN FREQUENIES OF SPECIES A IN(3R)a IN FIELD POPULATIONS.[a]

Population	+/+	+/a	a/a	N	Freq. +	Freq. a	χ^{2b}
Goldens Bridge, NY	0	12	88	100	0.060	0.940	0.407
Chickamauga Lake, TN	0	12	88	100	0.060	0.940	0.407
Guntersville Lake, AL	0	10	91	101	0.050	0.950	0.274
Stuttgart, AR	1	10	89	100	0.060	0.940	1.288
College Station, TX	10	46	44	100	0.330	0.670	0.162
Lake Charles, LA	25	54	21	100	0.520	0.480	0.668
Chatahoochee State Park, AL	19	48	21	88	0.489	0.511	0.736
Lake Seminole, GA	28	47	30	105	0.490	0.510	1.145
Lake Octahatchee, FL	56	12	6	74	0.838	0.162	12.032
Kanapaha Bot. Garden, FL	120	4	0	124	0.984	0.016	0.033
Lake Panasoffkeed, FL	100	0	0	100	1.000	0.000	
Myakka State Park FL	115	0	0	115	1.000	0.000	

[a]Kaiser et al. 1988a
[b]Significant at the 0.05 probability level

and in the left arm of chromosome 3 (Fig. 1d,e). Also, the breakpoints for the fixed inversions, In(2L)c and In(3R)e, were confirmed.

One of the important effects of hybridization is sterility, so to study this we dissected the ovaries and testes from F1 hybrids and observed them for abnormalities. Some hybrids had no reproductive organs, although most had some form of atrophied organs (Figure 4). When the atrophied testes were dissected, the contents revealed small amounts of deformed sperm (Figure 5). Some of the F1 hybrids were backcrossed and pre- and post-zygotic mortality were observed in all of the crosses (Table 2).

One of the more interesting aspects of the hybridization studies was the differential mortality expressed in the F1 hybrids (Kaiser et al. 1988b). When species A females were mated to sp. B_1 males, only F1 females were produced because all the males died in the pupal stage (Table 3). When sp. B_2 males were used in the cross, females and males were produced in about a 3:2 ratio. The reciprocal cross (B♀ x A♂) resulted in a normal sex ratio.

DISCUSSION

We used cytology as a taxonomic tool in determining that a sibling species complex existed in *A. quadrimaculatus* (Table 4). This proved necessary because classical

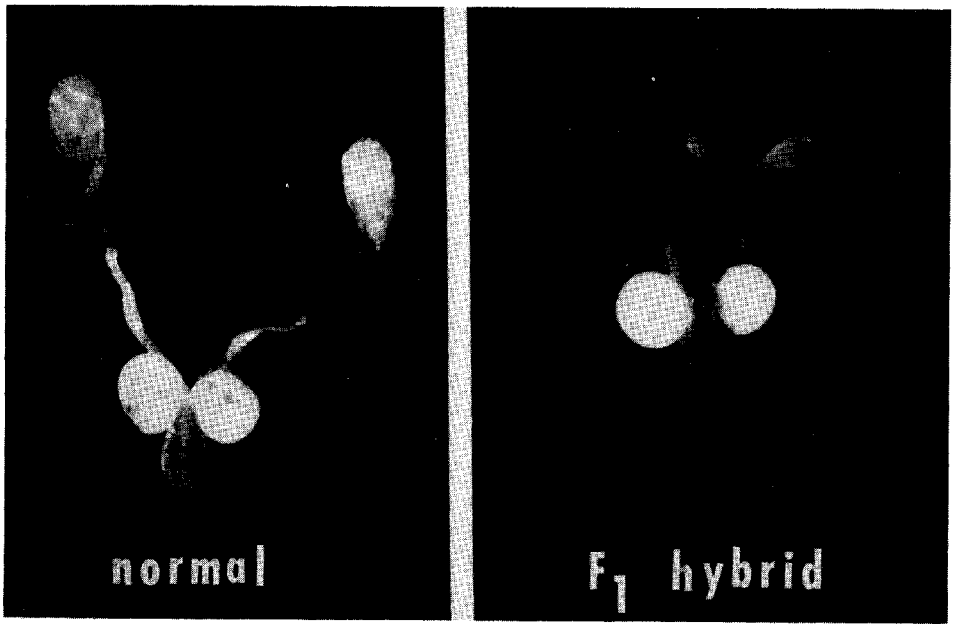

Fig. 4. Adult *Anopheles quadrimaculatus* reproductive organs (testes and accessory glands) from a normal species A male (left) and a species A x species B hybrid male (right).

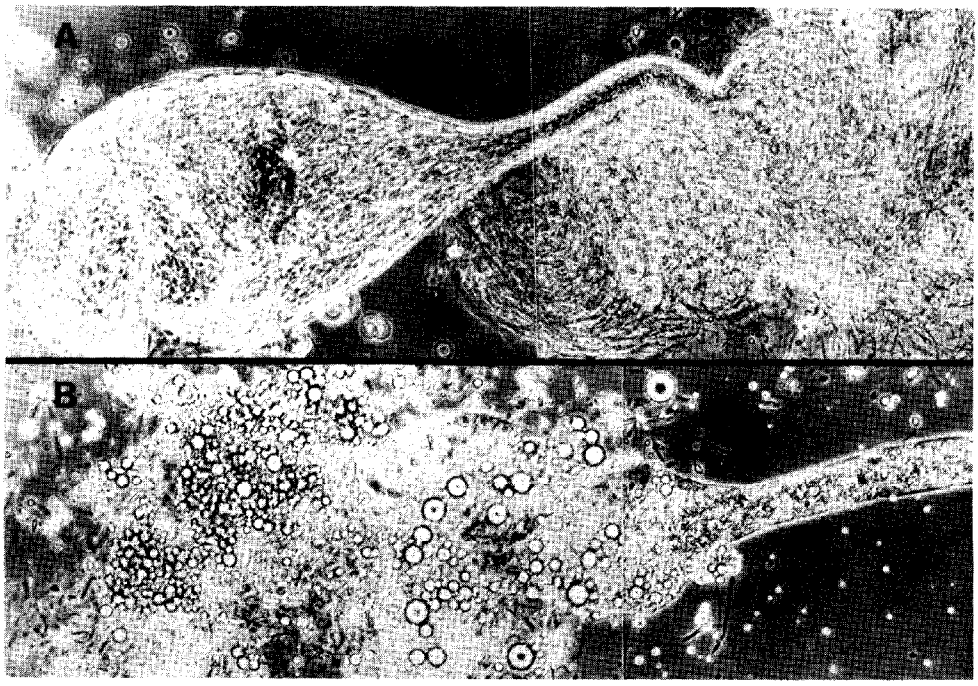

Fig. 5. Contents of adult *Anopheles quadrimaculatus* reproductive organ (testis) from a normal species A male (A) and a species A x species B hybrid male (B).

TABLE 2. SUMMARY OF DATA FROM 1 OF 4 BACKCROSS SCHEMES WITH *ANOPHELES QUADRIMACULATUS* SPECIES A AND B THAT DEMONSTRATES PRE-ZYGOTIC MORTALITY[1].

Cross	Adult Phenotype[2]	Maternal Genotype	Generation	# of Matings	# of Families	Mean Fecundity	Mean % Fertility
Control							
XArXAr x XArYA	*ro*	*ro/ro*		60	30	190.3 ± 22.4	91.5 ± 6.7
XB + XB + x XB + YB	*ro*$^+$	*ro*$^+$/*ro*$^+$		64	24	189.4 ± 39.3	87.1 ± 14.7
Parental							
XArXAr x XB + YB	*ro*♀	*ro/ro*	P_o	72	19	183.1 ± 49.7	74.1 ± 23.2
XB + XB + x XArYA	*ro*♀	*ro*$^+$/*ro*$^+$	P_o	65	20	153.5 ± 33.1	81.1 ± 22.8
Backcross (AB♀xAA♂)							
XArXB + x XArYA	*ro*$^+$♀	*ro/ro*	F_1	109	24	115.2 ± 58.4	13.2 ± 11.2
XArXAr x XArYA	*ro*♀	*ro/ro*$^+$	BC1	73	5	252.0 ± 26.9	36.8 ± 25.9
XArXB + x XArYA	*ro*$^+$♀	*ro/ro*$^+$	BC1	56	9	175.1 ± 88.0	12.9 ± 8.1
XArXAr x XArYA	*ro*♂	*ro/ro*$^+$	BC1	18	0		—
XArXAr x XB + YA	*ro*$^+$♂	*ro/ro*$^+$	BC1	14	0		—
XArxA x XArYA	*ro*♀	*ro/ro*	BC2	FM[3]	14	260.5 ± 60.0	77.8 ± 20.4
	ro♂	*ro/ro*	BC2	FM	10		88.5 ± 6.0
	ro♀	*ro/ro*$^+$	BC2	FM	6	195.7 ± 80.0	93.3 ± 4.8
XArXB + x XArYA	*ro*$^+$♀	*ro/ro*$^+$	BC2	FM	10	219.8 ± 70.6	76.9 ± 20.0
XArXAr x XArYA	*ro*♂	*ro/ro*$^+$	BC2	19	5		77.7 ± 26.9
XArXAr x XB + YA	*ro*$^+$♂	*ro/ro*$^+$	BC2	11			7.8 ± 0
XArXAr x XArYA	*ro*♀	*ro/ro*	BC3	FM	10	200.8 ± 63.5	63.6 ± 16.3
	ro♂	*ro/ro*	BC3	FM	10		79.1 ± 28.1
	ro♀	*ro/ro*	BC4	FM	10	210.4 ± 76.0	81.9 ± 17.1
	ro♂	*ro/ro*	BC4	FM	10		91.7 ± 4.7

[1]Kaiser et al. 1988b.
[2]Rose eye (*ro*).
[3]Females and males were allowed to mate freely in a cage.

TABLE 3. CROSSES BETWEEN MEMBERS OF THE *ANOPHELES QUADRIMACULATUS* SIBLING SPECIES COMPLEX.

Cross ♀ ♂	No. of Families	Mean % Hatch	Mean % Survival	Female	Male	Ovary	Testis
A x B_1	6	92.7 1.2	37.8 ± 5.3	258	—	aberrant	—
A x B_2	4	78.5 ± 6.4	80.4 ± 17.3	346	241	aberrant	aberrant
B x A	6	76.4 ± 23.3	67.6 ± 17.4	195	185	aberrant	aberrant
A x C	4	36.0 ± 23.1	62.9 ± 26.6	—	103	—	absent
C x A	5	65.5 ± 17.9	24.4 ± 20.5	—	29	—	absent
B x C_1	2	52.9 ± 35.5	43.2 ± 9.6	39	22	normal	normal
B x C_2	3	80.6 ± 10.7	12.3 ± 7.9	40	—	absent	—
B x C_3	7	42.6 ± 24.7	11.2 ± 5.2	—	67	—	absent
C x B_1	3	76.0 ± 28.8	30.1 ± 23.4	43	75	normal	normal
C x B_2	4	75.3 ± 17.0	1.3 ± 1.0	6	—	absent	—

taxonomic methods were not suitable for dealing with a sibling complex. There are drawbacks to using cytology since polytene chromosomes are not likely to be available in most important insect pests. Surprisingly, this was the case with a new member of the *A. quadrimaculatus* sibling complex because the quality of the ovarian polytene chromosomes was not suitable for taxonomic purposes. Fortunately, we were able to use another technique, isozyme analysis, to distinguish species A and B from the new species that was provisionally called species C (unpublished data). This example highlights the fact that programs with several taxonomic techniques should be employed when investigating new species or established ones that may include siblings.

TABLE 4. POPULATION OF FREQUENIES OF *AN. QUADRIMACULATUS* SPEIES A, B AND C BASED ON CYTOLOGICAL EXAMINATIONS OF THE OVARIAN NURSE CELL POLYTENE CHROMOSOMES.

Population	Sp. A	% A	Sp. B	% B	Sp. C	% C	Total
Goldens Bridge, NY	100	1.00	0	0.00	0	0.00	100
Lake Chickamauga, TN	100	1.00	0	0.00	0	0.00	100
Kentucky Lake, TN	5	0.05	100	0.95	0	0.00	105
Guntersville Lake, AL	100	1.00	0	0.00	0	0.00	100
Wheller Lake, AL	59	0.47	67	0.53	0	0.00	126
Nozuvww Nwr, MS	48	0.32	100	0.68	0	0.00	148
Stuttgart, AR	100	1.00	0	0.00	0	0.00	100
Lake Octahatchee, FL	74	0.08	904	0.92	0	0.00	978
Lake Seminole, GA	103	0.96	4	0.04	0	0.00	107
Chattahoochee St. Pk., AL	88	0.88	12	0.12	0	0.00	100
Montgomery Co., AL	19	0.12	145	0.88	0	0.00	164
Lake Charles, LA	100	1.00	0	0.00	0	0.00	100
College Station, TX	100	1.00	0	0.00	0	0.00	100
Dead Lake, FL	41	0.41	53	0.53	6	0.06	100
Gainesville, FL	155	0.99	1	0.01	0	0.00	156
Levy Co., FL	26	0.12	2	0.01	192	0.87	320
Myakka St. Pk., FL	100	1.00	0	0.00	0	0.00	100

ACKNOWLEDGMENT

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USE OF CHEMICAL CHARACTERS IN DEFINING POPULATIONS OF FIRE ANTS, *SOLENOPSIS SAEVISSIMA* COMPLEX, (HYMENOPTERA: FORMICIDAE)

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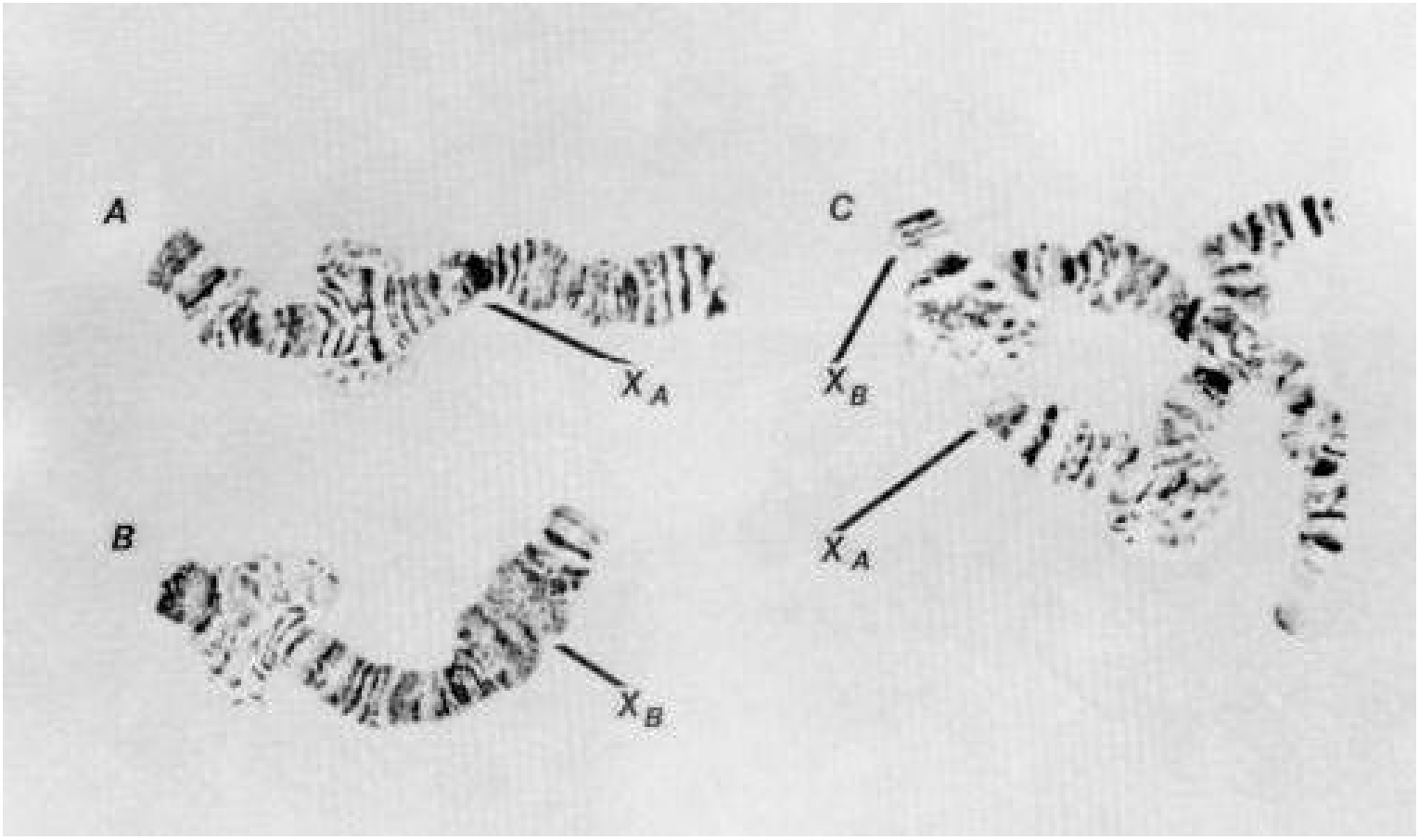
ABSTRACT

The fire ants, *Solenopsis invicta* and *S. richteri*, were accidentally imported into the United States in the first half of this century from South America. In their adopted habitat the imported fire ants have thrived causing considerable medical and agricultural problems in the nine widely infested states of the south and southeast. The red imported fire ant, *S. invicta* was considered the dominant ant in the infested areas, having displaced the black imported fire ant, *S. richteri*, into a small enclave in northeastern Mississippi. However, a large reproductively viable *S. invicta*/*S. richteri* hybrid population was recently discovered across northern Alabama and into Mississippi and Georia by chemical analysis. This paper reports on the use of three species-specific chemical characters (venom alkaloids, cuticular hydrocarbons, and trail pheromones) to define *S. invicta*, *S. richteri*, and hybrid populations in the United States. In addition, these characters have been applied to fire ant taxonomy in South America. We also discuss fire ant population dynamics in the United States and its implications on several models of hybridization. These results have important consequences regarding the species status of the two imported fire ants and the taxonomy of fire ant populations in South America.

RESUMEN

Las hormigas *Solenopsis invicta* y *S. richteri* fueron accidentalmente importadas de Sur America a los Estados Unidos en la primera parte de este siglo. En su medio

A
C
B
C
In(2L)c
C
C
In(3R)e
C
D
39E
C
43B
E
C


A
X_A
B
X_B
C
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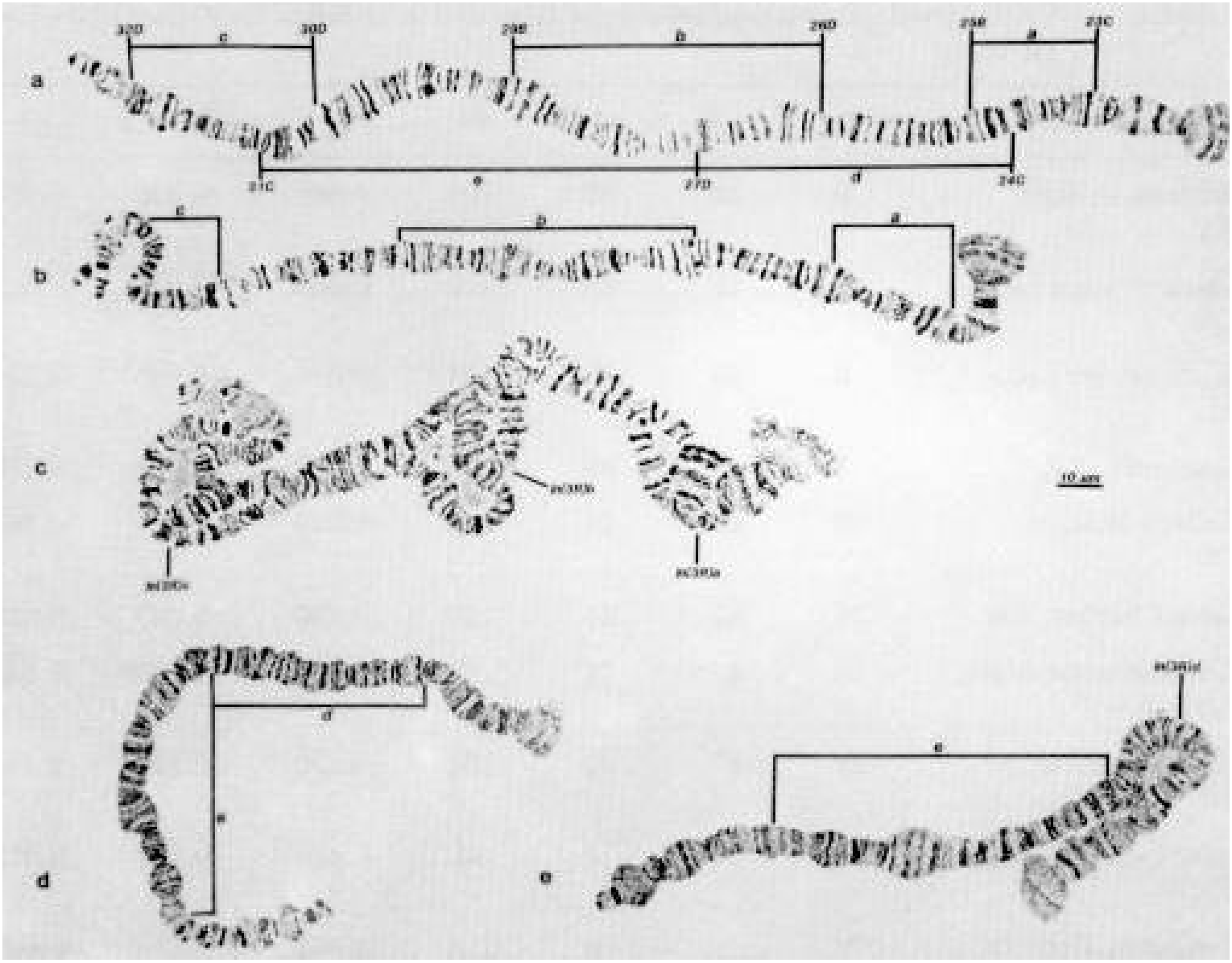
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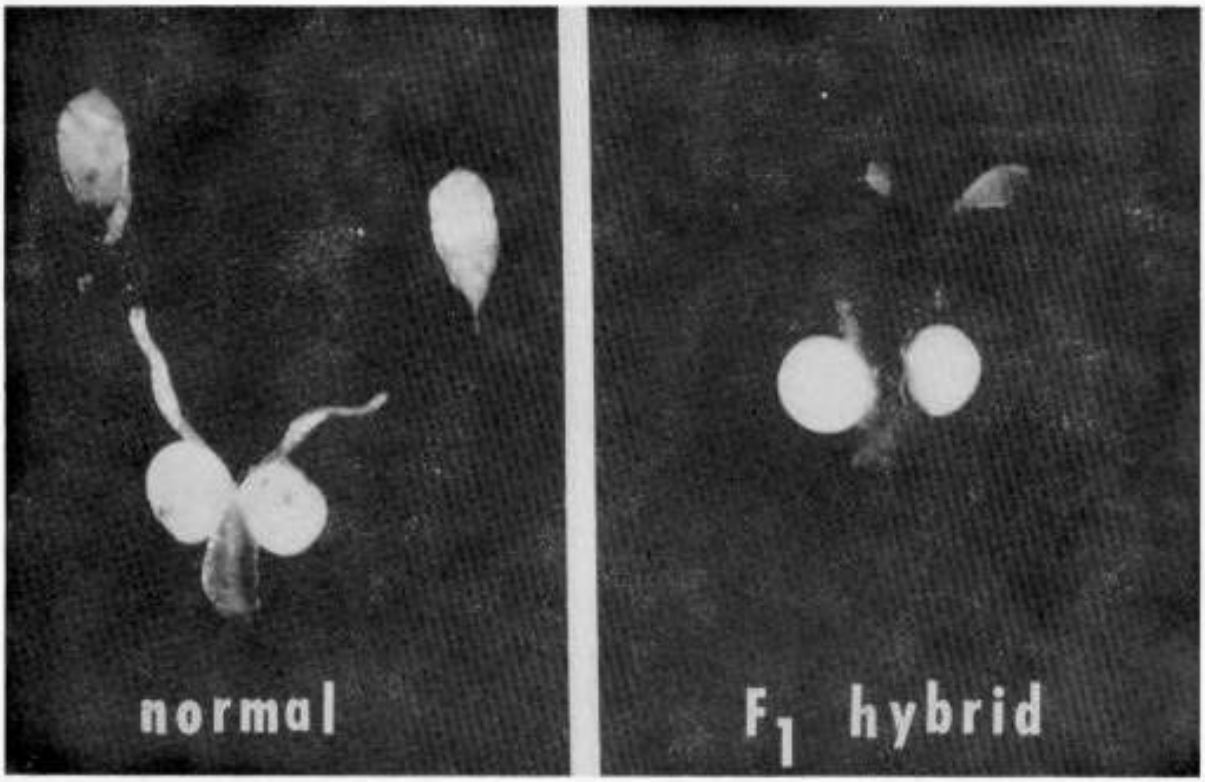
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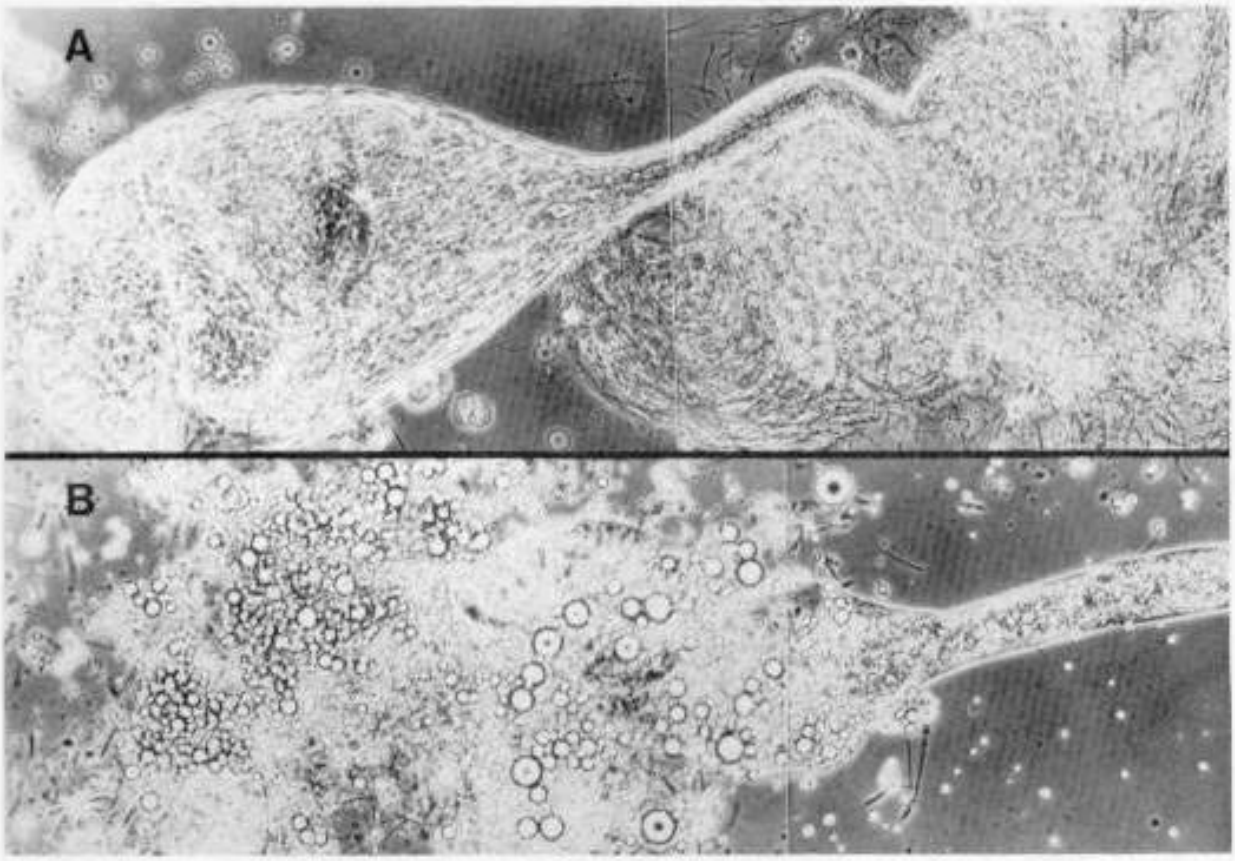
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USE OF CHEMICAL CHARACTERS IN DEFINING POPULATIONS OF FIRE ANTS, *SOLENOPSIS SAEVISSIMA* COMPLEX, (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

The fire ants, *Solenopsis invicta* and *S. richteri*, were accidentally imported into the United States in the first half of this century from South America. In their adopted habitat the imported fire ants have thrived causing considerable medical and agricultural problems in the nine widely infested states of the south and southeast. The red imported fire ant, *S. invicta* was considered the dominant ant in the infested areas, having displaced the black imported fire ant, *S. richteri*, into a small enclave in northeastern Mississippi. However, a large reproductively viable *S. invicta*/*S. richteri* hybrid population was recently discovered across northern Alabama and into Mississippi and Georia by chemical analysis. This paper reports on the use of three species-specific chemical characters (venom alkaloids, cuticular hydrocarbons, and trail pheromones) to define *S. invicta*, *S. richteri*, and hybrid populations in the United States. In addition, these characters have been applied to fire ant taxonomy in South America. We also discuss fire ant population dynamics in the United States and its implications on several models of hybridization. These results have important consequences regarding the species status of the two imported fire ants and the taxonomy of fire ant populations in South America.

