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PROGRESS REPORT
of the
ENTOMOLOGY RESEARCH DIVISION
AGRICULTURAL RESEARCH SERVICE
Section A

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1967, and June 30, 1968. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D.C.

July 1, 1968

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TABLE OF CONTENTS
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Page

Section A

Introduction		i
Area No. 1	Vegetable Insects	1
Area No. 2	Deciduous Fruit, Tree Nut, Grape and Berry Insects	25
Area No. 3	Citrus and Subtropical Fruit Insects	38
Area No. 4	Forage and Range Insects	54
Area No. 5	Soybean and Peanut Insects	76
Area No. 6	Corn, Sorghum, and Small Grain Insects	82
Area No. 7	Cotton Insects	107
Area No. 8	Tobacco Insects	140
Area No. 9	Sugarcane and Sugarbeet Insects	148
Area No. 10	Ornamental, Shrub, Flower, and Turf Insects ..	157
Area No. 11	Livestock Insects and Other Arthropods	164

Section B

Area No. 12	Insects Affecting Man, Households, and Industrial Establishments	199
Area No. 13	Bees and Other Pollinating Insects	228
Area No. 14	Analysis, Synthesis, Formulation, and Evaluation of Insect Control Chemicals .	241
Area No. 15	Identification of Insects and Related Arthropods	259
Area No. 16	Foreign Exploration, Introduction, and Evaluation of Biological Control Agents	278
Area No. 17	Insect Pathology	309
Area No. 18	Insect Physiology and Mode of Action of Insecticides and their Metabolites	321
Area No. 19	Fundamentals of Insect Sterility	340

INTRODUCTION

The Entomology Research Division conducts research on both destructive and beneficial insects in relation to the growing of fruits, vegetables, forage, grain, cotton, tobacco, sugarcane, sugarbeets, and ornamental crops; turf, pasture and range plants; the production of all classes of livestock and poultry; and the management of bees in relation to pollination and honey production. In addition, it conducts investigations on insects affecting man, households, and industrial establishments; the identification and classification of insects; the biological control of weeds; and on chemicals including insecticide residues on all raw agricultural commodities derived from crops and livestock. Basic research is conducted in insect pathology, physiology, biochemistry, nutrition, metabolism and genetics.

The Entomology Research Division has 409 professional scientists at 68 field locations in the United States, 9 in foreign countries, and one in the Virgin Islands. The Division and the Branch leadership staffs responsible for the administration of research programs throughout the country and abroad are headquartered at Beltsville, Md. Two Pioneering Laboratories, one on Insect Pathology and the other on Insect Physiology, both devoted to basic research, are also located at Beltsville. In addition, basic research is conducted by entomologists and chemists at Beltsville, Md., and Fargo, North Dakota, in cooperation with scientists of other disciplines. Insect identification research is conducted in Washington in close collaboration with the Smithsonian Institution.

The Division cooperates closely with State experiment station and university scientists. There is also cooperation with trade associations, industrial establishments, health agencies, and growers. Cooperation is also maintained with other research divisions in the Agricultural Research Service and with divisions of the Service concerned with plant and animal pest control and plant and animal quarantine programs. There is also cooperation with other research and regulatory divisions in the Department of Agriculture and with other agencies including the Departments of Defense, State, Interior, and Health, Education and Welfare, Atomic Energy Commission, World Health Organization, and the International Atomic Energy Agency.

Examples of significant accomplishments follow:

Imported parasite of the cereal leaf beetle established: During 1968 an egg parasite of the cereal leaf beetle was found to be established in Michigan. This was the result of cooperative work carried out by the Entomology Research Division's European Parasite Laboratory, the State Experiment Stations of Indiana and Michigan and the Plant Pest Control's Method Improvement Laboratory at Niles, Michigan. This is the first major accomplishment of the biological control work on the cereal leaf beetle that was started in 1963. During 1967 over 5200 parasites of cereal leaf beetle larvae belonging to three species and 1800 parasitized cereal leaf beetle eggs were collected in Western Europe and Yugoslavia and shipped to

the United States for propagation and release. A single adult of one species of larval parasites was recovered during the spring of 1968 at a locality in Michigan where the species was released in 1967.

Artificial diet developed for insect predator. Cage and small scale field tests have demonstrated the effectiveness in controlling the cotton bollworm by mass releases of the green lacewing, a predator on the eggs and young larvae. Present techniques for mass-rearing lacewings require rearing Angonmois grain moths to provide their eggs for feeding lacewing larvae. An artificial diet has been developed to provide food for lacewing larvae, possibly eliminating need for rearing grain moths. This will bring practical usefulness of this predator a step nearer for control of not only bollworm but other lepidopterous pests, aphids, and certain soft-bodied insects.

Imported natural enemy replaces chemical control of alligatorweed in Florida. The flea beetle, Agasicles spp., imported from Argentina in 1964-65 has effected sufficient control in Florida to permit cancellation of plans for use of chemicals to treat 2,200 acres of waterways in 1968. The insect has now been colonized in North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, and Texas. The research, introduction and colonization has been conducted in cooperation with the Army Corps of Engineers.

Synergists found to act like insect hormones. Certain synergists for insecticides have been found to produce hormone-like effects when applied alone to some insects. Application of one of these to the abdomens of yellow mealworms and milkweed bug nymphs disrupted growth and prevented development to the adult stage. Compounds previously known to have juvenile hormone-like activity have been available only for laboratory studies due to prohibitive costs for extraction or synthesis. This research has indicated biological activity of several compounds already available in commercial quantities.

Heat treatment effective against bee disease and pest. Holding bee hives and combs at 120° for 24 hours was effective in killing Nosema disease organisms. All stages of the greater wax moth were killed in 40 minutes at this temperature. Nosema-free bees placed in treated hives remained disease free and produced twice as much honey as Nosema-free bees placed in non-treated hives.

Partly sterilized insects potentially effective for pest suppression. Radiation dosages high enough to sterilize certain insects have resulted in a reduction of their vigor and ability to compete with untreated individuals in mating and sperm transfer. In studies with the cabbage looper sub-sterilizing doses of radiation were determined which would render them partly sterile but not reduce sexual competitiveness. When a 90-percent sterile male moth mates with a normal female, 10 percent of her eggs hatch but the progeny are totally sterile. Lower doses result in partial sterility in progeny that may persist for several generations. Release of partly sterile insects may be more practical for population suppression than totally sterile insects.

AREA NO. 1 VEGETABLE INSECTS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years FY 1968			Total
	Research Problem Area			
	204	205	701	
Arizona	2.0			2.0
California	4.0			4.0
Georgia	0.5			0.5
Idaho	0.1	0.1		0.2
Indiana	1.0			1.0
Maine	0.6	0.4		1.0
Maryland (Beltsville)	3.2	0.1	0.8	4.1
Oregon	0.3	0.3		0.6
South Carolina	5.0			5.0
Washington	2.9	0.2	1.7	4.8
Total	19.6	1.1	2.5	23.2

Intramural program is supplemented by extramural support representing (a) 0.6 SMY's at State Agricultural Experiment Stations^{1/}, (b) 1.6 SMY's at other U.S. institutions^{2/}, and (c) PL 480 funds in 0 countries.

^{1/} RPA 204 - 0.6; RPA 701 - 0.0

^{2/} RPA 204 - 0.8; RPA 205 - 0.8; RPA 701 - 0.0

Problems and Objectives

Vegetable crops are subject to heavy losses by insects through direct feeding damage and through transmission of plant viruses. Current grower practices involve the use of insecticides as the principal means of controlling insect pests. Attendant problems in the use of insecticides are the presence of undesirable residues on the marketable plant parts, environmental pollution, insect resistance to chemicals, toxic effect on beneficial insects, and recurring costs of insecticide treatments. Consequently, alternate methods for control of vegetable insect pests are urgently needed. Annual losses to insects are estimated at over \$185 million.

Major objectives of the research are to develop methods for control of insect pests by means of:

- 1. New, selective, nonpersistent insecticides that will minimize or avoid residues.
- 2. New approaches such as sterility, attractants, biotic agents, and bioenvironmental methods.
- 3. Insect resistant germ plasm in vegetable crops.
- 4. Integration of various of the above methods.

Progress - USDA and Cooperative Programs

RPA 204 - CONTROL OF INSECT PESTS

A. Basic Biology, Physiology, and Nutrition (2.0 SMY)

1. Cabbage

a. Cabbage looper. At Riverside, Calif., cabbage loopers were reared from egg to adult under various constant and cyclic temperatures. At the constant temperatures of 60, 70, and 80° F, the mean days required from egg to adults were 73, 41.7, and 21.0, respectively. At the cyclic temperatures of 40-50-60, 50-60-70, and 60-70-80° F, the developmental periods from egg to adult were greater than 134, 71, and 37 days, respectively. Under outdoor conditions during the same period, from early March to mid-May, the mean developmental period was 69.3 days.

A black strain of cabbage looper moths was started at Riverside from a single female mutant found in the laboratory culture. When this female was mated to a normal male, both male and female progeny segregated into 1:1 ratios for black and normal color. Additional work to determine the genetics of the mutant is underway.

Injection of cabbage looper pupae with nuclear polyhedrosis virus at Riverside showed that as pupal age advanced higher virus titers were required to prevent emergence of adult moths. Moths emerging from pupae

were frequently deformed, lacked the dark pigmentation of normal moths, and were shorter lived. When injected as adult moths, longevity, mating, and oviposition were not affected but egg viability was reduced.

Purified polyhedra from the cabbage looper and alfalfa looper fed to their own and reciprocal hosts at Riverside showed that the alfalfa looper virus was infectious to both hosts while the cabbage looper virus appeared more infective to its own host. The virus rods in polyhedra isolated from the cabbage looper are occluded singly in the polyhedral matrix while those from the alfalfa looper are occluded in bundles.

In laboratory studies on the effect of a recently discovered cytoplasmic polyhedrosis virus on various developmental stages of the cabbage looper, only 23 and 26% of male and female pupae, respectively, were of normal weight. Moth deformities increased as pupal weight decreased. Preliminary tests indicated that the virus is transmitted on the egg surface and that infection in adult females tends to reduce oviposition. Laboratory studies showed that polyhedra were inactivated by temperatures 70° C or higher for at least 8 hours and by alkaline solutions (Na₂CO₃) of more than 0.01 M for 10 minutes. Formaldehyde in the artificial diet lowered the activity of polyhedra. An aqueous suspension of polyhedra remained infective for 1 year under refrigeration.

2. Sweet Corn

a. Corn earworm. At Charleston, S.C., the incorporation of gibberellic acid in a semisynthetic diet at levels up to 5 ppm had no measurable effect on pupation success, pupal weights, or adult emergence. The modified diet was fed to the larvae throughout their entire development period.

Fifteen light traps were placed at levels varying from 25 to 1,050 feet on a 1,250-foot television tower at Pelham, Ga. The traps were designed so that any moth attracted to the trap had to be flying as high or higher than the trap. Weekly records indicate that Heliothis zea frequently fly at the 1,050-foot level. During the week of peak H. zea flight of August, over 90% of the total insect catch at the 1,050-foot level was H. zea.

Surveys conducted on St. Croix, V.I., during 1968 revealed that population levels of the corn earworm, fall armyworm, and sugarcane borer were sufficiently high to allow experimentation with suppression and eradication programs. Corn earworm moths tagged with P-32 and recaptured in virgin female traps demonstrated that most of the insects were concentrated in the vicinity of six corn fields on the Island; however, collections from 256 light traps scattered over the Island revealed the ability of the moths to range over the entire 84-square-mile landmass. The total natural corn earworm population during the month of radioactive captures on the Island was estimated at 9,152 males, or 109 males per square mile. Based on these results, attempts will be made on St. Croix

to eradicate the corn earworm by the sterile male release method.

3. Cucurbits

a. Banded cucumber beetle. At Charleston, S.C., an almost black, melanistic strain of the banded cucumber beetle was selected from several field-collected specimens. Preliminary data indicate that the dark color is controlled by a single recessive gene.

The addition of 50 ppm of cucurbitacin E (Semi-purified extract from bitter fruited watermelon) to the standard semisynthetic diet, increased the longevity of banded cucumber beetles and the number of eggs per female. The beneficial effect apparently was not due to the known feeding stimulant activity of the cucurbitacin since there was no significant difference in the consumption of the diet with or without the additive.

4. Strawberries

a. Spider mites. At Riverside, Calif., biweekly surveys from October 31, 1967, through May 14, 1968, to determine population fluctuations of the two-spotted spider mite showed that the populations declined to a low of less than 1 per leaflet on December 26, and reached a peak of 272 per leaflet on May 14 when the survey was terminated.

5. Potatoes

a. Aphid predators and parasites. Tests at Presque Isle, Me., showed that some kinds of diapause in the 7-spotted ladybird can be broken without harmful effects to ovipositing females by exposing the adult female to a continuous source of low-intensity incandescent light. However, inadequate food in the larval stage resulted in diapause in the adult female which incandescent light did not break. Some but not all of this kind of diapause disappeared from beetles that overwintered in hibernation cages over grassland out-of-doors. Winter survival of the adult beetles in these cages was influenced by age and condition of beetles when placed in the hibernation cages. Well-fed beetles maturing in early to midsummer survived better than those fed insufficiently for a period of 2 or 3 weeks before being placed in the cages. Winter survival of beetles maturing in September was influenced greatly by their age and by abundance of food in the larval stage and after maturation before being placed in the cages. There was no winter survival among specimens placed in cages as newly emerged adults, little survival when the adults matured from inadequately fed larvae (1 - 10%), and variable survival (16 - 53%) of adults moderately to well fed as larvae and fed for varying periods as adults. An average of 750 green peach aphids of all sizes was required for a newly hatched larva to develop to the pupal stage. The numbers required were 6.4, 15.9, 122.8, and 604.9 for development of the first to fourth instars, inclusive.

Marked improvements in oviposition of 7-spotted ladybird beetles resulted from improvement in techniques of mass rearing the larvae and in the storage, handling, and care of the adults. Both the numbers of egg clusters and eggs per cluster deposited by individual female beetles were larger in 1968 than in 1967. Progressive reduction in egg viability with age of females remained a problem.

Introduced from northern India, Aphidius smithi, a braconid parasite of the pea aphid has become established at Yakima, Wash., and other northern locations. Its unusual hardiness was demonstrated when parasites were captured in flight at Yakima during December and January when the air temperature was 4.4° C.

b. Green peach aphid. A mechanical trap utilizing yellow baffle plates for impaction surfaces and bottles of a dilute formaldehyde preservative was developed at Yakima, Wash., for determining hourly, as well as longer, periods of flight activity of the green peach aphid. Most flights occurred during daylight and twilight hours of the day; temperature was a lesser deterrent than wind to aphid flight.

c. Wireworms. In Autumn, sampling studies in 22 fields on 17 farms in Charleston County, S.C., wireworm populations in the various fields ranged from 0 to 5.0 larvae per square foot with an average of 1.6. The relative abundance of the various species was as follows: Conoderus falli, 69%; Glyphonyx sp., 15%; Conoderus amplicollis, 10%; and Conoderus vespertinus, 3%. One Melanotus communis and two undetermined elaterid larvae were found. Light trap studies at Charleston in and around an isolated corn field showed that although a few southern potato wireworm adults penetrated as much as 2 miles into woodlands, 83% of the adult population remained in the cultivated fields.

6. Cross Commodities

a. Cabbage looper. Population counts made at Charleston, S.C., during the spring season, on randomized field plots of lettuce and cabbage showed that 96% of the larvae recorded were on the cabbage. Very few looper eggs were observed on the lettuce foliage indicating that cabbage was preferred for oviposition.

b. Green peach aphid. At Purdue University, studies under a grant to determine the influence of electromagnetic energy on the green peach aphid revealed the following: (1) nymphs were more responsive to shorter wave lengths of monochromatic light ranging from 450 to 650 μ than were adults; (2) adult aphids ranging from 1 to 19 days in age responded similarly regardless of age except that older aphids tended to respond less to short wave lengths and more to longer wave lengths; and (3) the preconditioning effects of exposure to alternate light and dark did not affect their response to monochromatic light.

c. Spider mites. At Pennsylvania State University under a grant, a method was developed for rearing spider mites on homogenates of bean which will be used in the study of nutritional factors affecting spider mite reproduction and longevity. In a comparison of methods for separation of amino acids and proteins, thin layer chromatographic techniques were superior to alternate methods including cellulose acetate strip electrophoresis.

B. Insecticidal and Cultural Control (2.2 SMY)

1. Beans

a. Mexican bean beetle. In a field plot test at Charleston, S.C., five weekly spray applications of Gardona, Dursban, and malathion reduced larval populations by 99, 98, and 94%, respectively. A spray containing a 1-400 dilution of Pyrocyde (1.4% pyrethrum) was ineffective.

Of 21 pesticides screened for toxicity to the Mexican bean beetle at Beltsville, Md., Gardona, Azodrin, azinphosmethyl, carbaryl, carbophenothion, diazinon, dimethoate, endosulfan, and parathion gave good control as 1- and 7-day residues. Other materials showed high toxicity by 1-day residues and poor control by 7-day residues.

b. Western bean cutworm. DDT was the most effective of several materials applied to bean fields by ground and air equipment in the Twin Falls and Rupert, Idaho, areas for control of western bean cutworm. Trichlorfon also gave promising control.

2. Cabbage

a. Cabbage looper. Nine of 57 experimental compounds compared in laboratory tests in Charleston, S.C., showed a high order of toxicity. In field tests during the 1967 fall season, UC-34096, Dursban, and Stauffer N-4446 each at 1 lb/acre provided good plant protection against light populations of the cabbage looper and the fall armyworm. A low volume spray of naled failed to provide adequate cabbage looper control on cabbage. In 1968 spring season tests, Ortho 9006, SD-15289, Azodrin, and ENT-27325 were the most effective of the compounds tested.

3. Cucurbits

a. Banded cucumber beetle. Six of 80 experimental compounds screened in the laboratory in Charleston showed promise for soil applications to control larvae.

b. Greenhouse whitefly. At Beltsville, Md., 3 applications of Azodrin, azinphosmethyl, parathion, or naled were the most effective of 8 spray schedules in reducing whitefly populations on greenhouse cucumbers. Single applications of the same materials were the most effective of

4 additional compounds. In tests with soil systemics, Union Carbide UC-21149 and dimethoate were the most effective in reducing whitefly populations.

In tests of sprays or aerosols, azinphosmethyl, Azodrin, and parathion were highly effective against whitefly nymphs up to 6 days old and dimethoate against nymphs up to 4 days old. Azinphosmethyl and Azodrin also killed a high percentage of pupae. Residual toxicity of azinphosmethyl and Azodrin gave complete kills of nymphs hatching from eggs of all ages up to 10 days after treatment whereas parathion and dimethoate killed nymphs up to 8 days after treatment. In aerosols, parathion and tepp were the most toxic of several materials to nymphs up to 6 days old but lacked residual toxicity against eggs.

4. Peas

a. Pea aphid. Soil applications of UC-21149 to alfalfa, the overwintering host of the pea aphid suppressed aphid populations through three plant growth cycles in the presence of the braconid parasite, Aphidius smithi, in eastern Washington. Neither UC-21149 nor parasites alone was as effective for aphid control.

5. Potatoes

a. Aphids. At Presque Isle, Me., both disulfoton and UC-21149 gave satisfactory all-season control of aphids on Katahdin potatoes. When applied as foliar sprays at 0.2 lb active ingredient/A, parathion was somewhat more effective than endosulfan; however, 3 applications of each were required to hold down aphid populations to predesignated low levels.

At Presque Isle, a slight but highly significant early season reduction in aphid population resulted from mulching Katahdin potatoes with aluminum foil. The difference was not significant when only 50 to 95% of the soil was covered with the aluminum. The reduction soon disappeared, presumably because the potato foliage expanded to cover the aluminum and eliminate its repellent effect upon flying aphids. Throughout the remainder of summer the aphid populations were about the same in mulched and unmulched plots and at harvest tuber yields were not affected by treatment.

At Presque Isle, no mortality occurred during 1-week periods among foxglove aphids caged on new foliage of 1-week-old Katahdin potato plants grown from daughter tubers harvested from plants grown in plots sidedressed with disulfoton or in plots receiving planting furrow treatments of UC-21149. However, there appeared to be a depressive effect upon reproductive rate of the aphid from the UC-21149.

Katahdin potato tubers grown in untreated soil at Presque Isle in 1966 were planted in 1967 in potted soil into which was mixed disulfoton,

UC-21149 or Bay-68138. After the plants emerged, foxglove aphids were caged weekly for 4 weeks on new leaves of the growing plants. All treatments proved highly toxic to the aphid during the 4-week test period. All adult aphids died before depositing nymphs.

In studies at Yakima, Wash., approximately 5,500 peach trees, representing the principal overwintering source of the green peach aphid within a 275 mi² area in the Columbia Basin of eastern Washington were sprayed with an insecticide in the spring for 3 consecutive years to prevent migration of the aphid to potatoes and other susceptible crops. In 1967, the green peach aphid population on potatoes on the leeward side of the sprayed area during the season was 97% less than in 1965 when the trees were not sprayed.

b. Wireworms. In 3 years' tests at Yakima, Wash., Dyfonate was as effective as diazinon or parathion as a soil treatment for the control of wireworms.

In field plot tests at Charleston, S.C., Bay-77488, Bay-78182, Dursban, and carbofuran were equal to parathion in reducing larval populations of the southern potato wireworm in the soil.

6. Sweet Corn

a. Corn earworm and fall armyworm. At Tifton, Ga., Gardona was shown to be extremely toxic to corn earworm moths. Sugar water solutions containing varying rates of Gardona gave up to 100% mortality in 24 hours when as little as one microgram was fed per moth. Dosages greater than 10 micrograms per moth gave 100% mortality in all replications. A dosage of 0.5 microgram per moth produced 80% mortality in 24 hours.

In laboratory bioassays of new experimental materials against fall armyworm larvae, seven gave 100% mortality 24 hours after exposure to leaf discs dipped in a solution containing one ounce active ingredient per 25 gallons of water.

In field evaluations, the following low volume formulations and new materials gave effective control of corn earworm and fall armyworm: Endosulfan-malathion, DDT-malathion, Gardona, malathion, Azodrin, Shell SD-7438, carbofuran, and Mobam.

Fall armyworms collected from the field at Tifton and in south Florida were determined resistant to *p,p'*-DDT. Moths obtained from St. Croix, V.I., and from Brownsville, Texas, remained as susceptible to *p,p'*-DDT as the Tifton laboratory strain which has been reared in the laboratory for over 100 generations. An LD₅₀ in excess of 12,050 micrograms per 30 milligrams larval weight was demonstrated for field-collected fall armyworms compared to an LD₅₀ of 10 micrograms per 30 milligrams for the susceptible strain.

7. Tomatoes

a. Greenhouse whitefly. In experiments at Beltsville, corn cobs impregnated with dichlorvos in 1962 showed insecticidal toxicity when tested 6 years later, in 1968. Freshly impregnated corn cobs were more active than commercially available resin strips or urethane foam impregnated in the laboratory.

Populations of whiteflies on greenhouse tomatoes were destroyed by continuous exposure to corn cobs impregnated with dichlorvos or to aerosol treatments containing tepp, sulfotepp, dichlorvos, or parathion applied 6 times at 4-day intervals.

Tests with corn cobs impregnated with dichlorvos and one of 3 dispersants indicated that a highly refined kerosene oil was superior to xylene or methylene chloride.

C. Biological Control (4.5 SMY)

1. Beans

a. Mexican bean beetle. In preliminary laboratory tests at Charleston, S.C., the fungus pathogen Metarrhizium anisopliae caused 100% mortality of Mexican bean beetle larvae. Beauveria bassiana, and Spacaria rileyi caused moderate mortality alone as did Beauveria combined with Bacillus thuringiensis. Limited field tests with these pathogens were unsuccessful.

2. Cabbage

a. Cabbage looper. Preliminary field cage studies at Riverside, Calif., showed that the recently discovered cytoplasmic polyhedrosis virus of the cabbage looper can infect and reduce a population under semi-natural conditions. Among several criteria measured, the first test showed an 83% reduction in total pupae, and mean pupal weights were lowered by 12 and 32 mg for males and females, respectively. Dissection of cabbage looper larvae collected from Brawley and Irvine, Calif., and reared individually at Riverside showed that Voria ruralis and Copidosoma sp. were the most abundant parasites. A high percentage of larvae infected with nuclear polyhedrosis virus were also parasitized.