RESUMEN

Las hormigas *Solenopsis invicta* y *S. richteri* fueron accidentalmente importadas de Sur America a los Estados Unidos en la primera parte de este siglo. En su medio

adoptado las hormigas han prosperado causando considerables porblemas médicos y agrícolas en los nueve estados del sur y del sudeste. La hormiga roja importada *S. invicta*, fue considerada la hormiga dominante en las áreas infestadas, habiendo desplazado a la hormiga negra importada, *S. richteri*, hacia un pequeño enclave en el noreste de Mississippi. Sin embargo, se descubrió por medio de análisis químicos, una población híbrida reproductivamente viable de *S. invicta*/*S. richteri* en el norte de Alabama y dentro de Mississippi y Georgia. Este papel reporta sobre el uso de caracteres químicos que son específicos de ciertas especies (veneno, alcaloides, hidrocarbones cuticulares, y feromonas de rastro) para definir poblaciones de *S. invicta*, *S. richteri*, e híbridos en los Estados Unidos. Además, estos caracteres han sido splicados a la taxonomía de las hormigas en Sur America. Nosotros también discutimos el dinamismo de poblaciones de las hormigas en los Estados Unidos y sus implicaciones en varios modelos de hibridación. Estos resultados tienen consecuencias importantes en cuanto al estado de las especies de las dos hormigas y la taxonomía de la población en Sur America.

The taxonomy of the *Solenopsis saevissima* complex has had a tumultuous history, and is currently undergoing another revision (James Trager, University of Florida). Creighton (1930) reviewed the early history, and lumped 16 extant forms into 8 subspecies or varieties. One of these was *S. saevissima* var. *richteri*, a fire ant originally from Argentina that was collected near Mobile, Alabama in the late 1920's. Wilson (1952) considered most of these subspecies as unstable hybrid variants and consolidated the complex into two species, *S. interrupta* and *S. saevissima*, and one subspecies, *S. saevissima* var. *richteri*. Ettershank (1966) went even further and preserved only *S. saevissima* and *S. interrupta*. The most recent taxonomic study (Buren 1972) recognized *S. saevissima* and *S. interrupta* as valid species and raised two subspecies, *S. richteri* (the black imported fire ant) and *S. quinquecuspis* to species rank and described two new species, *S. invicta* (the red imported fire ant) and *S. blumi*. Buren (1972) and MacConnell et al. (1976) state that there are a large number of undescribed forms in the *saevissima* complex.

The following senario for the introduction of fire ants into the United States has been developed based on the earliest records of fire ant infestations (see Lofgren 1986). Black fire ants were accidentally imported into the Mobile, Alabama area around 1918. They were subsequently identified as *S. saevissima* var. *richteri* (Creighton 1930). In the 1940's a red form of imported fire ant was observed around Mobile, Alabama. Based on the population size at that time, it was estimated that this form was probably introduced in the 1930's. The natural spread of both imported fire ants occurred naturally by mating flights (estimated at a 5-6 miles per year) and most importantly, through transportation in infested sod and/or nursery stock by man. The latter led to the disjunct distribution of the ants reported by Bruce et al. (1949) and Culpepper (1953). Initial reports on the interaction of the two forms around Mobile, Alabama, indicated that the red form competitively dominated the black form (Wilson & Eads 1949). Most surveys of imported fire ant populations did not distinguish between the red and the black form, which were named *S. invicta* and *S. richteri*, respectively (Buren 1972). As a result, we know little about the population dynamics of the two species during their rapid expansion over the last three to four decades (see Lofgren 1986 for fire ant distribution maps). However, in 1974 *S. richteri* was reported to occupy only a small triangular area in northeastern Mississippi and northwestern Alabama with an acute apex at Meridian, Mississippi (Buren et al. 1974). *S. invicta* was purported to occupy all other infested areas of the South.

Chemical Characters and Fire Ant Taxonomy

Venom Alkaloids. The poison sac of fire ants of the *S. saevissima* complex contains primarily *cis* or *trans* 2-methyl-6-alkyl or alkenyl piperidines (MacConnell et al. 1970, MacConnell et al. 1976). The length of the alkyl or alkenyl group varies from C_{11} to C_{17} and may contain one double bond. To simplify the nomenclature of these compounds in this paper we will specify different alkaloids by their sidechain length and whether or not they have a double bond. For example, *trans*-2-methyl-6-(cis-6-n-pentadecenyl)-piperidine will be designated as *trans*-$C_{15:1}$ (see Fig. 1). The venom alkaloid profiles of female sexuals of the two imported fire ants found in the United States, *S. invicta* and *S. richteri*, and the two native species, *S. geminata* and *S. xyloni*, are very similar, and consist primarily of *cis* and *trans* $C_{11:0}$ alkaloids (Brand et al. 1973). However, wo er alkaloid patterns for these species are species-specific (Brand et al. 1972; MacConnell et al. 1971). MacConnell et al. (1976) addressed the chemotaxonomic potential of the venom alkaloids by analysis of 13 New World fire ant species (most undescribed) nd concluded that they do have taxonomic value, especially when used in conjunction with classical taxonomy. The species-specificity of the alkaloids has provided a useful tool for the study of a variety of research problems on fire ants (Vander Meer 1986a).

Cuticular Hydrocarbons. There is ample precedence for the use of cuticular hydrocarbons as taxonomic tools (Carlson & Service 1980, Carlson & Bolton 1984). In fire ant research, they have served as useful markers in investigations on a variety of problems not directly related to taxonomy, such as myrmecophile integration (Vander Meer & Wojcik 1982) and nestmate recognition (Vander Meer in press).

The chemical compositions of the cuticular hydrocarbons of *S. invicta* and *S. richteri* are species-specific (Lok et al. 1975; see Fig. 2). They are composed of normal, methyl, and dimethyl branched compounds. Analyses of various *S. invicta* life stages (Vander Meer et al. 1982; Vander Meer unpublished) have shown that the five major hydrocarbons of *S. invicta* are ubiquitous to the species, even being found in nest soil. Preliminary investigation of the cuticular hydrocarbon patterns from pinned specimens of 10 members of the *S. saevissima* complex, including the two imported fire ant species, indicate that although qualitatively they have the same components (at least 30 compounds), there are ample quantitative differences so that all are readily distinguished (Vander Meer unpublished). The cuticular hydrocarbons of *S. geminata* and *S. xyloni* (not members of the *saevissima* complex) are normal and monounsaturated compounds, also with species-specific patterns (Vander Meer 1988).

Fire Ant Hybridization in the United States

Biochemical Evidence. Vander Meer et al (1985) presented chemical evidence for the natural occurrence of hybridization between *S. invicta* and *S. richteri*. The two species are readily distinguished by both their venom alkaloids, in which *S. richteri* produces only a little of the C_{15} alkaloids that dominate the *S. invicta* pattern (Fig. 1), and cuticular hydrocarbon profiles (Fig. 2). We serendipitously discovered intermediate chromatographic patterns while investigating the chemistry and specificity of the trail pheromone. Further studies showed patterns of alkaloids and hydrocarbons intermediate to those of the parental species (see Vander Meer et al. 1985, Ross et al. 1987). The colonies that produced workers with these intermediate patterns were designated as natural hybrids. A mathematical index of similarity was developed using the quantity of alkaloids or hydrocarbons, or both. The index placed a quantitative value on the direction of hybridization (0.00 to 1.00 where 0.00 = *S. invicta* and 1.00 = *S. richteri*).

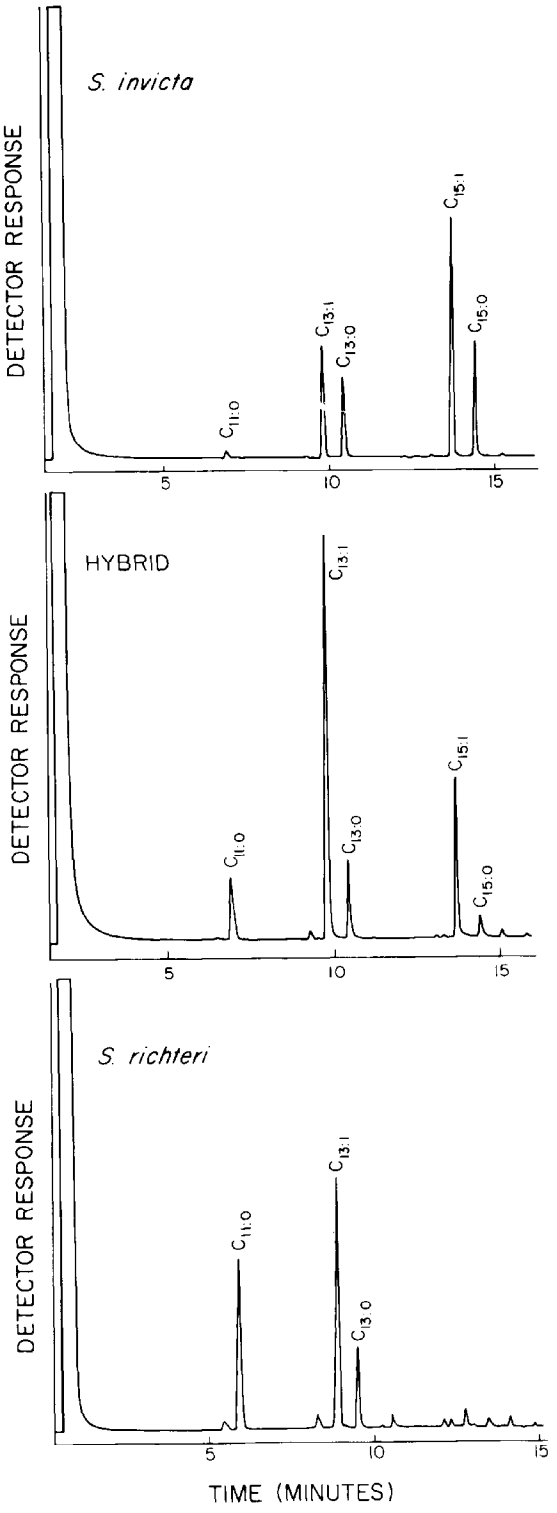

Fig. 1. Gas chromatograph (GC) trace of *S. invicta*, *S. richteri*, and hybrid venom alkaloids. All indicated alkaloids are *trans*. See Ross et al. (1987) for the GC conditions.

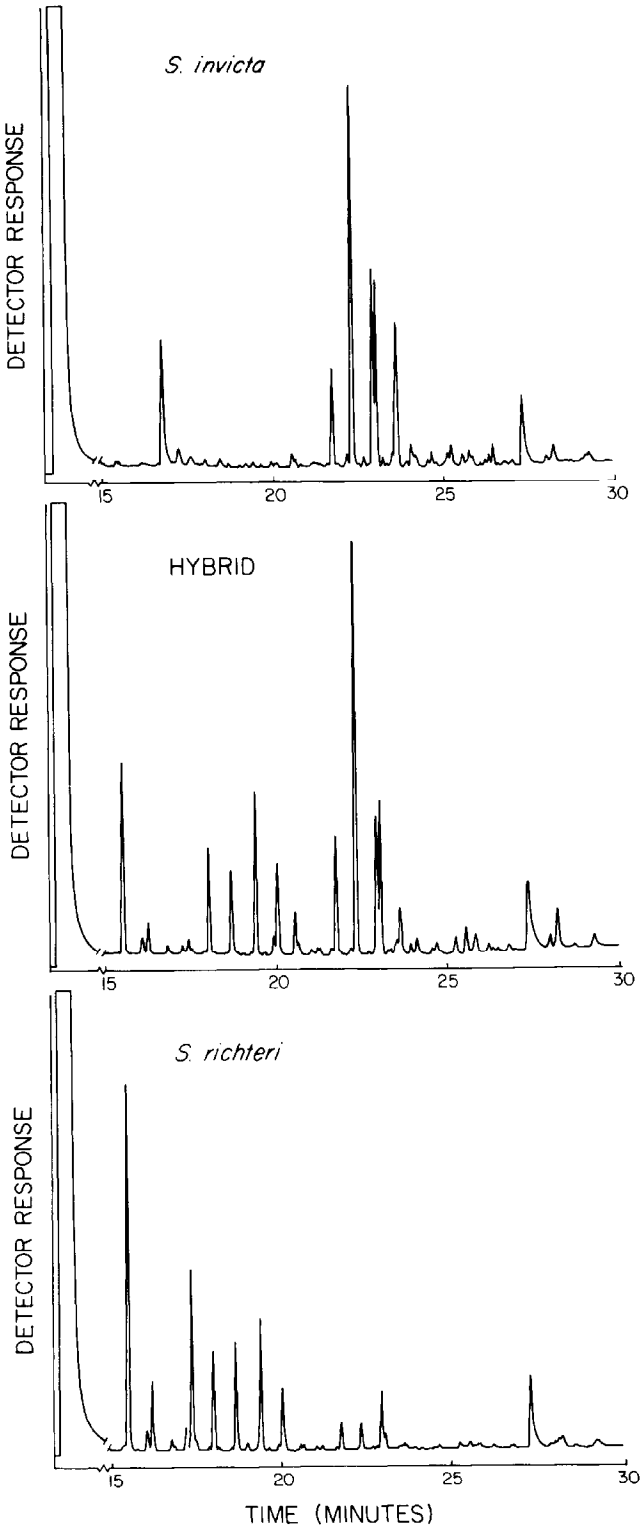

Fig. 2. Gas chromatograph (GC) traces of *S. invicta*, *S. richteri* and hybrid hydrocarbons. See Ross et al. (1987) for the GC conditions.

Values obtained for alkaloids did not necessarily correspond to those for hydrocarbons (Ross et al. 1987, Vander Meer unpublished), indicating the independence of the two biochemical characters.

Genetic Evidence. Genetic data in support of hybridization between the two species were obtained from enzyme electrophoresis (Ross et al. 1987). Four loci were found to differentiate the two parental forms: *alpha-glycerophosphate dehydrogenase-1* (*Agp-1*, EC 1.1.1.8); *Octanol dehydrogenase* (*Odh*, EC 1.1.1.73); *Esterase-2* and *Esterase-4* (*Est-2,4;* EC 3.1.1.1). These enzyme profiles confirmed that hybridization occurred in nature and that hybrid genotypes predominated in the hybrid zone indicating that F_1 hybrids are viable.

The biochemical and genetic methods were highly concordant in distinguishing between the two parental forms and in identifying hybrid colonies. Both methods showed that extensive gene introgression is occurring over a distance of at least 120 km within the limits of the study zone in northeastern Mississippi (Ross et al. 1987).

Behavioral Evidence. The recruitment strategy of the fire ant is complicated (Wilson 1962) and governed by a hierarchy of behaviors (Vander Meer 1987). The source of the recruitment pheromone is the Dufour's gland (Wilson 1959). In *S. invicta*, workers are initially attracted to a trail (attractant pheromone), followed by incitement to trail (inducer pheromone), and finally the actual following of the trail (orientation pheromone). A bioassay that measured the response of workers to a point source of Dufour's gland extracts clearly differentiated the two parental types (Vander Meer 1986b, Vander Meer in press). Bioassays using Dufour's gland extracts of each parent species and hybrid workers, demonstrated that hybrids were capable of responding to either parental type. Similarly, parental workers responded well to extracts of hybrid Dufour's glands. This behavioral evidence supports hybridization between the two species. Gas chromatographic examination of Dufour's gland extracts of the parental forms and their hybrid showed clear separation between the parents and an intermediate pattern for the hybrid (Vander Meer et al. in press).

Hybrid Morphology. The fact that hybrids of *S. invicta* and *S. richteri* have gone undetected for such a long time is related to the morphological similarity of the hybrid to that of *S richteri*. Consequently, morphological traits or cursory observations in the field made detection of hybrids very unlikely (Vander Meer et al. 1985, James Trager, University of Florida, unpublished).

Hybrid Population Dynamics. Initially, hybridization between *S. invicta* and *S. richteri* was thought to be a localized phenomenon, occurring between Meridian and Starkville, Mississippi. However, further analyses of collections made throughout Alabama and Georgia now indicate that the range of the hybrid is extensive throughout northern Alabama and northwest Georgia (Fig. 3). Populations of the hybrid in Alabama and Georgia do not have the parental *S. richteri* at their northern border. Since it is not likely that migration of the magnitude required by their current distribution could occur by natural mating flights, we postulate that man has been largely responsible for movement of the hybrid just as in the early spread of the imported fire ant from the Mobile, Alabama area. In addition to rapid dissemination, the hybrid had to be highly competitive with its *S. invicta* parent to the South in order to maintain its territory.

Hybridization in the Past. We developed methods for the analysis of venom alkaloids from alcohol preserved specimens (Vander Meer & Lofgren in preparation). Using these techniques we analyzed samples collected from Meridian to Starkville, Mississippi in 1964. Alkaloid patterns characteristic of both parental types and their hybrid were

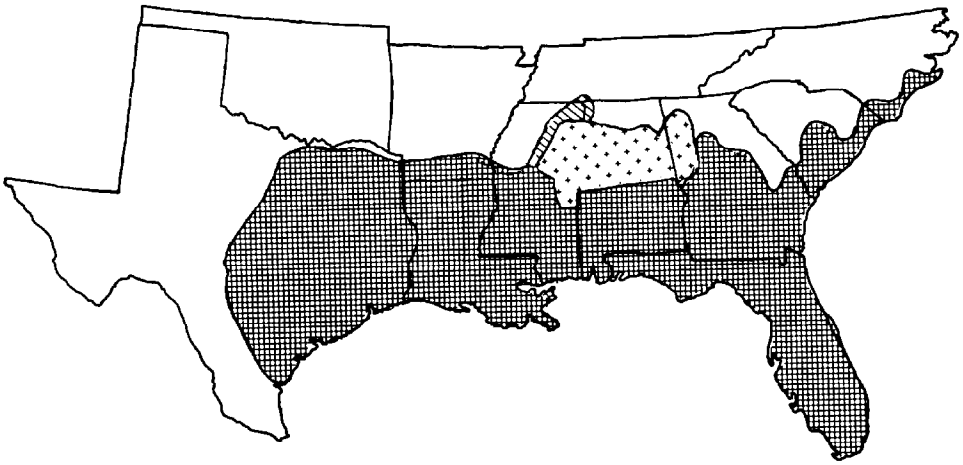

Fig. 3. Map of the Southern United States showing the current population range of *S. invicta* (squares); *S. richteri* (diagonal line) and the hybrid (plus marks).

found (Fig. 1). The results demonstrated that hybridization was occurring around and north of Meridian, Mississippi. In addition, analyses of samples collected around Mobile, Alabama in 1949 show evidence of hybridization. We conclude that hybridization has been occurring for a long time wherever the two forms met.

Hybridization Models. Based on the above results, we can account for the current zone of hybridization. Several models are available for consideration.

A) The dispersal-dependent model of neutral introgression (see Moore 1977) is based on the lack of a barrier to gene flow without any competition between parental forms. This model can be discarded based on known fire ant characteristics: 1) a high reproductive rate for both species, and 2) they are very competitive, consequently, under this model, secondary contacts and introgression would result in the appearance of rapidly decaying clines at the site of first contact.

B) In a dynamic equilibrium model there is selection against the hybrid, which is balanced by gene flow from between the parental populations. Mitigating against this model is the large population of hybrids located in Alabama and Georgia (Fig. 3), where there is no parental species on its northern boundary. Based on this model these hybrid populations should collapse.

C) A third model invokes the advance of a competitively superior genotype (*S. invicta*), with the hybrid zone moving in the direction of the inferior competitor (Barton & Hewitt 1985), which in this case would be *S. richteri*. This model cannot explain the hybrid populations in Alabama and Georgia (Fig. 3) where the inferior competitor is absent. However, in Mississippi we are observing a northward movement of hybrid, with subsequent displacement of *S. richteri*.

D) The bounded hybrid superiority model dictates that there is an advantage to recombinant genotypes in habitats intermediate to those favored by the parental forms. This model is consistent with hybridization occurring wherever the two parental types meet, with subsequent population equilibrium based on habitat suitability; i.e. the hybrid could not maintain itself in the southern half of the infested area, where the subtropical *S. invicta* has an environmental advantage. Further north, for instance Meridian, Mississippi, the hybrid is capable of maintaining its territory against *S. invicta*. This model also explains the persistence of the hybrid populations in northern Alabama and Georgia (see Fig. 3). It does not deal with the apparent advance of the hybrid in northern Mississippi, displacing *S. richteri*.

Strictly interpreted, no one model adequately explains all of the data and observations made on the fire ant populations in the United States. However, an asymmetric bounded hybrid superiority model would satisfy all the known observations. The hybrid is bounded to the south by *S. invicta*, which is being held in check by the hybrid at a geographic point where an equilibrium has been reached balancing differences in competitiveness and environmental conditions. To the north, in Alabama and Georgia, the hybrid only has to compete with environmental conditions; therefore the hybrid is asymmetrically bounded. However, in northern Mississippi, the hybrid is competing with both the environment and *S. richteri*. The observed northward displacement of *S. richteri* by the hybrid indicates that the hybrid may successfully outcompete *S. richteri* through its entire range, ultimately eliminating this parental form from the United States.

The Question of Species. Buren (1972), reinstated *S. richteri* as a species and classified *S. invicta* as a new species based primarily on the observation that in the United States, where both forms had ample opportunity to hybridize, no hybridization was found and there was little phenetic variability. Our discovery of hybridization through chemical characters, coupled with hybrid morphology that is almost identical to *S. richteri*, negates the criteria used to specify *S. invicta*. Obviously, where the two forms have the opportunity to mate they are successful at producing viable hybrids (Vander Meer et al. 1985, Ross et al. 1987). Therefore, if hybrids are not found in their native South America there must be pre-mating isolation mechanisms that prevent gene flow. Our laboratory is currently surveying likely areas of Brazil and Argentina for *S. invicta*/*S. richteri* hybridization. Although there are good reasons to question the validity of *S. invicta* as a separate species, it will remain in good standing until definitively proven otherwise.

The Fire Ant in South America

S. invicta and *S. richteri*. The same biochemical characters (venom alkaloids and cuticular hydrocarbons) developed for the study of fire ant hybridization in the United States are currently being applied to specimens collected by our laboratory in South America. The holotype for *S. invicta* was collected in Cuiaba, Mato Grosso, Brazil. Chemical analysis of samples from Cuiaba did not show the typical chromatogram pattern (chromatotype) that is associated with *S. invicta* found in the United States. However, chromatotypes identical to United States' types were found in samples from Caceres, Mato Grosso, Brazil about 200 km west of Cuiaba. Perhaps, the great agricultural related changes made by man in that area of Brazil, coupled with the weedy nature of the fire ant (Tschinkel 1986) has altered the fire ant species distribution from what it was in 1972. *S. invicta* chromatotypes have also been found in northern Argentina, although we cannot be certain of the exact origin of *S. invicta* populations in the United States, Argentina may be the most likely source.

The type locality for *S. richteri* is Buenos Aires, Argentina (Buren 1972). Chromatotypes near Buenos Aires were discovered, which were identical to those of *S. richteri* found in the United States (collections by D. P. Wojcik & D. P. Jouvenaz, USDA). The Buenos Aires area is a likely source for *S. richteri* in the United States, although other areas have not yet been extensively investigated.

Studies are planned to survey additional areas in Argentina to determine whether *S. invicta* and *S. richteri* meet to form hybrids.

Other *S. saevissima* Complex Members. Based on hundreds of chromatographic analyses of samples collected in Brazil and Argentina, we can conclude that at the present time, there are several distinct chromatotypes. Additional samples are being collected in order to determine the variation within populations. In the future we expect

to combine the biochemical tools with morphological and genetic characters to further investigate this important but difficult area of fire ant taxonomy and population dynamics.

Conclusion

The difficulty of the *S. saevissima* complex taxonomy has been recognized for a long time. For example Creighton (1930) wrote, "In approaching the subject of *S. saevissima* one has the unpleasant feeling that he is entering a battle-field strewn with unexploded missiles and that there is a strong possibility that one of these taxonomic duds may, through tampering, bring the investigator to grief". Wilson (1952) also commented that the *S. saevissima* complex "has consistently presented some of the more knotty problems of ant taxonomy". We hope that the chemical characters we have developed will aid in sorting out this very complex taxonomic problem.

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HYDROCARBONS FOR IDENTIFICATION AND PHENETIC COMPARISONS: COCKROACHES, HONEY BEES AND TSETSE FLIES

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ABSTRACT

The hydrocarbon components of Asian and German cockroaches showed consistent differences by gas chromatography (GC) that did not depend on geographic origin, sex or age, and that did reliably identify individuals of these otherwise morphologically similar species. European honey bee workers and drones showed consistent GC patterns. Race-specific similarities in GC patterns were present in Africanized workers and drones from Central and South America. Principal components analysis separated data from different races. Comb waxes reflected the genetic ancestry of the workers that produced that wax. GC data was used to construct phenetic comparisons of 26 species and subspecies of tsetse flies using dried museum and fresh specimens.

RESUMEN

Cuando se usó la cromatografía de gas (CG), los componentes de hidrocarbón de las cucarachas Asiáticas y Alemanas demostraron consistentemente diferencias que no dependían de origen geográfico, sexo o edad, y que con seguridad identificaban a individuos de esas especies que son además morfológicamente idénticas. Obreras y drones de la abeja Europea consistentemente demostraron patrones por CG. Similaridades en patrones por CG específicos de las razas, estuvieron presente en obreras Africanizadas y drones de Centro y Sur America. Análisis de componentes principales separó datos de diferentes razas. Cera del panal reflejó el antecedente genético de las obreras que producieron esa cera. Se usaron datos del CG para construir comparaciones fenéticas de 26 especies y subespecies de moscas tsetse usando muestras secas y frescas obtenidas del museo.

There are often life forms difficult to identify in the comparison of insects species that are reproductively isolated, but closely related, or of subspecies, of sibling or incipient species, or even of races and morphs. The following is a description of the use of readily obtained hydrocarbon compounds from the cuticle of closely related insects as markers for insect taxa (genera and species) and populations (races), with an excursion into systematics. It appears that synthesis of these materials is often genetically determined, and is not affected by geographic origin or host of the specimens. Thus, it is hypothesized that the hydrocarbons may reliably identify individuals of otherwise morphologically similar species. Insects possess cuticular compounds of many chemical classes for waterproofing purposes, and most of the external materials may be removed by a brief rinse of a fresh or dried specimen in a nonpolar solvent such as hexane. After isolation of the hydrocarbons, gas chromatography (GC) is employed for rapid quantitative and qualitative analysis of the hydrocarbons. The specimen is retrieved undamaged.

Materials and Methods

German cockroaches, *Blattella germanica* (L.) were collected in the field in Alaska, and at several locations in Florida, Alabama and Louisiana, killed by freezing, and then dried. They also were obtained from colonies in Gainesville, Florida, as were Asian cockroaches, *B. asahinai* (Mizukubo), the latter from collections made in Florida. *B. vaga* (Hebard) were obtained from a colony at Virginia Polytechnic University, Blacksburg, Virginia.

European honey bees *Apis mellifera* (L.), were collected randomly in 1983 from commercial type colonies in Florida (FL) and Georgia (GA). African materials were collected in Zimbabwe. Drones from Argentina (ARG) were collected in Argentina. Comb wax samples were empty, non-sex brood cells that were freshly constructed without an artificial foundation. Wax was obtained from suspected Africanized colonies near Bakersfield, California. Africanized comb was collected in Panama and Venezuela.

Tsetse flies were obtained from colonies located in Europe, from several museums, or collected in Africa (unpublished data).

Extraction, column chromatography and GC analyses were carried out for individual insects as previously described (Carlson & Bolten 1984). The hexane extract was passed through a short column of silica gel to separate polar lipids from the nonpolar hydrocarbon fraction. GC analysis then separated the hydrocarbons by vapor pressure, or essentially by their molecular weight. A modern 15 to 30 meter by 0.3 mm id fused silica capillary column with a nonpolar bonded phase was used that may temperature programmed up to 325° to complete each analysis in 10 to 30 min. A 1.8 m x 2 mm. packed column of 3% OV-1 was used for tsetse data. The data were best recorded using a micro computer based system for rapid data analysis. Only a small portion (1/10 to 1/50) of an insect equivalent was usually a sufficient quantity for analysis.

The relative retention time is presented as a four digit number without a decimal (Kovats Index, KI). For example, the sequence of elution for hydrocarbons of 31 carbons chain length was: alkadiene, KI 3043; alkene, KI 3065 to 3075; n-alkane, KI 3100; internally branched methyl alkane, KI 3135, 5-methyl isomer, KI 3152; 3-methyl isomer, KI 3175. All hydrocarbons were identified by GC mass spectrometry and were consistent for the range of compounds seen in insects described here. Peak ratios were calculated from GC data by dividing the integrated area of one consistently appearing peak by another. In honey bees, the intensity of the KI 2900 peak was divided by the intensity of KI 2935 to produce R0. Other ratios were R2= 3075/3265, R3= 3265/3743, R4= 3265/3765. Also, the totals of three methyl-branched alkanes in honey bee drones were summed in Table 1. For waxes, R2, R3 and R4, plus the total compounds eluting after KI 3300 were summed in Table 2.

Cockroaches

Analysis by GC gave excellent results in identification problems with the three *Blattella* species found in North America. An undescribed species of flying cockroach was recently reported in Lakeland, Florida that evaded ready identification by experts. GC analysis showed immediately (Fig. 1a) that all males, females and nymphs possessed very similar patterns of hydrocarbons, with a group of large GC peaks at 29 to 30 carbons in chain length and a group of smaller peaks at 31 to 32 carbons in chain length, but with little other material. This species, later identified from males as *B. asahinai* were subsequently found in Pinellas and Hillsborough Counties. Very different patterns were found in *B. germanica* males, females and nymphs (Fig. 1b), in which a group of small GC peaks appeared at 27 to 28 carbons together with a group of large peaks at 29 to 30 carbons, with very little material at 31 to 32 carbons. Retention indeces (KI)

TABLE 1. PEAK RATIOS (R) OBTAINED FROM GC ANALYSIS OF AFRICAN, AFRICANIZED AND EUROPEAN HONEY BEE DRONES: (MEANS ±S.D.).[a]

R Values	Zimbabwe (n=9)	Argentina (n=29)	Florida (n=21)	Georgia (n=18)
Ro	0.6 (0.1)b	0.2 (±0.1)a	1.7 (±1.3)a	0.8 (±0.5)b
R2	0.2 (±0.01)a	0.4 (±0.1)a	0.7 (±0.06)c	1.1 (±0.1)b
R3	5.2 (±1.0)a	6.3 (±1.1)a	19.2 (±5.3)b	8.0 (±1.6)a
R4	3.0 (±0.2)a	3.1 (±0.4)a	18.1 (±4.4)b	20.0 (±5.1)b
% of Methyl alkanes[b]				
	18.0 (±2.3)b	25.6 (±5.6)a	n.a.	12.6 (±3.8)b

[a]SD: standard deviation
[b]KI 2735, 2935, 3135

were assigned to all prominent peaks that were internally consistent with the mass spectra of selected specimens. Patterns of GC peaks were consistent in all specimens from the Continental U.S. and the Orient (Carlson & Brenner 1988). No changes could be ascribed to location, food, or degree of resistance to pesticides. An indication of the genetic basis of these components is shown in the pattern found in a hybrid nymph of *B. asahinai* and *B. germanica* (Fig. 1c), as this nymph appeared to contain a mixture of materials from both parents. All hybrids examined to date display this intermediate pattern regardless of age or direction of crossing (unpublished data).

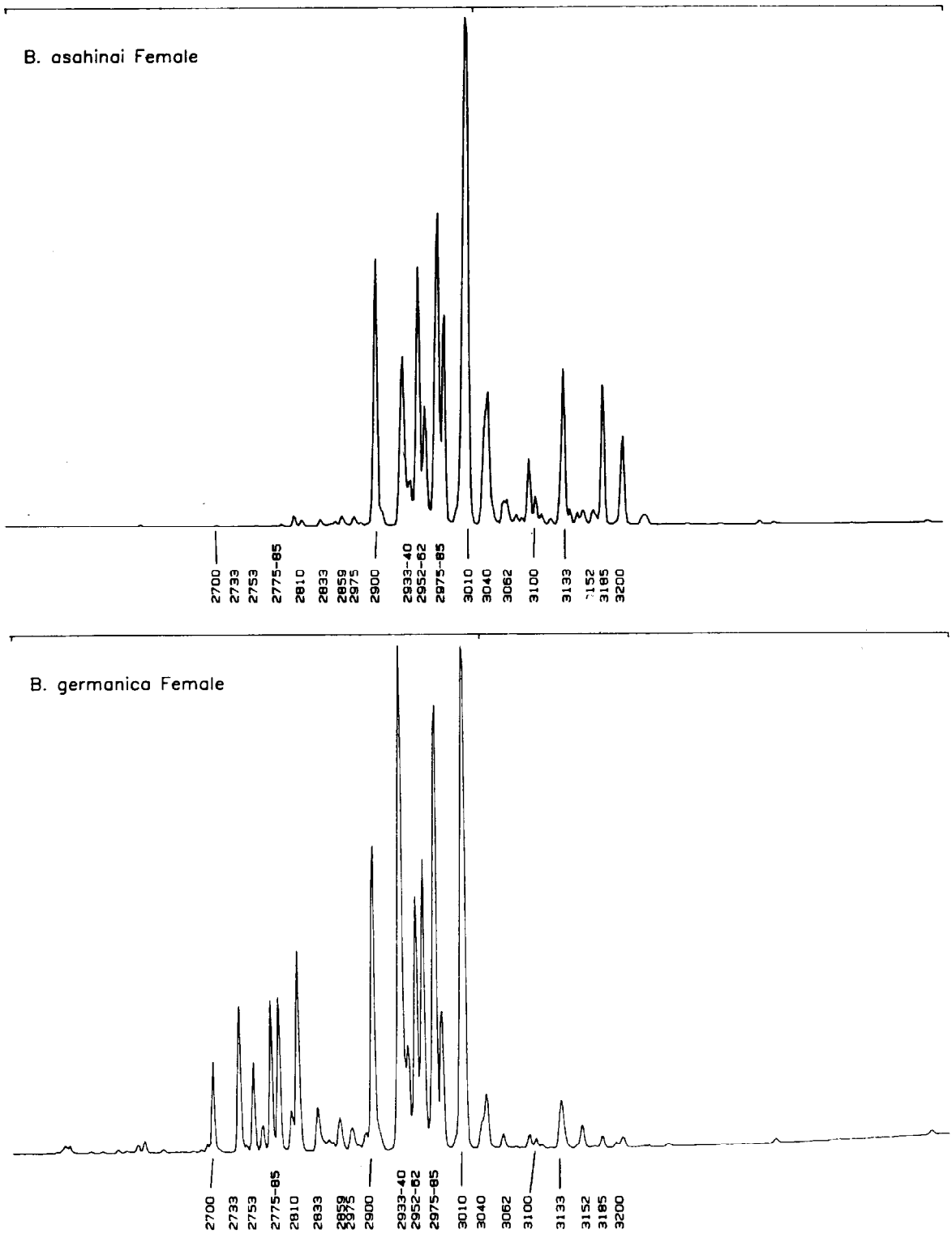
However, the hydrocarbon patterns of *B. vaga* were internally consistent, but quite unlike *B. asahinai* or *B. germanica* patterns, with methyl-branched components eluting in a wide range between standards of 32 carbons and 42 carbons, considerably after those mentioned above (Fig. 1d). This interesting variability within one genus has implications for the use of hydrocarbon data in phenetic comparison, as in the tsetse fly study mentioned below.

Principal components analysis was utilized to study the data from *B. germanica* and *B. asahinai*, and 12 GC peaks were selected for cluster analysis using the percent composition of each peak. The clusters were well separated. A scoring system was devised that used the 95% confidence intervals of the quantity of each of the 12 peaks for 120 specimens. All instars and adults of *B. asahinai* scored +7 to +12 with a mean of +10.5, whereas the *B. germanica* scored –7 to –12, with a mean of –10.5 (Fig. 2). Blind samples were successfully identified (40/40 = 100%) as were individual oothecae, legs, wings and cast skins by the same scoring system (25/25 = 100%, Fig. 3).

TABLE 2. PEAK RATIOS (R) OBTAINED FROM GC ANALYSIS OF AFRICAN, AFRICANIZED AND EUROPEAN/FLORIDA HONEY BEE COMB WAX: (MEANS).

R Values	Zimbabwe	Panama	Florida 1[a]	Florida 2
R2 3075/3265	0.18	0.19	0.33	0.44
R3 3265/3443	18.30	24.98	56.23	48.60
R4 3265/3465	3.65	3.30	17.04	24.30
% of total, HC larger than KI 3300				
	5.4	6.4	1.2	1.2

[a]FLA 1 and FLA 2 are from same colony.


B. asahinai Female
2700
2733
2753
2775-85
2810
2833
2859
2975
2900
2933-40
2952-62
2975-85
3010
3040
3062
3100
3133
3152
3185
3200
B. germanica Female
2700
2733
2753
2775-85
2810
2833
2859
2975
2900
2933-40
2952-62
2975-85
3010
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3100
3133
3152
3185
3200

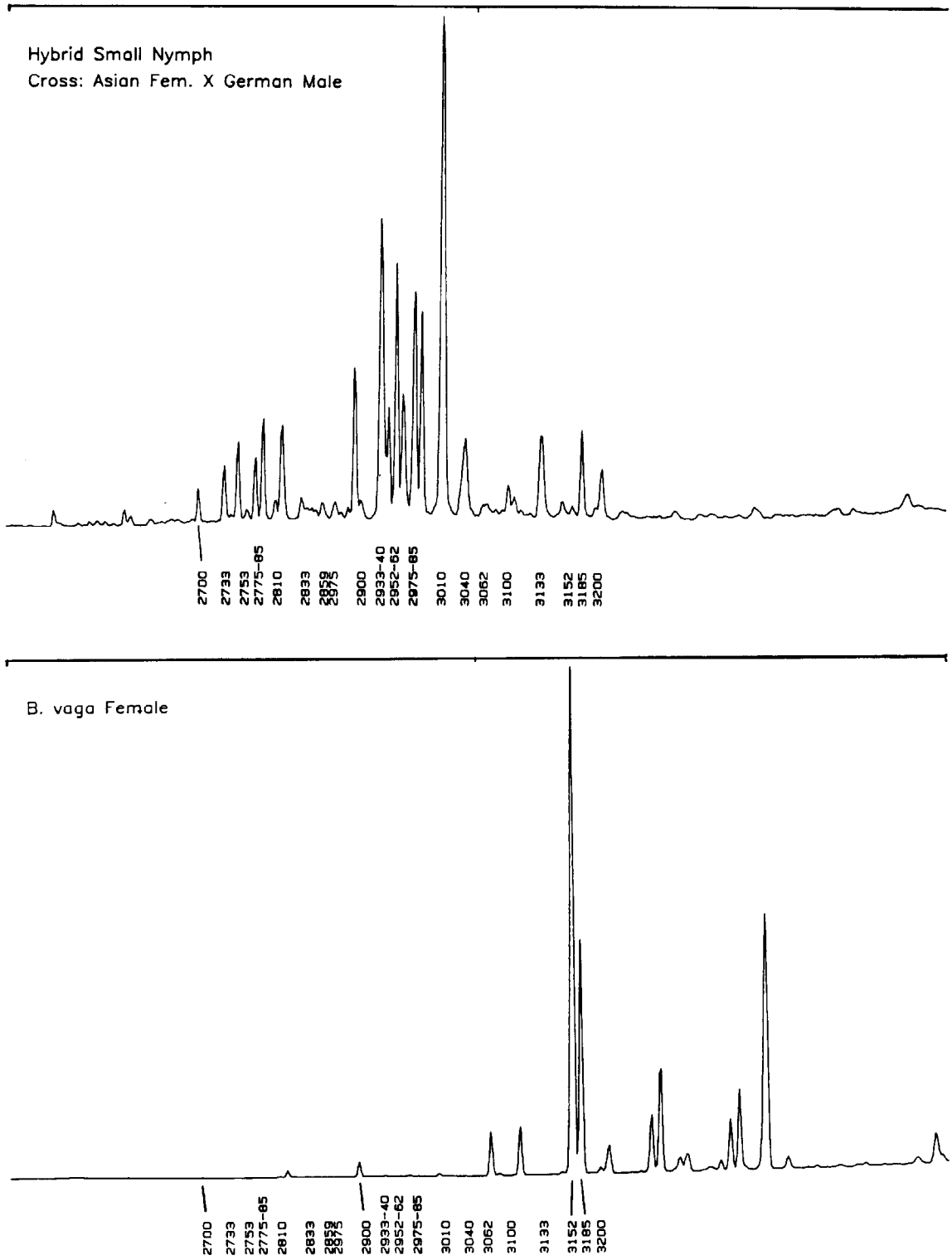


Fig. 1. GC of hydrocarbons from: a. female *Blattella asahinai*. b. female *B. germanica*. c. hybrid of *B. asahinai* and *B. germanica*. d. *B. vaga*.

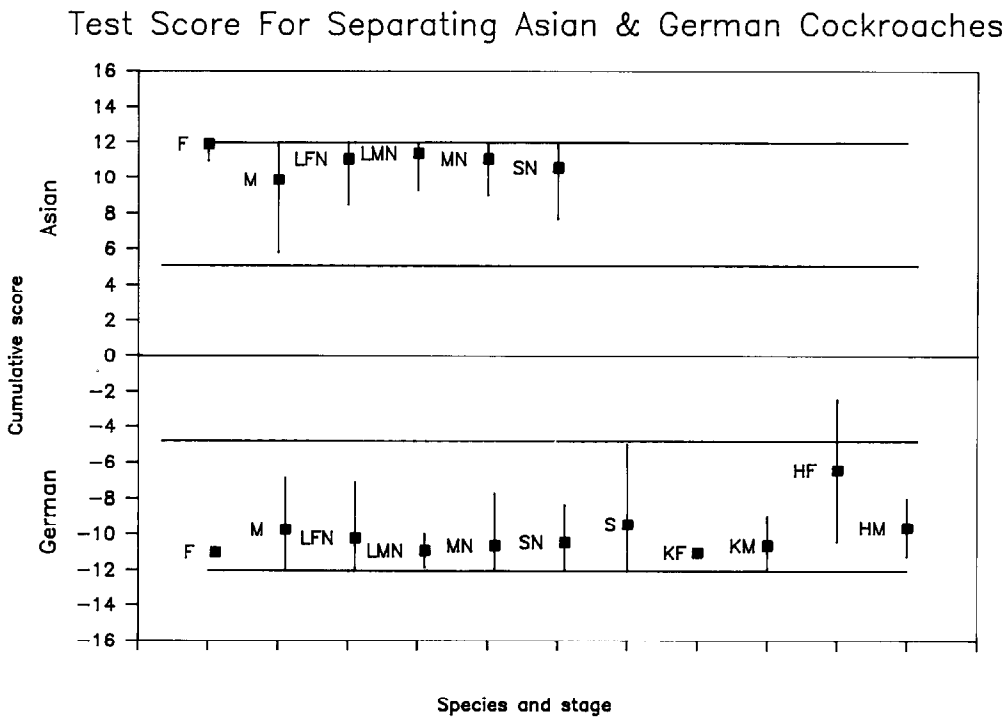


Fig. 2. Mean Cumulative Scores (x) and 99% confidence intervals of observations for various stages or strains of German (– values) and Asian cockroaches (+ values): F,f = female, M,m = male, N = nymph, LMN = large male nymph, MN = medium nymph, SN = small nymph, K = Alaska, S = wild German, H = Hydroprene treated.

Honey Bees

The synthesis of hydrocarbon compounds by the honey bee, *Apis mellifera*, is apparently under genetic control. Several series of lipids are always present within every individual of each species regardless of race. Honey bees and waxes of several races, and species including giant honey bees contained four major classes of long chain hydrocarbons of 21 to 43 carbons, alkadienes, alkenes, n-alkanes and methyl-branched alkanes (unpublished data).

Cuticular hydrocarbons of honey bee workers of Africanized (AV) and European ancestry (EU) were found to be different by GC (Carlson & Bolten 1984). The hydrocarbons included a homologous series of C35 to C43 alkenes and alkadienes that totalled 22% in AV and 1 to 3% in EU bees. Ratios between peaks allowed identification without measuring every peak (Carlson & Bolten 1984). Principal components analysis was used to map data from ten GC peaks of AF and EU bees (Fig. 4), (Lavine & Carlson 1987).

Sting shaft hydrocarbons of EU bees were analyzed and evaluated for their chemotaxonomic suitability (McDaniel et al. 1984). Examination of F1 hybrids from artificially inseminated EU queens with feral Venezuelan drone sperm resulted in workers with GC patterns that appeared to be Africanized by R2 and R4 values (unpublished data).

Honey Bee Drones

The hydrocarbon patterns determined for all drones were grossly similar as all drones had about as much methyl-branched alkanes as n-alkanes. However, both R2

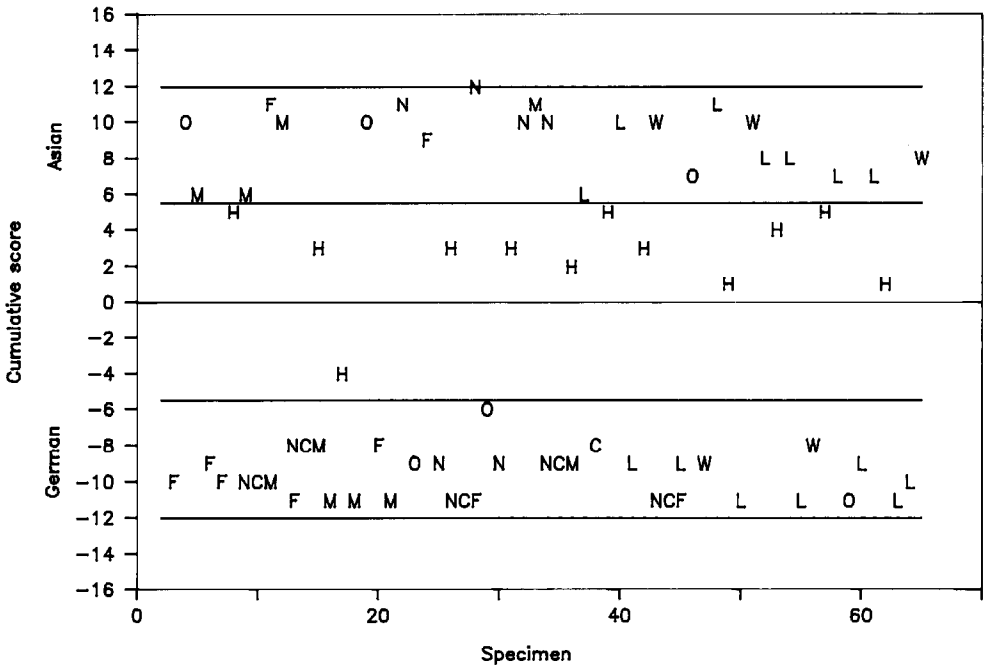


Fig 3. Plot of Cumulative Scores for 65 German (– values) and Asian (+ values) cockroach specens submitted blind. M = male, F = female, N = nymph, L = leg, C = cast skin, W = wing, H = hybrid, NC = New Caledonia, O = oothecae.

and R4 of FL (Fig. 5a) and GA European drones were significantly different from both ZIM and ARG (Fig. 5b) drone values (Table 1) ($P < 0.01$, student's t-test). No overlapping values were observed.

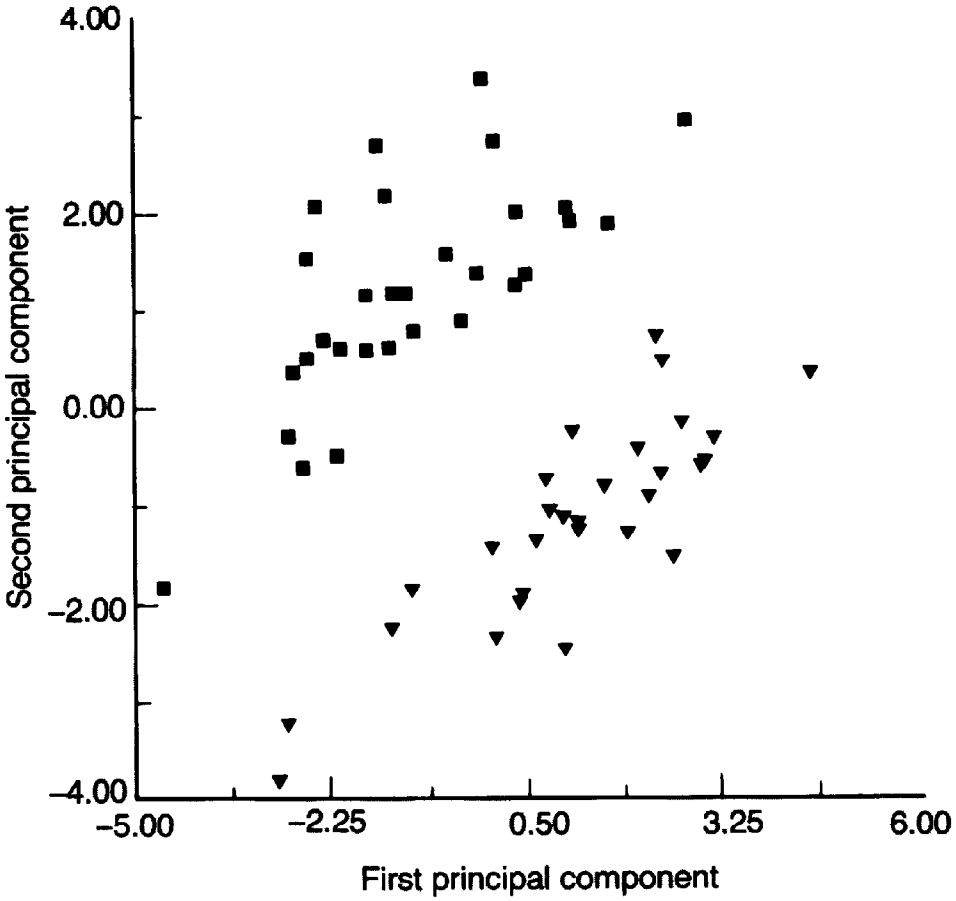
Comb waxes

The hydrocarbon (HC) composition of honey bee waxes resembled that of the bees that are presumed to have secreted that wax.

Fresh FL wax values were R2 = 0.33, R3 = 56 and R4 = 17, and the HC that eluted after KI 3300 totalled 1.2% in several samples (Table 2, Fig 6a). In contrast, fresh Panama wax (Fig. 6b) showed R2 = 0.19, R3 = 24.9, and R4 = 3.3, with the total HC that eluted after KI 3300 at 6%. Mexican and California wax patterns were similar to Florida patterns.

Differences in HCP of Africanized and European workers and drones can be evaluated by comparison of R2 and R4 values. The use of HCP for identifying drones include the same advantages as in identifying HCP of workers: dried specimens can be stored indefinitely, they are undamaged by extraction with solvent, and statistically useful numbers result from replicated GC analysis. Effects of location, genetic background, diet and age of drones are unknown, but it appears that HCP from at least two populations of European drones from the Southeastern US were alike enough to differentiated from Africanized and African drones.

Similarly, wax samples dissolved in hexane can be separated by chromatography for characterization by GC, and the collected fractions can be retained indefinitely for future reference.



A principal components representation of the pattern space defined by the 10 GC peaks for Venezuelan foragers.
The first two principal components account for 65% of the total cumulative variance. The squares represent Africanized foragers, and the inverted triangles are European foragers.

Fig. 4. Cluster analysis of data from Africanized and European honey bees.

Tsetse Flies

Unique patterns and quantities of cuticular alkanes were seen in the GC data of replicated samples of 26 species and subspecies of *Glossina* of both sexes. The distinctive patterns allowed visual assignment of most chromatograms as to sex and species in 10 species studied first (Carlson 1981). This effort was expanded to include specimens of all 26 species available, excluding only four rare forest species for which only type specimens were available (unpublished data). Early collections of flies from the British Museum (1899 to 1909) were used with results equally as good as for modern specimens, and all specimens were recovered undamaged. Patterns of hydrocarbons from old and recent specimens showed little change for individual *G. m. morsitans* males (1909 vs. 1983), *G. m. morsitans* females (1985 vs. 1907), and *G. p. palpalis females* (1977 vs.

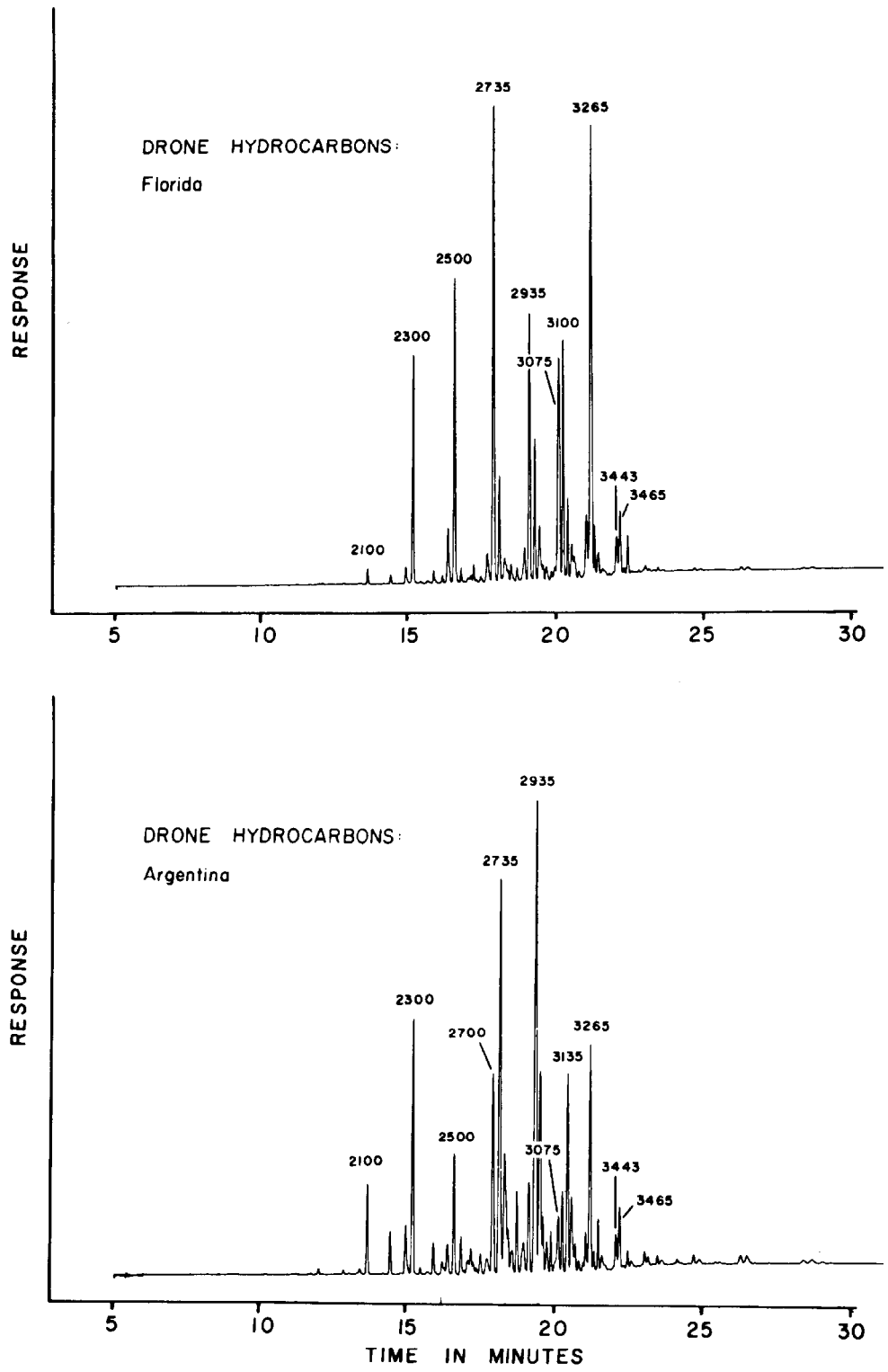


Fig. 5. GC of hydrocarbons from: a. Florida drones b. Argentina drones.

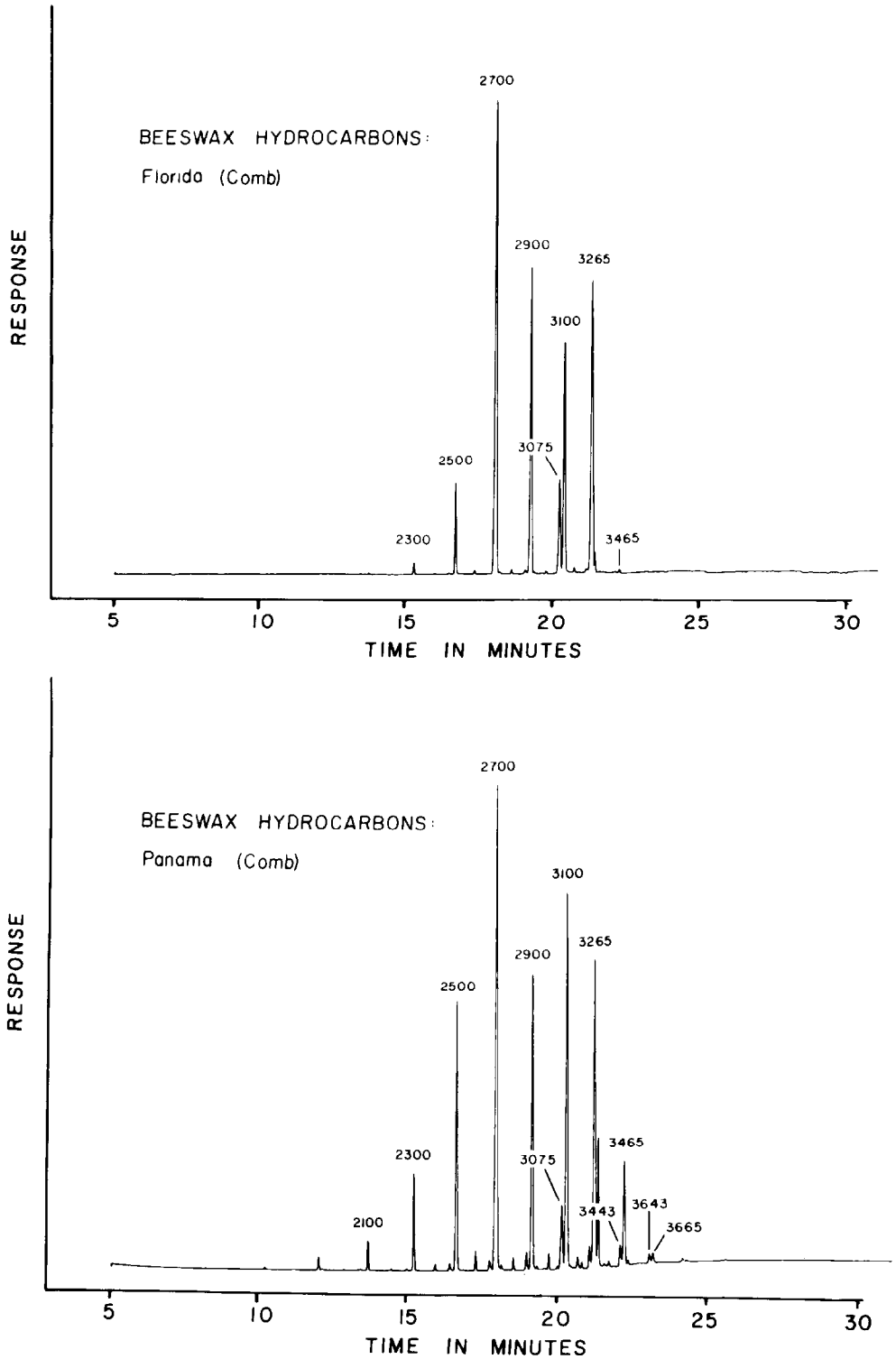


Fig. 6. GC of hydrocarbons from: a. Florida comb wax. b. Panama comb wax.