Laboratory feeding experiments at Charleston, S.C., confirmed that a red strain of the bacterium Serratia marcescens, originally isolated from corn earworm eggs, was pathogenic to 2nd-instar larvae of the looper. Highest mortality, 80%, occurred when the larvae were not allowed to choose between treated and untreated meridic diets. None died when the larvae were given a choice, indicating that the bacterium is repellent to larvae. In spring and fall season tests on cabbage, Bacillus thuringiensis dusts and sprays provided plant protection equal to that provided by the most effective of currently recommended insecticides.

3. Lettuce

a. Cabbage looper. At Mesa, Ariz., collections from various cultivated hosts showed that the tachinid fly, Voria ruralis, parasitized 42% of the larvae of the cabbage looper during January-March, 30% during April-May, 5% July-September, and less than 1% from October-December. In the same collections a polyhedral virus destroyed 34% of the larvae in January-March, 22% in April-May, 46% July-September, and 84% in the period October-December, when parasitism by Voria was lowest.

4. Potatoes

a. Aphid predators. A procedure was developed at Presque Isle, Me., that proved satisfactory for applying coccinellid eggs to potato plants in the field. The eggs were applied in a coarse water spray containing 0.125 percent of agar to prevent settling in the tank during application and to enhance adhesion of the eggs to the foliage. The egg-spray mixture was applied under a constant line pressure of about 3 psi. There was little difference in percent egg hatch whether sprayed on potato foliage or on soil. The hatch of sprayed eggs was only slightly if any less than that of eggs not put through the sprayer. Based on results of earlier studies, this technique should prove suitable for field application of chrysopid eggs also.

5. Sweet Corn

a. Corn earworm. In laboratory studies at Charleston, S.C., the fungi Spicaria rileyi and Metarrhizium anisopliae caused 83 and 61% mortality, respectively, of corn earworm larvae. Beauveria bassiana was ineffective. A disease destroying corn earworm eggs of the laboratory culture was diagnosed as Serratia marcescens. In a fall season field test a Bacillus thuringiensis dust and two Heliothis polyhedrosis virus sprays were all less effective in protecting tomatoes than chemical insecticides.

b. Fall armyworm. At Lafayette, Ind., quantitative studies with fall armyworm granulosis virus indicated an LD₅₀ of 0.2 µg of freeze-dried virus inclusion bodies for fourth-instar fall armyworms. It appears that both age and body weight of the larvae are important factors in their susceptibility to the virus. Efforts are underway to determine the effects of simultaneous infection with the fall armyworm granulosis and nuclear polyhedrosis viruses.

At Lafayette, a histopathological study of two virus diseases of the fall armyworm revealed that the granulosis virus attacks only the fat body, causes a proliferation of cells, and requires a relatively long time to produce mortality; however, the nuclear polyhedrosis virus attacks a wide variety of tissues, does not cause a proliferation of cells, and produces mortality in a relatively short period of time.

A study was conducted at Tifton, Ga., to determine the effect of age and fertility of host eggs on their suitability for parasitism by Trichogramma evanescens Westwood. The study demonstrated little or no variation in the suitability of Cadra cautella (Walker) eggs during the first 60 hours of the incubation period. All the host eggs in this age range were equally suitable for oviposition and development by the parasite. During the last 12 to 24 hours of the incubation period during which the morphological characteristics of the head of the host became evident, the eggs were progressively less acceptable for oviposition and development by the parasite.

Eggs from virgin females were often found to be suitable hosts for the parasite; however, the percentage that were suitable varied considerably. The causes for the variation were unclear.

Eggs 1-5 days old from tepa-sterilized fall armyworm moths were equally susceptible to parasitism by Trichogramma evanescens as 1- and 2-day-old control eggs. Since normal fall armyworm eggs usually hatch on the third day, chemosterilized eggs are available for parasitism at least twice as long as the normal eggs. These results indicate excellent possibilities for integration of Trichogramma with the use of chemosterilants.

6. Cross Commodities

a. Green peach aphid. A fungus epizootic that completely destroyed a greenhouse infestation of the green peach aphid in Charleston, was identified as Acrostalagmus aphidum.

b. Leaf beetles. Microscopic examination of several species of leaf beetles at Charleston disclosed protozoan infection in 60% of the banded cucumber beetles, 56% of the spotted cucumber beetles, and 54% of the bean leaf beetles sampled. No protozoa were found in striped cucumber beetles or Mexican bean beetles.

D. Insect Sterility, Attractants, and Other New Approaches to Control (5.4 SMY)

1. Cabbage

a. Cabbage looper. Studies at Riverside, Calif., showed that when sealed within 3x5-cm polyethylene bags (.05, 0.10, and 0.25 mm thickness) vapors of the synthetic sex pheromone of the cabbage looper were capable of permeating the walls of the container and attracting male moths. The thicker bag containing 100 mg of pheromone was the best of several combinations tested. Blacklight traps baited with the polyethylene bag dispensers were equally as effective as those baited with jar-sand dispensers.

In a series of experiments at Riverside, caged laboratory-reared cabbage looper males responded to synthetic sex pheromone emanating from a polyethylene dispenser up to 320 feet downwind when wind velocity was 2.5 - 5 mph and temperature was 60-63° F. When marked moths were released 350, 500, and 1000 ft downwind from the pheromone source 30, 28, and 18%, respectively, were recovered. When moths were released 160, 650, 1325, and 2020 ft from the nearest of 4 traps aligned 650 ft apart, 32, 15, 5, and 2%, respectively, of the moths were recovered. At Riverside, the efficiency of electrocutor grid traps against the cabbage looper declined during periods of high humidity in the fall. After undergoing modification of design, the grid trap baited with synthetic sex pheromone was 3.6 times more effective than a similarly baited blacklight trap during the 6-month period from December 1967 through May 1968.

A low-cost cylindrical pheromone trap constructed from hardware cloth was developed for trapping cabbage looper males at Riverside. A packet, which included sand impregnated with synthetic pheromone inside a polyethylene bag and insecticide-impregnated cheesecloth wrapped around a screen frame, was suspended from the inside top of the cage. Catches of male moths in the screen pheromone trap were comparable to those in a blacklight trap baited with pheromone.

2. Cucurbits

a. Banded cucumber beetle. At Charleston, S.C., a total of 235,245 virgin females were produced for extraction of crude sex pheromone. It was found that the yield of crude pheromone from the beetles could be increased considerably by sacrificing the females in the afternoon rather than in the morning. Extracts from 168,245 females were fractionated by chemists of the Pesticide Chemicals Research Branch. Of 61 fractions bioassayed, 13 were more attractive than the crude extract. Tests of various dispensers of the pheromone in field traps disclosed that glass jars containing paper toweling impregnated with the pheromone remained attractive for up to 68 days and were as efficient as more elaborate dispensers.

3. Lettuce

a. Cabbage looper and other caterpillars. The Mesa, Ariz., laboratory placed in operation 415 blacklight traps baited with the synthetic sex pheromone (cis-7-dodecen-1-oi-acetate) of the cabbage looper on March 6, 1967, on a 2240-acre ranch at Red Rock, Ariz. The traps have removed many millions of moths with the result that populations of the cabbage looper, corn earworm, beet armyworm, and salt marsh caterpillar are much lower at Red Rock than in a similar lettuce-producing ranch 12 miles away at Picacho. Dissection of trapped cabbage looper females showed a slight reduction of mating at Red Rock, and field counts on lettuce showed a reduction in the number of eggs and larvae of the cabbage looper, corn earworm, and beet armyworm.

Although trapping data and counts of eggs and larvae on lettuce indicate a reduction in moth population at Red Rock, the intensive insecticide program followed at both ranches has made it impossible to evaluate the effectiveness of light traps as a control. Arrangements are being made to maintain several untreated fields of lettuce at Red Rock for a better evaluation.

The Mesa laboratory operated from early April to early June around the perimeter of an isolated 87-acre lettuce field at Bonita, Ariz., 82 newly designed mechanical traps baited with synthetic sex lure of the cabbage looper. The traps captured approximately 25,000 cabbage looper males and reduced moth populations by about 90% below those in a ranch 12 miles away, but did not decrease the incidence of mating nor lower egg and larval infestations on lettuce.

4. Potatoes

a. Green peach aphid. At Yakima, Wash., proprietary defoliant sprays, applied to the foliage of peach trees in autumn when oviparous forms of the green peach aphid were being produced on the trees caused most of the leaves to drop prematurely before the egg-laying aphids crawled to the twigs to deposit overwintering eggs. The surviving aphids were concentrated at the rate of more than 40 per leaf on the few remaining leaves where they fell prey to syrphid fly larvae.

Studies were continued at Presque Isle, Maine, to determine the possibility of suppressing populations of the green peach aphid and spread of leaf roll virus in potatoes by eliminating Canada plum in two agricultural districts of northeastern Maine. The distribution of Canada plum in an isolated area of about 100 mi² in the Lee district and in a non-isolated area of 315 mi² in central Aroostook County was determined visually during the spring flowering period by aerial and ground survey teams. The thickets were destroyed chiefly by application of foliar or soil herbicides. In the nonisolated area about 475 thickets were found in 1967 and about 255 more in 1968. Approximately 500 of these were in rural areas. In the isolated area a total of about 100 thickets have been found, of which approximately 42 were found in 1966, 15 in 1967, and 43 in 1968. About 70 thickets were located in the buffer zone around the isolated area. The effects of Canada plum removal are being evaluated by monitoring populations of flying aphids, aphid populations on host plants, and spread of leaf roll in potatoes.

b. Wireworms. At Yakima, Wash., a sex pheromone was extracted from virgin females of the Pacific Coast wireworm and male beetles responded to crude extracts containing only a very small part of the total female equivalent.

5. Tomatoes

a. Drosophila. The Beltsville, Md., laboratory continued field studies for areawide suppression of Drosophila melanogaster in 1967 in cooperation with the Campbell Soup Co. at Rancocas, N.J. Twenty-three weekly releases, each averaging 2.5 million tepa-sterilized flies, were made between April 14 and September 13 in a 25 mi² area. The release area contained about 125 acres of commercially grown tomatoes with comparable check fields outside the area. Trapping records from the tomato fields revealed that area releases initially suppressed drosophila development in the tomato fields by 86%. In midseason more native flies were trapped from fields in the release area but following a change to direct field releases the ratio of released to native flies was reversed ranging from 0.5 to 59%. Isolated females collected from tomato fields in the release area showed an average 13% reduction in oviposition and 18% reduction in adult progeny compared to females from check areas. Drosophila eggs laid in slit tomatoes placed in fields in the release area indicated an average reduction of 55% for the season. Females isolated from samples taken each week from the tepa-treating cages to determine the effectiveness of the tepa treatments on flies being released showed a sterilizing efficiency of 94-99% and averaged 97% reduction in oviposition. Reduction in adult progeny varied from 94-100% and averaged 99% for the season.

In studies on the effects of marking Drosophila melanogaster flies for identification in a release program, tests at Beltsville indicated that dyed and undyed males were equal in mating competitiveness--6.3 vs 6.8 matings, respectively; also that longevity of dyed and undyed flies were about equal, 36 vs. 34% survival after 21 days.

6. Sweet Corn

a. Corn earworm and fall armyworm. At Tifton, Ga., research was continued on sex pheromones of the fall armyworm and corn earworm. The active principle of the female fall armyworm was isolated in pure form and identified. In an unreplicated test, the synthetic at the rate of 1 µg per ml per planchett per square yard per day for 10 days was placed in a section of the greenhouse in which a susceptible variety of corn was growing. Indications were that the pheromone had some confusing effect on the emerging insects, thereby reducing mating as well as larval damage. Further greenhouse and field studies are planned. A laboratory procedure was developed for the collection and isolation of the sex pheromone of the corn earworm. Active fractions from laboratory-reared female moths, as well as from moths caught in light traps, elicited copulatory attempts from males. The sex pheromone is located in the last abdominal segment in extremely small amounts. Work is now in progress to produce enough female moths from laboratory cultures as well as from field collections to attempt chemical characterization of the pheromone.

Irradiation of laboratory-reared corn earworm pupae, 1-2 days from eclosion, with cobalt-60 at the rate of 17.5 kr for females and 33.0 kr

for males gave almost 100% sterility. Males emerging from pupae treated with 33.0 kr were mated with nontreated females and less than 1% of the eggs oviposited hatched. Oviposition from the females emerging from pupae treated with 17.5 kr was reduced to about that of the untreated control and egg hatch was reduced to less than 2½% of the eggs oviposited. Adults, less than one day old when irradiated, were sterilized with dosages of 17.5 kr for females and 33.0 kr for males. Less than 2% of the eggs laid by pairing of irradiated normal moths hatched, but these did not develop. Mating competitiveness of irradiated moths varied from slight to as much as 25% reduction during repetitive trials.

Other tests with cobalt-60 at Tifton indicated that 20 kr effectively sterilized both sexes of fall armyworms. Competitive trials showed copulation unaffected by treatment but sperm from treated males were not competitive with sperm from untreated males. Evaluation of competitiveness between tepa and cobalt-60 sterilized insects indicate no significant differences.

b. Southwestern corn borer. At State College, Miss., male southwestern corn borer pupae were subjected to gamma irradiation to determine the effect of different levels of radiation on the emergence of three pupal stages, early, mid, and late. Results to date show that the early pupal stage is much more sensitive to radiation than either the mid- or late-pupal stages. Early pupal stages one to two days old tolerated no more than 5 kr without serious emergence problems. Mid- (4 to 5 days old) and late- (7 to 8 days old) pupae withstood levels as high as 20 kr without seriously affecting normal emergence.

E. Evaluation of Equipment for Insect Detection and Control (0.5 SMY)

1. Broccoli

a. Cabbage looper. At Forest Grove, Oreg., single applications of DDT-parathion or mevinphos-parathion each followed by two applications of tepp-naled and three applications of mevinphos gave effective control of cabbage looper on broccoli. Sprays were applied at the rate of 10 or 15 gallons per acre with a Bell 47D-1 helicopter between August 9 and October 2, 1967.

2. Potatoes

a. Southern potato wireworm. A study of light trap catches at Charleston, S.C., over a period of 12 years, disclosed very little difference in the numbers of beetles taken during nights when the moon was full and nights when the moon was new.

3. Sweet Corn

a. Corn earworm and fall armyworm. A modified Steiner trap in which the

virgin females were used to attract males and prevent their escape through the use of STIKEM was used to survey natural populations of corn earworms and fall armyworms. A height of 3 feet was determined to capture significantly more numbers of males than any other height. An increase in the number of females used as bait increased the catch but not in proportion to the increase in number of females. Females 3 days old were most attractive. Fed females caught significantly more males than non-fed females, and virgin females caught significantly more males than mated moths.

4. Cross Commodities. Studies at Forest Grove, Oregon, showed that Handy Oil RPM (supplied by the Standard Oil Company of California) has the same flow characteristics as malathion, Technical ULV at normal room temperatures, thus providing an innocuous substitute for nozzle calibration tests.

F. Varietal Evaluation for Insect Control (4.8 SMY)

1. Cucurbits

a. Cucumber beetles. Studies at Charleston, S.C., disclosed that striped and banded cucumber beetles have a similar preference for varieties of summer and winter squash. Seedlings of Zucchini and Cocozelle summer squash and Hubbard winter squash were most damaged by both species of cucumber beetles. Yellow Crookneck, Yellow Straightneck and Scallop varieties of summer squash and Table Queen variety of winter squash were least damaged. Direct comparisons of the relative damage to squash, cantaloupe, and watermelon varieties, disclosed distinct differences not only between varieties within a crop but also between crops. Taste tests of the seedlings indicated that the differences within crops were largely, if not entirely, due to the presence or absence of the bitter principle. Differences between crops, however, were not so easily explained. The relative resistance of squash probably was partly due to larger seedlings that could tolerate more feeding; however, the pronounced non-preference by the beetles for Yellow Crookneck and Table Queen squash suggests unknown chemical or physical factors that influence the beetles' choice of a host. Low levels of the bitter principle detectable by the beetles but not by taste tests may be involved in some of the varieties tested.

2. Lettuce

a. Cabbage looper. In screening tests at Riverside, Calif., involving 116 selections of Lactuca sativa and other Lactuca spp. for possible ovipositional preference by the cabbage looper, oviposition was low on 11 of the selections. One selection had 91% less eggs than the most preferred entry.

3. Strawberry

a. Spider mites. At Riverside populations were surveyed biweekly in a 68-entry strawberry plot from November 1967 through May 1968. On 8 of the entries, the mean number of mites per leaflet was less than 50% of the plot mean. On 40 of the entries, the mean per leaflet was higher than the plot mean.

4. Sweet Corn

a. Corn earworm and fall armyworm. At Tifton, Ga., the improved 81-1 and 471-U6 sweet corn inbreds that make up Walter's White were further evaluated. The improved inbreds are intended to be released to Crops Research Division, Charleston, S.C., for final testing and possible incorporation into their breeding program. A selected yellow of these inbreds is also being developed for release.

Twenty-five inbred lines of corn were evaluated at Tifton for corn earworm injury and injury was correlated with 9 characters, namely: food utilization, feeding stimulant, husk length, husk tightness, duration of fresh silk, days to mid silk, rainfall and irrigation during silking, coded husk protection, and solar radiation during fresh silk. Indications were that husk characters and laboratory feeding stimulation and food utilization were most closely and consistently related to injury. These showed the most promise for use in correlated variables in a selection program. When ears of corn were infested with corn earworm, fall armyworm, and tobacco budworm alone and in all combinations, indications were that most of the damage to the heavily injured corn was inflicted by the corn earworm. When the ears were harvested, earworm was the most prevalent.

5. Sweetpotato

a. Soil insects. At Charleston, S.C., selection from a random breeding population yielded sweetpotato clones with outstanding resistance to injury by soil insects. Several surpassed the previous standard (La 3-64) in resistance to the Diabrotica-wireworm-Systema complex and in addition possess resistance to white grubs which La 3-64 lacks. Grub resistance like sweetpotato flea beetle resistance, appears to be unconnected with resistance to the Diabrotica-wireworm-Systema complex. Four lines, however, were resistant to all three classes of insect injury.

6. Spinach

a. Green peach aphid. At the North Central Regional Plant Introduction Station, Ames, Iowa, a technique was developed for screening large numbers of spinach plants for resistance to the green peach aphid. Of a total of 2265 spinach plants (representing 73 accessions) evaluated, none were resistant to aphid attack.

7. Tomato

a. Tomato fruitworm. At Charleston, S.C., a search for fruitworm resistance in tomatoes turned up 148 promising accessions from approximately 500 screened. Real differences in fruitworm susceptibility were found among several advanced tomato breeding lines, but the level of resistance of the best was relatively low. Some evidence indicated that the resistance of these lines may not be improved by selecting within them.

8. Turnip

a. Turnip aphid. Greenhouse tests at Charleston, S.C., indicated that turnip aphid resistance in turnips may be related to light transmission through the foliage. Studies of the inheritance of turnip aphid resistance in turnips indicated an increased level of resistance in all of the crosses in which Shogoin (the resistant standard) was one of the parents. The progeny of a cross between two susceptible parents, however, were superior to Shogoin. The reason for this is not understood, but it is considered unlikely that it was due entirely to hybrid vigor.

RPA 205 - CONTROL OF DISEASES

A. Insect Vectors of Diseases (1.1 SMY)

1. Potato

a. Green peach aphid. Studies in India (PL 480 project A7-ENT-33) revealed variation among clones of the green peach aphid in ability to transmit potato leaf roll and potato virus Y. However, sexual forms of the aphid have not yet been found in nature or produced in the laboratory. Until experimental colonies of the sexual forms can be established from one of these two sources, emphasis is being placed on determining optimum conditions for the aphid to acquire and transmit the two viruses.

2. Tomato

a. Tobacco whitefly. Studies were initiated in Israel (PL 480 project A10-ENT-21) to investigate the periodic acquisition related factor (PARF) of tomato yellow leaf curl virus, a semi-persistent virus, by its vector, the tobacco whitefly. Although viruliferous insects became progressively less efficient in transmitting the virus after initial acquisition, they were unable to reacquire the virus until transmission from the initial acquisition had completely ceased. Inability to reacquire the virus during the persistence period appeared connected with some active process in the insect's body. Homogenates prepared from crushed viruliferous whiteflies and fed to virus free whiteflies before they were given an acquisition feeding reduced their ability to transmit the virus. The PARF factor was found in bodies of whiteflies 24 hours after they had completed a 24-hr acquisition feeding but not in those at the close of

the feeding period nor in those examined 7 days after acquisition feeding which was near the end of the cycle of inoculative loss in the insect. Transmission efficiency of non-viruliferous insects was reduced when they acquired the factor before acquisition feeding. Application of the factor to viruliferous insects after acquisition feeding did not reduce the mean number of transmissions per insect.

3. Cross Commodities

a. Leafhoppers. In studies on Indian Jassidae with particular reference to Circulifer and related genera and their importance as vectors of plant virus diseases (PL 480 A7-ENT-22), 24 genera of Cicadillidae were collected from agricultural crops, fruits, ornamentals, and weeds in the area near Ludhiava, India. Included were at least 3 species of Circulifer, one of which was tentatively identified as C. tenellus. Although symptoms resembling curly top virus have been observed in India, transmission by leafhoppers to indicator hosts for positive identification has not been made.

RPA 701 - INSURE FOOD PRODUCTS FREE OF TOXIC RESIDUES

A. Insecticide Residue Determinations (0.2 SMY)

1. Cabbage. At Charleston, S.C., spray applications of Azodrin at 0.5 lb active ingredient/acre resulted in residues of 0.69, 0.57, 0.38, 0.23, and 0.07 ppm 1, 5, 8, 15, and 22 days after treatment. At 1 lb/acre residues ranged from 1.1 ppm after 1 day down to 0.17 ppm after 22 days.

2. Cucumber. A 10% granular formulation of Union Carbide UC-21149 was put into the soil of greenhouse-grown cucumbers at rates of 22.4, 44.8, and 89.6 kg of active ingredient/hectare. Cucumbers, cucumber foliage, and greenhouse soil were sampled 11 and 26 days after treatment. Combined residues of UC-21149, its sulfoxide and sulfone in ppm in cucumbers at the successive sampling periods were 7.8 and 4.6 for 22.4 kg/hectare; 12.6 and 6.2 for 44.8 kg/hectare; 12.5 and 13.6 for 89.6 kg/hectare. The combined residues in ppm in cucumber foliage for the same periods were 131 and 149 for 22.4 kg/hectare; 399 and 268 for 44.8 kg/hectare; 467 and 400 for 89.6 kg/hectare. Soil residues for the same periods were 20.2 and 20.7 ppm for 22.4 kg/hectare; 70.1 and 9.0 ppm for 44.8 kg/hectare; 71.0 and 32.1 ppm for 89.6 kg/hectare.

3. Potatoes. At Yakima, Wash., foliage sprays of carbofuran were applied to potato plots at the rate of 1.12 kg of active ingredient/hectare 54 and 89 days after planting. Potato samples taken 45 days after treatment showed no detectable residues of carbofuran.

In tests with ultra-low volume spray applications at Yakima, oxydemeton-methyl plus a red dye was applied at 0.75 lb/acre to a potato field. Residues collected on filter papers placed horizontally in the plants

6 inches above ground level indicated deposits of 0.44 lb/acre with a roller-pump applicator with spinning discs, 0.08 with a spray applicator with hoods, and 0.05 with conventional equipment.

At Yakima, carbofuran was applied to the soil at planting time at 2.5 lb/acre. Tubers taken after 69 days and at harvesttime (83 days after treatment) contained no detectable residues (less than 0.01 ppm).

4. Cross Commodities. Greenhouse grown tomatoes, lettuce, and cucumbers at Beltsville, Md., were analyzed for dichlorvos, which had been applied as a 10% aerosol at the rate of 1 gram active insecticide per 1,000 cubic feet or as 5% dichlorvos impregnated on corn cobs for periods of 16 days. The concentration of dichlorvos in the air 2 hours after release of 10% aerosol was about 0.12 µg/liter. Air samples from the impregnated corn cob treatment contained about 0.3 µg/liter after 16 hours of continuous exposure; concentrations in the air when 16-hour nightly exposures were made over the testing period varied from about 0.05 to about 0.02 µg/liter. Tomato and cucumber fruits retained less dichlorvos than their foliage (<0.02 ppm in fruit). Less than 0.013 ppm was found in lettuce under all test conditions.

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AREA NO. 2 - DECIDUOUS FRUIT, TREE NUT, GRAPE AND BERRY INSECTS

USDA and Cooperative Programs

Location of Intramural Work	Scientist Man-Years FY 1968		
	Research Problem Area		
	204	205	Total
<u>Deciduous Fruit</u>	:	:	:
California	: 0.9	: 2.2	: 3.1
Georgia	: 1.1	:	: 1.1
Indiana	: 2.9	:	: 2.9
Ohio	: 0.7	:	: 0.7
Washington	: 6.0	:	: 6.0
West Virginia	: 1.0	:	: 1.0
<u>Total</u>	: 12.6	: 2.2	: 14.8
<u>Tree Nuts</u>	:	:	:
Georgia	: 3.0	:	: 3.0
Louisiana	: 1.0	:	: 1.0
Ohio	: 0.1	:	: 0.1
<u>Total</u>	: 4.1	: 0.0	: 4.1
<u>Small Fruits</u>	:	:	:
Ohio	: 0.2	:	: 0.2
<u>Total</u>	: 0.2	:	: 0.2
<u>Total</u>	: 16.9	: 2.2	: 19.1

Intramural program is supplemented by extramural support representing
(a) 0.5 SMY at State Agricultural Experiment Stations. 1/

1/ RPA 204 - 0.5 SMY

Problems and Objectives

Insects and mites attacking deciduous fruits, tree nuts, grapes, and berries reduce yield, lower quality, and contaminate the crop with insect parts. Some spread plant diseases that reduce the productivity of the host plant. Losses to these crops exceed \$238.5 million annually. Present control methods rely principally on control with insecticides which increase production costs and may leave objectional residues. Development of resistance to certain insecticides by insects and mites is also a serious problem.

Major objectives of the research are to develop and evaluate alternative ways to control insect and mite pests by use of:

1. Insecticides that will minimize or avoid objectionable chemical residues.
2. Insect sterilization techniques.
3. Parasites, predators, pathogens, or other biological agents.
4. Attractants, repellents, and other response stimuli.
5. Ecological and cultural management practices.

Progress - USDA and Cooperative Program

RPA 204 - CONTROL OF INSECT PESTS

A. Basic Biology, Physiology, and Nutrition (5.2 SMY)

1. Deciduous Fruits

a. Codling Moth. Facilities at Yakima, Wash. permit preparation and handling of 32 trays of artificial medium per day for rearing codling moth. The median yield over a 3 month period was about 125 moths/tray.

Basic studies on the influence and significance of photoperiod and light on diapause and development of the codling moth are being conducted under a grant to Washington State University. Larvae exposed in a field chamber for 10 days, starting on November 20, were placed in constant dark to complete development. The last light experience during field exposure was at hour 18 after dawn. The controls (no light supplementation) yielded 40% pupation, continuous incandescent yielded 31% pupation, 1 hour of incandescent yielded 79%, 6 spaced flashes of light yielded 66%, and a single flash yielded 63% pupation.

b. Peach Tree Borer. At Fort Valley, Ga., cast skins of emerged peach tree borers and lesser peach tree borers were collected from 34 sample trees between April 30 and November 18. More (826) lesser peach tree borers emerged during the season than did peach tree borers (92). Lesser peach tree borer emergence began in early May and continued at a high level until October. Emergence of peach tree borers began about July 1

and continued at a low level until early September when it peaked sharply then declined to the end of that month. Cocoons of the peach tree borer were collected from the orchard and subjected to altered light cycles in environmental chambers. Two days was sufficient to entrain emergence from the orchard's solar day to the altered light cycles. Adult emergences occurred in synchrony with the altered light cycles (3:00 a.m. to 4:00 p.m. and 9:00 a.m. to 10:00 p.m.), about an hour after "sunrise." Females emerging and calling males first in one light cycle reentrained their time of calling within 2 days to synchronize with the opposite light cycle when placed in that opposite cycle after their first call.

Under a research grant to North Carolina State University an array of synthetic diets were evaluated for rearing the peach tree borer throughout the larval stage. None of the diets allowed growth beyond the third instar.

c. Plum Curculio. At Fort Valley, Ga., the preoviposition period of the female plum curculio was determined for laboratory conditions of 80° F, 60% relative humidity and constant light. Oviposition began on the fifth day. The model preoviposition period was 11 days; 54.7% of the females had oviposited by the thirteenth day. One percent failed to oviposit and 15% had died at the end of 25 days of adult life. A time lag exists between mating and oviposition by plum curculios and decreases as the females age. For those few females that made fertile matings at six days of age, two days elapsed before oviposition began. One day elapsed between mating and oviposition by those females mating between seven and ten days of age. From eleven days of age onward, mating occurred and oviposition began within 24 hours of the time the females were placed with males.

d. Pear Psylla. Studies at Riverside, Calif., have shown that entrance of pear psylla (Psylla pyricola) adults into diapause depends upon reduction in day length during nymphal stages prior to the last instar. Adults in southern California assumed this resting form in late summer rather than autumn, thus restricting population increases. In northern California, longer periods of reproduction and denser populations have been noted.

Under a grant to Washington State Agricultural Experiment Station basic studies on the behavior of the pear psylla (Psylla pyricola Foerster) are being conducted. Females laid an average of 207 fertile eggs each when mating was delayed for 14 days after their emergence and 231 each when delayed 28 days. An average of 501 eggs were produced by females mated every 7 days. Females kept from mating until 14 or 28 days after their final molt did not live as long as those allowed free mating.

e. Scales and Mites. At Fort Valley, Ga., two seasons of life history study of the white peach scale indicated that 4 generations and a possible partial fifth generation occur each year. Peaks of crawler activity occurred in late April, the end of June, mid August, and early October.

A partial fifth generation occurred in late November in 1967.

At Athens, Ga., greenhouse plantings of apples, peaches, and pecans were established by the Georgia Agricultural Experiment Station for the rearing of field-collected eriophyid mites for study under a cooperative agreement. A detached leaf method is being employed for biological studies and studies of host plant resistance.

2. Tree Nuts

a. Shuckworms. At Albany, Ga., the hickory shuckworm has been reared through 7 continuous generations on a diet of soaked pinto beans, wheat germ, tortula yeast, ascorbic acid, mold inhibitors, agar and water. Pecan foliage and pecan nuts have been used to stimulate moths to mate. Eggs were obtained on a year-round basis by the use of foliage during the winter months and by the use of pecans when available. Moths reared on an artificial diet will oviposit from 2 to 452 eggs, mate from 1 to 5 times, and live 20 to 30 days when held in a glass house. Eighty-five to 92 percent of the eggs hatch. Shuckworm moths began emerging from naturally infested pecan shucks March 21. Peak emergence was from April 10-13 and ended May 6. A total of 5,545 moths emerged, of which 47.7% were males and 52.3% were females.

Data taken from a blacklight trap which automatically changes the killing-collecting containers at hourly intervals revealed that maximum hickory shuckworm moth activity was during the first three hours after sunset. More males were caught during the 1st hour after sunset and females during the 3rd. Moth catch progressively declined for 5 hours, then both sexes showed increased activity during the 9th hour after sunset after which activity again declined.

3. Small Fruits

a. Grape Berry Moths. Segregation of 32 non-diapausing grape berry moths at Wooster, Ohio, from an original field culture produced 48 first-generation nondiapausing and 136 second-generation nondiapausing moths. Female moths laid eggs on grape berries in the laboratory, and first-instar larvae that hatched from these eggs were transferred to a modified alfalfa diet media.

B. Insecticidal and Cultural Control (3.6 SMY)

1. Deciduous Fruits

a. Codling Moth. During the 1967 growing season experiments conducted at Vincennes, Ind., for control of codling moth showed the following percent efficiency at harvest: Azinphosmethyl 95.7, carbaryl 88.1, ryania 55.0, granulosis virus 45.3, Thuricide (semi-weekly applications) 34.9 and (weekly) 0.0, and released Trichogramma 24.1.

b. Peach Tree Borers. At Vincennes, Ind., superior oil, parathion, and endosulfan exhibited a high degree of toxicity to eggs of lesser peach tree borers. Dimethoate, demeton, and azinphosmethyl were of moderate toxicity, and binapacryl, dicofol, and tetradifon were low in toxic effect.

c. Pear Psylla. At Yakima, Washington, applications of three spray oils, PGSO-1, PGSO-2, and Volck Supreme, and two experimental compounds, were very effective against the pear psylla.

d. Spider Mites. Three applications of Sea-Born, a seaweed extract, reduced two-spotted spider mites on lima bean test plants at Kearneysville, W. Va. The seaweed extract did not appear to be directly toxic to Tetranychus urticae. The population reduction may have been the result of some adverse nutritional effect.

2. Tree Nuts

a. Miscellaneous Insects and Mites. At Shreveport, La., a single application of Geigy GS-13005 (.44 lb/100 gal) and demeton (.25 lb/100 gal) gave excellent control of the mite, Eotetranychus hicoriae and a yellow aphid on pecan. Dicofol (.37 lb/100 gal) gave excellent mite control but was ineffective against yellow aphids. Parathion (.30 lb/100 gal) gave fair control of both insects one day after treatment but mites were numerous thirteen days after treatment. Sulfur (4.0 lb/100 gal) gave only limited mite and aphid control.

At Albany, Ga., 5 conventional ground sprayer applications of azinphosmethyl at the rate of 1/2-lb actual/100 gal were compared with 5 ULV aerial applications at the rate of 1 lb actual/acre for control of the black pecan aphid and the hickory shuckworm on pecan. Both treatments provided excellent control of aphids and shuckworm when compared to untreated checks.

At Shreveport, La., Bidrin was poured into holes in the trunks of pecan trees at the rate of 2 ml per inch of trunk diameter. The material was translocated to leaves in a quantity sufficient to kill spittlebug but not phylloxera.

At Wooster, Ohio, two spray applications of a combination of carbaryl and malathion gave satisfactory control of walnut curculio and butternut curculio on butternut trees.

Soil applications of either dieldrin or heptachlor, at 10 pounds actual per acre, applied in late summer, gave significantly better control of emerging walnut husk maggot adults than the untreated check. There was no significant difference between the two insecticides.

3. Small Fruits

a. Mites. At Wooster, Ohio, European red mites were controlled on Concord grapes by the addition of dicofol to either the regular prebloom and petal-fall sprays or to the petal-fall and first-cover sprays.

C. Biological Control (3.4 SMY)

1. Deciduous Fruits

a. Codling Moth. At Vincennes, Ind., codling moth eggs from 0- to 2-day old were readily parasitized by Trichogramma minutum. Two to 3-day old eggs were parasitized in low numbers, while eggs over 3 days old did not support development of Trichogramma. In laboratory and field tests both Trichogramma minutum and T. cacoeciae, emerging from Angoumois grain moth eggs, readily parasitized codling moth and red-banded leaf roller eggs. Both parasite species are capable of development to functional adults in eggs of each of these two hosts. Codling moth eggs from a laboratory colony were affixed to apple trees in which T. minutum and T. cacoeciae were released, to adjacent trees, and to 1-foot stakes in the ground cover. The two species parasitized 53 and 80 percent, respectively, in the release tree but only 3 and 17 percent in trees 20 feet away. Eggs placed on stakes at 5-, 25-, and 50-foot distances from the release trees had 80, 51, and 67 percent parasitization by T. minutum and 59, 59, and 40 percent by T. cacoeciae. Natural parasitization in a non-release orchard was 5 percent.

b. Spider Mites. Cages covered with muslin cloth were used at Kearneysville, W. Va., to study the activity and population differences in pure cultures of Trichogramma urticae and mixed cultures of T. urticae and Amblyseius fallacis mites on bean plants. It was found that the cages provided isolation from invasion of A. fallacis up to 6 weeks. Mixed populations of T. urticae and A. fallacis, starting with 5 A. fallacis/cage, added to a T. urticae population of 300 motile forms/leaf, averaged less than one T. urticae motile form/leaf 30-35 days after addition of A. fallacis. Data indicated that one A. fallacis could eliminate up to 13 T. urticae.

c. Lady Beetles. Studies at Wenatchee, Washington, have shown that the adults of the convergent lady beetle, Hippodamia convergens, were very susceptible to residues of azinphosmethyl, binapacryl, carbaryl, DDT, diazinon, endosulfan, Morestan and parathion. Mortality was essentially total at 24 hours at both normal field rates and one-half field rates. The contact toxicity at both levels of binapacryl, endosulfan and Morestan at 24 hours was 50% or less while DDT resulted in 75-80% mortality. The contact toxicity of azinphosmethyl, carbaryl, diazinon and parathion was 100% at 6 hours. Dosage-mortality curves, established through topical application, indicated toxicities in the order azinphosmethyl>parathion>carbaryl>diazinon>DDT>endosulfan>binapacryl. Morestan was not evaluated topically due to solubility problems.

D. Insect Sterility, Attractants, and Other New Approaches to Control
(5.4 SMY)

1. Deciduous Fruit

a. Codling Moth. At Yakima, Wash., in 1966, traps baited each week with 10 female codling moths were placed every 200 feet for 1,000 feet in each cardinal direction from a 15-acre apple orchard in which 580,334 sterile male moths were released. With the exception of the trap line east of the orchard, there was a steady decrease in catch as the distance from the orchard increased. There was an increased catch in the trap 1,000 feet east of the orchard over the one 800 feet east of the orchard.

In 1967, 8288 irradiated sterile codling moths of each sex per acre were released from a helicopter into a 93-acre apple orchard near Yakima. No insecticide sprays for codling moth were used in this block, but ethion, oil, and tepp were applied to 83 of the 93 acres to control spider mites. At harvest, 0.26% of the apples in the release orchard and 0.24% of the apples in a similar orchard treated with insecticide were damaged by the codling moth; however, most of the damage in the release orchard occurred near stacks of propiles or piles of cut applewood.

The effects of different doses of gamma radiation on codling moth longevity and oviposition have been tested at Yakima. Longevity of adults irradiated at 30-35 krads as pupae or adults is greater than that of untreated adults or those treated with 25 or 45 krads. Irradiated females mated to untreated males produced fewer eggs than untreated checks or untreated females mated to irradiated males.

At Yakima, blacklight and sex pheromone traps compete with one another when the two are in close proximity. Blacklight trap catches reflect this competition in reduced catches of male codling moths. The female moth catch is unchanged. Blacklight traps, in the absence of competitive pheromone traps, catch 5 to 10% more males than females. However, when a competition exists, the ratio of males to females is reversed (5 to 10% more females than males).

No codling moth or red-banded leaf roller toxicants were applied to a 20-acre block of apples located within a 200-acre commercially productive orchard at Vincennes, Ind. Twenty traps, containing 5 virgin females of each of these 2 species, were maintained in the block throughout the season. A total of 209 male codling moth and 16,777 male red-banded leaf rollers was recovered from the traps. Harvest counts showed that 4.4% of the fruit in the experimental block was injured by codling moth and 6.9% by red-banded leaf roller; damage to the grower's block was 0.2% and 0.3%, respectively. The value of sex traps as a survey tool was demonstrated and will be used as an aid to properly timing insecticide sprays next season.

b. Peach Tree Borers. A total of 5,600 female equivalents of lesser peach tree borer extracts was fractionated by the Pesticide Chemicals Research Branch at Beltsville. Sixty-seven fractions were bioassayed by the Vincennes, Ind., laboratory. In field tests, 11 fractions caught males. These are the first fractions of the pheromone of the lesser peach tree borer demonstrating activity.

Marked laboratory-reared males were released in the center of the 45-acre orchard at Patoka (Vincennes) Ind., to determine the efficiency of 60 traps, each containing 6 virgin females. Seven releases (a total of 3,206 marked males) were made. The average recapture percentage was 81.62%, with a range of 69.44 to 91.71%. Eight releases, consisting of a total of 4,987 marked males, were made at different distances from the Patoka peach orchard. The following percentages of released males were recovered: 1/4 mi-47.10%, 1/2 mi-43.12%, 3/4 mi-22.55%, 1 mi-21.87%, 1-1/2 mi-17.01%, 2 mi-14.74%, 3 mi-4.37%, and 4 mi-6.07%. Seven additional marked males were recovered in traps outside the designated trap area at the Schultz peach orchard, about 7 mi northeast of the Patoka orchard. Of the 7 moths, 3 were caught at 3 miles from the release point, 2 at 4 miles, 1 at 9 miles, and 1 at 10 miles.

At Vincennes, Ind., glass beads, filter paper, sea sand and Myverol (distilled monoglycerides of lard) were used as carriers for crude extracts of lesser peach tree borer females. Each of the carriers were placed in separate gallon-size sticky traps and charged with 100 female equivalents of borer extract. The filter-paper traps caught more males than the combined total of the carrier traps (1,192 to 663). The Myverol traps were the least effective, not only in the number of males caught but also in the duration of effectiveness. Although the traps with glass beads and sand did not catch as many borers as the filter paper traps, they caught moths for 16 to 18 days, compared with 4 days for the filter-paper traps. Glass beads and sea sand show promise as carriers for the sex pheromone of lesser peach tree borers.

c. Plum Curculio. At Fort Valley, Ga., continued study of the effect of gamma radiation on the plum curculio revealed that males were not sterilized below 8 kr, but at that dosage longevity was greatly decreased. Eggs from normal females mated to irradiated males were just as infertile 10 days after the mating as immediately after the mating. Females treated with 8 kr were completely sterile and their production of eggs was greatly reduced. The number of larvae emerging was reduced by 90% when treated males (6 kr) were placed in competition with normal males for normal females at the ratio of 40:1:1, respectively.

d. Red-banded Leaf Roller. A synthetic red-banded leaf roller sex attractant (ENT 28959), in amounts of 10, 100, 1,000, and 10,000 female equivalents was placed on filter-paper tubes in field traps at Vincennes, Ind. The tubes were placed in gallon-size cardboard traps, coated with Stikem, and hung on apple and peach trees inside a 1/2-acre cage. Two

checks, one containing 5 and the other 10 virgin females per trap, were used. On alternate days, red-banded leaf roller males were released into the cage. Although the virgin-female traps were removed after 2 weeks, 90% of the total catch was caught by the 2 checks. Although the synthetic sex lure trap catches were low, the lures were still effective in catching males up to 21 to 25 days. As the strength of the synthetic lure was increased tenfold, there was about a twofold increase in male recoveries over the next lower test dosage.

Female red-banded leaf roller moths were used as lures in 43 traps located in 2 apple orchards at Kearneysville, W. Va. An average of 8.5 males per trap were captured in a two-week period. A synthetic pheromone (ENT 28959), used at 50 female equivalents per trap, retained slight moth attractivity through a period of 6 days in field traps. One-gallon-size cartons were found to be more efficient as traps than one-quart or one-pint cartons.

On the day of their emergence, virgin female red-banded leaf rollers were each individually caged and placed in gallon-size sticky traps, which were placed in apple trees at Vincennes, Ind. Younger females were more attractive than older ones and were most attractive during the first 2 days of adult life. As the moths aged, there was a gradual decrease in attractiveness. On the 9th and 10th days, females were about half as attractive as the newly-emerged adults.

e. Other Fruit Insects. Studies of trap recovery of the oriental fruit moth during the 1967 growing season under a grant to Colorado State University have shown the sex attractant trap to be superior to the standard liquid bait trap. It was found that the moths will disperse more than 300 feet from a release point in a 24-hour period.

Standard bait sprays of corn hydrolysate and malathion (WP), were applied to 16 apple trees at Wooster, Ohio, at weekly intervals in 1967. Apple maggot infestation was only 19% as compared to 100% the previous year when unsprayed.

Sticky board traps baited with a corn protein hydrolysate-dibasic ammonium phosphate bait mixture were effective in determining the peak abundance dates of the cherry fruit fly and the black cherry fruit fly in cherry orchards near Wooster, Ohio.

2. Tree Nuts

a. Shuckworms. At Albany, Ga., tests were conducted to determine the merits of various blacklight trap designs for catching hickory shuckworm moths. A standard 15-watt blacklight trap operating on alternating current caught slightly more total insects by weight and slightly more shuckworms than a similar trap operating on direct current. A trap with 5 15-watt bulbs and a 20" diameter funnel equipped with a fan caught 40% more insects by weight and 8% more shuckworm moths than a similar trap without a fan.

A trap with 5 15-watt bulbs caught 61% more insects by weight and 179% more shuckworm moths than a trap with a single 15-watt bulb.

During 1967 a relatively isolated 8-acre pecan orchard of 94 trees near Albany, Ga., was equipped with 33 evenly distributed standard 15-watt blacklight traps. The total calculated number of moths removed from the orchard by the traps was 4,834. Shuck infestation at harvesttime was 14.6%. The nut crop harvested from trees about .3 mi from the test orchard was about 80% infested.

b. Pecan Weevil. Under a grant to Texas A&M University, a study on the use of ionizing radiation and chemosterilants is being made for pecan weevil control. The study is being hindered by the occurrence of high mortality within the control population.

c. Walnut Insects. Under a grant to the California Agricultural Experiment Station at Berkeley, studies on the navel orangeworm, Paramyelois transitella (Walker), on walnuts, indicate that pheromone traps are a useful tool for evaluating field populations when only irradiated males are released. There is evidence that the pheromone traps lose their effectiveness when both males and females are released. Releases of up to 20,000 sterilized individuals per acre have not resulted in adequate overflooding, apparently because the laboratory strain is not adequately competitive with wild moths.

At Wooster, Ohio, standard bait sprays of corn protein hydrolysate and malathion (WP), were applied at weekly intervals. Walnut husk maggot infestation in English walnuts was only 5% compared to 50% the previous year when unsprayed.

3. Small Fruits

a. Blueberry Maggot. At Wooster, Ohio, blueberry maggot appeared to be completely controlled by weekly spray applications of a standard bait spray of corn hydrolysate and malathion (WP). The original infestation had been reduced with bait sprays for the past 4 years.

b. The grass spittle bug Philaneus spumarius Linnaeus as nearly grown nymphs on strawberries near harvesttime were effectively controlled by endosulfan sprays (1.0 lb/100 gal) in tests at Beltsville, Md.

E. Evaluation of Equipment for Insect Detection and Control (0.3 SMY)

1. Deciduous Fruits

a. Codling Moth. A blacklight trap, mounted on the roof of a 7x7x7-ft wood and screen walk-in cage, has proved to have value as a monitoring instrument for measuring codling moth and other insect activity in orchards at Vincennes, Ind. This trap, set in an unsprayed

apple orchard, provided capture records that were more reliable than 20 bait jars at 10 stations for demonstrating emergence peaks and being an index to the numbers and activity of codling moth in the orchard. The cage trap also enabled us to collect insects alive for our research use.

At Albany, Ga., equipment has been developed for collecting and separating pecan shucks from leaves and trash in the field. About 2,050 pounds of shucks infested with hickory shuckworm larvae were returned to the laboratory in plastic lined paper bags sealed and refrigerated at about 40° F. The shucks are being removed from refrigeration periodically to provide moths for the laboratory. Moths were available from April 21 through June 20 at a rate of 114.7 per day. Mortality from storage did not appear excessive. Equipment has also been developed for collecting moths emerging from pecan shucks, immobilizing and sexing them.

F. Varietal Evaluation for Insect Control

Under a grant to the Kentucky Agricultural Experiment Station some of the essential oils extracted from leaves of strawberry clons that were classed as mite resistant exhibited about 100 times the repellency to feeding mites as extracts from susceptible clons. Some of the amino acids that have been extracted from strawberries have been identified and found to differ in their concentration in mite susceptible and mite resistant clons. The strawberry variety Citation is the standard for susceptibility and Surecrop for resistance. The unnamed variety #21169 is more resistant than Surecrop.

RPA 205 - CONTROL OF DISEASES

A. Insect Vectors of Diseases (1.1 SMY)

1. Deciduous Fruits

a. Pear Psylla. An experiment initiated 5 years ago in evaluating the actions and effects of pear psylla, Psylla pyricola, as a vector of pear decline virus was completed at Riverside, Calif. Field cages, screen covered, 9 feet high, initially enclosing 20 pear trees each, were employed. The trees were of a pear decline susceptible type, Bartlett variety on oriental pear rootstocks. Of 40 trees exposed to pear psylla transferred from pear decline infected sites, 13 became infected with pear decline.

Insect transmission work with pear psylla, Psylla pyricola, and pear decline virus revealed an uniquely useful indicator tree for pear decline disease in the Magness pear variety. Three and four-year-old field trees, grown on own-roots, were inoculated by caged viruliferous psylla. The symptoms of disease appeared during the following year and consisted of a pre-mature leaf drop beginning in mid-August, which left the trees bare before the time of normal autumn leaf fall. In subsequent years, the

affected trees showed little new terminal growth and appeared to be in a slow-decline condition. Check trees fed upon by non-viruliferous psylla remained healthy.