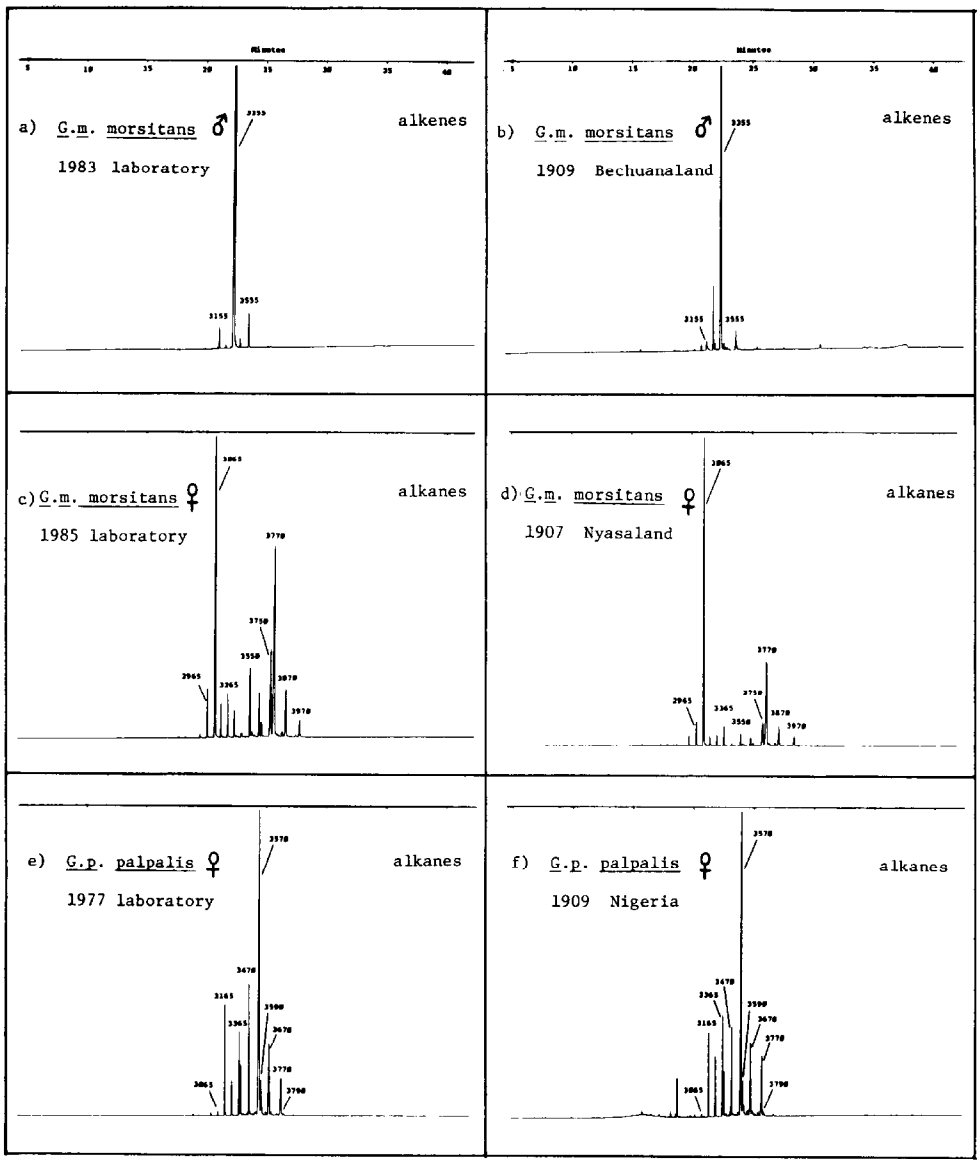


Fig. 7. GC of hydrocarbons from old museum and recently collected tsetse flies.

1909) (Fig. 7). Thus it appears that hydrocarbon patterns are highly conserved in tsetse flies and these materials, the endpoint of genetically determined biosynthetic pathways in the insect, may be utilized for phenetic comparisons by some scheme. Percent composition data was accumulated for two to 10 specimens or groups of each species using packed column GC. While recognizing that use of percent composition data for multivariate statistical treatment can introduce bias, an effort was made to get useful indicators for phenetic relationships by selection of common peaks within each group. These peaks represented most of the materials found, and only a few small peaks were not represented. Each GC peak was used as a "character"; five arbitrarily set "character states" were possible depending upon peak area; a= major (>20%), b= large (10-20%), c= intermediate (5-10%), d= small (2-5%), and e= very small, trace or no detectable peak (0-2%). In the example given here, 8 of the 11 GC peaks were the same

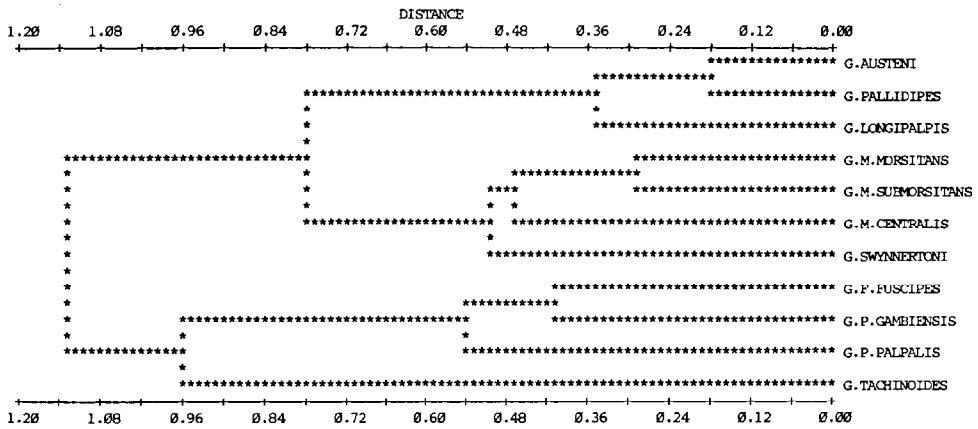


Fig. 8. Phenogram of *Glossina morsitans* and *G. palpalis* male tsetse based on UPGMA Cluster Analysis of 11 GC peaks via Nei (1978) Unbiased Genetic Distance.

for the *G. morsitans* and the *G. palpalis* group males. Phenograms for males of 11 species of these two groups based on UPGMA cluster analysis of GC peaks only were constructed using BIOSYS-1. Complete phenetic separations were found between the two groups (Fig. 8), that agreed quite well with recognized group classifications based on traditional morphological and genetic information. The other species have also been evaluated by this method for a study to be published elsewhere.

Conclusion

Hydrocarbon patterns obtained by GC appear to be very useful in identification in species complexes or within populations of morphologically indistinguishable insects. This is helpful particularly where traditional taxonomic methods are difficult or highly technical and time consuming as with nuclear or mitochondrial DNA. Hydrocarbon analysis can be readily performed with modern GC equipment particularly when long-lasting bonded phase capillary columns are used, and an answer obtained in less than an hour. Also, the specimens are not damaged by extraction with hexane. If further chemical separation is desirable, as to isolate and identify specific alkene and alkadiene isomers, this can be done by well established chromatographic methods for characterization by GC and GC mass spectrometry. We have found that badly damaged specimens, usually cockroaches, have been submitted for identification after a forceful collection experience. Fortunately, the cuticular components are unaffected by such crushing, and good results from GC are still to be expected.

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EVALUATIONS OF QUALITY OF IRRADIATED MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WEIDEMANN) (DIPTERA:TEPHRITIDAE), AT THE RELEASE SITE IN MIAMI, FLORIDA DURING AN ERADICATION PROGRAM IN 1985

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), was introduced accidentally into southern Florida in March 1985. Eradication efforts included four bait-sprays followed by releases of sterile flies four times per week for 12 weeks. The flies to be released were reared and sterilized in Hawaii and shipped as pupae by commercial air freight to Miami. Quality evaluations consisting of pupal weight, sex ratio, emergence rate, flight ability and mating propensity of the flies were made before release. In general, the quality of the flies received in Miami was found to be satisfactory throughout the shipment period.

RESUMEN

La mosca del mediterráneo, *Ceratitis capitata* (Weidemann), se introdujo accidentalmente en el sur de la Florida en Marzo de 1985. Esfuerzos para erradicarla incluyó cuatro rocios-cebo seguido por la liberación de moscas estériles cuatro veces por semana por 12 semanas. Las moscas liberadas se criaron y esterilizaron en Hawaii y transportadas a Miami como pupa por via aérea comercial de carga. Antes de liberar las moscas, se evaluaron sus cualidades por el peso de las pupas, la proporción de sexos, taza de salida de los adultos, la abilidad de vuelo, y la propensidad al acoplamiento de las moscas. En general, la calidad de las moscas recibidas en Miami fue satisfactoria durante el período de transports.

The Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (medfly), is an agricultural pest in Europe, Southern Africa, Central and South America, Australia and

Hawaii. Occasionally it has been found in the contiguous United States. In such cases, the pest usually has been eradicated by bait spray programs (Christenson 1958). The sterile insect technique (SIT) has been used for eradication of medflies in certain experimental situations and most recently in Mexico and Central America (Schwarz et al. 1985). It is now being used as part of eradication efforts for small introductions in the United States (Florida 1985, 1987, California 1987).

The SIT involves a coordinated program to rear, sterilize, and release male flies in sufficient numbers to "overflood" the wild population while retaining their ability to deliver their load of sterile sperm to fertile, wild females. A rearing and handling procedure resulting in the production of effective sterile flies is needed for the technique to successful (Calkins et al. 1982).

The medfly was accidentally introduced into Miami, Florida in March 1985. After four aerial sprays of malathion and Staley's sauce bait, (A. E. Staley Manufacturing Co., Atlanta, Ga), the sterile insect technique was used to ensure eradication of the pest. Approximately 5 million sterile flies, reared in and shipped from Hawaii each of 4 days per week, were released over an 208 km^2 area. A total of 200 million flies were released in the control area from May 5 to July 16, 1985.

Reported here are results of laboratory tests conducted to evaluate the quality of sterile medflies shipped from Hawaii to Miami, Florida during 1985 for release in the medfly eradication project in Dade County, Florida.

Materials and Methods

The Medflies used in this project were reared at the California State-USDA,-APHIS Medfly Laboratory in Honolulu, Hawaii (Tanaka et al. 1970). Two days prior to emergence, pupae were mixed thoroughly with neon red dye powder (Day-Glo Color Corp., Cleveland, OH) to mark emerging flies and packaged in 4-litre polyethylene bags creating a state of hypoxia. The flies then were irradiated with 12 Krad from a Co^{60} source (Ohinata et al. 1977). No adverse effect on mating competitiveness of flies treated with Day-glo dye has been found (Holbrook & Fujimoto 1970). Nine polyethylene bags containing pupae were then placed in cardboard boxes and six artificial ice packages (Blue Ice®) were included to keep the pupae cool during shipping. The boxes were shipped by commercial air carrier to Miami International Airport. When the boxes of pupae arrived at the laboratory after about 14 h, they were opened and a dial thermometer was inserted into each bag to measure the temperature of the pupae. A 50-ml sample of pupae was taken from each bag. These samples were combined and thoroughly mixed before being used for the following tests: sex ratio, eclosion rate, flight ability, and mating propensity, as called for by standard quality control protocols.

The pupae from each shipment were removed from the bags and placed in 35x60x30 cm cardboard boxes and moved into a 12 m long house trailer for emergence. The trailer was kept dark with the temperature maintained at $24 \pm 1°C$. Each box contained ca. 30,000 pupae from which flies began to emerge about 24 h later. Flies were provided with cakes of a mixture of sugar and yeast hydrolysate (3:1) or sugar cubes coated with yeast hydrolysate. Water was supplied by an agar block made of Gelcarin (G.P. 812) placed on the screened lid. Three days later, the boxes were transferred to a cooling trailer (4.5°C) where flies were immobilized. They were then removed from the boxes and placed into a machine designed especially for aerial release of medflies. The processes of removing flies from boxes and releasing them from the air each required 45-60 min. The temperature was maintained at 4.5°C during both operations.

A house trailer was modified to serve as the quality control laboratory. Rooms had individual heating, air conditioning and lighting systems to provide suitable conditions for holding and testing the insects.

To determine sex ratios, five sub-samples of 100 pupae were taken from the combined sample from each shipment and placed in petri dishes. The pupae were held in the quality control (QC) laboratory at 27 ± 1°C and 14L:10D. Four days later, the number of flies that emerged was counted and the sex ratio calculated.

Percent emergence and percent fliers were determined from each shipment by using a combined test. An individual flight test unit consisted of a petri dish (9 cm diam) and a tenite butyrate cylinder 10 cm high and 9 cm (outside diam.). The cylinder was painted black on the outside so that light could enter only at the top. Before each use, the inside of each cylinder was lightly coated with unscented talcum powder to prevent the flies from crawling out. This powder was removed from a 1 cm band around the inside bottom of the tube and the tube was placed into the petri dish. A sample of 100 pupae was placed in the petri dish, and the flight cylinder was put in place. A 1x10 cm paper strip folded accordion-like was placed against one side of the cylinder in the petri dish as a resting place for newly emerged flies. The test was replicated five times (100 pupae each) for each shipment. All five cylinders were placed inside a large screen cage (60x60x50 cm). Flies were aspirated out of the screen cage daily to prevent them from falling back into the tubes at death. After 60 h, the number of unemerged, partially emerged, and deformed and normal appearing flies that remained in the cylinder were counted. The percent emergence and percent fliers were calculated by the following formula:

$$\% \text{ emergence} = 100 - (\text{unemerged pupae} + \text{partially emerged adults})$$

$$\% \text{ fliers} = \% \text{ emergence} - \text{flies remaining in the tube}).$$

The mating propensity test measured the ability of unmated sterile male flies, produced in a mass rearing laboratory, to copulate successfully with virgin females. This test was conducted weekly on shipments 8, 13, 19, 23, 28, 33, 38, 43, 48, 50, & 51 with a modification of methods described by Boller et al. (1977, 1981). About 3,000 to 4,000 pupae from the sample described above were placed in an emergence plexiglass cage (20x40x30 cm) together with sugar cubes and water. Males and females were separated and placed into holding cages within 4 h after emergence. The holding cage consisted of a 360 ml waxed paper cup with a single hole punched in the bottom through which a piece of cotton dental wick was passed. This paper cup was then placed over another 180 ml plastic cup filled with water. For aeration, a circular opening 10 cm in diam was cut in the lid of the cup and covered with a 14-mesh nylon screen. A small cup (30 ml) containing a mixture of sugar and yeast hydrolysate (3:1) was placed on the bottom of the holding cage to provide food for flies. Twenty-five male or 25 female flies were introduced into each holding cage. Each test consisted of five replicates. However, an additional cage for each sex were prepared to provide extra flies if needed, or if a male was inadvertently placed in a virgin female cage. The cages of males or females were inspected periodically and any cage that was discovered to contain mating pairs was discarded.

All of the cages with flies were held at 27 ± 1°C and 14L:10D. The percent relative humidity varied from 60 to 80%. The flies were held for 7 days; day 1 was counted as the day of emergence. The flies were placed in the dark room the night prior to the test. In the morning, 25 females and 25 males were introduced into a mating cage (20x30x40 cm) under a dim light in the dark room during a 5-min period. After five cages were thus filled, they were transferred back to the holding room. A timer was started, mating pairs were removed in 10 dram snap-cap vials at 10-min intervals.

The number of mating pairs that formed within 10 min, and subsequently for each 10-min interval, was recorded for 60 min. The test was then terminated and the number of remaining males and females (not including the dead ones) were counted. The mating index (MI) for each replication was calculated by summing the number of mating pairs

formed within a 10-min period and multiplying by a weight factor (Boller et al. 1981). The number of pairs in th test may not necessarily be 25, because some flies may have died during the test. The MI for five replicates was then averaged to get the mean mating index (MMI) for each shipment.

There were several experimental variables affecting mating propensity that were tested at the release site. A test was designed to study the influence of age of the flies on mating propensity. The test was conducted in the same manner as described above, but flies were held in containers for 14 days before being tested.

The effects of low temperatures on mating propensity was tested. Flies used for this test emerged in the QC laboratory and were placed in holding containers as described. About 60 h after emergence and at the same time flies from the same shipment were being released in the field, holding containers with flies were placed in the refrigerator trailer for 1 h at 4°C. The flies were then transferred back to the QC laboratory and the mating propensity test was performed. This was to verify whether flies that were treated similarly to those that were being released, would retain a high mating index.

An experiment was designed to determine whether the current procedure of aerial release affected the mating propensity. The aerial release took place in the morning between 0600-0900 hrs and lasted ca. 1 h. About 3,000 flies remaining in the release unit at the end of the release flight were collected and placed in a 20x30x40 cm plexiglass cage. As soon as flies recovered from the low temperature, they were sexed and placed in holding cages. The cages were placed in the QC laboratory and the mating test was conducted at the age of 7 days, 4 days after flies were collected from the aerial release.

A test was designed to study the influence of light on mating propensity. Flies used for this test from shipments 33, 38 and 43 emerged in the QC laboratory and were placed in holding containers as described above. The mating propensity test was conducted on the proper day. The treatment exposed the flies for 4 h to normal daylight, before being tested, whereas the other treatment was tested immediately following removal from the dark room, as is the standard procedure.

Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC) was used to analyze data of these tests and to compare them with data from the mating propensity tests conducted under specified conditions from the same shipment.

A charting method for means was used on data from shipments involving emergence rates, flight ability and mating propensity (Boller et al. 1981). The baseline in each case was developed from the specific data from all of the shipments to show correlations among tests that deviated from the average evaluation score. The control limits were determined by three standard deviations of the means.

Results and Discussion

A quality control manual developed by USDA in 1986, revised in 1988, gives the specifications and tolerances for pupal weight, sex ratio, emergence, flight ability and mating propensity at the release site (Brazzel et al. 1986). These specifications and tolerances are: *Pupal weight*—7 mg, if below 6.5 mg for more than 5-10 lots, a problem exists. *Sex ratio*—variation of 45-55% of either sex is considered normal. A trend of 40:60 or greater indicates potential problems and possible rejection by the client. *Percent emergence*—eclosion in excess of 80% is considered satisfactory, below 75% is not acceptable. *Percent fliers*—if less than 60% of the adults escape from the flight tubes, it is not considered satisfactory. *Mating index*—a mean mating index of 50 or greater is considered satisfactory.

The average weight of pupae, 8 days or older, from shipment numbers 15 to 51 was 7.9 ± 0.09 mg measured in Hawaii prior to shipment (Kobayashi, personal communica-

tion). The temperatures at the time the shipments arrived at the Miami laboratory varied from 20-22°C.

The sex ratio of flies shipped to Miami in 1985 was approximately 50:50 (male:female), and varied less than 5% from the mean.

The average percentages of emergence and percent fliers were 87.32 ± 0.78 (Mean ± SE) and 71.19 ± 1.37, respectively. Two of the first seven shipments had emergence rates below 85%. Five of the first seven shipments had flight ability rates below 75% and a total of seven shipments consisted of less than good fliers. The reason for the low percentages could have been that the personnel who conducted the quality control tests were not familiar with the new techniques, or the rapid increase in production in Hawaii may have resulted in procedures that were detrimental to the flies. In one case, talcum powder was not wiped from the bottom of the tube, and therefore, flies became coated with powder and could not fly from the tube. After the technique was corrected and the room temperature was adjusted, the percent emergence and percent fliers increased.

The MMI of flies emerging in the QC laboratory in Miami from all lots tested were higher than 63 except for shipment 48, which had an index of 50. The average MMI for 11 shipments tested in Miami was 67.8. Boller et al. (1981) studied the mating propensity of seven laboratory-reared strains of medfly from Europe and Africa, and reported that the MMI of the unirradiated flies from these strains varied from 17.1 to 62.4. The MMI of *C. capitata* has been improved by selection, and it could reach a plateau of 60-70 after four generations (Boller & Calkins 1984). They considered that any strain with an MMI higher than 60 would be classified as fast mating flies.

The influence of age on mating propensity apparently was not significant (MMI of 7- and 14-day-old flies were 63.10 ± 6.45 and 62.69 ± 9.39, respectively). The mean mating index of the laboratory reared *C. capitata* increases with age and reaches a plateau about 7-8 days after emergence (Boller et al. 1981).

The effect of low temperature (4.5°C) on mating propensity also was not significant for exposure of 1 hr. (MMI of control flies and treated flies were 70.66 ± 3.06 and 68.68 ± 2.14, respectively). Mating propensity of flies emerged in the QC laboratory versus flies taken from the airplane at the end of the aerial release were not significantly different (MMI 67.8 ± 8.6 for control flies and 67.3 ± 4.2 for the flies taken from the airplane).

The exposure of the test insects to light for 4 h before initiation of the mating tests reduced mating propensity by 22 to 32%. Those held in darkness until just before the test had an MMI of 75.85, while those exposed to the extended light period had an MMI of only 54.57. The quality of flies received in Miami was considered satisfactory for pupal weight, sex ratio, emergence rate, flight ability and mating propensity.

The specifications of fly performance at the factory door are more stringent, because at that point, the pupae had not undergone the stress of many hours in hypoxia or of shipping. Unfortunately, quality control data taken prior to shipment were not available to us.

The combination of bait-spray followed by release of sterile flies appears to be a biorational approach for eradication of small introductions of medflies. The release of sterile flies, although more expensive than numerous bait spray applications initially, effectively reduces the number of bait sprays required, eases the public reaction to pesticides being sprayed over populated areas and reduces the number of complaints for damage to automobile paint.

End Note

Mention of a commercial or proprietary product does not constitute an endorsement of that product by the USDA.

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MORPHOLOGICAL DIFFERENCES BETWEEN ADULT *ANTICARSIA GEMMATALIS* AND *MOCIS LATIPES* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Adults of *Anticarsia gemmatalis* Hübner, the velvetbean caterpillar, and *Mocis latipes* (Guenée), referred to as the "striped grass looper", exhibit similar external morphologies and are apparently sympatric in the Western Hemisphere. Intraspecific and interspecific differences in wing maculation, leg scales and spines, and genitalia are discussed, as well as differences in abdominal sterna of females. These differences are useful for species identification and for segregation of blacklight and pheromone trap catches.

RESUMEN

Adultos de *Anticarsia gemmatalis* Hübner, y *Mocis latipes* (Guenée), tienen morfología externa similar y son aparentemente simpátricos en el Hemisferio Occidental. Se trata sobre diferencias intraespecíficas e interespecíficas en la maculación del ala, escamas de las patas y espinas, y genitalia, así como de diferencias en la esterna abdominal de las hembras. Estas diferencias son útiles para identificar especies y para segregar las capturas de trampas de luz negra y de feromonas.

In parts of the Western Hemisphere, *Anticarsia gemmatalis* Hübner, the velvetbean caterpillar (VBC), and *Mocis latipes* (Guenée), referred to as the "striped grass looper" (SGL), are pests of numerous legumes and grasses, respectively (Herzog & Todd 1980, Dean 1985). Both species belong to the subfamily Catocalinae (Hodges et al. 1983) and appear to be spatially and temporally sympatric in the Western Hemisphere (Chapin & Callahan 1967, Herzog & Todd 1980, Dean 1985). From 1980 to 1983, behavioral observations and blacklight trapping of adult VBC were conducted in the presence of both species (Gregory 1986). Due to external morphological similarities between VBC and SGL, moth identifications were difficult to make initially. Both species are approximately the same size, have the same silhouette, and exhibit similar wing maculation. Worn specimens look nearly identical. Forbes (1954) discusses the wing maculation of both species but makes no comparisons. Also, both species exhibit similar leg scale morphology but previous descriptions of their leg scales are vague or inaccurate (Bethune 1869, Forbes 1954, Greene 1974). To facilitate future investiga-

tions, similarities and differences between these two species are presented in this paper. Also, morphological details about the other *Mocis* spp. [i.e., *M. disserverans* (Walker), *M. marcida* (Guenée), and *M. texana* (Morrison)] that occur in the United States are discussed.

Materials and Methods

In September of 1980 and 1981, 50 males and 50 females of VBC and SGL were captured at night with an aerial net at the University of Florida's Green Acres Research Farm, Alachua County, Fla. Most adults were captured on bahiagrass, *Paspalum notatum* Flugge. All adults were examined visually and with a 70X dissecting microscope. Velvetbean caterpillar adults were compared with colony adults maintained at the Insect Attractants, Behavior, and Basic Biology Research Laboratory, ARS, USDA, Gainesville, FL, and with written descriptions of VBC external morphology by Watson (1916), Forbes (1954), Greene (1974), and Leppla et al. (1977). Striped grass looper adults were compared with written descriptions of external morphology by Bethune (1869), Hampson (1913), and Forbes (1954). Adults of both species were compared with specimens at the Florida State Collection of Arthropods (FSCA), Division of Plant Industries, Gainesville, FL. Adults of the other *Mocis* spp. were examined from specimens in the Louisiana State University Insect Collection (LSUC). Genitalia of all moth species were dissected to confirm species and sex identifications. Hodges' (1971) terminology is used to describe wing elements, major wing areas, and leg segmentation.

Results and Discussion

Both VBC and SGL exhibited the same wing span (ca. 35-40 mm) (Forbes 1954) and similar wing coloration and maculation (Figs. 1 and 2). Dorsal VBC wing coloration was extremely variable and ranged from light gray to grayish black and from light yellowish

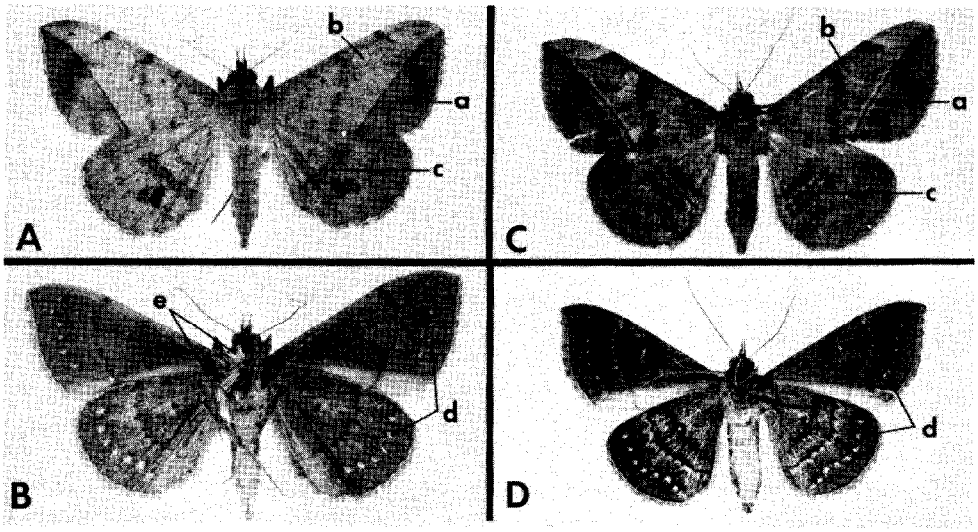

Fig. 1. *A. gemmatalis*. A. Male, dorsal. B. Male, ventral. C. Female, dorsal. D. Female, ventral. Legend: a = postmedial line, b = reniform spot, c = postmedial line, d = subterminal line, e = long setae on male legs. Adults collected by G. Strickland: male, 24-IX-1969, East Baton Rouge Parish, LA; female, 3-X-1970, East Baton Rouge Parish, LA. Both specimens are on deposit at the Florida State Collection of Arthropods.

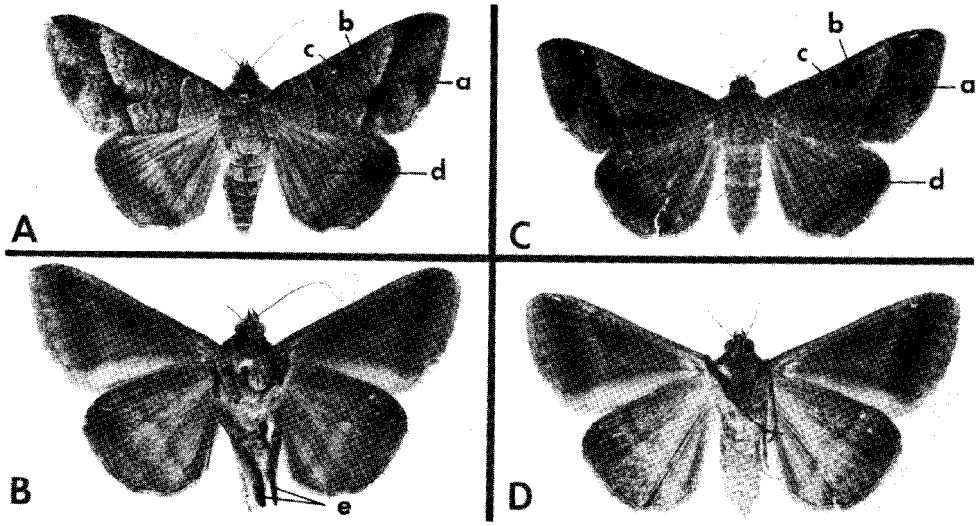

Fig. 2. *M. latipes*. A. Male, dorsal. B. Male, ventral. C. Female, dorsal. D. Female, ventral. Legend: a = postmedial line, b = reniform spot, c = subreniform spot, d = postmedial line, e = long setae on male legs. Adults collected by T. Dean: male and female, 31-X-1984, Alachua County, Gainesville, FL. Both specimens are on deposit at the Florida State Collection of Arthropods.

brown to brownish red; ventral wing coloration was less variable and tended to be light brown or cinnamon brown. Dorsal SGL wing coloration was less variable than VBC and ranged from light gray to blackish gray and from yellowish gray-brown to purplish gray-brown; ventral wing coloration ranged from light gray to light brown. Intersexual differnces in wing coloration and maculation of each species were negligible except that SGL females were sometimes more reddish than males as noted by Forbes (1954).

The main difference between the dorsal wing maculation of VBC and SGL was the placement of the forewing and hindwing postmedial lines. In both species, the union of these lines formed a straight line that crossed the forewing and hindwing when the wings were spread (Fig. 1, A and C, a and c; Fig. 2, A and C, a and d). On VBC this line began at the forewing apex and ran obliquely across the wing surface and onto the hindwing. On SGL this line began near the distal costal margin of the forewing and ran parallel to the outer wing margins; on the hindwing this line was vague.

Another difference between the dorsal wing maculation of VBC and SGL was the occurrence of the reniform and subreniform spots on the forewing. The reniform spot on VBC was figure-eight in shape, usually faint and sometimes obscure (Fig. 1, A and C, b); the subreniform spot was not visible. The reniform spot on SGL was generally evident, vaguely kidney-shaped and usually touched the subreniform spot (Fig. 2, A and C, b). The subreniform spot was usually distinct and circular, and sometimes opened onto the postmedial line (Fig. 2, A and C, c).

A notable difference between the ventral wing surfaces of the two species was the development of the subterminal lines. On VBC the subterminal line was a series of white dots that ran parallel to the outer wing-margins (Fig. 1, B and D, d). This line was not present on SGL (Fig. 2, B and D).

Both species exhibited sexual dimorphism in leg scales and interspecific differences in leg spines (Figs. 1-4). *Anticarsia gemmatalis* males had longer scales than females on prothoracic coxae and mesothoracic tibiae (Fig. 1, B and D, e; Fig. 3). These long scales on the males formed large hair pencils. Forbes (1954) reported that each

Fig. 3. Legs of *A. gemmatalis*. Top row, male. Bottom row, female. Left to right: prothoracic, mesothoracic, and metathoracic. Scale bar = 5 mm. Legs are from adults collected 24-IX-1986, East Baton Rouge Parish, LA.

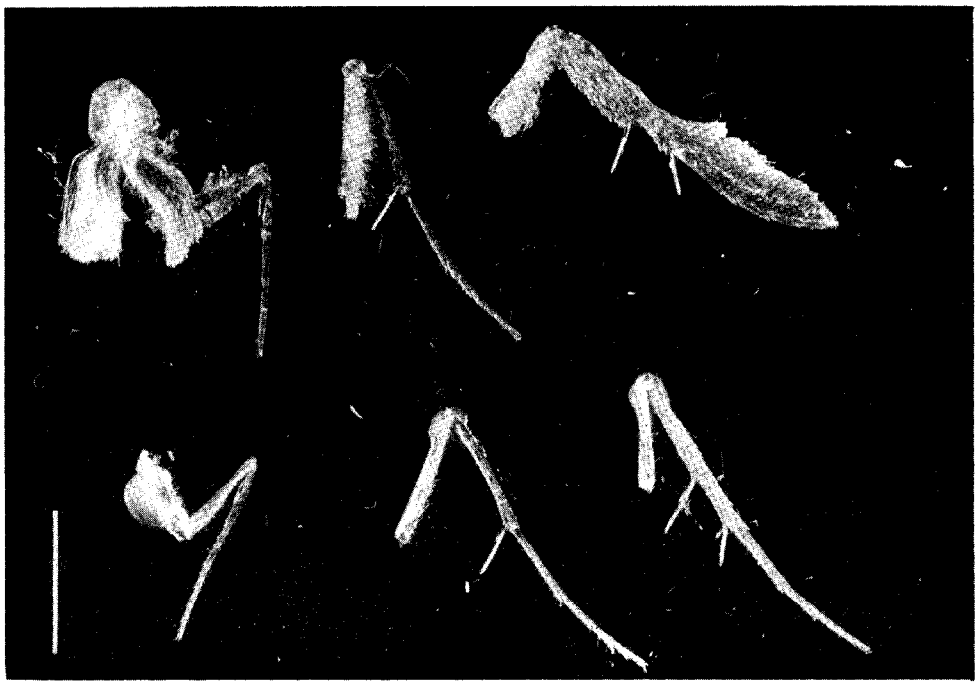

Fig. 4. Legs of *M. latipes*. Top row, male. Bottom row, female. Left to right: prothoracic, mesothoracic, and metathoracic. Scale bar = 5 mm. Legs are from adults collected 24-IX-1986, East Baton Rouge Parish, LA.

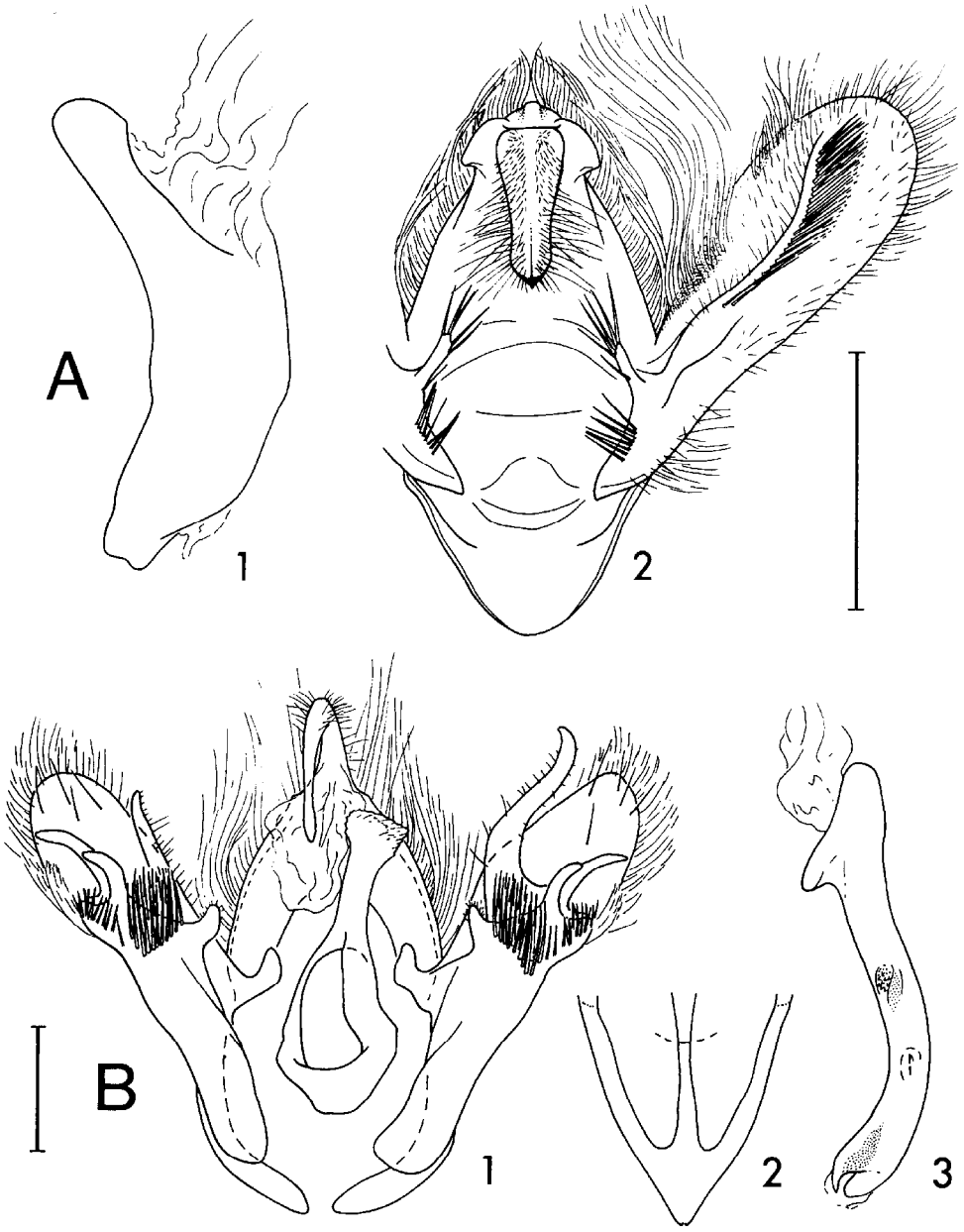

Fig. 5. Male genitalia with aedeagus removed. A. *A. gemmatalis*. 1. aedeagus, lateral view. 2. genitalia with uncus flattened, ventral view. B. *M. latipes*. 1. genitalia (vinculum distorted), ventral view. 2. vinculum in repose, ventral view. 3. aedeagus, lateral view. Scale bars = 1 mm.

mesothoracic tibia had a massive hair pencil but did not mention the coxal hair pencil. Greene (1974) reported incorrectly that long scales were present on the prothoracic femora and metathoracic tibiae. Males of *M. latipes* had longer scales than the females on the prothoracic coxae and on the femur, tibia, and tarsus of each metathoracic leg (Fig. 2, B and D, e; Fig. 4). The long scales on each prothoracic coxa comprised two distinct hair pencils (Fig. 4). The one on the left side of the coxa was attached to a sclerotized extension of the coxa, while the other one was attached to the coxa proper.

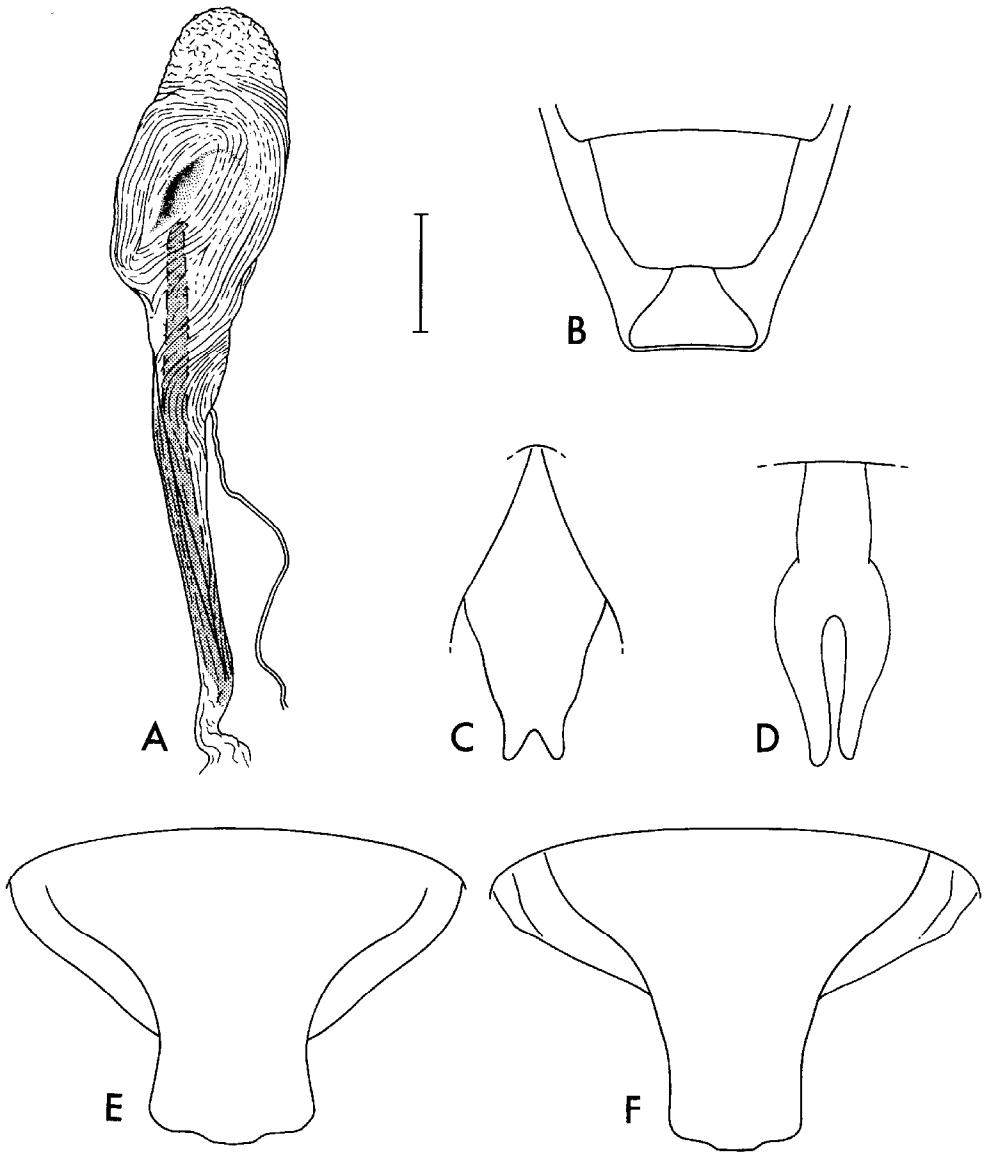

Fig. 6. Female genitalia. A, B. *A. gemmatalis*. A. bursa copulatrix, unmated. B. sixth visible abdominal segment (ventral view without ovipositor). C-F. *Mocis* spp., sixth visible ventral abdominal segment. C. *M. latipes*. D. *M. disserverans*. E. *M. texana*. F. *M. marcida*. Scale bar = 1 mm.

These two hair pencils appeared as one when the leg was held close to the body. The long scales on each metathoracic leg formed a dense fringe. Bethune (1869) noted that the metathoracic tarsi of SGL males were densely fringed, and Forbes (1954) reported the same for the tarsi and tibiae of *Mocis* spp., but neither mentioned hair pencils on the prothoracic coxae. Each mesothoracic tibiae of the *Mocis* spp. has a row of spines on the anterior face. Forbes (1954) reported that this character was present in the Catocalinae but absent in the Erebinae, in which he placed *Anticarsia.*

The dorsal postmedial line, ventral subterminal spots, reniform spot, and leg scale characteristics are useful for distinguishing VBC and SGL adults. On worn specimens, wing maculation usually fades before leg scale characteristics or the metathoracic tibial spines are lost. Genitalia dissections may be necessary on severely worn specimens. The male genitalia of VBC and SGL are shown in Fig. 5. The male genitalia of *M. latipes* cannot be flattened without distortion of the vinculum which is shown in repose in Fig. 5, B, 2. The appearance of the heavily sclerotized, asymmetrical processes on the valves will vary depending upon how much the valves are flattened. Similar processes distinguish the other three *Mocis* spp. from VBC. Both *M. latipes* and *M. disserverans* have a similar needle-shaped structure surrounding the aedeagus, but it is longer, sinuate, and pointed apically in *M. disserverans. Mocis marcida* and *M. texana* lack the needle-shaped structure but have a more heavily sclerotized area on each side of the aedeagus that is spined distally.

In the female genitalia of VBC, the ductus bursa is membranous with an elongate sclerotized rod-shaped structure extending into the bursa copulatrix (Fig. 6, A). In *Mocis* spp., the ductus bursa is a heavily sclerotized tube and only the bursa copulatrix is membranous. On VBC the last (i.e., sixth) visible ventral abdominal segment, excluding the ovipositor, is roughly rectangular in shape after the scales are removed (Fig. 6, B). In SGL, this sclerite is diamond-shaped and apically emarginate (Fig. 6, C), deeply cleft in *M. disserverans* (Fig. 6, D), and broad basally, narrowed distally, and apically sinuate in *M. texana* and *M. marcida* (Fig. 6, E and F, respectively).

Other moth species may be confused with VBC and SGL; therfore familiarity with the external and internal morphological characteristics of VBC and SGL can be useful for species identification and segregation of blacklight and pheromone trap catches. Both VBC and SGL have similar pheromones (Heath et al. 1983, Descoins et al. 1986) and are captured in VBC pheromone traps (Gregory, unpublished data).

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COMPARISONS OF CAT FLEA (SPHONAPTERA: PULICIDAE) ADULT AND LARVAL INSECTICIDE SUSCEPTIBILITY

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ABSTRACT

The resistance levels of a Florida strain of cat fleas, *Ctenocephalides felis* (Bouche), were evaluated using nine chemicals after colonization for approximately one (1984) and

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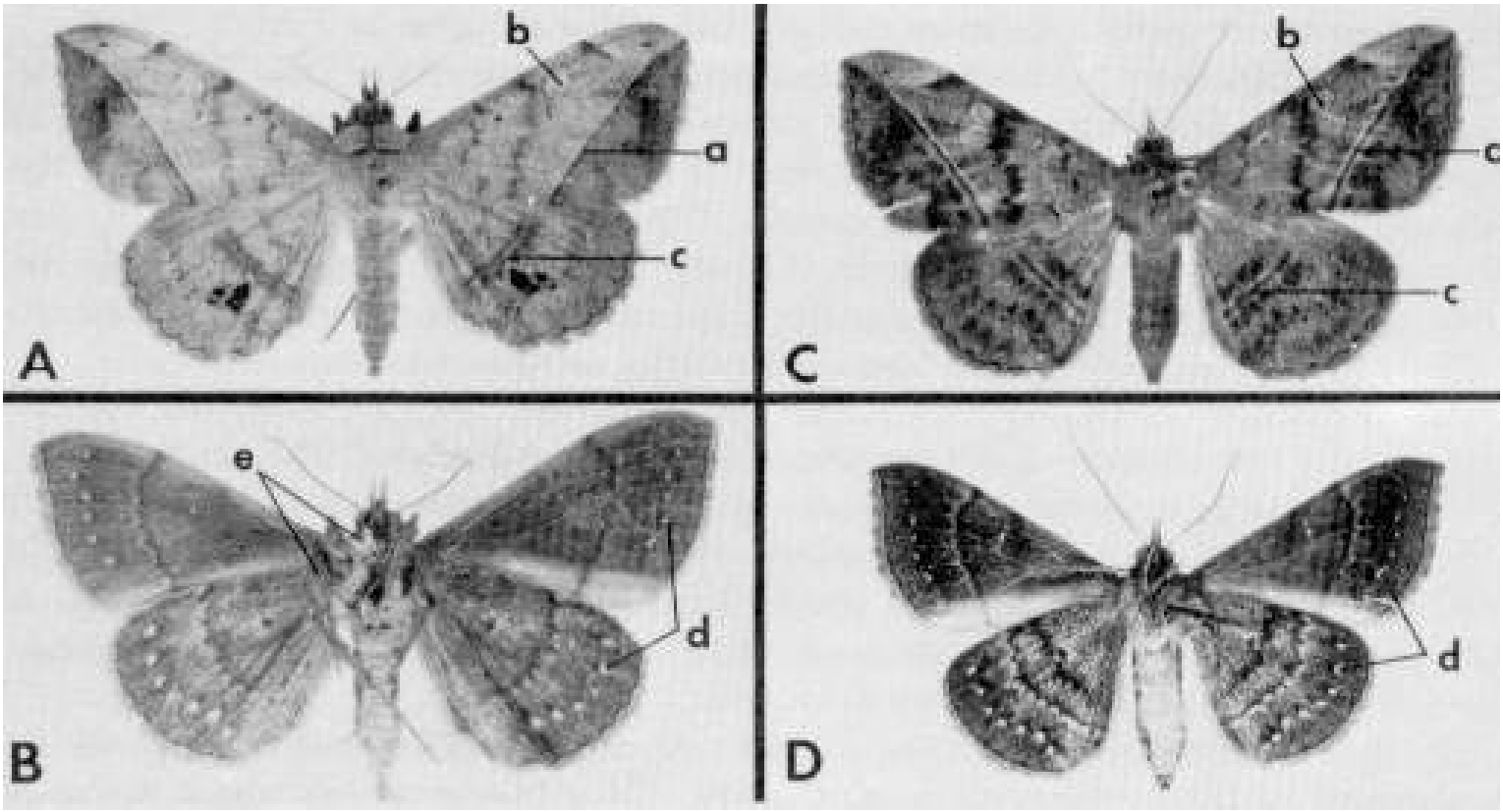
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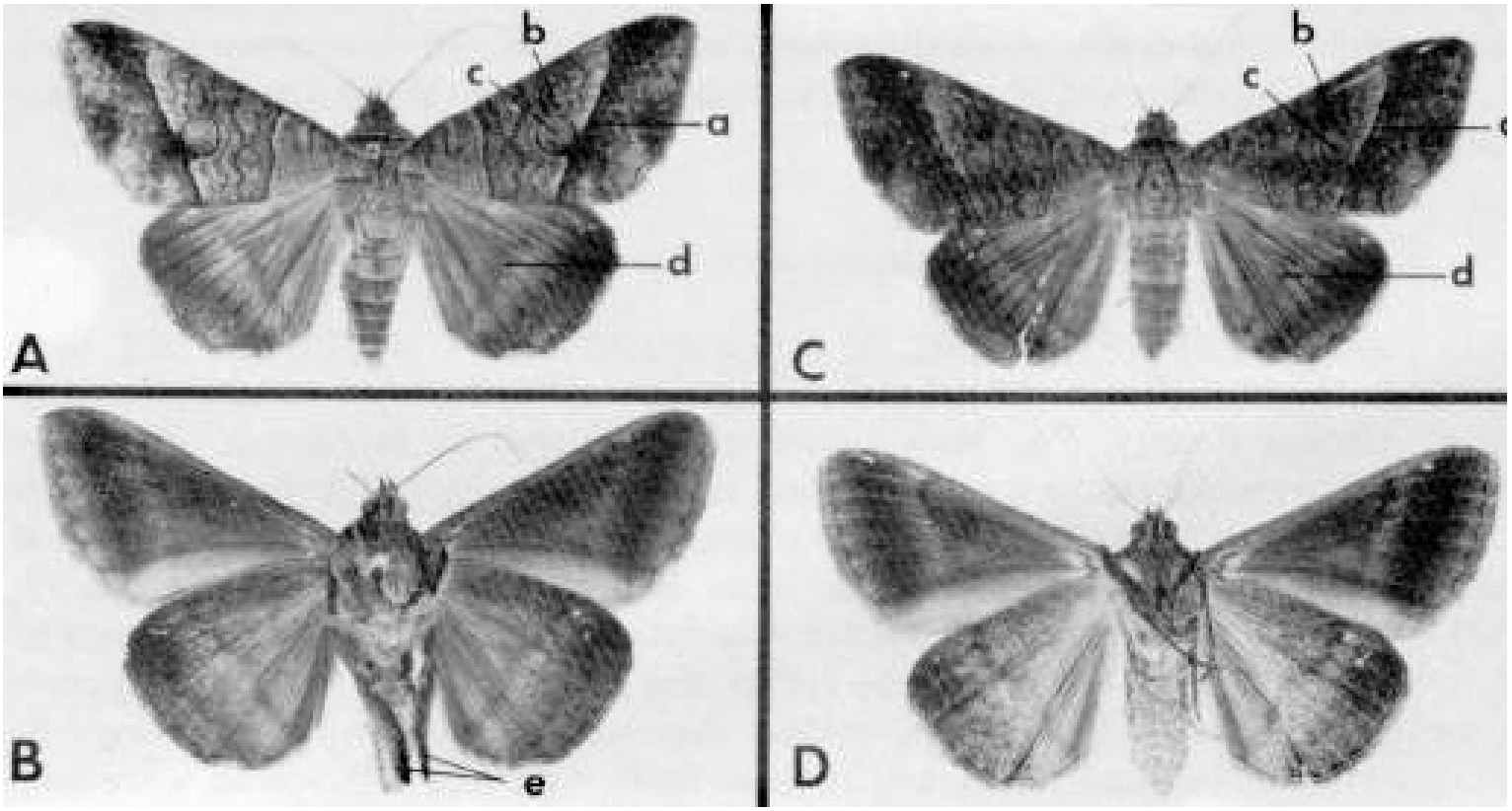
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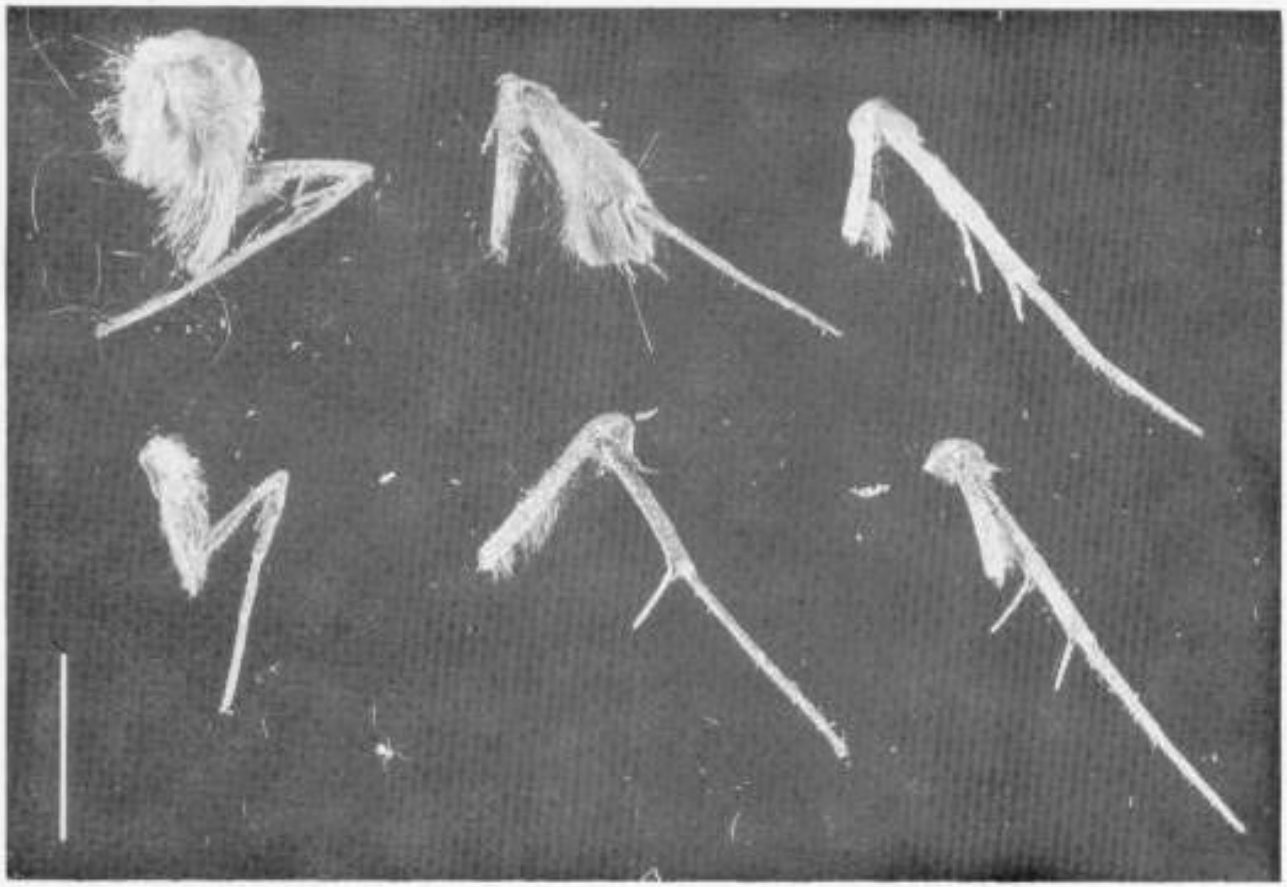

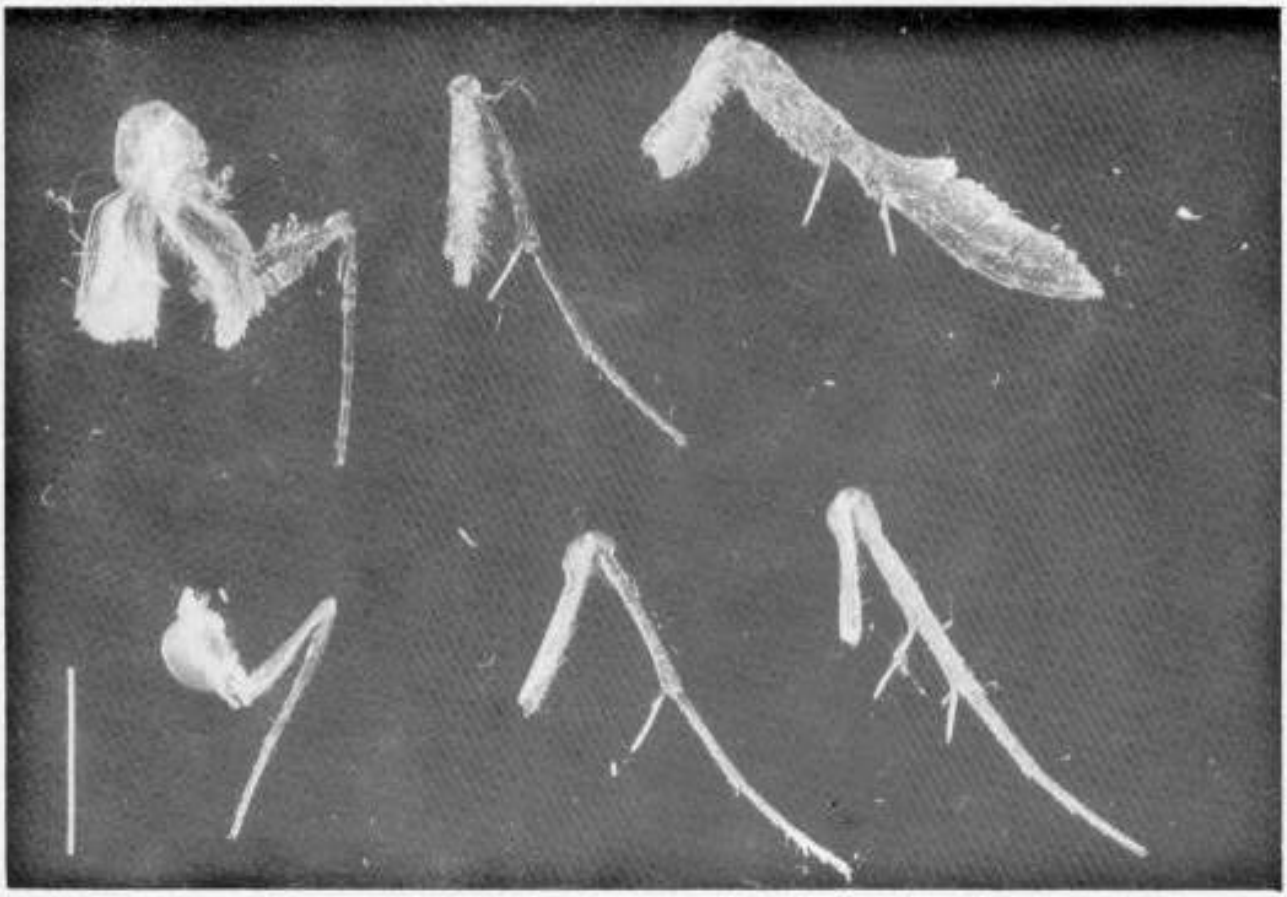

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COMPARISONS OF CAT FLEA (SPHONAPTERA: PULICIDAE) ADULT AND LARVAL INSECTICIDE SUSCEPTIBILITY

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ABSTRACT

The resistance levels of a Florida strain of cat fleas, *Ctenocephalides felis* (Bouche), were evaluated using nine chemicals after colonization for approximately one (1984) and

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two (1985) years. It was found that the fleas had lost some of their resistance to chlorpyrifos and malathion in 1985 compared to 1984. Moreover, higher resistance was detected to the three carbamates, bendiocarb, propoxur, and carbaryl. The increased carbamate resistance was attributed to the use of carbaryl dust to kill the fleas on cats after they were taken out of flea production. The resistance levels for propetamphos, diazinon, isofenphos and chlorfenvinphos did not change significantly.