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RPA 204 - CONTROL OF INSECT PESTS

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RPA 205 - CONTROL OF DISEASES

Insect Vectors of Diseases

Kaloostian, G. H. 1968. A leaf drop symptom associated with own-rooted Magness pear trees inoculated with pear decline virus by the pear psylla. Plant Disease Reporter. USDA, 52(5): 363-365.

AREA NO. 3 - CITRUS AND SUBTROPICAL FRUIT INSECTS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years FY 1968			
	Research Problem Area			
	204	205	701	Total
<u>Citrus</u>	:	:	:	:
California	: 2.3	: 0.7	: 0.0	: 3.0
Florida	: 2.0	: 0.6	: 0.0	: 2.6
Texas	: 1.9	: 0.0	: 0.1	: 2.0
<u>Total Citrus</u>	: 6.2	: 1.3	: 0.1	: 7.6
<u>Subtropical Fruit</u>	:	:	:	:
Florida	: 0.4	: 0.0	: 0.0	: 0.4
Hawaii	: 9.6	: 0.0	: 0.4	: 10.0
Mexico	: 4.0	: 0.0	: 0.0	: 4.0
<u>Total Subtropical fruit:</u>	14.0	: 0.0	: 0.4	: 14.4
<u>Total</u>	: 20.2	: 1.3	: 0.5	: 22.0

Intramural program is supplemented by extramural support representing PL 480 funds in 4 countries representing 311,982 U.S. dollars equivalent.^{1/}

1/ RPA 204

Problems and Objectives

Insects and mites attacking citrus and subtropical fruits reduce yield, lower quality, spread plant diseases, and contaminate fruit with insect parts. Insecticides required for their control leave undesirable residues and increase the cost of production. Losses to citrus alone exceed \$217 million annually. Reduction of these losses could result in net benefits of \$184 million annually by 1980. Three species of subtropical fruit flies that occur in Hawaii but not in the continental United States pose a serious threat to agriculture of the southern States. Another species is now established in Florida. Methods to avoid their introduction and to eradicate if introduced are necessary to avoid an estimated potential loss of \$42 million and the additional control costs.

Major objectives of the research are to develop and evaluate methods of control to utilize:

1. Chemicals that will minimize or avoid objectionable residues.
2. Nonchemical approaches to control including biological agents, attractants, and sterilization techniques.
3. Treatments to eliminate infestations in quarantined fresh fruits.
4. Chemical, biological, or ecological practices to avoid plant disease transmission by insect vectors.

Progress - USDA and Cooperative Program

RPA 204 - CONTROL OF INSECT PESTS OF FRUIT AND VEGETABLE CROPS

A. Basic Biology, Physiology, and Nutrition (5.1 SMY)

1. Citrus

a. Brown Soft Scale. High winds and heavy rainfall associated with Hurricane Beulah caused significant drop of citrus leaves infested with brown soft scale at Weslaco, Texas. This was followed by sharp increases in scale infestation and late seasonal population peaks.

Observations on sites of scale attachment revealed that in the early season they are much more abundant on the stems of citrus trees. In January, February, and March, 52%, 44%, and 69% more scale, respectively, were found on the stems than on the leaves. In May, a complete reversal occurred and the scale became more abundant on the leaves, and by June, 84% of the scale collected were found on the leaves.

In citrus groves at Weslaco, Texas, the 7 rows of citrus trees immediately downwind of salt cedar and sour orange windbreaks showed significantly lower scale populations than rows farther out. Observations on glass

"sticky plates" coated with petroleum jelly showed higher entrapment of brown soft scale crawlers and Texas citrus mites 180' from the windbreak than 30' away.

b. Thrips. A method of rearing citrus thrips on detached leaves was developed at Riverside, Calif. The life cycle at 86° F requires 13 to 14 days. This technique has made possible laboratory studies of this insect for the first time.

c. Gall Midges. At Allahabad, India, fall larval populations of gall midge, Dasineura citri Glover, affecting citrus varied from 10 to 12 each in citrus buds. Spring populations were similar. The incubation period of the egg in the autumn was 34 to 40 hours, the larval period extended 10 to 12 days, and the pupation period 4 to 6 days. Cocoons were found as deep as 4 inches in the soil at Allahabad. In dry periods droplets of water were highly attractive to the adults.

2. Subtropical Fruit

a. Caribbean Fruit Fly. At Orlando, Fla., 5 complete generations of Anastrepha suspensa have been reared under laboratory conditions with satisfactory increases in returns from numbers of eggs set on each succeeding generation.

b. Gall Midges. At Allahabad, India, under a PL 480 project, it was observed that mango buds infected by gall midge, Procytiphora mangiferae, never open naturally. Heavy infestations caused up to 100% loss of buds. P. mangiferae and Erosomyia indica populations were very low in the off-season of October and November. The latter is the most serious of the 3 species studied in India. It attacks the mango in 4 stages of development from preflowering shoot buds to small fruits. A DDVP aerosol spray was only partially effective in knocking down adult midges in population counts.

Research in Allahabad, India, under PL 480 showed that larvae of gall midges, Udumbaria nainiensia, attacking figs were able to survive long exposures (up to 25 days) in water. During heavy rains mature larvae leave figs very rapidly.

c. Subtropical Fruit Flies. At Hilo, Hawaii, Jerusalem cherry, wild momordica, and loquat were the most heavily infested hosts of 23 plant species collected during the period of June 1967 to May 1968. Cherries were infested by the Mediterranean fruit fly, momordica by melon fruit flies, and loquat by oriental and Mediterranean fruit flies. Mixed infestations of oriental and Mediterranean fruit flies were recovered from methley plum, mountain apple, litche, coffee, loquat, and guava. Soursop, ceylon gooseberry, acerola and tree tomato, which are known fruit fly hosts, failed to yield any infestations from small, limited samplings.

Mating behavior of fruit flies was observed at temperatures ranging from 58 to 68° F at elevations of 3,000 to 4,000 ft. Temperatures of 59 to 61° F inhibited oriental fruit fly mating and reduced mating of the melon and Mediterranean fruit flies. At 66 to 68° F the mating behavior of the oriental fruit fly continued to be inhibited but not so with the other two species.

At Honolulu, Hawaii, studies have shown that adult fruit flies must have sugar soon after emergence to survive, but may live several days without water. With 2-day old adult oriental fruit flies, mortalities were 9 to 10% for sugar only as compared with 98 to 99% for water only. Melon flies were able to survive 5 days with sugar but only 1 to 2 days with water but without sugar. In a normal Hawaiian environment (48 to 76% R.H.) Mediterranean fruit fly adults can do without water for 3 days; in an artificially-induced drier environment, only 1-1/2 to 2 days. The percent emergence from normal and irradiated medfly pupae placed in natural and dry environments 2 days before emergence was found to be almost identical.

Fruit flies also were fed Jello as a substitute for sugar and water in the adult diet. Oriental fruit fly egg deposition and hatch were the same on a Jello-hydrolyzed protein as in the sugar-water-HP diet. Melon fly females lived longer on Jello than on sugar and water. Male longevities were identical.

At Honolulu, Hawaii, preliminary isozyme surveys of oriental fruit flies were made. Electrophoretic analysis of acid phosphatases, amylases, leucine-amino-peptidases, and esterases in newly-emerged 2-week-old and 3-week-old white and yellow marked oriental fruit fly males and females indicated clear-cut differences only in the esterase system. Among 15 ester isozymen separated, there were 2 which were present in white and yellow strain females and in yellow strain males but not in white strain males.

At Honolulu, Hawaii, the dispersal and survival of a white-marked lab strain of the oriental fruit fly in the F₁₀₀ generation and a wild yellow-marked strain lab-reared for 10 generations were compared by means of 4 sterile releases. Recoveries from 32 to 36 methyl eugenol baited traps at selected sites on concentric circles 1/8, 1/4, 1/2, 3/4, and 1 mile out from the release sites showed that males of the white-marked strain were recovered in significantly greater numbers than those of the yellow strain in time and space.

B. Insecticidal and Cultural Control (2.7 SMY)

1. Citrus

a. Screening New Insecticides. Twenty-seven out of 44 experimental materials screened for effectiveness against citrus rust mite at Orlando, Fla., gave high mortality at 20 ppm. Ten were effective at 1 ppm or less.

b. Insecticide Sprays. Brown soft scale populations on potted citrus trees increased 7-1/2 fold between 7 and 8 weeks after treatment with methyl parathion, while the check showed a mean increase of 11.4% during the same period in tests at Weslaco, Tex. Trees treated with azinphos-methyl remained free of scale from the 4th through the 8th week before being reinfested.

Despite average reductions in the number of cotton sprays applied to cotton, only 1/3 the usual number during 1967, brown soft scale increased sharply in July and August in Rio Grande Valley, Tex., citrus groves. This was particularly apparent in several groves immediately north, or downwind, of cotton plantings.

c. Systemic Insecticides. At Riverside, Calif., 14 systemic insecticides were tested for control of spirea aphid, citrus thrips, and citrus red mite. Sweet orange seedlings in 1-gal pots received from 0.05 to .4 g actual toxicant applied to soil surface. Eight to 9 weeks after application, the highest dosage of Union Carbide UC-21149, disulfoton, dimethoate, demeton, schradan, Azodrin, and American Cyanamid CL-47031 produced control of the spirea aphid. Eleven weeks after application the highest dosage of UC-21149, Dansanit, dimethoate, Bay 37289, Azodrin, CL-47031, and phorate produced control of the citrus thrips. UC-21149 and schradan gave control of the citrus red mite for up to 28 weeks, Dansanit 32 weeks, and phorate 45 weeks. Disulfoton remained effective at 52 weeks with 85% mortality.

At Riverside, Calif., soil applications of UC-21149 were made to navel and valencia oranges and Lisbon lemon trees at 4.5 g active ingredient per tree in April 1966, 10 g in March 1967, 25.0 g per tree in Feb. 1968. Control of thrip larvae was 88 to 98% in the summer of 1967 compared to 35 to 100% in 1968. In 1967, 9 months after application, control of the citrus red mite was 0 to 90% compared to 97 to 100% in summer 1968. One month after the 1968 applications there was 93 to 97% reduction in terminals infested with citrus aphids. Trunk measurements of navel and valencia trees in December 1967 showed a slight increase in diameter over untreated controls. Lemon trees show slightly less growth than the controls.

UC-21149 applied as side dress treatment to mature navel orange trees at rates of 0.1 to 0.2 pound active ingredient per tree gave effective control of citrus red mite from 5 to 14 mos after application. During the first 5 months following application, control was inadequate, only 62% control being obtained with the higher dosage. At 14 months, 67 and 97% control was obtained at 0.1 and 0.2 pound dosages, respectively. After 15 months the higher dosage also became ineffective.

2. Subtropical Fruit

a. Screening New Insecticides. At Honolulu, Hawaii, 39 candidate

insecticides were evaluated topically and residually against fruit flies. Four experimental compounds were equal or superior to malathion in activity.

b. Insecticide-bait Sprays. At Honolulu, ten experimental materials combined with PIB-7 evaluated as bait spray deposits on guava foliage performed as well or better than the standard malathion. Carbofuran, Zectran, and Matacil were non-injurious to automobile finishes of acrylic lacquers and enamels.

At Honolulu, 2 protein hydrolysates, O.M. BHY (hydrolyzed blood protein) and O.M. HAP (hydrolyzed meat protein) were more attractive than the standards against both sexes of all 3 species of fruit fly.

In Hawaii, additional 1 mile² tests of undiluted PIB-7 plus technical malathion or naled in 19:1 ratios were applied at 15 oz/acre. Oriental fruit fly populations were reduced 75% and sterile medfly and melon fly populations were reduced 97 to 99%.

Malathion low volume concentrate at 1 oz/acre, with or without the addition of 8 oz of protein hydrolysate bait (PIB 7), effectively reduced Caribbean fruit fly populations in Miami, Fla., when applied to square-mile plots 3 times at weekly intervals during September. Malathion low volume concentrate at 1/2 oz plus PIB 7 at 8 oz/acre also reduced fly populations at this time of the year.

C. Biological Control (3.8 SMY)

1. Citrus

a. Parasites. Microterys flavus was the predominant parasite of brown soft scale during the year at Weslaco, Tex. During past years at most 5% of the parasites collected were M. flavus. This species became increasingly important this year. In January, February, and March, 55.4, 54.4, and 74.3%, respectively, of the parasites collected were of this species. Coccophagus lycimnia, which normally constitutes in excess of 90% of the parasites collected, dropped to 19.4% in March. Higher rainfall and humidity following the hurricane may be responsible for the increase in importance of M. flavus. Parasite levels also became higher than previously recorded. Some groves showed that 65% of the scale collected were parasitized in March.

Heavy parasitism of brown soft scale by Microterys flavus in the Vining Grove at Weslaco, Tex., led to studies indicating that scales from this grove are more susceptible to this parasite than other Rio Grande Valley scales, including the laboratory colony. Laboratory tests of comparative susceptibility showed that 30% of the Vining scale, 11% of the scale from the Guerra Grove in Roma, and none of the laboratory scale were parasitized when they were reared on citron melons and exposed to M. flavus. Brown soft scale obtained from heavily infested cotton at Brownsville, Tex.,

proved to be more susceptible. When this scale was transferred to citron melons and exposed to M. flavus, 100% of the scales were parasitized.

Observations on rearing Microterys flavus in the laboratory at Weslaco, Tex., revealed that the brown soft scale heavily encapsulates and melanizes foreign matter inserted in the scale body. The host defense mechanism appears to be the same when parasite eggs, glass wool fibers, or minuten madein are inserted in the body. Encapsulation of the parasite egg begins at the point of entry and progresses down the egg stalk to the egg. A heavy deposition of melanin that usually accompanies the encapsulation appears to be the principle factor responsible for the mortality of the eggs.

Studies on the ovipositional behavior of Encyrtus lecaniorum, a parasite of brown soft scale, revealed that a parasite with a life span of 8 days deposited as many eggs as those with life spans of 13 days. The mean number of eggs deposited per parasitized scale ranged from 1.58 to 2.56. From 77.5 to 84.2% of the scale exposed to parasites were parasitized. The parasite with the shortest life span had the highest rate of parasitism.

Following release of the California red scale parasite, Aphytis melinus, in a citrus grove at Roma, Tex., a significant decline in population of this scale occurred. Parasites of this genus have been recovered from the grove one year after release but have not been positively identified as to species.

Black scale collected from lemon, grapefruit, mulberry, oleander, and natal plum were heavily parasitized with a parasite identified as Eunotus sp., family Pteromalidae, at Weslaco, Tex. This species was reported from Puerto Rico from this host in 1964. These are the first records of black scale on citrus in several years in this area. Black scale is of little economic significance in the Rio Grande Valley area and Eunotus sp. appears to be the principle parasite.

In May 1967, approximately 11,500 Metaphycus helvolus, an encyrtid parasite of black scale, were released in 4 major citrus growing areas of Florida by personnel of the Orlando, Fla., station. Recoveries were made up to 7 months after the introduction. However, subsequent monthly samples have been negative, indicating that the parasites failed to overwinter successfully. The indigenous predator, Scutellista cyanea, was found to be of significant importance in suppression of 2nd and 3rd generation scale populations but ineffective against 1st generation black scale, Cheiloneurus sp., an internal encyrtid parasite, was found parasitizing 2nd and 3rd stage, and an entomogenous chytrid was found attacking all stages of black scale. This is the first report of these natural enemies occurring on black scale in Florida.

b. Predators. Under a grant at the University of California, Riverside,

the use of supplemental foods to increase populations of mite predators is being investigated. Amblysicus hibisci (Chant) and A. limonicus Garman and McGregor were fed various combinations and proportions of sucrose, yeast hydrolysate, lipids, etc. Water solutions of high concentrations of these foods caused decreased oviposition over the pollen diet, apparently due to a lack of chemical gustatory properties for initiating feeding.

c. Pathogens. The noninclusion virus of the citrus red mite was evaluated in the field at Riverside, Calif. Introduction of field infected mites in large numbers from an epizootic was effective in establishing new epizootics. Virus sprays at 0.05 and 0.1% on citrus seedlings in greenhouses also were effective.

Viability of the citrus red mite virus at room temperature in intact mites almost equalled mites held in deep freeze. Laboratory studies on the influence of temperature and drying on the virus disease show that it can be resuspended after drying at room temperature and holding for periods of 0, 1/2, 1, 2, and 4 hours before resuspension.

Of 7 species of Tetranychid mites tested at Riverside, to determine susceptibility to citrus red mite virus disease, only Tetranychus telarius (Boisduval) developed birefringent crystals indicative of the virus disease.

At Riverside, a Model 1 D-Vac vacuum insect net was useful for studying the noninclusion virus disease infecting the citrus red mite, Panonychus citri (McGregor) when populations are very low. Fifteen of 21 groves sampled showed virus infection. Seven of these had not been detected by the standard procedure of picking 20-leaf samples by hand.

The parasitic fungus, Hirsutella thompsonii, was isolated from the citrus rust mite at Orlando, Fla. Maximum growth and sporulation of the fungus was obtained on potato dextrose agar and Sabouraud dextrose agar. Vegetative growth of H. thompsonii was excellent in a 1.5% yeast extract, 0.05% bacto peptone, 2% dextrose liquid medium with artificial aeration. Methods are being developed for mass producing the pathogen.

2. Subtropical Fruit

a. Parasites. Opius oophilus, the egg-larval fruit fly parasite, continued to dominate parasite recoveries in Hawaii. High parasitism, especially by oophilus, occurred in litche, Jocote corona, coffee, loquat and rose apple. Opius tryoni and Opius fletcheri, parasites of the medfly and melon fly, respectively, continued to be recovered in fair numbers. O. tryoni and O. fletcheri were believed to be ineffective at one time.

At Hilo, Hawaii, it was determined that parasitized and unparasitized fruit fly pupae can be differentiated beginning 2-3 days after pupation. Distinguishing characteristics (by microscopic examination of pupae through

transmitted light) are the formation of the 3 body regions (head, thorax, and abdomen) 36-72 hours after pupation while the parasitized pupae are larva-form in structure. The segregation of parasites and flies before their emergences lessens the workload of later separation after emergences and also lessens the chances of injury or mortality to the adults caused by handling.

D. Insect Sterility, Attractants, and Other New Approaches to Control
(6.6 SMY)

1. Citrus

a. Sex Pheromones. California red scale, Aonidiella aurantii (Maskell), are being reared at Riverside, Calif., for sex pheromone research in large numbers on green lemons and on White Rose potatoes. Virgin females reared on potatoes produced sex pheromone essentially equal in attractiveness to those reared on lemons. However, females on lemons began producing pheromone 5 days earlier and continued for a longer period of time.

Studies of flight pattern of male California red scale were made at Riverside, using traps baited with virgin red scale females placed in lemon trees. Counts showed a distinct male flight peak just before or after sunset. Males were collected when late afternoon temperatures ranged from 86 down to 60° F. Light measurements show that peaks of male flight usually occurred while light was decreasing from approximately 200 down to 15 ft. Males were present when humidities ranged from 20 to 65%. Over twice as many males were captured in traps at heights of 8 and 12 feet as at 4 feet, indicating that traps for red scale field collections should be placed in the upper parts of the trees.

A small trap for field collection of California red scale males was developed. Red scale males were marked with Calco-oil dye and released in a mature orange grove. Tanglefoot-coated cards (3"x5") on 1-pint ice cream cartons baited with virgin females and placed in concentric circles around the scale release point trapped males 300 feet upwind and 620 feet downwind from the release point.

2. Subtropical Fruit

a. Sterilization Techniques. At Honolulu, Hawaii, complete dominant lethality was not obtained in medfly males irradiated at a dosage of 10.7 ± 0.7 kr 1 day before, 1 day after, or 2 days after emergence. Approximately 0.1 to 0.2% of eggs laid by normal females crossed with irradiated males hatched. More eggs were laid by females irradiated as adults than by those treated as 9-day old pupae. Eggs laid by irradiated females crossed with normal males did not hatch.

At Hilo, Hawaii, Mediterranean fruit flies irradiated at 10 kr as pupae were slower to respond to female overtures than normal males. This delay

of 1 to 2 hours would handicap them in mixed populations when females wanting to mate would be diverted to the more aggressive or responsive normal males.

Longevity of melon flies was affected adversely by irradiation to a greater extent than by chemosterilization with tepa. Normal male and female melon flies lived 58 and 54 days, respectively, before there was 50% mortality in the population; chemosterilized flies 52 and 53 days; and radiation-sterilized flies 38 and 33 days.

At Honolulu, 4 sterile releases of equal numbers of the dark and normal melon fly strains were made to compare longevity of the sterilized flies in the field. Trap recoveries from 54 cue-lure baited traps showed that the 2 strains were recovered in about equal numbers over the same period of time. Fifty-eight percent of the recaptured marked flies were taken more than 0.5 mile from the release site.

At Honolulu, low-cost paper bags and cardboard boxes were compared as aerial release containers for release of sterilized melon and Mediterranean fruit flies. At a loading rate of 3,000 pupae per container, bags containing X-shaped cardboard inserts, with sugar cubes as food, were superior to bags with excelsior and equal to the cardboard boxes. Water was not necessary as long as the flies were released within 2 days of emergence.

b. Sex Pheromones. At Hilo, released sexually mature, virgin Mediterranean fruit fly females were attracted in large numbers to live mature males enclosed in Steiner plastic traps. Native populations of Mediterranean fruit fly in the area had been completely displaced by the oriental fruit fly. Of 4,460 females released, 24% were recovered within 6 hours (1 female 1,000 feet away in 4 hours) and 40.3% within 4 days. One male per trap was as attractive as trimedlure, 5 males caught 3 times as many, and 25 sterile males 13 times as many as 1 sterile male. Sterile males were as attractive as normal males to females the first day. Thereafter, sterile males attracted 60% less than normal males. In tests where wild populations of Mediterranean fruit fly existed, the presence of mature males in the traps failed to capture any females.

c. Chemical Lures. At Honolulu, 66 candidate lures were evaluated in an olfactometer against adult fruit flies. Four were very attractive to the Mediterranean fruit flies in the liquid in glass traps but were only slightly so when tested on wicks.

Twelve of 14 fractions of angelica seed oil were found to show attraction in varying degrees to the male Mediterranean fruit fly.

At Honolulu, the approximate threshold concentration of methyl eugenol, trimedlure, or cue-lure for fly response in olfactometer wick tests was estimated by exposing lure concentrations ranging from 500 mg to 0.5

nanogram on 3/8x1/4 inch cotton wicks. Under olfactometer conditions the threshold concentration for attraction was within the range of 5 to 50 ng for oriental fruit flies, Mediterranean fruit flies, and melon flies for each attractant, respectively.

At Honolulu, Hawaii, it was found that male dark strain melon flies were as responsive to cue-lure as the normal (light colored) laboratory flies. Of an estimated 5,000 males of each strain, 2,316 dark and 2,639 normal males responded to cue-lure in the olfactometer. Male melon flies did not respond to cue-lure for the first 3 days after emergence; there was a slight response only the 4th day and a very strong attraction after the 5th day.

At Hilo, sexually mature virgin melon fly females responded to cue-lure in plastic traps only when they were starved in large out-door cage tests. In similar tests, sexually mature virgin females of the oriental and Mediterranean fruit flies responded to their respective lures (methyl eugenol and trimedlure) when food was provided and available to the flies. These responses occur only in the absence or scarcity of mature male flies.

d. Male Annihilation Techniques. In Hawaii, Thixcin E was superior to CAB-0-SIL and monoglyceride of lard as a thixotrope for preparing viscous male lure plus toxicant formulations for airplane distribution in male-annihilation programs. The shearing action of a blender is sufficient to disperse the Thixcin E in fruit fly lures to produce mixtures of the proper consistency without the use of heat.

At Honolulu, 4 musk chemicals extended the effectiveness of trimedlure for 1 week or longer but did not improve the effectiveness of medlure.

In a 2-mile² plot near Hilo, fiberboard bait stations saturated with naled in cue-lure reduced the male population 99%. In one 15-acre area, sterile flies survived at least 66 days after emergence, suggesting that the lure may be unattractive to some flies.

At Hilo, 4 ml of trimedlure on a 5/8" diameter wick in the center of a fiberboard wafer treated with naled caught 3 times as many Mediterranean fruit flies as a fiberboard bait station saturated with a solution of naled in trimedlure. The "wick-wafer" used only 1/7th as much lure but required 8 times as much insecticide. The "wick-wafer" provides flies with an acceptable but lethal resting surface near the lure source which the completely lure-treated wafer does not provide.