Comparing the 24 h toxicity of the nine compounds against larval and adult stages in 1985, chlorpyrifos, diazinon, chlorfenvinphos, and propoxur were more toxic to adults than larvae and propetamphos, malathion, and bendiocarb were more toxic to larvae than adults. The tolerance to isofenphos was similar for larvae and adults. Both stages responded equally to carbaryl and were completely resistant.

All organophosphates in 1985 exhibited delayed larval mortalities and affected cocoon formation of the larvae that survived the 24 h exposure. However, none of the organophosphates, or the carbamates, appeared to have any effect on adult emergence indicating that the delayed toxicity does not extend beyond larval stage.

RESUMEN

Se evaluaron los niveles de resistencia de una raza de pulgas de gato, *Ctenocephalides felis* (Bouche), de la florida, usando nueve productos químicos después de la colonización, por aproximadamente un (1984) y dos (1985) años. Se encontró que las pulgas habían perdido algo de su resistencia a clorpirifos y malatión en 1985 comparado con 1984. También se detectó más resistencia a los tres carbamates, bendiocarb, propoxur, y carbaril. El sumento de la resistencia a los carbamates se satribuyó al uso de polvo de carbaril para matar las pulgas en los gatos después de que se sacaron de la producción de p;ulgas. Los niveles de propetamfos, diazinón, isofenfos y clorfenvinfos no cambiaron significativamente.

cuando se comparó la toxicidad de 24 h de nueve compuestos contra las etapas larvales y de adultos en 1985, clorpirifos, diazinón, clorfenvinfos, y propoxur, fueron más tóxicos a los adultos que a las larvas, y propetamfos, malatión, y bendiocarb fueron más tóxicos a las larvas que a los adultos. La tolerancia a isofenfos fue similar en larvas y adultos. Ambas etapas respondieron igual al carbaril y fueron completamente resistentes.

Todos los organofosfatos en 1985 tuvieron una reacción demorada en la mortalidad de las larvas en 1985, y afectó la formación del capullo de las larvas que sobrevivieron la exposición de 24 h. Sin embargo, ninguno de los organofosfatos o carbamates parecen tener ningún efecto en la salida de los adultos, lo que indica que la toxicidad demorada no se extiende má alla de la etapa larval.

Insecticide resistance had been reported in the literature as causing control failures in field populations of fleas (Brown 1958, Brown & Pal 1971, Kilpatrick & Fay 1952, McDuffie et al. 1953). Other studies (Collart & Hink 1986, El-Gazzar et al. 1986, Fox et al. 1968, Fox & de Leon 1984, Miller et al. 1977) have attempted to demonstrate the presence or development of insecticide resistance in the cat flea.

In 1984 after 9-12 months of colonization, a Florida strain of the cat flea, *Ctenocephalides felis* (Bouche), was tested for susceptibility to a number of chemicals commonly used for flea control in the U.S. and compared to a California strain which had been colonized for about two decades. The results of this study were reported by El-Gazzar et al. (1986) and clearly showed that the Florida strain exhibited a higher degree of resistance to all nine chemicals compared to the California strain.

The present study was conducted to determine whether the Florida strain had lost any pesticide resistance during colonization after the 1984 evaluation. The second objective of this study was to determine if insecticide susceptibility was the same for both the adult and larval stage of the cat flea.

Materials and Methods

Cat fleas were reared at the USDA-ARS Insects Affecting Man and Animals Laboratory in Gainesville, Florida on domestic cats by the method described by El-Gazzar et al. (1986). Each cat was in flea production for 4 wk. During this period of production, 200 adult, mixed-sex fleas were placed on each animal per wk. After 4 wk, the cats were treated with a 5% carbaryl dust to kill fleas and to reduce the irritation and hair loss caused by the fleas. For the next 5 wk, animals not in production were housed in a separate animal building where no insecticides were applied. Insecticide susceptibility of adult fleas was first evaluated in 1984 after 9-12 months of colonization (El-Gazzar et al. 1986). A second evaluation with adult and larval fleas was carried out in 1985 after 21-24 months of colonization.

The insecticides used in this study were bendiocarb, carbaryl, chlorfenvinphos, chlorpyrifos, diazinon, isofenphos, malathion, propetamphos, and propoxur. The chemicals were tested as residues of technical materials dissolved in reagent grade acetone and applied to Whatman No. 1 filter paper. The range of concentrations tested was between 0.1 and 400 μg A.I../cm^2. Adult fleas were exposed to chemicals on treated filter paper strips (El-Gazzar et al. 1986). For each concentration, 100 adult fleas (1 to 6 h old were exposed to treated filter papers in 80 ml test tubes. Mortality was determined after 24 h exposure.

Larvae (100 ca. 3.5 days old) were exposed on filter paper discs 11.0 cm in diameter. The discs were pressed inside 120 ml paper cups so that they had vertical edges to prevent larvae from escaping. Ca. 0.5 g of larval diet was placed on each filter paper disc. Control groups were exposed to filter paper treated only with acetone. After 24 h, numbers of dead and live larvae were counted. Surviving larvae were placed in 250-ml waxed paper cups containing 75 g of larval rearing medium. The medium consisted, by volume, of 5 parts of sand and part of food (20:3:2, by weight, pulverized dry laboratory rat chow, dried blood meal, and Brewer's yeast). The cups were covered with organdy cloth and held at 27 ± 2°C and 70 ± 5% RH. After 5 wk, the cups were checked for cocoons and adults. Each test was replicated two to four times.

Percent mortalities of both adults and larvae were corrected for control mortalities by Abbott's formula (1925). Probit analysis (Finney 1971) was applied to the corrected percent mortalities to estimate the lethal concentrations (LC_{50} and LC_{90}). In the larval study, percentage reduction in pupal cocoon formation (based on the number of larvae that survived the 24 h exposure) and adult emergence (based on the number of cocoons) were calculated for all treatments. Probit analysis (Finney 1971) was then applied for estimating the lethal concentrations for cocoon formation (delayed larval mortality) and adult emergence (pupal and unemerged adult mortality).

Results and Discussions

The data on the nine chemicals tested are presented in Table 1. Based on the 1985 LC_{50} values for chlorpyrifos (0.72 μg/cm^2) and malathion (37.72 μg/cm^2), the Florida strain of fleas, had lost some resistance since 1984, requiring 39 and 38% less chemical, respectively, to achieve the LC_{50}. Meanwhile, this strain of fleas exhibited a greater degree of resistance to bendiocarb, propoxur, and carbaryl than in 1984, requiring 7.7, 2.7, and >11.9 times more material, respectively, to achieve the LC_{50}. The development of higher resistance to carbamates is probably attributable to the use of the 5% carbaryl dust on the cats after each flea production period. The high resistance developed by fleas to bendiocarb and propoxur indicated that there is probably cross-resistance between these two insecticides and carbaryl. The resistance level to diazinon, propetamphos, isofenphos, and chlorfenvinphos did not significantly change.

TABLE 1. LETHAL CONCENTRATIONS OF NINE INSECTICIDES TO CAT FLEA ADULTS IN 1985 COMPARED TO 1984.

Insecticides	*n*	1985 LC_{50} (μg/cm²) (95% C.I.)	Slope	1985 LC_{50}/ 1984 LC_{50}[a]
Chlorpyrifos	1,020	0.72 (0.60-0.87)	3.09	0.61
Diazinon	550	4.09 (2.39-6.97)	2.94	0.78
Propetamphos	580	6.24 (4.48-8.68)	3.09	0.93
Isofenphos	1,072	9.20 (6.68-12.49)	2.69	1.10
Malathion	669	37.72 (20.99-60.26)	1.74	0.62
Chlorfenvinphos	1,970	48.74 (41.21-56.61)	2.45	1.30
Bendiocarb	1,000	100.73 (31.36->500)	0.34	7.69
Propoxur	1,553	140.48 (23.07-unavail)	0.99	2.69
Carbaryl	1,000	>500[b]		>11.86

Total number of adults used for each compound ranged between 1200 and 1800.
[a]1984 values from El-Gazzar et al. (1986).
[b]Unachievable within the range of concentrations applied.

Flea larvae were exposed to the same nine chemicals, and the data are shown in Table 2. The larval stage was more tolerant than the adult stage to four of the chemicals. At the LC_{50} level, it took 1.7, 1.4, 1.7, and 1.5 times more chlorpyrifos, diazinon, chlorfenvinphos, and propoxur, respectively, to kill larvae than adults. However, of these four chemicals, only chlorpyrifos and diazinon differed significantly as determined by nonoverlap of the 95% C.I. Three compounds, propetamphos, malathion and bendiocarb were more effective against larvae than adults. At the LC_{50} level, it took 35, 71, and 54% less propetamphos, malathion, and bendiocarb, respectively, to kill larvae compared to adults. Carbaryl was completely ineffective against both larvae and adults.

Of the nine compounds evaluated, all organophosphates, but no carbamates, resulted in delayed larval toxicity, expressed in Table 3 as a significant dose response on cocoon formation of larvae surviving 24 h of exposure. The greatest effect on cocoon formation (delayed larval toxicity) was by chlorpyrifos (LC_{50} = 3.02 μg,/cm²). Diazinon, malathion and chlorfenvinphos required higher concentrations (35.1, 195.4, and 263.8 μg/cm², respectively) to reduce cocoon formation by 50%. None of the chemicals caused delayed pupal toxicity as indicated by no significant dose response in those that survived to pupation.

TABLE 2. LETHAL CONCENTRATIONS OF NINE INSECTICIDES TO CAT FLEA LARVAE AFTER 24 H EXPOSURE.[a]

Insecticides	*n*	LC_{50} (μg/cm²) (95% C.I.)	Slope
Chlorpyrifos	2,080	1.25 (0.96-1.68)	1.09
Propetamphos	1,780	4.02 (2.48-6.09)	1.92
Diazinon	1,400	5.82 (5.01-6.93)	0.71
Malathion	1,900	10.74 (5.57-15.86)	0.74
Isofenphos	1,200	13.15 (10.52-10.98)	1.57
Bendiocarb	1,200	46.39 (35.02-68.74)	0.32
Chlorfenvinphos	1,700	80.65 (unavailable)	1.01
Propoxur	1,200	207.70 (78.93->500)	0.32
Carbaryl	1,200	—[a]	—[a]

[a]Total number of larvae used for each compound ranged between 1200 and 1800.
[b]Unachievable within the range of concentrations applied.

TABLE 3. LETHAL CONCENTRATION OF NINE INSECTICIDES FOR CAT FLEA COCOON FORMATION (AS CRITERION FOR DELAYED LARVAL MORTALITY) AFTER 24 H EXPOSURE PERIOD.[a]

Insecticides	*n*	LC_{50} (μg/cm²) (95% C.I.)	Slope
Chlorpyrifos	2,080	3.02 (2.22-5.05)	0.87
Propetamphos	1,780	7.93 (3.03-97.84)	0.95
Isofenphos	1,400	17.81 (12.28-107.11)	1.39
Diazinon	1,900	35.10 (19.44-108.72)	0.43
Malathion	1,200	195.40 (76.19->500)	0.44
Chlorfenvinphos	1,200	263.80 (unavail.)	0.32
Bendiocarb	1,700	—[b]	0.16
Propoxur	1,200	—[b]	0.06
Carbaryl	1,200	—[b]	0.02

[a]Total number of larvae used for each compound ranged between 1200 and 1800.
[b]Unachievable within the range of concentrations applied.

The results indicated that of the six organophosphates chlorpyrifos, propetamphos, and isofenphos were the most efficient chemicals against both immature and adult fleas. The results also indicated that cat fleas can develop high levels of resistance to carbamates after relatively short periods of exposure to these chemicals and the use of carbamates should be avoided by researchers raising fleas for experimental purposes.

ACKNOWLEDGMENT

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PREDICTING THE PHENOLOGY OF *HELIOTHIS VIRESCENS* (LEPIDOPTERA: NOCTUIDAE) ON TOBACCO USING ACCUMULATED DEGREE-DAYS

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ABSTRACT

Degree-day (°D) estimates were calculated between *Heliothis virescens* (F.) population peaks of adult males and between larval infestation peaks on tobacco in Florida. The mean °D between the first and second and between the second and third adult peaks for 3 years was 512 and 583, respectively. The mean °D between the first and second larval peaks was 550. Similarly, there was a mean of 554 °D between the first and second larval damage peaks on tobacco. From pheromone trap data collected over 5 years, there was a mean of 212 °D from the first catch of *H. virescens* males in the spring to the first adult population peak.

RESUMEN

Se calcularon estimados de grado-día (°D) entre el auge de poblaciones de adultos machos y entre el auge de infestación por larvas de *Heliothis virescens* (F).) en tabaco en la Florida. El promedio °D entre el primero y el segundo, y entre el segundo y el tercer auge de adultos en 3 años, fueron 512 y 583 respectivamente. el promedio °D entre el primero y el segundo auge larval fue de 550. Similarmente, hubo un promedio de 554 °D entre el primero y el segundo auge larval dañino al tabaco. En trampas de feromonas colectadas durante 5 años, hubo un promedio de 212 °D en la primera captura de machos de *H. virescens* en la primavera, hasta el primer auge de población de adultos.

The tobacco budworm (TBW), *Heliothis virescens* (F), is a serious pest of cotton and tobacco in the United States. Because improved methods of predicting insect populations are needed in pest management programs, interest has developed in constructing realistic population models for TBW (Stinner et al. 1974, 1975, Harstack et al. 1976). Knowledge of field developmental time between population peaks of adult TBW and the immature stages is useful in constructing these models to predict potential TBW infestations. Earlier studies used calendar days to measure developmental times of the immature stages of TBW in the field (Brazzel et al. 1953, Neunzig 1969). However, when predicting the duration of immature stages over a range of fluctuating temperatures, degree-days (°D)—a measure of cumulative temperature units—are sometimes more useful and accurate. Wang (1960) reviewed the early methods of attempts to integrate temperature over time to predict the phenology of organisms.

Hartstack et al. (1973) used blacklight traps to determine population peaks of *H. virescens* and *H. zea* in a cropping system of cotton, corn, grain sorghum, and pasture or hay crops so that they could estimate the mean generation times in °D. The accuracy in calculating °D was increased when Allen (1976) presented a method of correcting for local geographic bias. Using his method, Hogg & Calderon (1981) estimated the developmental times in °D of the larval and pupal stages of *H. virescens* and *H. zea* on cotton under field conditions in Mississippi. They reported °D means of 225, 212, and 234 for TBW larvae, female pupae, and male pupae, respectively. Mitchell (1986) stated that

the more successful of the pheromone model systems developed to date have used °D as an important component and that simple systems, which can used with a minimum of technical training, can be developed using °D. The purpose of our study was to provide a method by which °D could be used to predict population peaks of TBW adults, larvae, and larval damage in tobacco fields in Alachua County, FL.

Materials and Methods

We determined °D between population peaks of both TBW adults and larvae and also between peaks of TBW damage in 12 tobacco fields. The °D values were calculated using the method with corrections for overestimating bias in Florida developed by Allen (1976). The minimum developmental threshold was assumed to be 12.6°C (Hartstack et al. 1973, Hogg & Calderon 1981). Climatological data for Gainesville, FL, were obtained from the University of Florida Agronomy Department and the National Oceanic and Atmospheric Administration. The weather station was located ca. 23 km from the tobacco fields.

Insect survey data were collected from four fields per year over a 3-year period (1978-1980). The tobacco fields (A-L) ranged in size from 2.3 to 7.6 ha (mean = 4.5) and were maintained by cooperating tobacco producers. Planting dates ranged from March 21 to April 10 (mean = April 1), and harvest began June 17 to July 3 (mean = June 24) of each year.

Adult male TBW populations were monitored in each field using cone-shaped traps constructed of 3.2 mm-mesh hardware cloth with an inner-truncated cone similar in design to those described by Hartstack et al. (1979). Each trap was baited with a laminated plastic Hercon strip containing 10 mg of a synthetic attractant for male TBW. The attractant used in 1978-79 (fields A-H) contained virelure [(*Z*)-11-hexadecenal/(*Z*)-9-tetradecenal, 16:1)] and the attractant used in 1980-85 (fields I-L) consisted of seven components [81.4% (*Z*)-11-hexadecenal, 2.0% (*Z*)-9-tetradecenal, 1.3% (*Z*)-9-hexadecenal, 3.2% (*Z*)-11-hexadecen-1-ol, 1.0% (*Z*)-7-hexadecenal, 9.5% hexadecanal, and 1.6% tetradecanal]. The traps were spaced equally within the fields at a density of one trap per ha except in fields C and D where 22 traps per ha were maintained throughout the growing season as a part of another study (Tingle & Mitchell 1981). All traps were maintained at plant height or at least 30 cm above ground level. Trapped moths usually were counted and destroyed twice weekly. Counts of feeding damage to plant terminals and larvae also were made twice weekly by visually examining 40 or 50 consecutive plants at each of five to nine preselected locations within each field (250 to 360 plants per field). After the tobacco plants were topped (i.e., flower buds removed), feeding damage assessments were made from secondary growth. The same plants were examined each time, but only fresh damage was recorded.

In addition to the trap and field data collected during 1978-1980, one or more survey traps for *H. virescens* were maintained continuously in this tobacco growing area from April 1, 1979 until January 30, 1986, except for the period August 20, 1981 to April 1, 1982. The accumulated °D from the first trap catch of TBW in the spring of each year (1980-1981 and 1983-1985) until the first detected peak of TBW adults were calculated from the trapping data.

Results and Discussion

According to pheromone trap catches of male moths, there were five adult TBW peaks each year beginning in mid-April with the last peak in early October. There were two larval peaks, as well as two damage peaks, in preharvest tobacco. The first TBW larval and damage peaks usually occurred in late April. Following a second adult peak

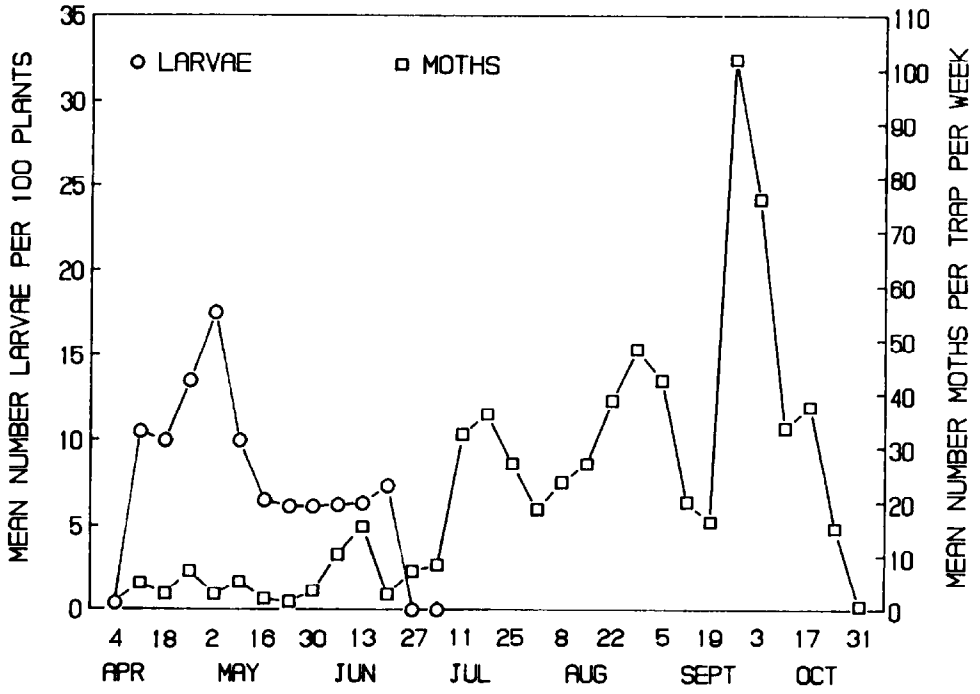

Fig. 1. Mean weekly counts of adult *Heliothis virescens* males collected per pheromone-baited trap and the weekly mean counts of *H. virescens* larvae per 100 tobacco plants in fields E, F, G, and H during 1979, Alachua County, Florida.

in early June, the second larval and damage peaks occurred in mid-June before harvest began (mid-June to early-July). The 1978 trap and field data are presented graphically in Figure 1 to illustrate the typical seasonal fluctuation of TBW adults and larvae in the tobacco fields.

Pheromone-trap catches of male TBW moths in tobacco over a 3-year period indicated means of 512 and 583 °D (50 and 41 days) between the first and second and between the second and third adult peaks, respectively (Table 1). Similarly, 550 and 554 °D (49 days) were calculated between the first and second larval and damage peaks, respectively. One trap per ha in the tobacco fields appeared to be sufficient for indicating adult population peaks. The trapping data obtained with a very high trap density (22 traps/ha) in fields C and D (1978) showed less distinct population peaks in early season when few moths were present.

Survey traps that were maintained in some fields past the 1978-1980 tobacco growing seasons indicated a fourth peak in the adult TBW population in late-August and a fifth peak in early-October, as reported by Jackson et al. (1984). The mean number of °D between the third and fourth adult peaks in fields A (1978) and E (1979) and in fields I and J in 1980 was 553 ±28 (35 ± 3.0 days). Similarly, there was an accumulation of 511 ±3 °D (37 ± 0.5 days) between the fourth and fifth peaks in field J (1980). Prediction of these later population peaks would be of greater importance in crops (e.g. cotton) that have longer growing seasons than tobacco.

We calculated from the 1980-1981 and 1983-1985 trap data a mean of 212 °D (35 days) from the first trap catch of TBW adults in March of each year to the first adult peak, occurring in April (Table 2). Potter et al. (1981) suggested using °D to predict emergence of TBW moths from overwintering pupae. Dependability of predictive models to forecast TBW emergence and population peaks on tobacco or other crops likely could be increased by using °D.

TABLE 1. MEAN CALENDAR DAYS AND ACCUMULATED DEGREE-DAYS (°D) BETWEEN PEAKS OF *HELIOTHIS VIRESCENS* ADULTS (MALES), LARVAE, AND TOBACCO PLANT DAMAGE CAUSED BY *H. VIRESCENS* LARVAE. ALACHUA COUNTY, FLORIDA.

	Days between peaks				°D between peaks			
	1 and 2			2 and 3	1 and 2			2 and 3
Year	Adults	Larvae	Damage	Adults	Adults	Larvae	Damage	Adults
1978[1]	481.2	51 ± 1.6	49 ± 1.5	43 ± 1.5	501 ± 15	604 ± 14	586 ± 19	622 ± 21
1979[2]	52 ± 2.3	50 ± 2.8	52 ± 4.0	38 ± 1.7	559 ± 16	550 ± 22	577 ± 39	531 ± 24
1980[3]	51 ± 1.5	47 ± 2.9	47 ± 2.7	43 ± 0.9	476 ± 16	496 ± 9	498 ± 21	597 ± 24
Mean ± S.E.	50 ± 1.2	49 ± 1.2	49 ± 1.5	41 ± 1.7	512 ± 25	550 ± 31	554 ± 28	583 ± 27

[1]Fields A-D.
[2]Fields E-H.
[3]Fields I-L; survey traps placed in fields I and L too late in season to detect first adult peak.

TABLE 2. CALENDAR DAYS AND ACCUMULATED DEGREE-DAYS (°D) FROM FIRST TRAP CATCH OF *HELIOTHIS VIRESCENS* MALES IN SPRING UNTIL THE FIRST ADULT POPULATION PEAK IN A TOBACCO GROWING AREA OF ALACHUA COUNTY, FLORIDA. 1980-1981 AND 1983-1985.

Growing season	TBW trap catch dates		°D to first adult peak	
	First moth	First peak	Days	°D
1980	3-13	4-10	28	207
1981	3-20	4-17	28	193
1983	3-4	4-15	42	232
1984	3-14	4-23	40	207
1985	3-9	4-15	37	225
Mean	3-12	4-16	35	212
±S.E.	±3	±2	±3	±7

In summary, cumulative °D between population peaks of TBW adults or larvae in tobacco provide a means for predicting the occurrence of possible future infestations of this pest on tobacco. Similarly, there is a close relationship between the accumulated °D and sequential plant damage peaks in tobacco caused by TBW larvae. Although the variations in °D between peaks were somewhat higher than we would have liked, these variations probably would have been less had the data been collected more frequently than the one to three times per week. Our sample intervals did not permit statistical comparison of °D and calendar days for predicting TBW population and damage peaks. However, the mean °D values presented in Table 1-2 do provide reliable indicators, which can used by crop production consultants and others advising on the control of TBW in tobacco. Therefore, pheromone trap catch data can be used to forecast the occurrence of TBW infestations in tobacco. The timing of larval population peaks and potential damage to tobacco by the TBW also can be predicted at least one generation in advance using °D data. Thus, pest management advisors can decide in advance the necessity for damage monitoring and the type of control needed. Optimal timing of chemical control can be determined by using pheromone trap data and °D summations as have been reported for the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (Gargiullo et al. 1985). The possible reduction in the quantity of pesticide used and the frequency of application now required to control TBW in tobacco also would enhance opportunities for integrating biological control strategies with conventional methods of control.

ACKNOWLEDGMENTS

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SCIENTIFIC NOTES

DEVELOPMENT OF *HELIOTHIS VIRESCENS* (LEPIDOPTERA: NOCTUIDAE) LARVAE AFTER PARASITIZATION BY *MICROPLITIS CROCEIPES* AND *MICROPLITIS DEMOLITOR* (HYMENOPTERA: BRACONIDAE)

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Microplitis croceipes (Cresson) is an important parasitoid of *Heliothis* spp. in the U.S.; *M. demolitor* Wilkinson was imported into the U.S. from Australia (Shepard et al. 1983). The following study was conducted primarily to determine how many times *Heliothis virescens* (F.) larvae molt after being parasitized by *Microplitis* spp.

Heliothis virescens larvae were reared in plastic cups (25-ml) on an artificial insect diet (King & Hartley, 1985). Adult parasitoids were held in wooden cages (0.14 cu m) with Plexiglas® tops and ventilated in the back with organdy fabric. Undiluted honey was streaked on the top of the cage as a food source, and a water source was provided by means of soaked cotton balls held in plastic cups (25-ml). Rearing methods for the parasitoid colony are detailed by Powell & Hartley (1987).

Host larvae were parasitized by exposing them singly on a small brush to several (15-20) female parasitoids (1-5 d-old) held in a plastic container (500-ml). Use of two to four such containers was rotated during manual exposure of hosts to wasps. This method improved the likelihood that each larva was parasitized because oviposition was observed. Multiple ovipositions in the same host were not allowed. Determining the occurrence of molting was facilitated by lightly powdering each host's entire cuticle with Day-glo® fluorescent pigment (DAY-GLO Color Corp., 4515 St. Clair Ave., Cleveland, OH 44103); when larvae molted, the dye was cast with the exuviae.

While in the first instar (ca. 24-h old), 36 host larvae were exposed for parasitization to *M. croceipes* and 36 were exposed to *M. demolitor*. The same number and procedure were used for three additional groups of larvae when they reached the early second, third, and fourth instars. In addition, 36 non-parasitized larvae for each instar group were held as controls and treated in the same manner as test larvae. Larvae were checked during morning hours to observe the occurrence of molting. When molting was observed, control and test larvae were re-powdered and developmental times (to the nearest day) between molts were recorded. Each larva was held individually in plastic cups containing diet (total of 36 control and 144 test larvae).

Studies were conducted at 26 ± 2°C, 60 ± 10% RH, and a photoperiod of 15L:9D. Data were analyzed using a least squares analysis of variance procedure with an *a priori* significance level (α) of 0.01.

The percentage of *H. virescens* larvae developing to different instars after parasitization by *M. croceipes* and *M. demolitor* is illustrated in Fig. 1. When host larvae were in later instars at the time of parasitization by either *Microplitis* spp., they molted fewer times than larvae parasitized as earlier instars. For example, 94% of the hosts exposed to *M. demolitor* in the second instar molted twice more to develop to the fourth instar, while 93% of those exposed in the fourth instar molted only once more. The

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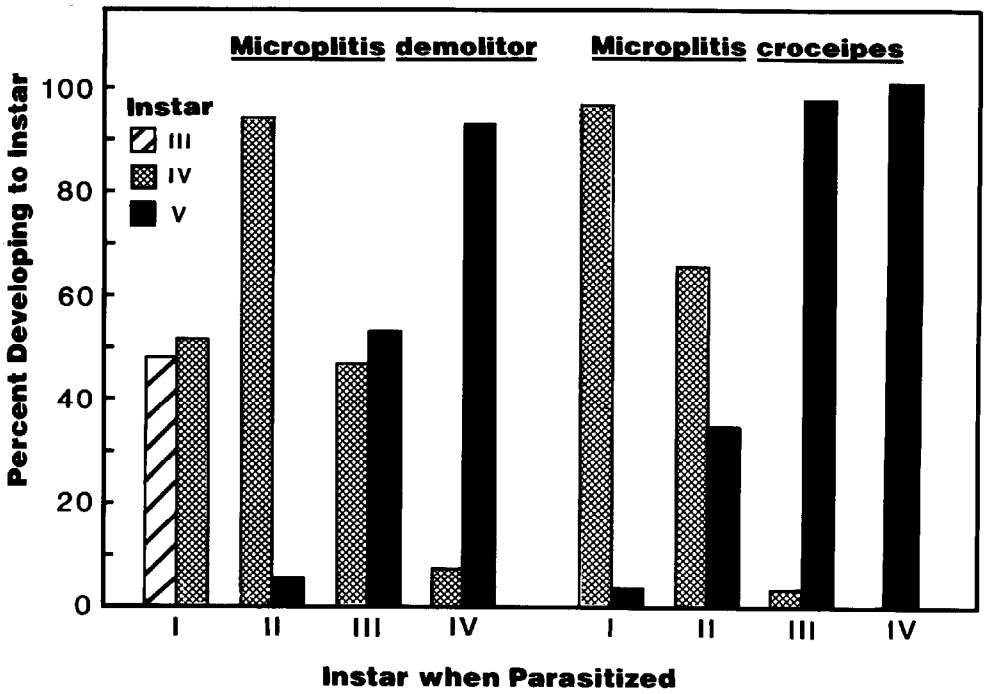

Fig 1. Percentage of *Heliothis virescens* (F.) larvae with final development to the third, fourth, or fifth instar after parasitization by *Microplitis demolitor* Wilkinson or *M. croceipes* (Cresson) while in the first, second, third, or fourth instar.

results reported here for *M. croceipes* generally agree with those of Lewis (1970). The host generally is known to have five instars, and parasitization did not result in a greater number of instars. Host larvae parasitized by *M. croceipes* showed a sharp decline in number of larvae developing only to the fourth instar, and an increase in number of those developing to the fifth instar. A greater percentage of larvae in first and second instars that were parasitized by *M. croceipes* reached the fifth instar than did first and second instars parasitized by *M. demolitor*. Thus, *M. demolitor* was more efficient than *M. croceipes* in preventing early instars from reaching late instars.

The feeding habits of late instars often are such that they escape the effects of insecticides and/or predators; therefore, preventing development to late instars would be a useful management tool. However, when larvae are parasitized, feeding rates usually are reduced greatly anyway, so the larva's presence in a crop does not necessarily indicate damage. Information on host instar at time of parasitoid pupation would be useful in ecological studies where predicting instar at time of parasitization is important.

Developmental times for *M. demolitor* were significantly faster ($P<0.01$) for *H. virescens* larvae parasitized in the first and second than third and fourth instars (Table 1). *Microplitis croceipes* tended to develop fastest in larvae parasitized in the third instar, agreeing with data of Hopper & King (1984). They found developmental time for *M. croceipes* to be fastest in the preferred third instar. For a thorough discussion of preference, acceptance, and fitness components of *M. croceipes* attacking *H. virescens*, see Hopper (1986). *Heliothis virescens* larvae held as unparasitized controls developed to pupae in ca. 13 d (Table 1). No differences were expected among the four groups of non-parasitized larvae; pooled data yields 13.1 ± 0.1 d (n = 120 larvae).

A closer look at developmental times when host larvae were exposed in different instars reveals that as the number of molts increases from one to two to three, the

TABLE 1. MEAN TOTAL DEVELOPMENTAL TIMES (D) OF *MICROPLITIS DEMOLITOR* AND *M. CROCEIPES* PARASITOIDS (N = NUMBER) FROM TIME OF EXPOSURE OF *HELIOTHIS VIRESCENS* HOSTS IN THE FIRST, SECOND, THIRD, OR FOURTH INSTAR TO PUPATION AND COCOON FORMATION, AND OF NON-PARASITIZED *H. VIRESCENS*.[a]

	Host instar when exposed to parasitoid							
	First		Second		Third		Fourth	
Treatment	n	$\bar{x} \pm SE$	n	$\bar{x} \pm SE$	n	$\bar{x} \pm SE$	n	$\bar{x} \pm SE$
M. demolitor	33	7.4 + 0.1 a	35	7.2 + 0.1 a	34	7.9 + 0.1 b	27	8.6 + 0.1 c
M. croceipes	31	8.5 + 0.1 a	32	8.2 + 0.1 ab	33	8.0 + 0.1 b	33	8.3 + 0.1 ab
Non-parasitized[b]	32	13.2 + 0.1 a	26	12.9 + 0.1 a	32	13.1 + 0.1 a	30	13.1 + 0.1 a

[a]Means followed by the same letter within a row are not significantly different ($P<0.01$).
[b]Mean time for non-parasitized larvae with data pooled = 13.1 ± 0.1 d.

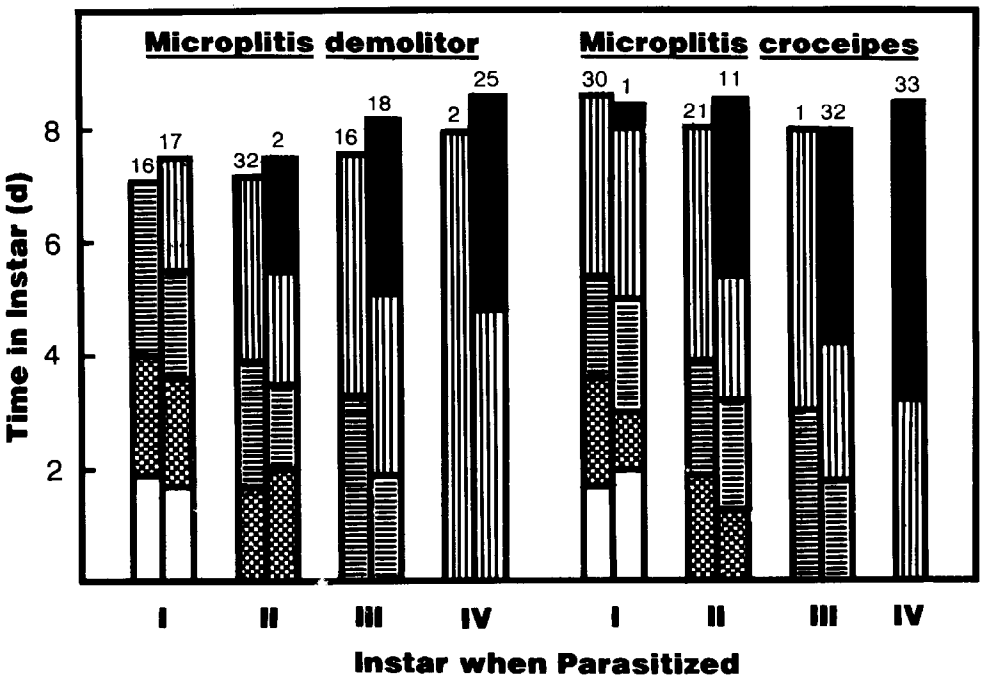

Fig. 2. Cumulative developmental times to pupation of *Microplitis demolitor* Wilkinson and *M. croceipes* (Cresson) when *Heliothis virescens* (F.) was parasitized in instars I through IV. Each bar is divided to show ca. times the host spent in different instars (I—no pattern, II—checkered, III/horizontal lines, IV—vertical lines, and V-solid). The number above each column indicates the number of observations.

average time spent per instar decreases respectively, from 4.1, to 2.5, to 1.9 d (n = 126 obs.) for *M. demolitor*, and from 4.1, to 2.7, to 2.1 d (n = 128 obs.) for *M. croceipes* (Fig. 2). In extreme cases, two fourth instar hosts exposed to *M. demolitor* produced parasitoid cocoons without molting; and one first instar exposed to *M. croceipes* molted four times, and produced a parasitoid pupa shortly after the last molt. I thank M. M. Ford and C. S. Jany for assisting with this study and Day-glo Color Corp. for providing the fluorescent pigment.

Endnotes

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agric. and does not imply its approval to the exclusion of other products that may also be suitable.

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GATHERING OF FUNGAL HONEYDEW BY *POLISTES* SPP. (HYMENOPTERA: VESPIDAE) AND POTENTIAL TRANSMISSION OF THE CAUSAL ERGOT FUNGUS

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Ergot honeydew is a sweet, viscous exudate found on flowering cereal heads infected by *Claviceps* spp. (Ascomycetes). The honeydew is a complex mixture of mono-, di- and oligosaccharides resulting from fungal conversion of host phloem constituents (Mower & Hancock 1975a,b). Infective fungal conidia are protected from desiccation by the exudate (Mower & Hancock 1975a), which also assists in dissemination of the fungus by attracting insects that feed on it (Langdon & Champ 1954, Moreno et al. 1971, Carter 1973).

Many insect species are known or suspected vectors of ergot conidia. Diptera and Coleoptera comprise the majority of insects on record (Atanasoff 1920), although other orders, including Hemiptera, Lepidoptera and Hymenoptera have been reported also (Atanasoff 1920, Langdon & Champ 1954, Parris & Moore 1961, Mongolkiti et al. 1969, Moreno et al. 1971, Sharma et al. 1983). The present report describes foraging behavior of several species of *Polistes*, confirms their exploitation of ergot honeydew as a nutritional resource and implicates these wasps as potential vectors of the fungus.

Observations were made from 14 July to 18 August 1987, in a five-ha field of mixed grasses in Baton Rouge, Louisiana, between 1600 and 2000 hours at temperatures of 27° to 32° C. Fifty individuals were observed. The majority were *Polistes fuscatus* (F.) and *Polistes metricus* Say (n = 40), but *Polistes dorsalis* (F.) (n = 10) also were recorded. Observation periods for individual wasps rarely exceded 10 min and were terminated by observer intervention or by flight of the wasp beyond following distance. Individuals of all three species were seen collecting droplets of honeydew on infected florets of dallis grass, *Paspalum dilatatum* Poir, which was abundant in the field and had a high incidence of infection by *Claviceps paspali* F. L. Stevens & J. G. Hall. Infection was determined by visual inspection of florets for the presence of honeydew droplets. Similar foraging activities on inflorescences of vasey grass, *Paspalum urvillei* Steud., and bahia grass, *Paspalum notatum* Flugge, led to observations of honeydew-producing fungal infections on these grasses as well. Individual wasps alternated freely between these grasses while collecting honeydew during a single observation period. All three grasses are reported hosts for *C. paspali* (USDA 1960).

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Wasp foraging behaviors appeared to be focused exclusively on locating and collecting ergot honeydew. Typical foraging activities began with flight to and contact with infected grass heads. Wasps often landed at the top of a floret and walked downward in a spiraling pattern, imbibing honeydew as it was encountered. Movement to adjacent florets on the same plant was accomplished by crawling or by short, hopping flights. Occasionally, individuals were seen to carry droplets in their mouthparts during flight.

Wasps quickly differentiated infected, uninfected and infected-senescing inflorescences, often prior to contact. Only active infections elicited honeydew-searching behaviors. Between periods of active foraging, individuals always flew to grass heads bearing no sign of infection or to vasey grass leaf sheaths and groomed antennae, eyes, forelegs and gaster extensively. With one exception, grooming behavior was observed only on uninfected grasses.

Polistes are known to utilize considerable quantities of carbohydrate-rich liquids in nest cell provisioning and larval rearing (Rau 1928, Rabb 1960). Sources of carbohydrates reported include floral and extrafloral nectar and aphid honeydew (Rabb 1960). Rabb (1960) also reported heavy foraging of dallis grass heads by *Polistes* spp. in July and August but made no mention of accompanying fungal infection, although his observations typify those reported here.

Eight individuals, six *P. fuscatus* or *P. metricus* and two *P. dorsalis*, collected while foraging on infected grasses, were examined microscopically for the presence of fungal conidia. Conidia were confirmed from each individual on the antennae, tarsi, mouthparts and in the gut. Conidial presence suggests that these wasps might readily transmit the ergot fungus from infected to uninfected grasses. Langdon & Champ (1954) reported *C. paspali* transmission to dallis grass by the green fly, *Pyrellia coerulea* (Wied.) and found fungal inoculum both externally and in the gut of these flies. In a controlled study, Moreno et al. (1971) confirmed transmission of *Claviceps purpurea* (FR.) by adult cabbage looper, *Trichoplusia ni* (Hubner), in barley.

By selecting only uninfected grasses as grooming sites, *Polistes* increase the likelihood of successfully spreading fungal infection. Honeydew foraging activity by individuals that visit several grass species also would promote greater host distribution of the fungus.

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DAILY AND SEASONAL FLIGHT OF MALE *PARANTHRENE DOLLI* (LEPIDOPTERA: SESIIDAE), MONITORED BY PHEROMONE TRAPS

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Interest in the management and culture of cottonwood, *Populus deltoides* (Bartr.), has drawn attention to one of its major pests, generally known as the cottonwood clearwing borer, *Paranthrene dollii* (Neum.). In nurseries, the larvae damage the stools and switches of cottonwood by boring into the wood and pith, and constructing open tunnels up to 16 cm in length (Solomon & Abrahamson 1972). Culling of potentially usable cuttings in cottonwood nurseries has ranged from 12% (Solomon et al. 1976) to 20% (Nebeker 1980-unpublished data) because of *P. dollii* damage. Current control methods for *P. dollii* involve insecticide applications and mechanical means, such as removal and burning of infested stools, switches and cuttings. Additional information on the biology and behavior of the species is needed to aid in the development of pest management program in cottonwood nurseries. Therefore, this study was conducted to determine the daily and seasonal flight periods of adult males.

Flight periods of males in 1980 and 1981 and daily flight activity in 1981 were determined by using pheromone traps in Mississippi. Pherocon 1-C® traps (Zoecon Corp., Palo Alto, Calif.) were baited with male sex attractants (Nielsen et al. 1979), (*Z*,*Z*)-3,13-octadecadien-1-ol (ZZ-ODDOH) and (*E*,*Z*)-3-13-octadecadien-1-ol (EZ-ODDOH). the two alcohol isomers were prepared separately at a concentration of 2 mg/ml of hexane, and then blended at a 1:3 ratio of ZZ-ODDOH and EZ-ODDOH. Aliquots of 0.25 ml of the prepared mixture were impregnated into rubber stopper (no. 8753-DD2, A. H.

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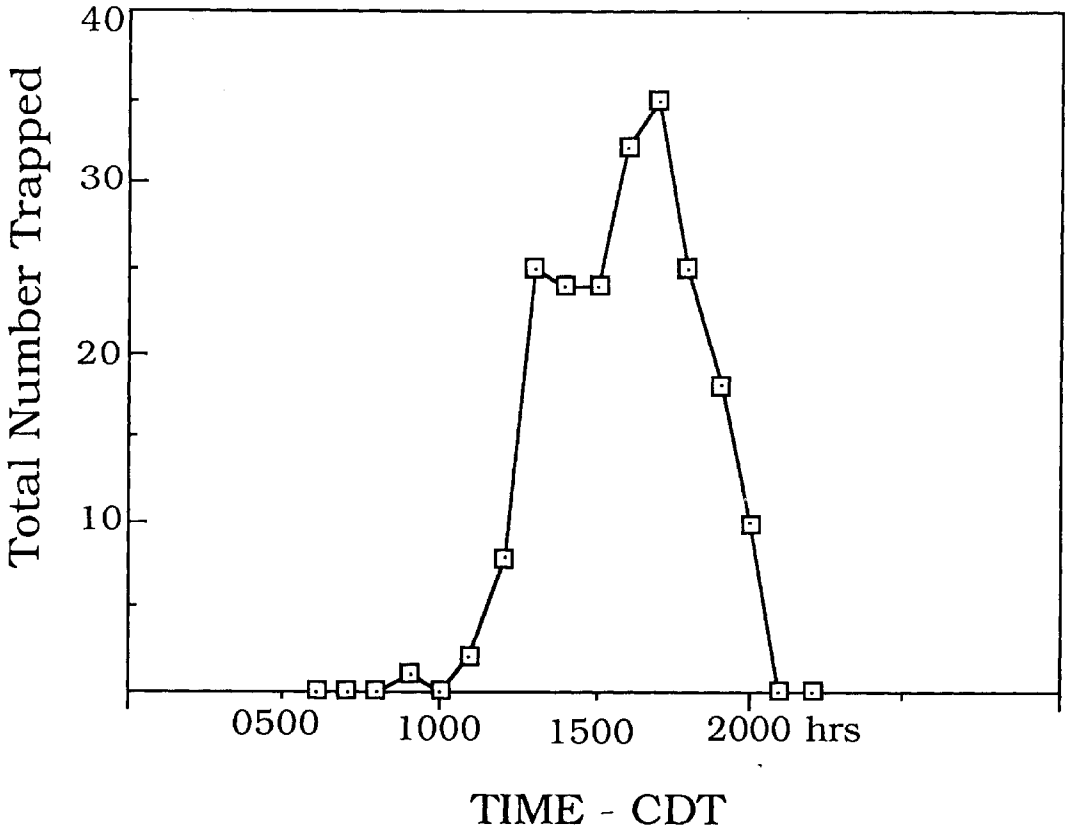

Fig. 1. Hourly flight activity of male *Paranthrene dollii* monitored at the Mississippi Forestry Commission Nursery, Winona, MS. on May 4-5 and June 6-7, 1981. CDT = Central Daylight Time.

Thomas, Co.) dispensers and were suspended by paper clips from the inside top of the Pherocon traps about 2 cm above the sticky liner.

Baited traps were suspended at 1.5 and 0.75 m heights on wooden stakes and uniformly distributed (average 60 m apart within the cottonwood clonal material) throughout the Mississippi Forestry Commission Nursery, Winona, and at Stoneville MS. They were placed at the respective sites from May 1980 through November 1981 and from May through November 1980. Trap heights were alternated at each visit. Traps were visited twice a week from May to October 1980 and once a week from April to November 1981. Ten traps were monitored at Winona and 2 at Stoneville. The attractant was replaced every 14 days from April through October, and monthly from November through March. During this winter period only 4 traps were monitored at Winona. Moths were removed with forceps, brought to the laboratory, and cleaned with paint thinner to facilitate counting and identification. Trap bottoms (liners) were changed each month or when necessary.

The daily flight activity of males was determined in 1981 (May 4-5 and June 6-7) by hourly trap counts from 0600 to 2200 hrs (CDT) (Fig. 1). Hourly flight studies revealed that male activity began as early as 0900 hr. Flight activity increased sharply at noon, peaked around 1700 hr and ceased by 2100 hr (CDT). No males were captured during the night. The daily rhythm of activity corresponds closely to the time of female calling between 1300-1800 hrs (Eroles-Harkins 1983).

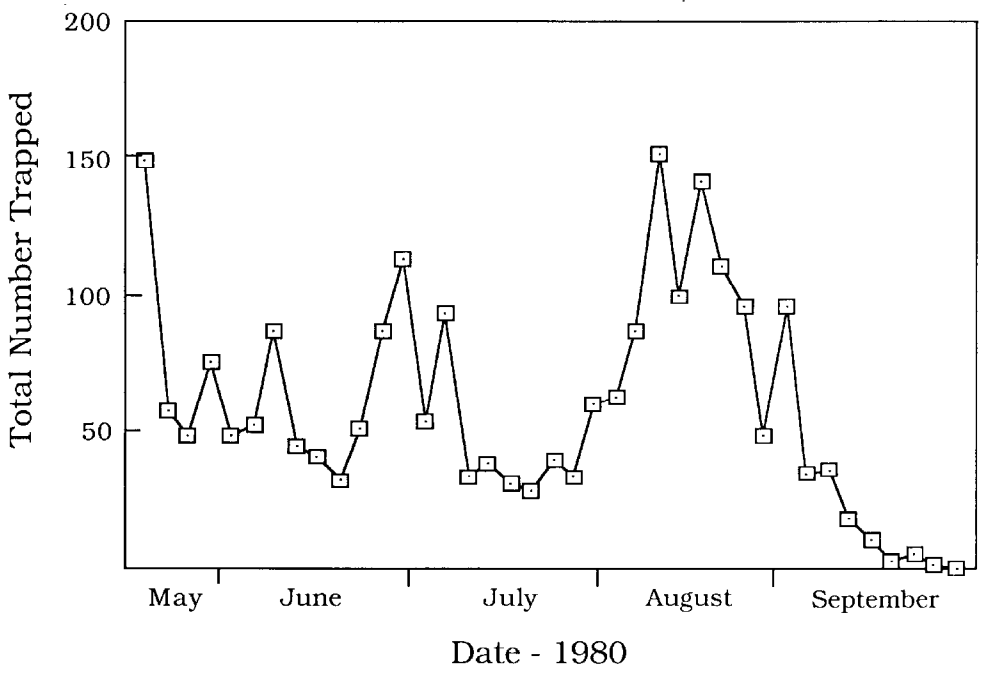

Fig. 2. Seasonal abundance of male *Paranthrene dollii* caught in the pheromone baited traps (10 traps) at the Mississippi Forestry Commission Nursery, Winona, MS.

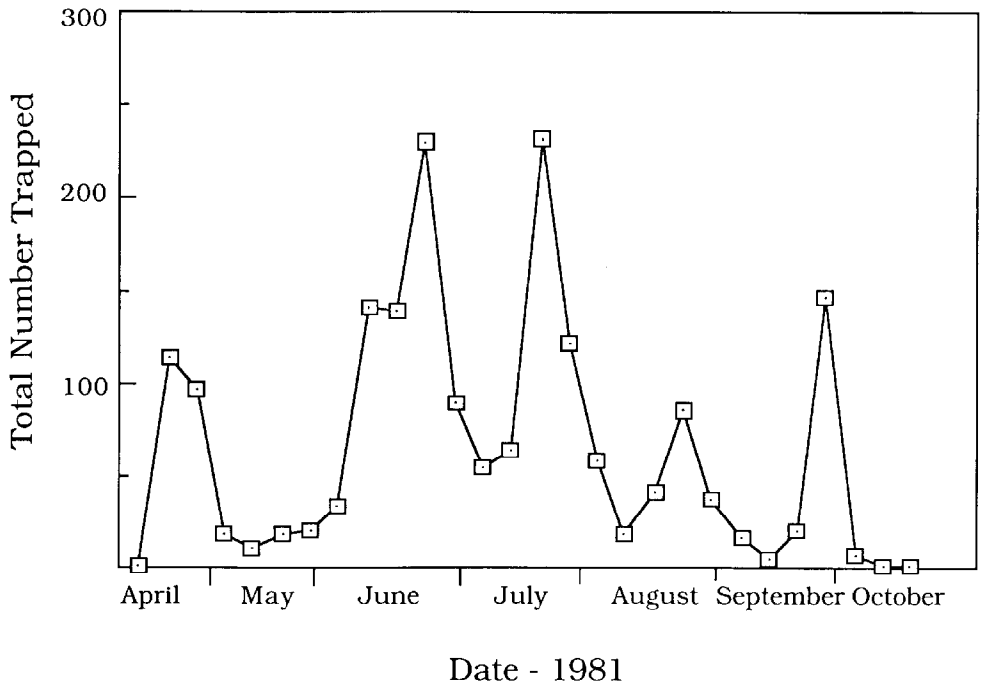

Fig. 3. Seasonal abundance of male *Paranthrene dollii* caught in pheromone baited traps (10 traps) at the Mississippi Forestry Commission Nursery, Winona, MS.

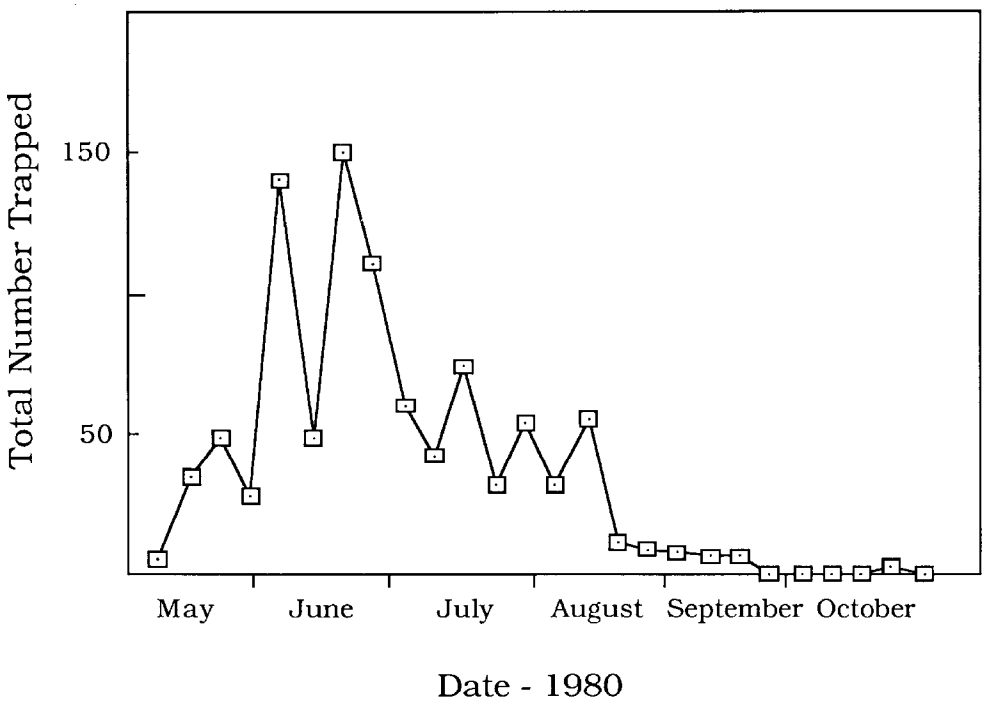

Fig. 4. Seasonal flight of male *Paranthrene dollii* monitored in pheromone baited traps (2 traps) at Stoneville, MS.

A total of 917 males were captured at Stoneville in 1980 and 4,085 males were trapped at Winona, MS during 1980 and 1981. There was no significant difference ($p = 0.05$) between trap catches at the two different heights. Several adult flight peaks were observed from April through October during 1980 and 1981 (Figs. 2-4). Based upon trapping throughout 1981 flight activity of *P. dollii* begins in late March or early April with three or more major seasonal peaks, and ceases in October. Sharp et al. (1978), using pheromone traps, recorded two periods of flight of *P. dollii* in Florida from April through June and again from September to early December. This more closely suggests two generations per year in Florida rather than a 2-year generation suggested by Sharp et al. (1978). We suggest that *P. dollii* has only one generation per year in northern Mississippi. This conclusion is based on the results of this trapping study and the results of Eroles-Harkens (1983). This is not in agreement with Sharp et al. (1978) citing Englehart (1946), where they suggest that *Paranthrene* (Hubner) species have a 2-year or longer life cycle.

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FEEDING BEHAVIOR AND DIETARY SUBSTRATES FOR REARING LARVAE OF THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA*

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Interrelationships between the nutrient and physical composition of larval diets for tephritid fruit flies were first investigated by Mourikis (1965). He discovered that Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), larvae ingest mostly liquid food released from fruit by their mouth hooks and that larvae aggregate at communal feeding sites, presumably to cooperate in liquefying the diet. Many complex diets have since been formulated to mass rear larval fruit flies, such as the Mediterranean fruit fly (Mitchell et al. 1965), *Dacus* spp. (Schroeder et al. 1972), and *Anastrepha* sp. (Spishakoff 1966, Kamasaki et al. 1970). Alternative substrates for these diets have been adopted at different times and places due to the availability of local materials, cost, associated rearing methods and equipment, and assumptions about larval feeding behavior. Consequently, substrates have remained the most variable and undefined of these requirements (Tanaka et al. 1969). The original carrot, sugarcane bagasse, citrus pulp and ground rice hull media have been replaced with diets based on wheat plus an acrylic polymer thickening agent (Tanaka et al. 1969), cottonseed flour with or without a fibrous nylon substrate (Schroeder et al. 1970, 1971), corn cob grits or agar (Burditt et al. 1975), sawdust (Aliniazee & Brown 1977), wheat (Barnes 1979), wheat and soy flour (Schwarz et al. 1985), and corn meal plus agar (Zucoloto 1987). Vargas et al. (1983) compared sugarcane and sugarbeet bagasse, and wheat mill feed as dietary substrates for mass rearing Mediterranean fruit fly larvae. They found no differences in the yields of pupae, although those from sugarbeet bagasse diet were significantly lighter. We conducted this study to determine if larvae of the Caribbean fruit fly, *A. suspensa* (Loew), shred the dietary substrate and ingest particles, and if they can reared on a liquid diet supported by tissue paper, artificial sponge, or powdered cellulose.