E. Evaluation of Equipment for Insect Detection and Control (0.5 SMY)

1. Subtropical Fruit

a. Traps for Survey and Detection. In Hawaii various modifications of

the standard plastic trap baited with trimedlure were tested. Catches of Mediterranean fruit flies were reduced 72% by substitution of 1% naled in the lure for lindane-chlordane (LC) powder in the bottom of the trap; 21% using both naled and the LC powder; 55% by confining the wick in an open end glass tube to reduce volatilization and, 34% by using fiber squares saturated with lure. An Israeli trap with a bottle designed to save lure caught 59% less flies than the standard when it contained DDVP and 35% less when the LC powder was used. As many as 100 flies were attracted but paused for 1 or more hours on adjacent foliage and exterior of high lure-output traps compared to low output traps. With a slight decline in temperature in the late afternoon, most flies left the foliage and entered the nearest trap.

In Hawaii, studies to assess the efficiency of various types of fruit fly traps showed that sticky traps performed significantly better than the standard plastic medfly trap in catching wild medflies. When medflies approach trimedlure in standard traps, many alight on foliage nearby or on the outside of the trap and are slow to enter. Sticky traps take advantage of this behavior by providing a sticky surface near the lure to hold the flies when they alight.

Cue-lure, in clear plastic traps, operated more effectively as an attractant for the male melon fly when the traps were hung at 1/10 foot or less than 6 feet above ground level than when placed between 6 and 15 feet. Cue-lure on fiberboard squares 3 feet above ground level was superior to squares placed on bare soil.

F. Insect Control Treatments for Commodities Regulated by Plant Quarantine (2.4 SMY)

1. Subtropical Fruit

a. Fumigation Treatments. At Honolulu, combinations of methyl bromide with post-treatment refrigeration treatments were tested for control of the Mediterranean fruit fly, Ceratitis capitata and the oriental fruit fly, Dacus dorsalis, in papayas and oranges in corrugated fiberboard cartons or field boxes. Fumigation at the dosage of 2 lb/1,000 ft³ for 2 hrs at 70° F required refrigeration for 11 days at 45° F or 3 days at 35° F. Fumigation at the dosage of 2 lb/1,000 ft³ for 2.5 hrs at 70° F required refrigeration for 4 days at 37° F, 6 days at 45° F, or 10 days at 55° F. Fumigation at the dosage of 2 lb/1,000 ft³ for 3 hrs at 70° F required refrigeration period of 3 days at 45° F and 6 days at 55° F.

In Hawaii, J. C. Carter #1 avocados tolerated fumigation with methyl bromide at the dosage of 2 lb/1,000 ft³ for 2.5 hrs at 70° F and post-treatment storage at 45° F for 6 days.

At Honolulu, 20-min dip treatment in water at 118° F, before or after fumigation with methyl bromide at the dosage of 1 lb/1,000 ft³ for 2 hours

at 70° F, increased the mortality of oriental fruit fly, D. dorsalis, eggs and larvae in fumigated papayas from 88 to 99%. The mortality of melon fly, D. cucurbitae, larvae in fumigated papayas was not increased significantly by the dip treatments.

Tolerance tests were conducted on papayas given the hot water treatment to control decay organisms and then cooled before fumigation with methyl bromide at the dosage of 2 lb/1,000 ft³ for 3 hrs at 70° F and post-treatment refrigeration at 55° F for 6 days. The number of decayed fruits appeared to increase with the stage of coloring when treated. The marketable period at temperatures about 80° F was 2.3 to 3.9 days for fumigated fruits and 2.6 to 5.1 days for untreated fruits. Refrigeration at 45° F scalded both untreated and treated fruits.

At Hoboken, N.J., 6-hour fumigations with ethylene oxide, in combination with carbon dioxide or freon, gave complete kill of nonestivating snails but not of estivating ones.

b. Modified Atmosphere. At Honolulu, mortality of D. dorsalis eggs and larvae in papayas was 82% when fruits were stored in a modified atmosphere containing 0.2 to 1.2% oxygen and 13% carbon dioxide for 5 days at 55° F. Mortality was 60% when the oxygen concentration was 5% and the carbon dioxide concentration was 11%, and 72% when the oxygen concentration was 15% and the carbon dioxide concentration was 3 to 5%.

c. Vapor Heat. At Honolulu, Burpee cucumbers and N-52 tomatoes did not tolerate quick run-up vapor heat treatment. Surface pitting was observed in 80% of the cucumbers and aroma and flavor were less characteristic. The treated cucumbers were spongy, resulting from the separation of the carpels. The flavor and aroma of treated tomatoes were reduced, seeds were darkened, and the texture of the pulp was grainy.

d. Gamma Radiation. At Honolulu, minimum doses of gamma radiation in the Hawaii Development Irradiator required to prevent the development of adults from eggs and larvae of Dacus dorsalis Hendel was found to vary with the bulk density of papayas in the cartons. The mean minimum dose was 25.3 and 29.0 kr when the bulk density was 22.0 and 24.6 lb/ft³, respectively. The minimum dose of 22.2 kr was not sufficient to prevent adult emergence when the bulk density was 24.1 lb/ft³. The minimum dose of about 24 kr of gamma radiation appeared to kill the eggs, larvae, and pupae of the mango weevil in limited tests. A larva survived when the irradiated mangoes were refrigerated at 45° F for 5 days, but the larva was weak and died within 24 hours. There were no survivors from mangoes absorbing minimum dosages of 30.1 to 34.5 kr. The bulk densities of the cartons with mangoes were 27.8 to 33.6 lb/ft³.

RPA 205 - CONTROL OF DISEASES OF FRUIT AND VEGETABLE CROPS

A. Insect Vectors of Diseases (1.3 SMY)

1. Citrus

a. Tristeza. Increasing numbers of melon aphids above 200 per test plant did not result in greater transmission of tristeza virus to Key lime seedlings at Orlando, Fla. Tests using 200, 400, or 800 aphids per plant gave transmission rates of 20.5, 23.0, and 20.5%, respectively, in 83 test plants per series.

b. Exocortis. Exocortis virus was transmitted to one Etrog citron out of 6 when lubber grasshoppers were allowed to feed on an exocortis-infected Etrog citron plant then transferred to healthy plants at Orlando, Fla. Symptoms of exocortis appeared after 134 days.

c. Stubborn. Tests have been undertaken at Riverside, Calif., to determine if there is an insect vector of stubborn disease of citrus in parts of the U.S. where this disease apparently is spreading naturally. Tests have shown that several species of psyllids feeding on plants in or near citrus will feed on citrus and survive for up to 4 weeks. Two species of psyllids, the potato or tomato psyllid, Paratrioza cockerelli, and an Acacia psyllid, Psylla uncatoides, are being tested as vectors. Greenhouse grown Duncan grapefruit or Madam Vinous sweet orange variety are being used as indicator plants. Successful transmission would be expected to become apparent in 3 months to 1 year.

RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC RESIDUES
FROM AGRICULTURAL SOURCES

A. Fumigant Residue Determinations (0.5 SMY)

1. Papayas

At Honolulu, total bromides were determined in papayas packaged in cartons, fumigated with methyl bromide at the dose of 2 lb/1,000 ft³ for 3 hours at 70° F and refrigerated at 55° F. Residues were 11.3 ± 1.1 ppm and 13.1 ± 0.9 ppm when the fruits were analyzed on 1 and 3 days after treatment respectively. Residues were 17.5 ± 1.3 ppm and 15.0 ± 1.3 ppm when fumigated at the dosage of 4 lb/1,000 ft³ for 3 hours at 70° F.

Total bromide residues in papayas were 368 ± 36 ppm at 2 hours, 236 ± 40 ppm at 1 day, and 66 ± 6 ppm at 3 days after treatment in corrugated fiberboard cartons with EDB at the dosage of 24 oz/1,000 ft³ for 2 hours at 70° F. Residues in the shredded newspaper used in the cartons were 513 ± 2 ppm, 211 ± 28 ppm, and 58 ± 8 ppm, respectively. Residues in single-faced corrugated fiberboard liners for pineapples in field boxes were 136 ± 11 ppm, 71 ± 8 ppm, and 40 ± 5 ppm on 2 hours, 1 day, and 3 days, respectively, after treatment at the dosage of 12 oz/1,000 ft³ for 3 hours at 70° F.

Publications - USDA and Cooperative Program

RPA 204 - CONTROL OF INSECT PESTS OF FRUIT AND VEGETABLE CROPS

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Insecticidal and Cultural Control

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Insect Sterility, Attractants, and Other New Approaches to Control

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- Hart, W. G., Fujimoto, M. S., Kamakahi, D., and Harris, E. J. 1967. Attraction of melon flies, Dacus cucurbitae Coquillett, to lures on foliage or cane fibers at various heights. J. Econ. Entomol. 60: 1139-42.

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- Tashiro, H., and Chambers, D. L., 1967. Reproduction in the California red scale, *Aonidiella aurantii* (Homoptera: Diaspididae). I. Discovery and extraction of a female sex pheromone. *Ann. Entomol. Soc. Amer.* 60: 1166-70.

Evaluation of Equipment for Insect Detection and Control

- Hart, W. G., and Myers, V. I. 1968. Infrared aerial color photography for detection of populations of brown soft scale in citrus groves. *J. Econ. Entomol.* 61: 617-24.

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RPA 205 - CONTROL OF DISEASES OF FRUIT AND VEGETABLE CROPS

Insect Vectors of Diseases

- Norman, P. A., and Sutton, R. A. 1967. Host plants for laboratory rearing of the melon aphid. *J. Econ. Entomol.* 60: 1205-07.
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AREA NO. 4 - FORAGE AND RANGE INSECTS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968				
	Research Problem Area				Total
	112	207	701	906	
<u>Alfalfa</u>	:	:	:	:	:
Maryland (Beltsville)	:	2.6	0.6	:	3.2
Arizona	:	1.1	:	:	1.1
Nebraska	:	0.7	:	:	0.7
Pennsylvania	:	0.3	:	:	0.3
Washington	:	:	0.1	:	0.1
Total Alfalfa	:	4.7	0.7	:	5.4
<u>Clover</u>	:	:	:	:	:
Nebraska	:	1.0	:	:	1.0
Oregon	:	0.1	:	:	0.1
Pennsylvania	:	0.5	:	:	0.5
Total Clover	:	1.6	:	:	1.6
<u>Grass</u>	:	:	:	:	:
Arizona	0.9	:	:	:	0.9
Georgia	:	0.6	0.6	:	1.2
Montana	3.5	0.4	0.4	:	4.3
Nebraska	:	0.3	:	:	0.3
Oregon	:	0.9	:	:	0.9
Pennsylvania	:	0.2	:	:	0.2
Washington	:	:	0.1	:	0.1
Total Grass	4.4	2.4	1.1	:	7.9
<u>Ornamentals</u>	:	:	:	:	:
Mississippi	:	:	:	3.0	3.0
Total Ornamentals	:	:	:	3.0	3.0
Total	4.4	8.7	1.8	3.0	17.9

Intramural program is supplemented by extramural support representing (a) 0.7 SMY at State Agricultural Experiment Stations^{1/} and (b) PL 480 funds in 1 country representing 85,065 U. S. dollars equivalent.

^{1/} RPA 112 0.7.

Problems and Objectives

Numerous insect pests that attack forage and range plants in the United States lower seed production, reduce the yield and quality, and decrease the abundance of range plants. Certain insects are involved in the transmission of forage-crop diseases. These losses are estimated at more than \$350 million annually. Among the more important insect pests are grasshoppers, lygus and other plant bugs, stink bugs, seed chalcids, the alfalfa weevil, root borers, spittlebugs, leafhoppers, and a variety of aphids. Insecticides are used to control some of these insects but they are often costly and may create residue hazards in meat and milk as well as adversely affect wildlife.

Major objectives of the work are to develop new or improved control methods by utilization of:

1. Chemical control methods that will avoid harmful residues.
2. Biological control methods - parasites, predators, and pathogens.
3. Germ plasm to develop insect resistant varieties.
4. Knowledge of insect biology and ecology to develop control measures.
5. Sex attractants, sterilization techniques, and other new approaches.

Progress - USDA and Cooperative Program

RPA 112 - RANGE MANAGEMENT

A. Basic Biology, Physiology, and Nutrition (0.9 SMY)

1. Grasshoppers. In central and southeastern Arizona studies on factors influencing grasshopper populations and plant damage were continued on several intermountain rangelands. 1968 spring vegetation was abundant and favorable for grasshopper survival and development, but spring temperatures were below normal and development was about two weeks later than in 1967. Spring populations on three study areas showed a slight increase and more variability in 1968 over 1967: 1968 populations per square yard ranged from 2.2 to 7.9 as compared to from 2.0 to 3.7 in 1967. Late summer populations on four study areas in 1967 differed little from those of 1966, ranging from 3.9 to 8.0 and 2.9 to 8.2 per square yard, respectively. In 1967 damage to forage on an area averaging 6.6 grasshoppers per square yard was approximately 85%. Damage in 1968 is expected to be less due to an abundance of weedy food plants.

Under a research grant to Kansas State University, it was observed that the species of grasshoppers in cultivated pastures appeared earlier and developed more rapidly than those in native grasslands. There were no significant changes in food plant preferences during the different life stages. Slant-faced grasshoppers (Acridinae) were generally grass feeders and their ingestion percentage often ranked with the abundance of grass species. The Oedipodinae (band-wings) were grass feeders or mixed feeders

preferring grasses. The spine-breasted grasshoppers (Cyrtacanthacridinae) were classed as forb feeders or mixed feeders preferring forbs.

A study was initiated under a cooperative agreement with the University of Idaho of the food habits of rangeland grasshoppers. The crop contents of more than 1,000 grasshoppers from different areas have been analyzed and coded for data processing.

B. Insecticidal and Cultural Control (1.5 SMY)

1. Grasshoppers. At Bozeman, Mont., 38 candidate insecticides were screened by topical application to Melanoplus sanguinipes. Compared to a malathion standard, ten experimental compounds were superior at equal dosages.

In small plot field tests Dursban was effective at 2 ounces per acre, and SD 8447 was promising at 8 ounces in comparison to malathion at 10 ounces per acre.

In repeated ULV applications by air to 80-acre plots of rangeland, carbaryl at 8 ounces actual per acre averaged 95.9% control with a sticker and 98.6% without a sticker. Azinphosmethyl applied at 3 to 6 ounces actual per acre gave controls ranging from 95 to 99%. Bay 39007 applied at 6 ounces actual per acre averaged 97.7% control. Dursban applied at 1 ounce actual per acre did not give satisfactory control. Technical malathion at 8 fluid ounces per acre gave 96.1% control.

C. Biological Control (1.5 SMY)

1. Grasshoppers. Studies on the pathogens of grasshoppers and their potential use in grasshopper control have involved 11 specific or groups of pathogens in the laboratory while field studies, both natural and applied, have involved a virus and a protozoan. Field studies have established that the incidence of disease can be increased significantly through the application of pathogens on grasshopper baits.

A study of the natural occurrence of Nosema locustae, a protozoan, in grasshoppers in Camas County, Idaho, is in the sixth year. It is providing information on natural spread of infections which will be useful in predicting the course of epizootics after disease applications.

At San Carlos, Ariz., in September 1967, 24.8% of the adults of Trachyrhachis mexicana were parasitized by the nemestrinid fly, Trichopsidea clausa. In three areas of extreme southern Arizona adults of Morseiella flaviventris and Boopedon nubilum were parasitized by the nemestrinid, Neorhynchocephalus sackenii, at rates of 10.3, 43.3, and 43.2%. Three hundred ninety-two larvae of N. sackenii were collected and redistributed at San Carlos. In 1968, parasitism of spring grasshoppers at San Carlos by flesh flies was less than 1%.

Research under a cooperative agreement with Montana State University showed that the composition of a polyhedral virus of grasshoppers was 80.2% protein, .9 to 1.0% deoxyribonucleic acid, 0.6 to 1.9% polysaccharide and the balance apparently contains lipid fractions. A crystalline virus of grasshoppers was less complex, containing about 67% protein and 29% ribonucleic acid.

Under a cooperative agreement with Montana State University six major classes of lipids were isolated from the total lipids of grasshoppers infected with Malamoeba locustae and disease free grasshoppers. Free fatty acids, sterols, diglycerides, wax esters, and polar lipids composed 25% of the total lipid and tryglyceride the remaining 75%. Myristic, palmitic, palmitoleic, stearic, oleic, and linolenic acids are the principal fatty acids. There was no appreciable quantitative or qualitative difference in the fatty acid composition of the fat bodies of infected or disease-free grasshoppers.

At Bozeman, Mont., the search for a suitable larval media for rearing parasitic flies of the genus Blaesoxipha has been unsuccessful to date, although animal liver formulations seem to be the most promising. Laboratory infections of American grasshoppers by the European Anthomyiid fly, Acridomyia sacharovi, have not been successful.

D. Insect Sterility, Attractants, and Other New Approaches to Control
(0.0 SMY)

1. Grasshoppers. Under PL 480 research at The Hebrew University, Jerusalem, Israel, broadcasting recorded masticating sounds of the desert locust to nymphs of the same species produced a weak but insignificant feeding response. This is contrary to previous results when a definite response occurred.

E. Evaluation of Equipment for Insect Detection and Control. (0.3 SMY)

1. Grasshoppers. Three types of spinner nozzles were compared at Bozeman, Mont., by droplet size and efficiency of control of grasshoppers on 160-acre rangeland plots. Technical malathion was applied by aircraft at 6 fluid ounces per acre. All nozzles gave highly satisfactory control. The Micronair, however, had the least portion of its volume (20%) in drops under 100 μ in diameter and showed the largest ground deposit as compared to the Fischer (EA-12) and turbaero (Bals spinner) atomizers. All airplane tests were in cooperation with Plant Pest Control Division.

F. Varietal Evaluation for Insect Resistance (0.2 SMY)

1. Grasshoppers. Of 26 grass varieties tested for resistance to feeding by Melanoplus sanguinipes at Bozeman, Mont., six were non-preferred and resulted in less than normal weight gain by the grasshopper. They were Stipa viridula (green stipa), Agropyron dasystachyum (P-15581), Festuca ovina var. duriuscula (Durar), F. arundinacea (Kenmont), Dactylis glomerata (Pennlate), and Bromus inermis (Lincoln).

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

A. Basic Biology, Physiology, and Nutrition (1.9 SMY)

1. Alfalfa

a. Alfalfa Weevil. In Maryland adults of the alfalfa weevil were much later moving into alfalfa fields in the fall of 1967 than in previous years. A similar pattern appeared to be true throughout many of the Eastern and Notheastern States. In both Maryland and New Jersey larval populations were much lower in the spring of 1968 and peak development delayed by 2-3 weeks.

Survival of alfalfa weevil adults, with the synthetic hormone, 10, 11 epoxfaresenic acid methyl ester, was good when alfalfa was supplied after treatment, but when treated weevils were placed on sugar water after treatment survival was very low. Tests with additional synthetic hormones show that some compounds required a much lower dosage to break diapause and initiate normal oviposition (10 μ g/weevil as compared to the present 100 μ g/weevil) and had additional effects such as a pronounced juvenilization of larvae and carryover of this effect in progeny of treated adults.

In the laboratory at Beltsville, Md., Sonora alfalfa, grown in flats and infested when 2-3 weeks old successfully supported populations of up to 500 alfalfa weevil larvae per flat. It was shown that 1000 adults per week could be produced by this method.

The addition of alfalfa pollen to cages containing both stems and leaves for food increased alfalfa weevil egg production by 35%. When weevils were confined with alfalfa stems only there was very little oviposition; the addition of pollen increased egg production by 96%. Parafilm "straws" with a diameter similar to alfalfa stems were 53% as effective as alfalfa stems and leaves.

Studies were conducted at Beltsville to determine the effect of rhythm and photoperiod on oviposition. A periodicity effect was not evident as no differences were detected in the total eggs laid by weevils when examined at 8 a.m. and at 8 p.m. Under a regime of 16 hours light and 8 hours darkness (16L 8D), significantly more eggs were laid than under 16D 8L. Significantly less eggs were laid under conditions of constant light (24L) and constant darkness (24D) than under 16D 8L. Also egg laying essentially ceased after 100 days under constant conditions but continued up to 120 days under the variable conditions.

The western strain of the alfalfa weevil has now reached Dawson County, one of Nebraska's prime alfalfa districts. Studies were initiated in 1967 to determine whether the weevil will develop in greater numbers and become more of a pest in this new location than it has been in other areas during its past history in Nebraska. Its seasonal occurrence generally followed

the same pattern as it does elsewhere in the United States. The rate of parasitism by Bathyplectes curculionis fluctuated from 6.1% to 53.5% during the period of weevil development and averaged 15% for the season.

b. Spotted Alfalfa Aphid. At Lincoln, Nebr., significant differences were found between the sexual and asexual forms of the spotted alfalfa aphid in regard to adult weights, post reproductive period, nymphs produced per day, total number of nymphs produced, and total length of life. The reproductive capacity and length of life was greater among the asexual biotype.

c. Pea Aphid. At Tucson, Ariz., four new biotypes of the pea aphid were differentiated by studying populations from Bakersfield, Calif., (BPA); Corvallis, Oreg., (CPA); Mesa, Ariz., (MPA); Reno, Nev., (RPA) at 60, 68, 76, and 84° F in temperature controlled chambers. The longest nymphal stage at 60° was demonstrated by the MPA biotype followed by CPA at 68, 76, and 85°. The shortest nymphal stage at 60° was shown by the CPA biotype followed by BPA at 68 and 76°, and RPA at 84°. Additional evidence for separating the biotypes was obtained when the four populations caused differential seedling survival among four pea aphid resistant alfalfas. Highly significant differences in seedling survival and mean seedling height were obtained among varieties and aphid biotypes.

At Tucson, Ariz., fecundity of the pea aphid decreased when aphids were transferred from broadbean to alfalfa and increased when transferred from alfalfa to broadbean, irrespective of the number of days exposed to the initial host. The most significant changes occurred when aphids were confined to initial hosts for very short (5-10 days) or very long (40-80 days) periods.

d. Lygus. At Tucson adult longevity of lygus bugs was markedly reduced when reared on fresh beans without sugar supplements. However, there was no statistical difference in egg deposition, hatching of nymphs, or nymphal mortality when bugs were reared on beans treated with solutions of 10% sucrose, 10% honey, or tap water. The length of the life cycle was similar between bugs reared on fresh beans treated with 10% sucrose and those treated with tap water.

At Mesa, Ariz., weekly lygus bug surveys in alfalfa and cotton fields showed no nymphs and very low adult populations in cotton fields. Populations were higher in alfalfa fields remote from cotton than in fields adjacent to cotton. Highest populations were observed in alfalfa in August. The data suggest that very little migration of lygus from alfalfa to cotton occurred during the period July 1, 1967, to June 30, 1968.

Under a grant to the University of California at Davis, an enzyme, endopolygalacturonase, produced in the salivary gland of lygus bugs was indicated as a primary cause of the plant damage which results from lygus bug feeding. At dilutions of 1:500,000 the enzyme digested more than 5 mg of plant tissue per hour. Extracts from homogenized whole bugs on the

salivary glands were active but extracts from bugs from which the salivary glands were removed showed no activity. Females showed, on the average, twice the activity of males.

e. Seed Chalcid. At Mesa, Ariz., field populations of the alfalfa seed chalcid entered initial diapause in late August and reached full diapause during the last week in November. Termination of diapause entered initial stages in the middle of April, peaked about the middle of May, and receded during middle of June. There was some evidence that a very small percentage of the population remained in diapause more than one year.

In studies under a grant to the University of Wyoming, attempts to induce the alfalfa seed chalcid to oviposit in several artificial media have been unsuccessful. Treating the media with extracts of different chemicals from alfalfa had little effect on ovipositional response.

f. Aphids. At Las Cruces, N. Mex., research under a grant to New Mexico State University to develop chemically defined diets for aphids showed that a temperature of 69° F was optimum for rearing the cotton aphid. Optimum concentrations of zinc in the diet were 0.0001 and of iron 0.001%. Manganese was detrimental at all concentrations. Omission of some salt mixtures resulted in an increase in growth or longevity. Calcium showed a synergistic effect with one or more elements in the salt solution but little effect in the absence of salt mixtures.

2. Clover

a. Sweetclover Root Borer. Field observations on Walshia micecolorella, sweetclover root borer, indicate that it completes but one generation per year in the vicinity of Lincoln, Nebr. As during the previous year, the insect was abundant only in sweetclover nurseries where plantings have been made, in close proximity to each other, each year for several years. It appears that this concentration and continuation of sweetclover may be an important factor in building up infestations.

b. Sweetclover Weevil. Studies at Lincoln, Nebr., in 1966 indicated that coumarin may be a natural attractant for the sweetclover weevil. However, additional studies using coumarin baited traps produced erratic results indicating that while coumarin may show some attractiveness, it probably is not solely or primarily responsible for attracting weevils to sweetclover.

3. Grass

a. Bromegrass Seed Midge. At Lincoln, Nebr., larvae of a bromegrass seed midge entered diapause inside the florets about the time the grass seeds matured in early July. Laboratory studies indicated that this diapause cannot be broken, even after exposure to low temperatures, until the larvae are subjected to approximately the same moisture conditions which

initiate seed germination. Adult emergence will take place in approximately 10-14 days after the initial application of the moisture.

b. Billbugs. At Corvallis, Oreg., adults of the billbug, Sphenophorus venatus confluens overwintered in plant debris in or near orchardgrass fields. The billbugs fed in April, mated and oviposited eggs throughout May. Eggs were laid inside the stems or near a leaf sheath. Larvae usually fed inside the stem until larval growth became restricted by the size of the stem. Larvae that hatched from eggs deposited on the leaves fed briefly then moved to the roots where they completed their development. Pupae were first found in early August. A few teneral adults were observed in mid-August which marked the beginning of emergence of new adults. Newly emerged adults remained inactive for 6 to 14 days before searching for a suitable overwintering site.

c. Sod webworm. At Corvallis, Oreg., 60% of the sod webworm, Crambus trisectus, larvae reared on Shorey's artificial diet, reached the pupal stage. Males and females laid fertile eggs. Chewings fescue leaves were superior to roots as larval food for C. trisectus. After 25 days, 95% of the leaf-fed larvae were alive compared to 40% of the root-fed larvae. The average weight of root-fed larvae was 15 mg, where, for leaf-fed larvae it was 62 mg. Mortality was 35% for individually reared larvae and 56% for group-reared larvae. No cannibalistic activity was observed.

In other experiments at Corvallis, additives were highly effective in reducing growth of microorganisms in sod-webworm cultures with no adverse affects on larvae. The most effective additive was a mixture of 1.25 gm methyl p-hydroxybenzoate, 0.63 gm sorbic acid, and 1.25 ml of 40% formaldehyde in 400 ml of water.

At Corvallis, Oreg., first instar larvae of C. leachellus cypridalis and C. trisectus were reared at 24° C - 16 hour and 24° C - 10 hour photophases. After 48 days larvae of both species in the 16 hour group developed without interruption. In the 10 hour photophase all trisectus larvae entered diapause, but reaction of cypridalis was variable. Induction of diapause in cypridalis was clearly dependent on an interaction of temperature and photoperiod but not in the case of trisectus where photoperiod acted independently of temperature. Photoperiod was the key factor in termination of diapause of two sod-webworm species. Diapausing larvae were exposed to 24° C - 10 hour and 24° C - 16 hour photophases. In the short day treatment the trisectus larvae were still in diapause after 50 days, but all cypridalis larvae terminated diapause after 37 days although larval growth was retarded. In the long day treatment both species terminated diapause after 24 days. Clearly, the short photo-period exerted a strong influence on breaking diapause in cypridalis although the photo-period effect diminished with prolonged exposure to temperatures favorable for growth.

B. Insecticidal and Cultural Control (1.2 SMY)

1. Alfalfa

a. Alfalfa Weevil. At Beltsville, Md., laboratory screening 9 out of 17 experimental insecticides were promising to control the alfalfa weevil. Encapsulated phorate and parathion were compared with granular formulations at 1 and 2 pounds per acre. Adult weevils were exposed in a chamber for 4 hours every 5 days until mortality dropped below 50% or until 40 days elapsed. Phorate capsules and granules at both rates remained effective through this period (80% mortality or higher) while parathion capsules and granules at the 1 pound rate were ineffective after 20 days and only moderately active at 40 days at the 2 pound rate.

Topical treatment of larvae and adults of the alfalfa weevil with the same insecticides showed that there was a highly significant correlation in response at the LD-95, and a positive but not significant correlation at the LD-50. This indicates that adults, which are much easier to store and handle, can be used in the laboratory as an indication of larval insecticidal activity in the field. There was no difference found in the response of field and first generation laboratory reared adults to topical treatments of three insecticides.

Phorate, carbofuran, and Dursban were applied on March 26 in granular form at 1 and 2 pounds per acre for alfalfa weevil control. A second application of carbofuran and Dursban was made to the same plots on April 9. Larval feeding damage was evaluated on May 8. Carbofuran at the 2 pound rate gave very effective control at both application dates. Carbofuran and phorate gave good control at the rates and dates applied. Dursban did not effectively control the weevil in these tests.

Sprays of carbofuran, Geigy GS-13005, and phorate at 1, 1, and 1/2 pound per acre respectively, were more effective against the alfalfa weevil than methoxychlor, azinphosmethyl, methyl parathion, or a mixture of malathion and methoxychlor at recommended rates.

Eight insecticides were applied at comparable dosages to small plots of alfalfa at Beltsville. Fifteen days after treatment carbofuran, Bay 39007, and Union Carbide UC-34096 gave very effective control of the alfalfa weevil.

b. Army Cutworm. At Lincoln, Nebr., sprays of diazinon at 1 and 1/2 pound, carbaryl at 1-1/2 pound, and trichlorfon at 1 pound per acre applied to alfalfa, significantly reduced the population of army cutworm. Trichlorfon at 1/2 pound and carbaryl at 1 pound per acre were not effective. However, the best treatments only effected a 66% reduction and no differences in alfalfa growth was observed.

2. Grass

a. Billbugs. Post-harvest field burning was ineffective in reducing billbug populations in orchardgrass in tests at Corvallis, Oreg. A total of 409 larvae was found in pre-burning samples, whereas, 383 larvae were found in post-burning samples. Among larvae found in post-burning samples, only five were injured by burning.

C. Biological Control (0.9 SMY)

1. Alfalfa

a. Alfalfa Weevil. Tetrastichus incertus was recovered from alfalfa weevil larvae collected in Crawford, Mercer, and Washington Counties, Pa., and in Highland and Pulaski Counties, Va. The parasite was recovered in only 2 of the 10 New Jersey survey fields and did not exceed 2% parasitism. Six of the 10 fields in Maryland showed the presence of the parasite; an increase over last year. The highest incidence of parasitism for a single Maryland field was 24%.

Bathyplectes curculionis was recovered from 8 of the 10 Maryland survey fields. One field showed a peak parasitism of 60% and an average parasitism of 49% over a 2-1/2-month period starting April 18. This high level of parasitism occurred at the same time as maximum larval development this year. This synchronization of parasite and host populations appeared to be due to the delayed development of alfalfa weevil larvae due to cool temperatures. In New Jersey B. curculionis was found in all 10 fields with a peak of 31% parasitism in one field.

b. Egyptian Alfalfa Weevil. At Mesa, Ariz., no recoveries were obtained of the egg parasite, Peridesmia discus or the larval parasite, T. incertus of the Egyptian alfalfa weevil during the spring survey of 1968, one year after release of the parasites.

c. Pea Aphid. Research under a grant to the University of Kentucky showed that the parasite, Aphidius smithi, is a significant factor in the control of the pea aphid. A single female A. smithi exposed to 50 and 100 aphids parasitized 85 and 92%, respectively.

2. Clover

a. Aphids. A parasite, Aphelinus semiflavus, previously unknown in Nebraska, was detected in 1965, 1966, and 1967, but only when greenhouse cultures of the sweetclover aphid were started from field collected aphids in the fall. Aphid mummies caused by two other species (Praon palitans and Trioxys utilis) are occasionally detected in the field while mummies of Aphelinus are not. Natural field parasitism of the sweetclover aphid and the spotted alfalfa aphid by A. semiflavus varied from 4 to 24% as determined by laboratory rearing of field collected aphids. In laboratory

rearing studies A. semiflavus developed from egg to mummy in 5-8 days (temperature 22°C at night, 31° C day) and required another 6-9 days in the cocoon before adult emergence. The parasite developed equally well on three species of Therioaphis.

3. Grass

a. White Grub. In 1962 field plots in Nebraska were treated with milky disease spores (a mixture of Bacillus popilliae and B. lentimorbus) for control of the white grub, Phyllophaga anxia. Although B. popilliae attacked the grub in the laboratory, subsequent field infection had not been noted until one of these plots was visited, with state cooperators, in the fall of 1967. At that time several grubs with typical milky disease symptoms were found in a meadow adjacent to the one containing the treatments. Further laboratory investigation in the Department of Entomology, University of Nebraska, demonstrated the presence of spores of B. popilliae in the blood of these grubs. This observation indicates the establishment and spread of B. popilliae under field conditions.

b. Rhodesgrass Scale. Research at Weslaco, Tex., under a contract with Texas A&M University showed that aircraft releases of adult and preimaginal stages of Neodusmetia sangwani, a parasite of the Rhodesgrass scale, resulted in 50.0 and 47.8 establishment, respectively. A greater number of established colonies was found with air drops at 1 x 2 mile than 1 x 1 mile release densities.

D. Insect Sterility, Attractants, and Other New Approaches to Control. (0.0 SMY)

1. Alfalfa

a. Alfalfa Weevil. Research under a grant to Virginia Polytechnic Institute, Blacksburg, Va., showed that extracts from alfalfa using either a 1:1 acetone - distilled water mixture or distilled water alone elicited an attractant or arrestant response from the alfalfa weevil. Both extracts were deactivated by heat or when left overnight in an uncovered dish at 3-5° C. When stored at this temperature in a covered container activity was retained for 12-15 days.

E. Evaluation of Equipment for Insect Detection and Control (0.1 SMY)

1. Alfalfa

a. Alfalfa Weevil. At Beltsville, Md., flaming of dormant alfalfa to control the alfalfa weevil was conducted in the fall and spring. Evaluations on April 30, indicated that both fall and spring flaming and a combination of the two gave effective control up to that time. However, weevil development was delayed this spring, and evaluations on May 2 showed that high numbers of eggs were present and a late infestation could be

expected. Stubble treatment after early harvest both with an insecticide and by flaming did not give effective control on the second crop. Plots harvested on May 13 required an insecticide spray prior to harvest to adequately control the weevil, suggesting that under conditions more favorable to weevil development flaming would have to be supplemented with an insecticide treatment.

F. Varietal Evaluation for Insect Resistance (4.3 SMY)

1. Alfalfa

a. Alfalfa Weevil. At Beltsville, Md., laboratory screening and selection for resistance to the alfalfa weevil were concentrated on seven populations which previously had shown the highest level and frequency of resistant selections, and/or with good agronomic characteristics. Under laboratory conditions significant progress has been made for resistance to larval survival and development and adult feeding preference.

Small plot and nursery studies with the most promising laboratory selections in the 1966-67 program gave very promising results in the spring of 1968 both at Raleigh, N. C., and at Beltsville, Md. In the management trials the alfalfa weevil resistant selections yielded as high or higher than the commercial varieties Cherokee, Iroquois, Saranac, and Vernal. There were significantly lower numbers of larvae collected from the resistant selections MS Hp5 and MS Hp 6 than from other entries.

In the spring of 1968 seed of AWPx₃ was made available to alfalfa breeders as a source of alfalfa weevil resistant germplasm. This material was selected for resistance to larval development, adult feeding, and oviposition in laboratory tests.

Large cage field studies were established in July 1967 to compare the response of the alfalfa weevil to four alfalfa entries selected for resistance. Promising results were obtained with two entries selected for larval and/or adult feeding resistance. Entries selected primarily for oviposition resistance in the laboratory failed to express adequate resistance under these conditions.

The response of larvae from eight populations of the alfalfa weevil to alfalfa clones selected for resistance to Maryland weevils was compared in the laboratory at Beltsville, Md. Adults collected from the field in California, Utah, Montana, North Carolina, Ohio, Maryland, Pennsylvania, and New York were parents of the larvae used in the study. There was no significant difference in the response of weevil populations from the eight locations under conditions of this test. Larvae of all populations of the eastern strain developed faster than those of the western strain. Larvae from North Carolina and New York developed faster than those from Ohio, Pennsylvania, and Maryland.

b. Egyptian Alfalfa Weevil. In field plots at Mesa, Ariz., 50 plants representing four genetic sources were selected free of damage under moderate to heavy Egyptian alfalfa weevil infestation. All plants have been placed in an isolated block for production of seed and recurrent selection tests.

c. Aphids. At Tucson, Ariz., progenies of 11 pea aphid resistant experimentals and varieties of alfalfa were evaluated for resistance to biotypes ENT-A and ENT-B of the spotted alfalfa aphid. Seedling survival ranged from 94% for Syn. BB to 59% for Syn. EE in tests against ENT-A. Washoe and Dawson had 82 and 80% seedling survival, respectively. In tests against ENT-B, seedling survival ranged from 95% for Syn. BB to 73% for NS-39. Washoe and Dawson had 84% seedling survival.

At Tucson progenies of new Nevada and Utah experimental alfalfa were evaluated for resistance to ENT-A. The experimentals were developed for resistance to diseases, nematodes, and the spotted alfalfa aphid. Four of the six experimentals from Nevada (MSE-5, MSF-5, MSE-6, MSF-6) had resistance equal to the resistant check. Seedling survival ranged from 90-99%. Two Utah experimentals (U-5156, U-5157) had only moderate resistance.

At Mesa over 500 plants representing eight different genetic sources were screened for antibiosis to ENT-A, and 110 showed high resistance, 120 moderate resistance, 20 low resistance, and 250 were classed as susceptible.

In tests at Tucson 15 superior plants from a 24-clone polycross combination of Chilean alfalfa were selected for high level of resistance to ENT-A of the spotted alfalfa aphid. An additional 17 plants from Hairy Peruvian believed to have tolerance to lygus bugs were selected for high resistance to ENT-A. Thirteen progenies of Sonora parentage were selected from a new two-clone, high forage producing combination for resistance to ENT-A.

At Tucson 53 superior alfalfa plants were selected from a number of 2-clone combinations for combined resistance to ENT-A of the spotted alfalfa aphid and the Bakersfield, Calif., and Mesa, Ariz., biotypes of the pea aphid. All 2-clone combinations were developed in Arizona from selections out of Mesa-Sirsa alfalfa. Several plants were also selected for combined aphid resistance from two new experimentals developed in California.

In greenhouse tests at Tucson antibiosis reaction of pea aphid resistant alfalfa plants was non-significant between populations of the pea aphid reared on broadbean and alfalfa. Populations of pea aphids (Bakersfield, Calif., biotype) reared separately on alfalfa and broadbean in the greenhouse were held on susceptible and pea aphid resistant alfalfa plants for 10 days. Reproduction and survival of the original test population averaged 1.0 and 2.8 aphids per resistant plant from the population reared on alfalfa and broadbean, respectively. On susceptible alfalfa plants reproduction averaged 75 and 48 per plant from the population reared on alfalfa and broadbean, respectively.

A study was initiated at Lincoln, Nebr., to determine the effects of feeding by the pea aphid and the spotted alfalfa aphid on the yield and quality of three alfalfa varieties and one experimental synthetic. The alfalfas used were Vernal and Ranger which are susceptible to both aphids, and Dawson and Kansas State-10 which are resistant to both aphids. Three cages were each planted to four replications of the four entries. One was infested with pea aphids, the second with spotted alfalfa aphids, and the third was kept free of insects. In general, alfalfa infested by either aphid contained less carotene, protein, and digestible dry matter and more fiber than noninfested alfalfas. The resistant alfalfas in the pea aphid cage supported fewer aphids, produced more forage and contained more carotene than the susceptible alfalfas. Fiber, protein, and digestible dry-matter contents of the four alfalfas were not significantly different. The resistant alfalfas in the spotted aphid cage supported fewer aphids, produced more forage, contained less fiber and more carotene and protein than the susceptible alfalfas. Digestible dry-matter contents of the four alfalfas were not significantly different.

d. Lygus. At Mesa, Ariz., 3000 plants representing 32 different genetic sources were screened for resistance to field populations of Lygus. Twenty-five plants were selected free of lygus bug injury.

At Tucson, Ariz., 16 ratios (1:2, 3:2, 5:2, 7:2, 1:4, 3:4, 5:4, 7:4, 1:6, 3:6, 5:6, 7:6, 1:8, 3:8, 5:8, 7:8) of number of lygus nymphs to alfalfa buds were used to determine the ratio that gave normal development and survival of nymphs. Utilizing lygus susceptible alfalfa plants, ratios of 1:8, 1:6, and 1:4 gave zero, 25 and 25% mortality of nymphs, respectively, the lowest among all ratios tested. The 7:2 ratio gave 100% nymphal mortality. A ratio of one nymph to eight buds was used in testing 30 selections for lygus resistance. Five plants showed evidence of resistance when several nymphs died after a 14-day testing period, whereas, all nymphs on susceptible check plants survived and matured.

Research at Manhattan, Kans., under a grant to Kansas State University, has shown that seedling plants of alfalfa varieties can be used to test for resistance to lygus bugs. Seedlings in the cotyledon stage were killed more readily than those in the unifoliolate stage. Plant damage was greater under 16 hours of light than under 0, 8, or 24 hours. Late instar lygus bug nymphs caused more damage than an equal number of adults. The synthetic alfalfa variety KS-10 that has resistance to the pea aphid and spotted alfalfa aphid was more resistant to lygus bugs than other varieties tested.

e. Seed Chalcid. At Mesa, Ariz., a significant reduction in alfalfa seed chalcid populations was found in all new synthetic alfalfas developed for resistance to this insect. Populations were eight times greater on susceptible check entries. A significantly fewer number of diapausing larvae was found in all resistant experimentals, suggesting that resistance either prevents initiation of diapause or terminates it prematurely.

f. Miscellaneous Insects. At University Park, Pa., the third cycle of selection of alfalfa for resistance to spittlebug and the potato leafhopper was completed this spring and compared with previous cycles. There was a trend toward increased resistance to spittlebug and to wilting leaflets caused by potato leafhopper.

Studies were made at Lincoln, Nebr., to determine the relationships among leafhopper feeding injury, yield, and quality of alfalfa clones varying in reaction to this insect. Two field cages were each planted to three replications of 12 alfalfa clones. One cage was infested with 20 adult leafhoppers per plant, and the other was kept free of insects. In general, clones in the leafhopper-infested cage, regardless of visual damage score, produced less forage, contained less moisture, carotene, fiber, and protein than the same clones grown in the noninfested cage. The mean percent reduction from the noninfested controls for each of these characters, with the exception of fiber content, was less for the more resistant clones than the more susceptible clones.

At Tucson, Ariz., over 1600 plants were screened for resistance to root, stem, and leaf diseases. Nearly 250 plants were selected from this group for superior agronomic characteristics. Seed of this group was produced and the progeny screened for resistance to leafhoppers, Empoasca sp., and the spotted alfalfa aphid, resulting in the selection of 12 superior plants.

At Manhattan, Kans., under a contract with Kansas State University, a method was developed for screening alfalfa in the unifoliolate stage for resistance to the potato leafhopper. Among 28 varieties and four experimental alfalfas the winter hardy and variegated floral types were more resistant. One synthetic variety with resistance to the pea aphid, spotted alfalfa aphid, and with some resistance to the potato leafhopper has been approved for seed increase.

At Reno, Nev., under a cooperative agreement with the University of Nevada attempts, using thin layer chromatography, to identify the compounds in steam distillates of alfalfa that may be related to aphid resistance have been disappointing. When put through a charcoal column, the distillate loses most of its activity indicating that these compounds are probably bound to the charcoal. Attempts are underway to elute them from the charcoal.

Under a contract with the Research Triangle Institute, Research Triangle Park, N. C., a satisfactory technique, using thin layer chromatography, has been developed to analyze and separate into individual components the saponins in insect-resistant and susceptible varieties of alfalfa.

At Raleigh, N. C., under a cooperative agreement with North Carolina State University, several extracts from Lahontan alfalfa containing saponins were evaluated and two deterred feeding by the alfalfa weevil. Three saponin fractions from DuPuits alfalfa were tested and one showed deterrent activity. The others showed some attractancy at high concentrations.

2. Clover

a. Sweetclover Root Borer. Walshia miscecolorella was found to infest all of the biennial species and varieties of sweetclover observed at Lincoln, Nebr. While some differences seem to exist between varieties and lines in regard to borer infestations, further work is needed to determine the nature and importance of the differences.

b. Sweetclover Weevil. A feeding deterrent in sweetclover which appears to be primarily responsible for the resistance of Melilotus infesta to the sweetclover weevil has been isolated in pure crystalline form. It has been identified by physical and chemical methods as ammonium nitrate. Ammonium nitrate has been shown to act as a strong feeding deterrent for the weevil. This research was done under a grant to the University of Nebraska, Lincoln, Nebr.

3. Grass

a. Fall Armyworm. At Tifton, Ga., 441 bermudagrass clones were screened for resistance to first instar fall armyworm larvae, and 12 showed nonpreference.

At Tifton 1436 Pearl millet inbreds were screened as seedlings for resistance to first instar fall armyworm larvae. Approximately 4% were rated resistant, 28% intermediate, and 68% susceptible to larval feeding.

At Tifton, various lines of Pearl millet, peanut foliage, and Coastal bermudagrass with known resistance ratings to fall armyworms were analyzed for 6-MBOA (and BOA) content as possible indicators of resistance. The SPF data could not be related to the resistance ratings and since the 6-MBOA and BOA content, if any, is very low, these compounds appear not to be indicators of fall armyworm resistance in these crops.

b. Two-Lined Spittlebug. A highly variable collection of 404 clones of bermudagrass was screened at Tifton, Ga., for resistance to the two-lined spittlebug. Five percent were rated resistant, 47% intermediate, and 48% susceptible.

G. Insect Vectors of Disease (0.3 SMY)

At University Park, Pa., the common fungus fly, Bradysia sp., infesting greenhouses injured red clover and alfalfa seedlings and predisposed these plants to Fusarium roseum, and F. oxysporum f. sp. medicaginis, respectively. Control of Bradysia is essential in conducting insect-root rot investigations.

A study was made at University Park, Pa., to determine the relationship of Sitona hispidula, Fusarium, and bacterial wilt on alfalfa. S. hispidula reduced the growth of alfalfa and provided an entrance for Fusarium. A clear relationship was not established between S. hispidula and bacterial wilt.

RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC RESIDUES
FROM AGRICULTURAL SOURCES

A. Insecticide Residue Determinations (1.8 SMY)

At Tifton, Ga., Imidan residues disappeared on corn in 7 days to 3.37, 3.68, and 5.78%; on soybeans in 15 days to 0.32, 0.50, and 0.92%; and on Coastal bermudagrass in 15 days to 1.06, 1.33, and 1.07% of the residues detected after spray applications at rates of 4, 8, and 16 ounces per acre. Residues of Imidoxon after 7 days on corn and 15 days on soybeans and Coastal bermudagrass were not detected.

At Tifton, Dursban was applied at dosages up to one pound per acre to corn in the field. The residues declined rapidly in the field but remained rather constant in silage. Samples taken on the day of treatment with 1 pound per acre averaged 15 ppm of residue. On the following day, Dursban residues in chopped corn taken just prior to ensiling had decreased to about 4 ppm. During 140 days of storage in the silo the residues declined to about 2 ppm. Residues of Dursban in corn and corn silage treated at 4 and 8 ounces per acre were proportionally lower. No residues of the oxygen analog of Dursban were found in any of the samples. Aqueous seepage from the silos contained traces of Dursban. No residues of Dursban or its oxygen analog were detected in the milk from cows fed the corn silage or in their urine. Their feces contained up to 0.31 ppm of Dursban but no detectable oxygen analog. Cholinesterase activity of blood from the animals fed the treated silage appeared to be unaffected.

At Tifton plots of Coastal bermudagrass and corn treated with fenthion emulsifiable concentrate at 0.5, 1.0, and 2.0 pound per acre were sampled immediately after treatment and at 1, 2, 7, 14, and 21 days posttreatment. Residue analyses for fenthion and five of its metabolites (oxidation products) were performed. Extensive oxidation of fenthion to the metabolites (primarily to fenthion sulfoxide) occurred almost immediately in the field. Residues of the six compounds were detected in the grass and all but the fenthion oxygen analog were found in the corn after various intervals of weathering in the field. Residues of fenthion and the metabolites in corn ensiled in gallon jars were persistent and oxidized at a lower rate than in the field.