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Caribbean fruit fly eggs were derived from a colony established at the Insect Attractants, Behavior and Basic Biology Research Laboratory in 1973 and reared on a corn cob grits diet (Greany et al. 1976). For our experiments, adults were allowed to oviposit for 5 to 6 h periods and the eggs were collected in a 6-oz plastic container by washing them from the substrate with 0.03% sodium benzoate solution. The eggs were rinsed several times with the same solution to remove debris, pipetted onto black filter paper moistened with the solution and incubated for 48 h at 26 ± 1°C. Filter paper supporting 100 embryonated eggs was placed directly in each container of the diet or, after at least 80% of the eggs hatched, 100, 1- to 6-h-old neonate larvae were transferred with a camel's hair brush. All larvae were reared at 26 ± 1°C and 65 ± 15% RH with a 12 h photophase. Pupae were held for emergence in moist vermiculite.

The basic diet was composed of water (400 ml), corn cob grits (Grit-O-Cobs; grade 2040; The Andersons', Maumee, OH 43537; 137 g), sugar (30 g), torula yeast (20 g), raw wheatgerm (15 g), methyl-*p*-hydroxybenzoate (0.7 g), sodium benzoate (0.5 g) and 12 N HCl (2.5 ml). Diet was prepared by first dissolving the sodium benzoate in the water, adding the acid and pouring in the methyl-*p*-hydroxybenzoate dissolved in 5 ml of ethanol. Next, the sugar, finely ground wheatgerm and yeast were added successively. The mixture was then poured into a 4-liter mixer and combined with the corn cob grits. The ingredients were mixed for 5 min at low speed, checked for a pH of 3.5 and dispensed into rearing containers.

Feeding behavior was difficult to observe in the standard corn cob grits diet because the larvae burrowed beneath the surface. Consequently, diets made of white facial tissue dyed with a few drops of red food coloring or commercially available blue and green Kleenex® tissue paper were used. The tissue was shredded in a blender, combined with the other ingredients as before, and dispensed into 15 x 100 mm diam clear plastic petri dishes. Methods used to prepare this diet were similar to those developed for rearing larvae of the apple maggot, *Rhagoletis pomonella* (Walsh), (Neilson 1969) and sorghum shoot fly, *Atherigona soccata* Rondani, (Singh et al. 1983). Each dish received 100, 5- to 6-h-old neonate larvae that were observed from 2 to 5 h after lights-on every day throughout their development. These observations were facilitated by using a color video camera mounted on a stereo microscope.

To test alternative dietary substrates, the corn cob grits were replaced with tissue paper (50 g), cellulose (α-cellulose; Sigma Chemical Co., St. Louis, MO 63178; 150 g), artificial sponge or simply the diet-coated container walls (no dietary substrate). Preliminary evaluations were conducted in 100 ml Erlenmeyer flasks plugged with cotton after being filled with 25 ml of diet and 100 neonate larvae. Final tests were made using 140 x 190 x 20 mm plastic trays containing the filter paper with embryonated eggs. Data were combined from 4 tests, each replicated 3 times per substrate. Parameters included the rate of larval development, number of pupae recovered, individual weights of 120 pupae per substrate (10 per replicate at 2-3 days after recovery), adult emergence and a subjective evaluation of their quality. Means (± SD) were compared by analysis of variance ($p = 0.05$).

The addition of food coloring or use of dyed tissue paper had no apparent effect on larval developmental rates, pupal yields, pupal weights, adult emergence or fecundity. Video recordings indicated that, regardless of source, color was present in the 2nd instar but was most intense during the 3rd instar on day 5. Dyes produced brightly colored larvae, each with an easily visible digestive tract but no color was evident in the tissue or fat body. Prepupae, pupae and adults were colorless, probably because the dyes were excreted (Sharp & Ashley 1984).

Caribbean fruit fly larvae ingested both liquids and solid particles. This was accomplished by means of a feeding mechanism similar to that of the Mediterranean fruit fly (Mourikas 1965). We observed that neonate larva obtain food from the surface of the

diet, perhaps because they lack the anterior stigmatic plates and spiracles developed in the second instar that would allow them to remain partially submersed. By the third instar, fully developed larvae burrowed throughout the diet. All larval stages fed by means of highly sclerotized, paired mouth hooks that were cast back and forth to shred the medium. Ingestible liquids and particles were directed into the suctioning pharynx, a process presumably assisted by the secretion of saliva. Larvae fed on the diet without a supporting substrate but they apparently could not pupate, since most of them drowned during the prepupal stage.

Larvae reared on the paper, sponge, alphacel and corn cob grits diets had similar developmental rates, pupal yields, pupal weights and adult yields. The first pupae appeared on days 7 and 8, and most of the larvae pupated by day 10. Mean (±SD) pupal yields and weights were not significantly different on the respective diets. Yields averaged 34±18, 21±17, 41±30 and 41±30%, and the weights were 16.5±0.6, 15.0±0.9, 13.5±2.8 and 14.9±0.7 mg. Adult yields from these pupae were 89±9, 76±17, 74±17 and 85±9%; significantly higher for paper and corn cob grits.

The frequency distribution in weight categories ranging from 12-20 mg of 120 pupae from each diet indicated that all substrates produced a reasonable proportion of 14-16 mg pupae (Fig. 1). However, the alphacel diet resulted in significantly smaller and the paper larger pupae. The respective diets produced 1, 14, 27 and 7 pupae less than 12 mg. Using 14 mg as a threshold, 96, 72, 63 and 75% of the pupae from the respective diets were of acceptable weight. This is realistic, since Leppla et al. (1976) and Greany et al. (1976) repectively produced pupae with statistically equivalent mean weights of 12.7±1.7 mg and 13.2±0.2 mg on a sugarcane bagasse diet.

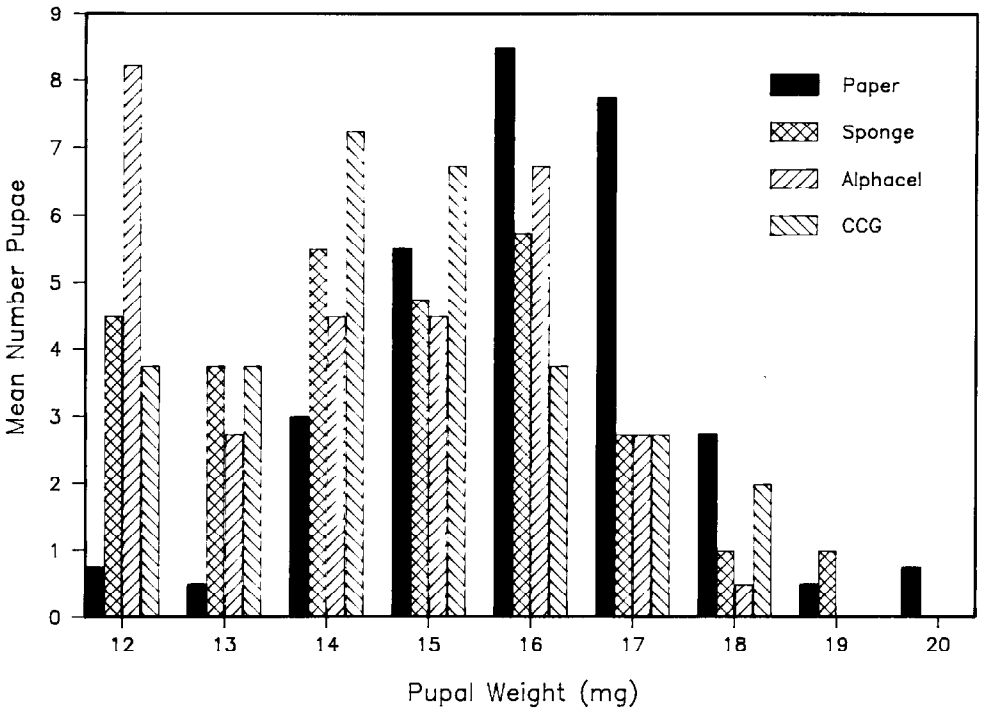

Fig. 1. Frequency distribution of the weights of Caribbean fruit fly pupae from larvae reared using tissue paper, sponge, α-cellulose or corn cob grits as dietary substrates. Of 120 pupae randomly sampled from each diet, 119, 106, 93 and 113, respectively, weighed 12-20 mg.

We conclude that Caribbean fruit fly larvae shred their dietary medium and perhaps require both liquid and particulate food. Ingestible substrates seem to produce larger pupae and increase survival (Zucoloto 1987), so nutritionally inert particles may be important for the regulation of feeding, digestion and absorbtion (Bignell 1978). Remarkably, Carroll (1986) found that Caribbean fruit fly larvae could survive and develop into reproductive adults on a diet of conspecific eggs, neonate larvae and probably associated microorganisms. Thus, virtually any substrate may be used that supports the larvae and diet, remains free of contamination and is practical. It is also desirable for the substrate to be inexpensive, readily available, easy to store and use, consistent, defined in composition and disposable or recycleable. Selection of a substrate for rearing fruit fly larvae, as with any other dietary ingredient, depends more on the quality of fly produced than on economics or expediency.

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SEASONAL OCCURRENCE OF *GNOPHOTHRIPS FUSCUS* (THYSANOPTERA: PHLAEOTHRIPIDAE) ON SLASH PINE IN FLORIDA

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Adults of the slash pine flower thrips, *Gnophothrips fuscus* (Morgan), feed from Dec-Feb on female slash pine, *Pinus elliottii* Engelm. var. *elliottii*, flowers, so reducing potential seed yields (Ebel 1961, DeBarr 1969, Hedlin et al. 1980). Adults also have been observed from Apr-Sep on current-year's shoots of slash pines of all ages and within the needle fascicles of seedlings (Hedlin et al. 1980). The immature life stages of *G. fuscus* have been described by Ranasinghe et al. (1985). We report on the occurrence of all life stages on young slash pine seedlings and of flying adults within the crowns of older (cone-bearing) slash pine trees in NE Florida.

One hundred young (2-5-yr old) slash pine seedlings growing in the understory of open-grown, mixed slash and longleaf, *P. palustris* Mill., pine trees in a seed production area near Olustee, FL were sampled from Apr 1979-May 1980. At 1-2-week intervals from Apr-Sep and monthly otherwise, an entire current year's (or most recently formed) shoot was collected from each of 15 randomly-selected seedlings. The numbers of thrips life stages present per 15-shoot sample, determined by microscopic examination, were plotted against collection dates.

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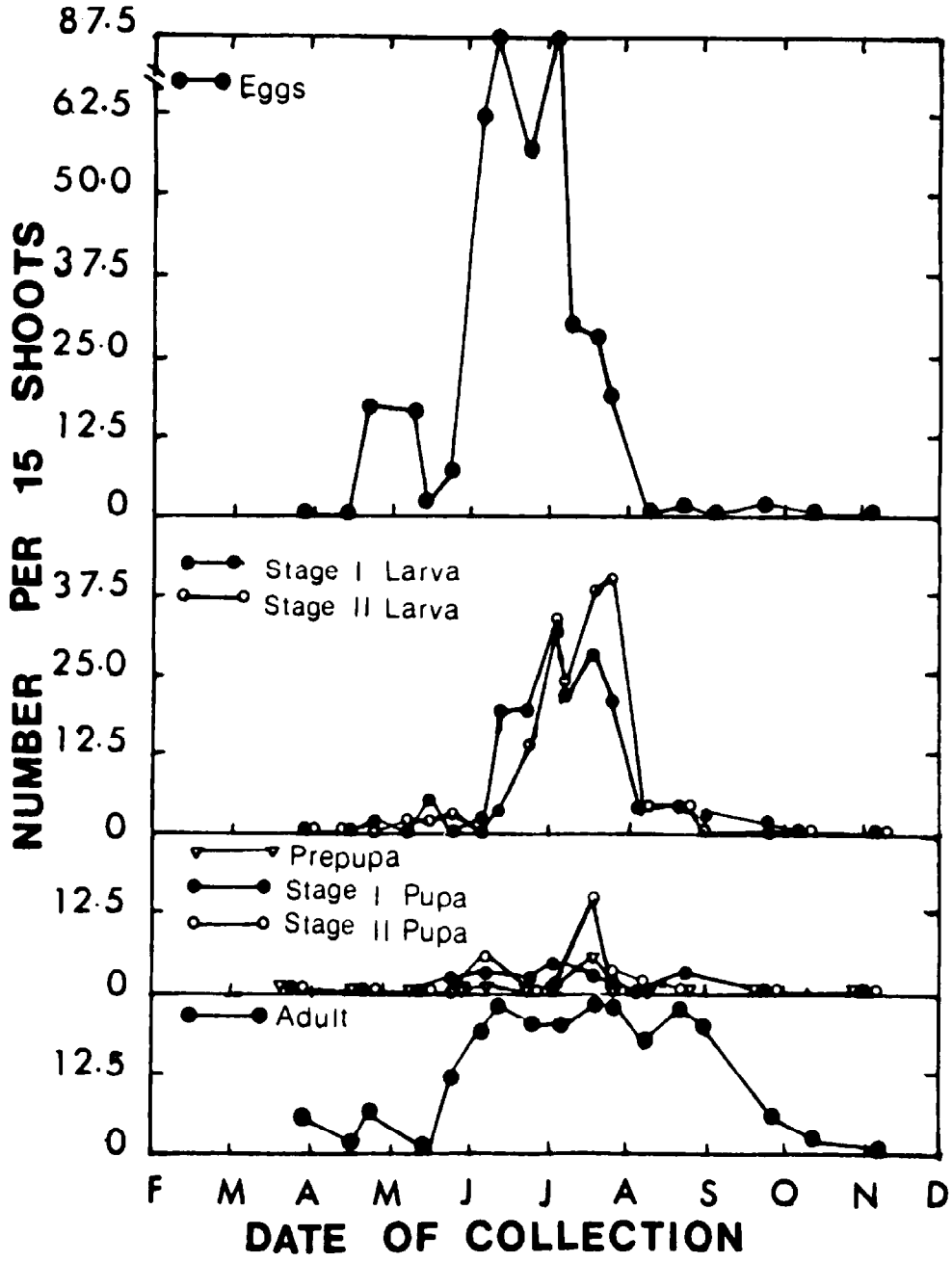

Fig. 1. Seasonal abundance of *G. fuscus* life stages on shoots of young slash pine seedlings near Olustee, FL from April 1979-May 1980. Note: Numbers of specimens shown in April and May are averages for 1979 and 1980.

The crowns of six older (17-20-yr old) slash pine trees growing in the overstory at the same site as the seedlings were divided into N, E, S and W quadrants. Sticky traps (yellow plastic discs 15-cm dia sprayed on one side with Tanglefoot® insect adhesive) were used to sample flying *G. fuscus* adults. One trap was suspended from a shoot in the middle of each quadrant in the upper crown by means of a thin nylon cord which permitted the disc to rotate in the wind. Exposed traps were replaced with fresh ones

monthly and trapping was conducted from Dec 1977-Nov 1979 (total of 1 trap x 4 quadrants x 6 trees = 24 traps per month). Mean monthly catches were plotted against collection dates and weather station air temperatures at nearby Lake City. Data were analyzed to determine if there were significant differences in the number of *G. fuscus* adults trapped among seasons, years, and quadrants (FUNCAT procedure, SAS Institute 1982).

On young slash pine seedlings, all stages of *G. fuscus* were collected in small numbers during spring (22 Mar-21 Jun), the numbers peaked in the summer (22 Jun-21 Sep), and declined during fall (22 Sep-21 Dec). None was found during the winter (22 Dec-21 Mar) (Fig. 1). Mean numbers collected per 15-shoot sample were 12.4 adults, 25.0 eggs, 9.0 stage-I larvae, 9.5 stage-II larvae, 0.5 prepupae, 1.8 stage-I pupae, and 1.9 stage-II pupae, respectively. An estimated three generations of *G. fuscus* can occur per growing season in NE Florida, based on laboratory rearing experience (Ranasinghe 1981).

All *G. fuscus* adults trapped within the crowns of older trees were macropterous. Mean catches were lowest during winter, peaked in the spring, and declined during the summer and fall. (Fig 2). Low catches coincided with colder winter temperatures and reported feeding activity on female slash pine flowers, suggesting that adults overwinter and feed on flowers within the crown of a given tree. Macropterous adults perhaps migrate to breed on fresh host material during warmer weather (Fig 2). Lewis (1973) reports similar behavior of many species of thrips. There were significant differ-

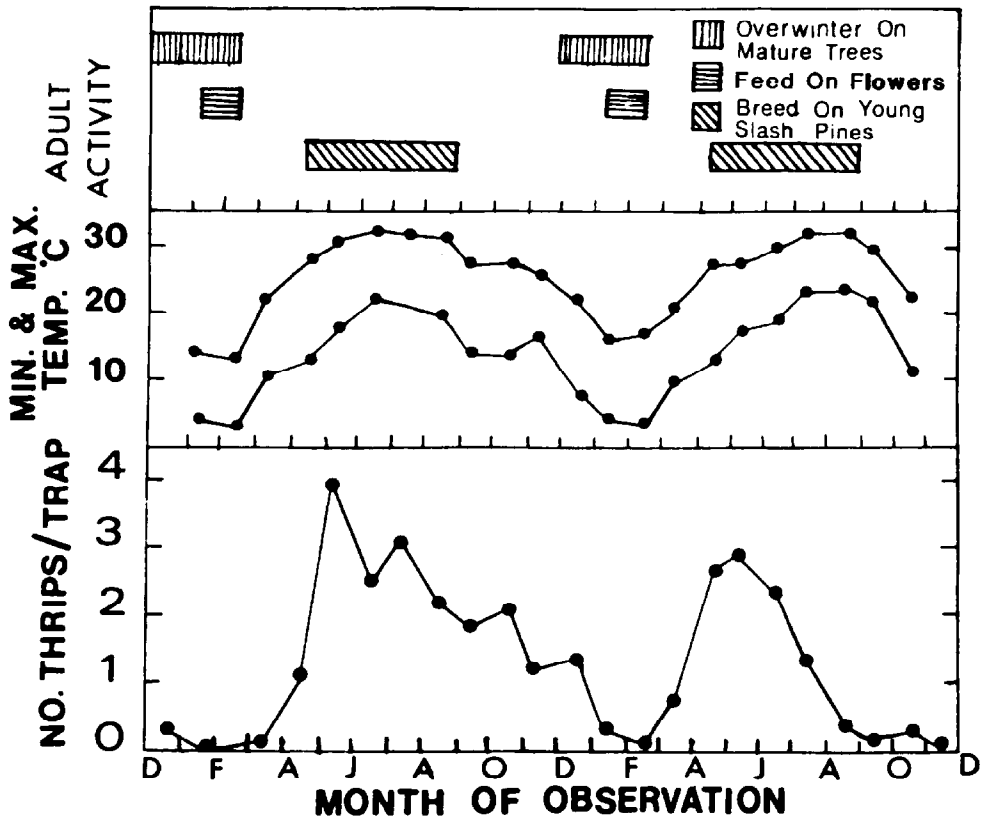

Fig. 2. Activities of *G. fuscus* adults (top), mean monthly air temperatures (middle) and mean monthly catches of *G. fuscus* adults per trap (bottom) in 24 sticky traps hung in the crowns of 6 cone-bearing slash pines near Olustee, FL from December 1977-November 1979.

ences (χ^2 = 79.42***, with 3 df) among seasons in the probability of a trap having a catch, but the year x season interaction also was significant (χ^2 = 57.15***, with 3 df). There were no such significant differences (χ^2 = 0.95, n.s. for 3 df) among quadrants, in agreement with DeBarr et al. (1975) who found no significant differences in the numbers of *G. fuscus*-attacked female slash pine conelets among quadrants. Further research is needed to determine seasonal changes in the density of this thrips in managed slash pine seed orchards.

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A MITE, *OGMOTARSONEMUS EREPSIS* (TARSONEMIDAE), IN OVIPOSITION INCISIONS OF THE PLANTHOPPER *PROKELISIA MARGINATA* (HOMOPTERA, DELPHACIDAE) ON THE SALTMARSH CORDGRASS, *SPARTINA ALTERNIFLORA*

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The upper surfaces of leaves of saltmarsh cordgrass (*Spartina alterniflora* Loisel) on the shores of the northern Gulf of Mexico frequently bear distinctive brown wounds. The largest of these wounds are deep and visible through the leaf, from the lower, abaxial, surface. These wounds are more frequent and larger on older, basal, leaves of

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The upper surfaces of leaves of saltmarsh cordgrass (*Spartina alterniflora* Loisel) on the shores of the northern Gulf of Mexico frequently bear distinctive brown wounds. The largest of these wounds are deep and visible through the leaf, from the lower, abaxial, surface. These wounds are more frequent and larger on older, basal, leaves of

cordgrass. Large wounds are made up of anastomosed patches of small linear wounds. Although many sorts of damage can cause cordgrass leaves to turn brown, most natural browning is caused by the mite *Ogmotarsonemus erepsis* Lindquist (Lindquist 1986). This mite is concentrated within healthy cordgrass leaves, and the only access to the interior is through a break in the leaf cuticle. Most access to the interior of green leaves in nature is via linear oviposition incisions made by the planthopper *Prokelisia marginata*. Incisions are made between the longitudinal ridges on the leaf. Leaves bearing no hopper incisions have no mites inside and no linear brown wounds. Mites lay eggs within a few days of colonizing an incision, but do not spread their activities more than a few millimeters from the incision. Isolated incisions have only small isolated brown areas, which do not grow larger in the absence of additional oviposition incisions by the planthopper.

Basal cordgrass leaves can remain green and healthy for several months in nature and accumulate a large number of hopper ovipositions. At the base of an old leaf, dense aggregations of spots will anastomose into a large blotch of brown, which grades into a narrower scattering of spots 2 or 3 cm up the leaf (Fig. 1). In heavy aggregrations of hopper eggs, the cuticle becomes so tattered that the leaf laminae sag apart, allowing the mites freer access to leaf tissue and spreading the browning more broadly than around isolated incisions.

Mite-free cordgrass can be produced from corms that have had dead leaves removed before potting in the greenhouse. As well, mites can become extinct from greenhouse cordgrass that has had old leaves removed promptly upon the first signs of senescence.

Mites have no apparent effect upon planthopper eggs, which have very high hatching rates in both the presence and the absence of mites. Similarly, neither obvious benefit nor risk to the egg parasitoid of the planthopper, *Anagrus delicatus* Dozier (Mymaridae) (Stiling & Strong 1982, 1983), is caused by the mite. Any difference in attractiveness to wasps between browned and mite-free oviposition sites must be slight, because eggs are usually parasitized before mites have much effect on incisions.

Oviposition incisions of the planthopper turn brown only after being invaded by the mite. Even very high egg densities of the planthopper cause no leaf discoloration or

Fig. 1. Section of cordgrass leaf with moderate level of infestation by *Ogmotarsonemus erepsis*. The larger dark patches toward the left end of the leaf are anastomosed individual wounds. The smallest linear flecks are isolated individual wounds. Virtually all of the wounds on this leaf were made by the mite in oviposition scars of the planthopper *Prokelisia marginata*. Leaf is 1.4 cm wide at right edge of the photo.

apparent damage when the mite is absent. Almost invisible to the naked eye without the mite, oviposition scars with the mite stand out as obvious brown flecks.

The age of an incision can be known by the developmental stage of its planthopper eggs. Hopper eggs with no discernible eyespots are less than three days old at summertime temperatures; well-developed eyespots appear within six days. The outlines of the head, thorax, and abdomen appear at approximately one week. The hopper egg hatches about 10 days after being laid, during the warm months of April through October.

At our study sites at St. Marks National Wildlife Refuge and Oyster Bay, Wakulla Co., Florida, most incisions in naturally growing corgrass contain mites by the time the eye has appeared in the host egg. Therefore, dispersing mites are abundant throughout the marsh. The distinctive brown spotting appears in all oviposition incisions of the planthopper within two weeks after mite-free cordgrass has been transplanted from the greenhouse to our study sites.

The mite lives and reproduces in places other than oviposition incisions of the planthopper, as is obvious from the rapid colonization of incisions at sites with few planthoppers and of mite-free cordgrass set in the marsh in habitats where cordgrass does not grow naturally. Nonetheless, mite colonization and the resulting brown spots are so reliable an indication of hopper oviposition incisions that planthopper eggs can be found at extremely low densities. For example, on offshore oyster bars where vigorous waves wash most nymphs off cordgrass before maturity, planthopper densities are extremely low. Even so, on the bars, oviposition scars invariably contain mites and are brown.

Examination of a large number of planthoppers has revealed no phoretic mites; mites disperse independently of the planthopper. The brown spots made by mites in oviposition incisions are readily distinguished from other sorts of injury to cordgrass leaves. On the offshore oyster bars, our standard sampling protocol is to examine a large number of leaves and to collect those with any brown flecks or spots for microscopic examination in the laboratory. With the naked eye in the field, we separate spotted leaves into two groups, those with longitudinal spots characteristic of mite-browned oviposition incisions and those with other sorts of spots (which are usually round or transverse). Later, in the laboratory, microscopical examination of all spotted leaves invariably confirms the field distinction. Very young eggs of the planthopper can be missed by this technique because oviposition incisions take a few days to be colonized by the mites and turn brown. The next week's sampling detects these eggs, after they and their incisions have aged but before the hopper eggs hatch. The mite's browning of the leaf allows study of parasitism rates by the wasp *Anagrus delicatus* at extremely low host egg densities (Stiling & Strong 1983, Antolin & Strong 1987).

The mite was identified by H. A. Denmark, Chief of Entomology, Florida Department of Agriculture and Consumer Services. It is preserved as Lot 85-04712 of the Systematic Entomology Laboratory, IIBIII, of the Insect Identification and Beneficial Insect Introduction Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705. The research was supported by grant BSR 8206856 from the National Science Foundation.

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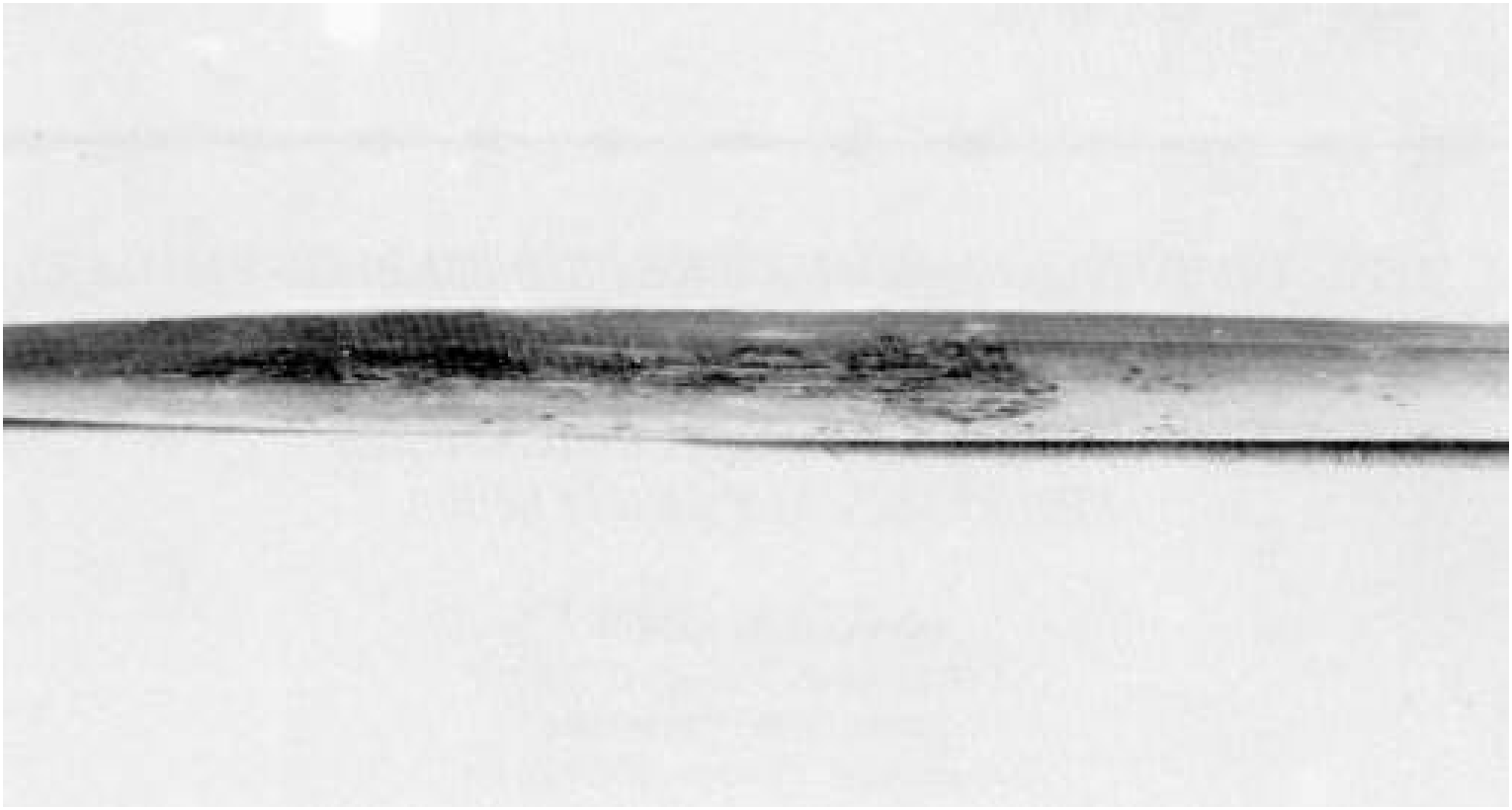

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