At Tifton biological assays to determine the relative toxicity of fenthion and five metabolites were performed using first instar fall armyworm larvae. In order of decreasing toxicity the compounds were: fenthion 0-analog (P=O,S), fenthion (P=S,S), fenthion sulfoxide (P=S,SO), fenthion 0-analog sulfone (P=O,SO₂), fenthion sulfone (P=S,SO₂), and fenthion 0-analog sulfoxide (P=O,SO).

At Tifton the feces from beef steers fed silage treated with Bidrin (15.7 ppm dry basis) for seven days contained no detectable residues of Bidrin or Azodrin (3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate), a

metabolite of Bidrin. Urine samples taken concurrently with feces contained no detectable residues of Bidrin but Azodrin residues ranged from 0.36 to 0.51 ppm.

Residue analyses were made at Tifton of milk and feces from cows fed silage containing 13 or 23 ppm (wet basis) of Gardona. The feces contained 0.17 ppm or less (wet basis) of residue. No residues were detected in the milk.

At Beltsville, Md., pigs were fed alfalfa hay containing 34 ppm of DDT, 28 ppm of heptachlor epoxide, or 150 ppm of malathion. Samples of blood were taken at 2-week intervals throughout the feeding period. The maximum residues found were about 0.01 ppm of heptachlor epoxide, and 0.56 ppm of p,p'DDT. No malathion or maloxon were found.

At Yakima, Wash., a 10% granular formulation of Union Carbide UC-21149 was applied to the soil of potted alfalfa plants and field plots at 1/2, 1, and 2 pounds per acre of active ingredient. The greenhouse alfalfa was sampled 24 to 70 days after treatment and the field alfalfa was sampled seven days after treatment. No UC-21149 was found in any of the samples. UC-21149 sulfoxide and sulfone residues were found in amounts up to 5.7 ppm in the greenhouse alfalfa and up to 0.87 ppm in the field alfalfa. A 10% granular formulation was applied to the soil of potted alfalfa plants at 1, 2, and 3 pounds per acre. Analysis of the alfalfa 51 days after treatment showed no UC-21149 residues, but the sulfoxide ranged from 2 to 14 ppm and the sulfone from 2 to 21 ppm.

Analysis at Yakima of rangegrass treated in Wyoming with ULV applications of Bidrin at 2 and 4 ounces per acre, showed residues of 13 and 51 ppm of Bidrin, respectively, four hours after application. In 45 days these decreased to 0.26 and 0.70 ppm.

At Yakima analyses of rangegrass treated with ULV applications of Bay 39007 at 6 ounces per acre showed 114 ppm on the grass immediately after treatment, 28 ppm after 7 days, and 6 ppm after 14 days. Azinphosmethyl applied at 4 ounces per acre showed 92 ppm immediately after treatment, 106 ppm after three days, 82 after seven days, and 47 after 14 days.

RPA 906 - CULTURE AND PROTECTION OF ORNAMENTS AND TURF

A. Basic Biology, Physiology, and Nutrition (0.7 SMY)

1. White-fringed Beetles. At Gulfport, Miss., visible increases in larval sizes were noted for white-fringed beetle larvae fed different diets, but larval development beyond the second instar did not occur with any diet tested. The use of 0.5% methyl-p-hydroxybenzoate appreciably retarded microbial growth for upwards of 40 days with no detrimental effect on the larvae.

Viability of white-fringed beetle eggs from 1 to 38 days old and stored under different conditions was studied at Gulfport, Miss. Sixty percent of the eggs from 17 to 38 days old held at 92% RH and 55° F hatched after five months storage.

White-fringed beetle eggs of different ages were exposed to temperatures of 8°, 20°, and 30° F for 1 to 14 days. Eggs exposed to 8° F for one day failed to hatch. At 20° F eggs 3 to 10 and 8 to 15 days old hatched after exposures of one and three days, respectively. Eggs 21 to 28 days old failed to hatch when exposed to 20° F. for one day. Eggs 8-15 and 21-28 days old hatched after 14 days' exposure to 35° F.

An artificial rearing method using a potato-soil medium was developed for the white-fringed beetle under a research grant to Auburn University, Auburn, Ala. The length of the larval period was 5.5 months compared with 10.7 months in the field. Survival to the adult stage as high as 50% has been achieved. Conventional artificial diets tested have been unsatisfactory as the antimicrobial agents used were lethal to the larvae.

B. Insecticidal and Cultural Control (1.3 SMY)

1. White-fringed Beetles. At Gulfport, Miss., 32 compounds were tested as soil insecticides against newly hatched white-fringed beetle larvae. At 5 and 10 pounds per acre Dursban and Stauffer N-4446 (ENT 27045) gave 100% control. Thirteen other materials gave 100% mortality at the 10-pound rate.

Potting soil was treated with 10% granular disulfoton at the rates of 1, 5, 10, and 25 pounds per acre. Peanuts were planted and adult white-fringed beetles introduced. The 25-pound rate (2.5 pounds actual) gave 100% mortality for 10 weeks. The 10-pound rate (1.0 actual) gave 100% control for seven weeks.

White-fringed beetle larvae collected in a treated nursery showed the presence of dieldrin and DDT when analyzed by gas chromatography. Soil from the same locations also showed the presence of dieldrin and DDT. Biological tests indicated that the progeny of larvae collected from the same sites were resistant to dieldrin. Larvae from untreated sites showed no trace of insecticides and larvae reared from adults collected from the same sites showed no apparent resistance.

C. Insect Sterility, Attractants, and Other New Approaches to Control (1.0 SMY)

1. White-fringed Beetles. At Gulfport, Miss., 35 chemical compounds were tested in a specially constructed glass olfactometer as attractants or repellents against adult white-fringed beetles. The beetles were not visibly attracted or repelled by any of the materials.

Grant research at the University of Georgia, Athens, Ga., showed that white-fringed beetle adults were not responsive to volatile components of intact or crushed leaves of favored host plants including peanuts, soybeans, aster, cocklebur, or ragweed. Twenty-four chemicals were also tested but none showed any repellent or attractant activity.

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RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

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RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC
RESIDUES FROM AGRICULTURAL SOURCES

Insecticide Residue Determinations

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AREA NO. 5 - SOYBEAN AND PEANUT INSECTS

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-Years FY 1968
Georgia	Peanuts	0.4
Iowa	Other Oilseeds	0.2
Mississippi	Soybeans	0.8
Missouri	Soybeans	2.0
<u>Total</u>		<u>3.4</u>

Intramural program is supplemented by extramural support representing 2.6 SMYs at State Agricultural Experiment Stations (soybeans 0.8; peanuts 1.3; and other oilseeds 0.5).

Problems and Objectives

Soybeans and peanuts are severely damaged by insects in the areas where these crops are grown in the United States. The increasing concentration of acreage in soybeans and possibly the adaptation of native insects to this crop are resulting in more serious insect problems. Basic information is lacking on the biology of many of these pests and on the damage they cause to these crops. Such information is needed to serve as a foundation for the development of satisfactory control methods. Some insecticides, although highly effective, cannot be used because they leave harmful residues.

Major objectives of the work are to develop new or improved insect control methods by use of:

1. Knowledge of the biology and ecology of the major pests.
2. Chemicals that will not result in residues in the crop or crop products.
3. Varieties and selections resistant to insects.
4. Insect parasites, predators, and pathogens.
5. Attractants, repellents, and other new methods of control.

Progress - USDA and Cooperative Program

A. Basic Biology, Physiology, and Nutrition (0.7 SMY)

1. Soybean Insects. In tests at Columbia, Mo., to determine cabbage looper damage to soybeans, small cages each containing four plants were infested with 1, 2, 4, and 8 second instar larvae. Although considerable variation in amount of foliar damage was observed, there was no significant reduction in yield. In greenhouse tests it was shown that three loopers per plant reduced the amount of foliage by 43%. Cabbage loopers feeding on soybean foliage required about 24 days to complete larval development. There was no evidence to suggest that Trichoplusia ni fed directly upon soybean pods.

Preliminary studies at Stoneville, Miss., on rearing the bean leaf beetle, Cerotoma trifurcata, under laboratory conditions indicated that eggs must be held under moist conditions to hatch. The larvae are negatively phototropic, and prefer the young secondary roots of soybeans.

2. Peanut Insects. At Tifton, Ga., preliminary tests of the effects of various temperatures and levels of relative humidity upon development of lesser cornstalk borer eggs indicate that humidity has relatively little effect upon either the rate of development or viability.

At Tifton, Ga., the lesser cornstalk borer has been reared in the laboratory on either a bean or a wheat germ diet. The bean diet is less expensive and produces more and larger insects. Temperature and humidity have

an effect on the number and size of pupae produced, as well as the longevity and fecundity of the adults. A laboratory colony of lesser cornstalk borers has been carried through three generations and more than 2000 eggs per day have been produced.

B. Insecticidal and Cultural Control (0.8 SMY)

1. Soybean Insects. At Columbia, Mo., four varieties of soybeans were treated prior to planting with 4, 6, and 8 ounces of disulfoton per bushel of seed. The heaviest rate of treatment caused a significant reduction in the number of plants which emerged. Stand reductions in plots treated with 8 ounces per bushel were Wayne variety 9.7%, Chippewa 14.5%, Hawkeye 16.2%, and Clark 76.9%. At Columbia, Mo., Clark soybean seed was treated with 2 ounces and 4 ounces of Azodrin per bushel by soak and slurry methods. Plant stands after emergence were 4.3 plants per row foot in the check as opposed to 3.2 plants per row foot and 2.8 plants per row foot with the 2 ounce and 4 ounce of Azodrin slurry treatment. In the soaking treatment at 2 and 4 ounces of Azodrin per bushel plant stand of 2.3 and 0.7 plants per row foot were found.

In tests at Stoneville, Miss., the systemic insecticides, disulfoton, phorate, Lannate, and Furadan, applied as a seed treatment or a granular treatment caused a reduction in the stand of soybeans. Phorate showed the greatest stand reduction in both the seed and the granular treatment.

Research with systemic insecticides for soybean insect control under a cooperative agreement with Mississippi State University showed that disulfoton, phorate, Temik, Landrin, and Hercules 13462 applied at planting time had no effect at the rates used.

2. Insects of Other Oilseeds. Research under a grant to Texas A&M University indicated that early planting of sunflowers helped to avoid extreme damage by the sunflower moth. Sunflowers treated with 2 or 3 applications of carbaryl, GS 13005, azinphosmethyl, or methyl parathion when the heads were in 50 to 100% bloom produced significantly more seed than the check.

C. Biological Control (0.4 SMY)

1. Soybean Insects. Weekly collections of green cloverworm larvae from June 1, to October 1, 1967, at Columbia, Mo., revealed six species of parasites. Three species of tachinid flies, Winthemia sp., Copecrypta sp., and one other unidentified tachinid, along with three braconids in the genera, Meteorus, Apanteles, and Rogas, were collected during the period.

In laboratory tests at Columbia, Mo., first instar nymphs of Chrysopa carnea were able to prey upon Heliothis zea eggs and larvae no older than 48 hours. Second instar nymphs could not successfully attack and kill three day old H. zea larvae.

Field collections of soybean insect predators showed that Nabis roseipennis and N. ferus were the most abundant during the early part of the season at Columbia, Mo. The life cycle of N. roseipennis from egg hatch to adult required about 20 days in the laboratory.

D. Insect Sterility, Attractants, and Other New Approaches to Control
(0.2 SMY)

1. Soybean Insects. At Columbia, Mo., H. zea adults reared from male pupae immersed in a water bath at 41° C for 1.5 hours were effectively sterilized. When treated males were caged with either treated or untreated females, no egg hatch occurred. No reduction in hatch was observed when only females were heat treated. Dissections showed that treated adult males do transfer spermatophores to females indicating sperm sterility.

E. Evaluation of Equipment for Insect Detection and Control (0.1 SMY)

1. Soybean Insects. A technique using time-lapse photography was devised at Columbia, Mo., to successfully record the frequency and duration of mating by corn earworm moths. An electronic flash unit and an exposure no more frequently than once each 90 seconds allowed the eyes of the moths to become dark adapted. Use of this short duration electronic flash unit eliminated the necessity of using special infra-red photographic methods.

F. Varietal Evaluation for Insect Resistance (1.2 SMY)

1. Soybean Insects. At Columbia, Mo., the two basic soybean genotypes backcrossed to each of seven distinct pubescence types were used in oviposition preference tests with the corn earworm. Pubescence types differed in the number and configuration of trichomes on leaves and stems. Results showed highly significant differences in oviposition preference between pubescence types as well as significant varietal interaction. Glabrous soybeans in both basic genotypes were least preferred and dense pubescence types were most preferred for oviposition.

Damage and oviposition of the corn earworm to soybeans at Columbia, Mo., were found to be proportional to the number of trichomes per square centimeter of leaf surface. Four distinct classes of pubescence were distinguishable and oviposition was directly correlated with these classes and increased with an increase in trichome density. Damage was significantly reduced in sparsely pubescent soybean types and evidence suggested that this relationship is maintained even when no choice of pubescence types is offered.

Laboratory data at Columbia, Mo., showed that glabrous type soybeans had significantly higher potato leafhopper oviposition than dense or sparse pubescence types. There was no significant difference between five other pubescence soybean types with regard to oviposition.

2. Peanut Insects. At Tifton, Ga., resistance of peanuts to thrips and the lepidopterous foliage feeder complex in the field was investigated among 343 plant lines. Six varieties were less preferred by the larval defoliators and three lines exhibited resistance (nonpreference) to thrips. For the third straight season this type of investigation showed that yield of lines decreased significantly with increased larval foliage feeding and that leaf feeding by thrips was not significantly associated with yield.

At Stillwater, Okla., research under a grant to Oklahoma State University showed that of 881 peanut lines evaluated, eight had significantly less thrips damage than the commercial variety Starr. Tolerance, antibiosis and preference factors were considered in determining resistance. A synthetic diet was found satisfactory to rear thrips for laboratory peanut resistance tests.

3. Insects of Other Oilseeds. At Ames, Iowa, two species of crambe (a new industrial oilseed crop), Crambe abyssinica and C. hispanica were screened for resistance to the turnip aphid, Hyadaphis pseudobrassicae and the green peach aphid, Myzus persicae. PI 247310 (C. abyssinica) showed a high level of resistance to the turnip aphid. All other accessions were susceptible to turnip aphid attack, although a few plants intermediate in reaction were noted in three other accessions. All crambe accessions were susceptible to the green peach aphid and no plants were selected for further evaluation.

4. Mustard, oilseed rape, and turnip rape insects. At Ames, Iowa, introductions of the oilseed crops oriental mustard (Brassica juncea), black mustard (B. nigra), yellow mustard (B. hirta), oilseed rape (B. napus), and turnip rape (B. campestris) were screened for resistance to the turnip aphid. Introductions of oriental mustard were extremely variable in their reaction to the turnip aphid with resistant, intermediate, and susceptible plants occurring in all accessions tested. Most of the black mustard plants that were evaluated were aphid resistant. However, all yellow mustard plants were highly susceptible. Of the 18 accessions of oilseed rape that were evaluated, only PI 171538 showed resistance to the turnip aphid. A total of 13 accessions of turnip rape were screened for aphid resistance. Out of 635 plants only one plant, a selection from PI 173868 was resistant. Aphid populations on this plant remained low, it grew normally and flowered whereas other turnip rape plants were either killed or severely stunted and did not flower. S₁ seed has been obtained from this plant.

G. Insect Vectors of Disease (0.0 SMY)

1. Peanut Insects. At Raleigh, N. C., under a cooperative agreement with the University of North Carolina, sticky board traps were placed between a clover pasture and a peanut field. Aphid migration to peanuts reached a peak during July. No predominant direction of flight or effect of wind was observed. There was no correlation between aphid numbers and the incidence of peanut stunt disease.

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AREA No. 6 - CORN, SORGHUM, AND SMALL GRAIN INSECTS

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-Years FY 1968
Georgia	Corn	10.0
Iowa	Corn	6.5
Mississippi	Corn	3.0
Ohio	Corn	2.0
South Dakota	Corn	5.9
Iowa	New crops	1.0
Indiana	Small grain	3.0
Kansas	Small grain	1.0
Louisiana	Small grain	1.0
Michigan	Small grain	3.0
Montana	Small grain	1.0
North Dakota	Small grain	2.0
Oklahoma	Small grain and sorghum	2.0
South Dakota	Small grain	4.1
Total		45.5

Intramural program is supplemented by extramural support representing (a) 1.2 SMYs at State Agricultural Experiment Stations, (b) PL 480 funds in one country totaling 7600 U. S. dollars equivalent.

Problems and Objectives

Many species of insects cause losses amounting to millions of dollars annually to corn, sorghum, and small grains. It is estimated that 25 species of insects cause an annual loss of \$900 million to corn alone. Some of the most destructive insects of grain are: European corn borer, corn earworm, greenbug, Hessian fly, wheat stem sawfly, cereal leaf beetle, and rice water weevil. Some progress has been made toward the solution of some of the insect problems encountered in the production of grain crops, but more effective, more economical, and safer insect control measures are needed. Reducing crop losses in grain due to insect damage could result in net benefits of at least \$1 billion annually.

Major objectives of the research are to develop and evaluate alternative ways to control insect pests through:

1. Research on their biology, ecology, and population dynamics.
2. Insecticides that will not leave residues harmful to animals, man, or beneficial insects.
3. Use of attractants, sterilants, or other new approaches.
4. Development of insect resistant varieties.
5. Methods to avoid or counteract insect resistance to insecticides.

A. Basic Biology, Physiology, and Nutrition (11.5 SMY)

1. Corn

a. European Corn Borer. At Ankeny, Iowa, tests conducted with corn borer resistant inbred Oh 43, which was released by the Ohio Agricultural Experiment Station in 1949, indicated that this inbred line has not mutated and/or lost its resistance to corn borer. Lines of Oh 43 maintained at Iowa State University and Ohio Agricultural Experiment Station continued to show comparable resistance to the borer.

At Ankeny tests conducted from 1965 to 1967 to determine if biotypes of the corn borer are developing that can successfully attack resistant inbred lines of corn, indicated that no such biotype has developed on at least one highly resistant inbred, CI.31A.

Data collected in 1966 and 1967 showed that over 90% second brood larval mortality occurred on resistant inbred lines within three days after egg hatch. Larvae from parental stock reared continuously (over 30 generations) on a meridic diet (laboratory strain) were less capable of attacking susceptible inbred inline WF9 than larvae reared from a field-collected population (wild strain).

At Wooster, Ohio, a mass selection program, under diapause-inducing conditions, for a non-diapause strain of European corn borer has produced a strain which has an incidence of over 93% non-diapause.

The 1967 early spring survey indicated a population of 3,833 borers per acre. A late spring survey conducted after corn fields had been disked and seeded to oats showed an average of 1,050 borers per acre. The mid-summer borer population in Boone County was 3,521 borers (third generation) per acre. The summer generation averaged 6,552 borers per acre. However, the post-harvest survey showed that early frost and harvesting operations had reduced the population 81%, to 1,250 borers per acre.

Tests conducted in bioenvironmental chambers with crosses between moths collected in Alabama, Maryland, and Minnesota showed that borers containing Minnesota genes under Montgomery, Ala., or Baltimore, Md., conditions will diapause. However, the same genetic compositions under Minnesota conditions will pupate. This indicates genetic environmental interactions are exerted in favor of the Minnesota genes under more southern conditions and in favor of the Alabama or Maryland genes under northern conditions. However, the progeny of the Minnesota male X Minnesota female cross failed to pupate to any degree at any of the three simulated locations. This agrees with field results reported in the past that the borer in Minnesota is a univoltine type. These tests also indicate that the day length and temperatures of south central Alabama may suppress pupation and cause diapause to occur.

Field experiments investigating the possible existence of biotypes of the European corn borer conducted at Ankeny, Iowa, Waseca, Minn., Portageville, Mo., and North Platte, Nebr., with borer populations from six geographical areas showed (1) a gradient of increasing survival from north to south for all six biotypes; (2) decreasing rate of pupation from north to south. (This is related to the decrease in day length.); (3) decreasing rate of development for all six biotypes from 55 days at Waseca (most northern location) to 38 days at Portageville (most southern).

b. Southwestern Corn Borer. In 1967, the southwestern corn borer spread to four additional counties in Alabama, two in Tennessee, and six in Kentucky. No new county infestations were found in Mississippi.

c. Corn Earworm. A study conducted during the summer of 1967 at Tifton to determine suitable crops for production of the corn earworm pupae showed that the production potential of several host plants was as follows: sweet corn, 19,600 pupae per acre; dent corn, 9,400 pupae per acre; cotton, 4,200 per acre; sesame, 2,800 per acre; and tobacco, 2,200 per acre.

Research conducted under contract by the University of Michigan indicated that light phasing will alter the circadian rhythms of insects, especially Heliothis zea.

d. Corn Rootworm. Laboratory studies conducted at Brookings, S. D., on oviposition preference of the western corn rootworm indicated: (1) soil sites composed of large particles were preferred over small particles;

(2) cracks in soil were preferred to large soil aggregates; (3) moist soil was preferred to dry soil; and (4) clumps of grass were preferred to either corn stalks or surface trash.

Studies of adult corn rootworm hemolymph proteins using polyacrylamide gel electrophoresis indicated a difference in major protein constituents between adult southern and western corn rootworms. Fatty acid analysis of Diabrotica eggs showed that the fatty acid composition of the lipids in all three species was qualitatively identical.

Thin-layer chromatographic analysis of lipids from southern, western, and northern corn rootworm eggs indicated that triglycerides were the predominant neutral lipid and the major phospholipids were phosphatidyl choline and phosphatidyl ethanolamine. Tests conducted at Brookings, S. Dak., indicated that maxillae inhibit feeding of the corn rootworm larvae in the absence of sucrose, a feeding stimulant.

Fifteen host plants, other than corn, have been found on which the western corn rootworm larvae can complete their immature stages and emerge as adults in the field. They are: sand lovegrass, weeping lovegrass, intermediate wheatgrass, barley, wheat, spelt, pubescent wheatgrass, tall wheatgrass, rice, green foxtail, yellow foxtail, foxtail millet, teosinte, eastern gamagrass, and Job's tears.

e. Miscellaneous Insects. At Tifton, Ga., investigations of the antennae sensors in the family Noctuidae and Saturniidae have demonstrated two groups of sharply tapered sensillae on the scape and pedicel of these moths. These sensillae occur in dome-shaped configurations and are distinguished by extremely thin walls and a heavy, doughnut-shaped mounting at the base. They respond to visible radiation. Recordings of these sensors, while irradiated with light, show a typical biphasic nerve potential which feeds back into the antennae and can be detected at any point along the main stem of the moth's antennae.

Studies being conducted in Zaria, Nigeria, Africa, under the AID/ARS Major Cereals Project indicate that instead of one species of shoot fly, there are at least three different types, probably species, of shoot fly damaging sorghum and millet in Africa. Stem borers causing damage on millet, sorghum, and maize are Busseola fusca and Sesamia.

Studies being conducted under the AID/ARS Major Cereals Project in Uganda, Africa, have indicated that the most serious insect problem on maize is the maize stalk borer, Busseola fusca. Insects attacking sorghum are the shoot fly, Atherigona varia siccato, stalk borers, Busseola fusca, Sesamia calamistis, and Chilo zonellus, and the sorghum midge, Contarinia sorghicola.

2. Small Grain and Sorghum Insects

a. Rice water weevil. A technique has been developed for mass rearing the rice water weevil, Lissorhoptrus oryzophilus, in the greenhouse which will make it possible to conduct studies on the host plant resistance and insecticidal control during the winter months.

Field studies conducted in Louisiana on Trichogramma indicated that Trichogramma parasitized 99+% of eggs laid by Chilo plejadillus and Diatraea saccharalis during July and August.

b. Greenbug. Field collected greenbugs from the Stillwater, Okla., area and areas in other States have been identified as biotype B. Biotype A, the original field strain, can no longer be found in the field.

Several differences in greenbug biotypes A, original field strain, and B, current field strain, were studied. In all cases biotype A fed in the vascular bundle, but biotype B fed in the parenchyma (mesophyll) area of the leaf. Insertion of the stylets in feeding was intercellular and in a curving path by biotype A, but it was intracellular and in a straight-line path by biotype B. Biotype B was capable of destroying wheats with resistance to biotype A. Differences in nutritional requirements of the two biotypes also were observed. When both were fed on the standard chemically defined diet, biotype A weighed an average .378 mg in 16 days, as compared no .472 mg in 16 days for biotype B.

c. Hessian Fly. A new Hessian fly biotype Race E was identified from a sample of Hessian fly collected from a Georgia population. It differs from the Great Plains race and Races A, B, C, and D, in that it is capable of infesting wheats having the H_3 gene for resistance and Turkey wheat, having no resistance, but it is incapable of infesting wheats having the H_6 gene for resistance and wheats such as Vermillion and Seneca, resistant to the Great Plains race but susceptible to Races A, B, C, and D. Race F, not yet identified in field populations, was developed in the laboratory by manipulating genes for virulence in the Hessian fly.

Race B was the only race present in a sample collected from an infested wheat field in east-central Missouri where Monon wheat has been growing for several years. In experimental plots in central Missouri, Race A was the predominant race with a small percentage of Race B present. In north-east Kansas the predominant race was Race A, whereas in central and western Kansas and the Great Plains race was predominant. Races A, B, and C were present in small numbers in western, northeastern, and central Kansas, respectively.

Samples from 450 certified wheat fields in 64 Indiana counties had average Hessian fly infestations of 6.1% for varieties Monon, Redcoat, Reed, Riley, and Riley 67, and 1.0% for Knox 62 and Benhur. All varieties were infested with Race B. During 1967 Hessian fly infestations in

Michigan averaged 19%. In Ohio the infestation averaged 2.2%. Only traces of fly were found in North Dakota. Race B was present in all States.

Tests conducted in South Dakota indicated that wheat yield in small field plots, artificially infested with larvae of Eleodes suturalis at planting time, can be reduced 10%.

Studies conducted on the effect of photoperiod on corn leaf aphid indicated that aphids lived longer and produced more progeny when reared on barley under long day condition compared with short day. On artificial diet, aphids produced more progeny under long day.

An outbreak of army cutworm occurred in South Dakota during 1968. A large proportion of the winter wheat and alfalfa fields in western South Dakota were infested with 1 to 15 larvae per square foot. Intensive spraying was needed to control the insect. A majority of the larvae pupated by May 20 and moths appeared in light traps installed to trace their movement by June 7. A high incidence of parasitism and/or disease was found in the immature forms.

d. Wheat Stem Sawfly. In the fall of 1967, estimates were obtained of wheat stem sawfly populations and damage in 20 counties of western North Dakota, using a system of sequential analysis of the sampling units. Percent fields infested ranged from 75 to 100%. Of 20 counties sampled, it was estimated that 16 counties had 100% of the fields infested. Greatest damage from sawfly stem tunneling and cutting occurred in the northwestern counties.

At Fargo, N. Dak., studies on the effect of wavelength of light on wheat stem sawfly oviposition and infestation showed that sawfly oviposition was associated with amount of light transmitted at wavelengths of 425 millimicrons or less. These wavelengths are included in the violet and ultraviolet portions of the solar spectrum. Increased transmission of solar radiation at wavelengths of 425 m μ , or less, resulted in increased sawfly oviposition, infestation, and damage.

Evidence has been found that wheat plants reflect ultraviolet light. Amount of ultraviolet light reflected by wheat plants appears to be a function of: wheat variety, wheat species, stage of development, and degree of stem elongation. Experiments are in progress to test the hypothesis that sawfly preference for oviposition sites is a response to amount of ultraviolet light reflected from the plant.

e. Cereal Leaf Beetle. Recent research at East Lansing, Mich., has shown that diapause of the cereal leaf beetle can be terminated by topical treatment with the synthetic juvenile gonadotropic hormone (trans trans 10, 11-epoxyfarnesenic acid methyl ester). This is considered to be a major breakthrough in the rearing of the cereal leaf beetle as previous techniques required that adult beetles be held for about 90 days at 38° F to break

diapause. Laboratory studies indicate the possibility that only the female cereal leaf beetle has an obligatory diapause.

Laboratory studies conducted at East Lansing, Mich., revealed that light had a marked effect on oviposition behavior of the cereal leaf beetle. Oviposition is obtained after as little as four weeks of cold storage when beetles are kept in a 16-hour photoperiod; whereas, as much as 16 weeks of storage is needed before oviposition occurs under the other photoperiodic conditions.

Studies have indicated that the egg stage of the cereal leaf beetle is the most resistant stage to cold temperatures, followed by the pupa, larvae, and active summer adult, respectively. Complete development from egg to adult requires an average of 92 days at 58° F, 52 days at 67°, and 28 days at 80°, 23 days at 90°. An age specific life table constructed from laboratory populations of the cereal leaf beetle showed that mortality was highest in the pupa stage (39%), followed by the egg (35%), and the larval stage (33%). The net reproduction rate was calculated to be 3.9 under the laboratory rearing conditions.

Studies indicate that the cereal leaf beetle can be effectively sterilized by beta gamma and X-ray irradiation. 2,000 r appears to be the minimum amount of radiation required to sterilize the adult beetle. Histological changes in the neurosecretory system of the cereal leaf beetle indicated that diapause in Oulema melanopus is a result of an inactive corpus allatum.

f. Miscellaneous Insects. The lesser cornstalk borer was reared successfully for the first time on a wheat germ artificial diet. Egg, larval, pupal, and adult development were adequate for mass production procedures. The sorghum webworm was likewise mass reared for the first time on a similar wheat germ diet in the laboratory. This diet was also successfully used to rear all forms of the sugarcane borer.

The long life cycle of most spiders studied may be a limiting factor in their mass production for use in biological control of sorghum insects. Phidippus audax, for instance, required about 300 days to complete development from egg to adult.

Fall armyworms reared on the artificial wheat germ diet for 17 generations fed readily when transferred to seedling sorghum plants, and damage to the plants was comparable to that caused by larvae reared on natural food (sorghum).

B. Insecticidal and Cultural Control (5.1 SMY)

1. Corn Insects

a. European Corn Borer. At Ankeny, Iowa, systemic crop protection chemicals, American Cyanamid CL-47470 (4 pounds per acre) and carbofuran

(4 pounds per acre) applied at planting time produced good corn leaf aphid control 70 days after treatment. These chemicals plus TD-5032 exhibited encouraging borer control. Fourteen compounds tested against first generation borers at various rates, mixtures, and in various formulations indicated that EPN, parathion + heptachlor, parathion, diazinon, Azodrin, Gardona, Velsicol VCS-506, Hercules 14503, Union Carbide UC-30045, and DDT are effective insecticides for corn borer control.

At Ankeny, Iowa, the second year of examining the effect of visible phytotoxicity from the application of diazinon on yield and quality of 20 inbred and 45 single cross corn varieties substantiated previous results. Differential phytotoxicity was obtained with no reduction in yield, test weight, or kernel weight. Genetic and environmental factors influenced foliar expression of phytotoxicity to diazinon.

Experiments conducted to determine the effect of plant population (13, 17, and 26 thousand per acre) and row width (15, 20, 30, and 40 inches) on borer control, and borer establishment showed differences in control or establishment for first- or second-generation borers.

Laboratory studies designed to select for borer tolerance to a chlorinated hydrocarbon (DDT), organophosphate (diazinon), and carbamate (carbaryl) crop protection chemicals indicated that after 12 generations of selection pressure the borers developed a degree of tolerance to each chemical tested. A slight cross tolerance between classes of chemicals was also noticed.

The toxicities of phorate and five of its metabolites to the borer were determined to provide the relationship between residues present and insect control. Residues of these compounds were determined for the various parts of the plant treated with 1 pound actual per acre 1, 3, 7, 14, 21, and 28 days after application and at harvest. Phorate, its sulfoxide, and sulfone were the most toxic to the borer and were present in the whorl, as determined by chemical analysis, in the greatest amounts.

b. Rootworms. At Brookings, S. Dak., a 3-year study showed that removing corn for silage by September 1 resulted in significantly higher yields the following year due to a reduction in rootworm populations and corn lodging. There was an increase of 12.2, 11.0, and 11.6 bushels per acre for 1965, 1966, and 1967 following early silage removal. This indicates that heaviest oviposition occurs after September 1 in South Dakota, and that the use of chemical control might not be necessary the following year in fields where silage has been cut by this date.

The effect of environmental factors on the overwintering eggs of northern and western corn rootworms was evaluated in the field using spring and fall plowing as a means of relocating the eggs vertically in the soil. Winter temperatures below -8° C were lethal to corn rootworm eggs. Dessication of eggs near the soil surface due to lack of late fall and winter precipitation resulted in a greater egg mortality than that resulting from the cold temperatures.

c. Miscellaneous Insects. Research conducted under contract by the National Cash Register Company, produced several microencapsulation formulations of foliar and soil insecticides (malathion and diazinon) and viruses (specifically, polyhedrosis virus) for testing against alfalfa weevil, corn borer, grasshoppers, corn earworm, cotton bollworm, and corn rootworm. Preliminary results indicate little or no increase in control using encapsulated materials.

2. Small Grain Insects

a. Greenbug. At Stillwater, Okla., spray applications of Dursban to small grains for control of greenbugs at rates of .25 and .125 pound per acre resulted in 96 and 99% control after seven days. Of 24 other new insecticides tested for greenbug control, Dasanit, Bay 78182, Geigy GS-13005, Bomyl, and Thiocron were as effective as parathion at comparable application rates.

Liquid disulfoton used as a seed treatment for greenbug control on wheat, oats, and barley was very effective for 13 and 8 weeks for the .25 and .125 pound per 100 pounds seed treatment rates. Seed germination was reduced at higher rates of application. This material shows promise as an insecticide for greenbug control. Liquid disulfoton at varying rates in combination spray shows good promise for spring greenbug control in small grains.

b. Cereal Leaf Beetle. At East Lansing, Mich., Bay 39007 and carbofuran gave excellent field control of adult cereal leaf beetle and larvae both as a seed treatment and as granular applications. All seed treatment rates of Bay 39007 (1, 2, and 4 ounces per 100 pounds of seed) and carbofuran (4 and 8 ounces per 100 pounds of seed) gave good control of spring adults and larvae. Granular application of one half pound per acre of Bay 39007 and carbofuran were also effective. Similar rates with Union Carbide UC-21149 killed significantly fewer larvae but the one pound rate was not different from Bay 39007 or carbofuran.

c. Rice Water Weevil. At Baton Rouge, La., 20 insecticides were evaluated for control of the rice water weevil as seed treatments. Dursban was the only material that gave satisfactory control and did not show observable signs of phytotoxicity.

Carbofuran as a granular formulation applied at 0.5 pound active ingredient per acre 24 hours pre- to five days post-flood provided excellent control of the rice water weevil. Chevron RE 5305 at one pound active ingredient per acre also provided satisfactory control.

Studies on the effect of time of application on control of the rice water weevil indicate that the application of granular insecticides on the soil surface just hours prior to flooding gave the best maximum and long lasting control. Treatment with 0.5 pound active ingredient of carbofuran was very effective against weevil larvae established in the root systems of the seedlings when applied within 16 days after flooding.

C. Biological Control (4.1 SMY)

1. Corn Insects

a. European Corn Borer. At Ankeny, Iowa, Bacillus thuringiensis formulations 90TS(S7-145), liquid, S7-144, liquid with a protective agent, and S7-147 (microencapsulated preparation), were tested in the laboratory and field as microbial insecticides for control of the European corn borer. Applications were made on a basis of viable spores per gram of formulation. At 25×10^6 viable spores per gram of diet, S7-145 and S7-146 formulations gave 79.5% and 89.0% mortality after 48 hours. S7-144 gave 41.1% mortality.

In field studies S7-144 gave 76% control; S7-145, 51%; S7-146, 61%; and DDT (check), 72%.

Horogenes punctorius and Macroncentrus grandii were the two prominent parasite species collected in a survey covering nine States in the Midwest. A few Pyraustomyia penitalis and Lydella thompsoni were also collected. The highest parasitism was 12.8% by M. grandii in North Dakota. No Trichogramma sp. were detected.

Perezia pyraustae infection rates in Boone County, Iowa, during the 1967 surveys were the lowest ever recorded. During this same period there was a slight increase in corn borer larvae per acre over the previous year.

At Brookings, S. Dak., seven chlorinated hydrocarbons and organophosphates were tested in vitro for compatibility with B. thuringiensis. Growth curves were established for nutrient broth cultures containing 0.7, 1.7, and 3.5 ppm (equivalent to 1, 2.5, and 5 pounds per acre actual) insecticide. Bacterial growth was prohibited with 3.5 ppm DDT, aldrin, and heptachlor. 1.7 ppm of the chlorinated hydrocarbons reduced bacterial populations by more than 50%. Growth curves were affected to a lesser degree at the 0.7 ppm level. Malathion, diazinon, and the epoxides of aldrin and heptachlor did not appear to affect growth when compared with the controls.

b. Fall Armyworm. At Lafayette, Ind., a histopathological study of two virus diseases of the fall armyworm, Spodoptera frugiperda, revealed that the granulosis virus attacks the fat body, causes a proliferation of cells, and requires a relatively long time to produce mortality; however, the nuclear polyhedrosis virus attacks a wide variety of tissues, does not cause a proliferation of cells, and produces mortality in a relatively short period of time.

A study was conducted at Tifton, Ga., to determine the effect of age and fertility of host eggs on their suitability for parasitism by T. evanescens. The study demonstrated there is very little or no variation in the suitability of Cadra cautella eggs during the first 60 hours of the incubation period. All the host eggs in this age range are equally suitable for oviposition and development by the parasite. During the last 12 to 24 hours

of the incubation period and associated with the period during which the morphological characteristics of the head of the host become evident, the eggs become progressively less acceptable for oviposition and development by the parasite.

Eggs from virgin females were often found to be suitable hosts for the parasite. However, the percentage of these eggs that were suitable varied considerably. The causes for the variation were unclear.

Eggs of one to five days of age from tepa-sterilized fall armyworm moths were found to be equally susceptible to parasitism by T. evanescens as one- and two-day-old control eggs. Since normal fall armyworm eggs usually hatch on the third day, chemosterilized eggs are available for parasitism at least twice as long as the normal eggs.

c. Corn Earworm. In feeding tests at Stillwater, Okla., involving adults of ladybird beetles, lacewings, (beneficial insects), corn earworms, and 5th and 6th instar corn earworm larvae (destructive insects), the jumping spider, Phidippus audax, showed preference for the corn earworm larvae over a 7-day feeding period. Some of the beneficial insects were eaten. Egg laying female spiders were the heaviest feeders.

2. Small Grain and Sorghum Insects

a. Army Cutworm. At Brookings, S. Dak., a rather large (120 x 240 μ), single, rod-type virus embedded in polyhedral bodies measuring 2.5 μ in diameter was isolated from field collected larvae of the army cutworm. Preliminary studies indicate that only the fat body cells were infected with the virus.

b. Cereal Leaf Beetle. During the spring and early summers of 1966 and 1967 field releases of Anaphes were made in various areas in southern Michigan. Several recoveries (parasites) were found in the spring of 1968, several miles from the release areas, indicating that Anaphes is now established in the cereal leaf beetle area.

A microbial analysis on several dying cereal leaf beetle larval specimens observed at East Lansing, Mich., revealed two potential pathogenic bacteria, Streptococcus faecalis var. liquefaciens, and Serratia marcescens. These as well as Aerobacter aerogenes and Proteus sp. which were previously reported from the cereal leaf beetle and later isolated from our laboratory cultures are also potential pathogens. The presence of these microorganisms suggest a strong possibility that the primary cause of mortality is linked with suboptimal environmental or rearing conditions.

Investigations conducted in Poland under PL 480 have led to the discovery of five additional parasites of Oulema gallaeciana overall parasitism recorded varied from 10 to 50%. A convenient morphological character of sexing cereal leaf beetle adults was found.

Several shipments of parasites collected under PL 480 in Yugoslavia were shipped to the United States for release in the cereal leaf beetle area. These shipments included about 4000 egg parasites (A. flavipes) and 3600 larvae parasites (unidentified).

D. Insect Sterility, Attractants, and Other New Approaches (2.9 SMY)

1. Corn Insects

a. Fall Armyworm. At Tifton, Ga., research was continued on sex pheromones of the fall armyworm, S. frugiperda. The active principle of the female fall armyworm was isolated in pure form and identified. When the synthetic was released in the greenhouse at the rate of one microgram per square yard for 10 days, it confused the adults to such a degree that mating and larvae damage was significantly reduced.

At Tifton, Ga., mating studies in which sterilized and fertile fall armyworm males were mated to normal females showed that the sequence in which the matings occur is very important. Females mated to a sterile male and then to a fertile male, oviposited 95% viable eggs, indicating that sterile sperm are not competitive with viable sperm. However, in the opposite mating sequence, results were not so clear. Oviposition from females mated to fertile males and then sterile males indicate an all or nothing type response. Approximately 45% of the females produced all sterile eggs after the second (sterile) mating while 45% produced all viable eggs.

b. Corn Earworm. One hundred percent sterility was obtained on laboratory-reared corn earworm pupae one to two days from eclosion, with irradiation dosages of 17.5 KR (females) and 33.0 KR (males). Adults, less than one day of age when irradiated, were also sterilized with dosages of 17.5 KR (females) and 33.0 KR (males). At Tifton, Ga., Cobalt-60 was used to sterilize male and female fall armyworms. Adults of both sexes were effectively sterilized with 20 KR. Competitive trials show copulation unaffected by these treatments but sperms from treated males are not competitive with sperms of untreated males.

c. Southwestern Corn Borer. At State College, Miss., studies on the effect of different levels of gamma radiation on the emergence of the southwestern corn borer from the pupa indicated that the early pupal stage is much more sensitive to radiation than either the mid- or late-pupal stages. The early pupal stage, one to two days old, cannot tolerate more than 5 KR without serious damage. Mid- (4 to 5 days old) and late- (7 to 8 days old) pupae can withstand levels as high as 20 KR without serious injury.

d. European Corn Borer. A sex pheromone of the European corn borer has been isolated from 150,000 female moths. The male moth is apparently extremely resistant to the sex pheromone.

2. Small Grain Insects

a. Cereal Leaf Beetle. Studies conducted on the cereal leaf beetle at East Lansing, Mich., indicated that mating was not seriously diminished by exposure of adults to 2000 r. Treatment levels of 4000 r and above of beta and X-rays reduced mating to a very low level. Egg hatch is affected by exposures of 500 and 1000 r, but no drastic reduction occurs until males are exposed to 2000 r. Survival was reduced by a factor of approximately one-half at 2000 r of beta and gamma radiation, but for the X-ray treatments the average survival time in days at 2000 r exposure level actually exceeded that in the controls. Reduction of 72.1, 72.5, and 84.4% were obtained in over-all hatching at the 9:1 ratio (male sterile to untreated females) for beta, gamma, and X-ray treated beetles, respectively. These tests would appear to indicate that, provided mass rearing can be achieved, the sterile male technique can be used to suppress or eradicate light cereal leaf beetle populations.

b. Southern Corn Rootworm. Fewer eggs are laid per female and egg viability was decreased when southern corn rootworm beetles were treated with apholate at 50 or 100 ppm either feeding or contact. Decreased egg viability is believed due to failure to transmit sperm to the female during copulation because of lack of seminal fluid.

E. Evaluation of Equipment for Insect Detection and Control (0.5 SMY)

A portable, battery operated, self-contained, recording weather station has been constructed for field use at Brookings, S. Dak. Ten sensor inputs record automatically for five minutes of every hour. Sensor inputs are for light intensity, wind speed, wind direction, rainfall, temperature (4 sensors), and humidity (2 sensors). Solar cells keep batteries charged. A roll of chart paper will contain the hourly recordings for 14 days.

Modified Steiner traps containing virgin females and STIKEM were used to survey natural populations of corn earworms and fall armyworms on the Island of St. Croix. A height of three feet was determined to capture significantly more numbers of males than any other height. Virgin females three days old were most attractive. Fed females caught significantly more males than non-fed females, and virgin females caught significantly more males than mated moths.

F. Varietal Evaluation for Insect Resistance (17.7 SMY)

1. Corn Insects

a. European Corn Borer. Tests conducted at Ankeny, Iowa, have shown that DIMBOA is a major factor in the natural resistance of corn to the European corn borer. A correlation coefficient of 0.76 has been established in plants between the concentrations of DIMBOA and field ratings for corn borer resistance levels. This indicates that a chemical analysis of plant

tissue for DIMBOA can be used to accurately predict the level of corn borer resistance in inbred and single cross hybrids.

Analytical results of seed at germination show that high concentration of two benzoxazinones, DIMBOA and a second benzoxazinone (DIBOA) occur in the embryonic corn plant. The endosperm and scutellum contain lower concentrations of the compounds. Biosynthesis of the benzoxazinones takes place throughout development of the plant; however, overall whole plant concentrations decrease as the plant matures. DIMBOA and DIBOA occur in all plant portions. Concentrations were generally highest in the roots of the plants, followed in decreasing order of concentration by the stalk, whorl, and leaf.

Greenhouse tests conducted with first instar larvae placed on first brood resistant and susceptible inbred corn plants show that larval migration is greater from resistant plants than from susceptible plants. Susceptible plants sprayed with the compound DIMBOA were also more repellent to the borer than unsprayed plants. These tests indicate that DIMBOA may be a non-preference chemical factor.

At Wooster, Ohio, 18 of 694 corn lines, from Michigan State Agricultural Experiment Station, indicated a good degree of resistance to European corn borer and leaf blight. Eight of 35 inbred lines tolerant to maize dwarf mosaic had a satisfactory degree of corn borer resistance. Nineteen of 59 S₃ lines from the Ohio breeding material had a good degree of resistance.

In continuing tests at Ankeny, Iowa, to locate additional sources of first-brood resistance, two of 33 South Carolina inbred lines, and one of 14 Georgia inbreds had a good degree of resistance; 17 of 300 plant introductions had a good degree of resistance.

Several lines from a WF9 synthetic, B14 synthetic, 2-ear synthetic, corn borer synthetic #3, and other sources had a good degree of resistance. These lines represent most of the new lines under development in the corn breeding project.

b. Corn Rootworm. In tests conducted at Brookings, S. Dak., the following lines have showed superior tolerance to the corn rootworm: HD2187, B57, CI21E, Mo22, B14, N38A, N8B, SD10, Oh05, A251, CI38B, Zap15, Zap13, Mex6, LRC3,1, and LRC3,6. One of these lines will be released as soon as a special genetic stock for tolerance to rootworm larvae. Two synthetics have been initiated involving these lines. The performance of the most promising lines was relatively comparable under irrigation and dryland where there was a moisture stress.

The number of lines tested during 1967 and percent with an acceptable level of performance within each type of material tested follows: (a) inbreds 139 - 22%, (b) single crosses 267 - 24%, (c) S₁ 169 - 40%, (d) S₂ 362 - 49%, (e) S₃ 135 - 37%, (f) S₄ 21 - 33%, (g) synthetics 30 - 60%. The

increased number saved in later generations indicates the positive effect of selection in previous generations. The synthetics represent corn belt maturity in Central and South American lines where numerous species of rootworms are indigenous.

c. Miscellaneous Insects. Research being conducted in India under PL 480 has led to the development of artificial rearing techniques for two stalk borers, Chilo zonellus and Sesamia inferens. Maize germ plasm with resistance to C. zonellus has also been found.

2. Small Grain and Sorghum Insects

a. Greenbug. Studies at Brookings, S. Dak., showed that cumulative amounts of greenbug feeding produced a quantitative, detrimental decline in chlorophyll content and rate of photosynthesis, an increase in respiration, and little effect upon fresh weight, dry weight, and leaf area. Resistant barley leaves maintained a higher chlorophyll content. Soluble protein content of barley leaves was not significantly altered by greenbug feeding. Peroxidase activity increased markedly at the site of infestation in both susceptible and resistant varieties. Phenolic content of infested leaves was maintained at a higher level than in the control leaves. Malic dehydrogenase activity and isoenzyme patterns remained unchanged during greenbug feeding.

One hundred and twenty-six wheats from Argentina tested in Oklahoma for greenbug resistance were all susceptible to biotype B greenbug. Three thousand World Wheat Collection entries also were susceptible to the same biotype.

Will barley, which is resistant to both greenbug biotypes and to the corn leaf aphid, was found to be susceptible to the yellow sugarcane aphid, Sipha flava.

b. Wheat Stem Sawfly. Hybrid material containing P. I. 94585 (solid-stemmed) selections were tested in the field at Conrad, Mont., for resistance to sawfly attack. Some of them show promise of maintaining stem solidness stability (resistance) with desirable agronomic characters. The rescreening of Turkish varieties for new sources of resistant germ plasm was continued. Many of the resistant and intermediate varieties have been found to be late or hollow-stemmed.

At Minot, N. Dak., dyed plastic sheeting was used as light filters to determine associations between several wheat plant characters and solar radiations at specific wavelengths. In the hard red spring wheat variety, Fortuna, stem solidness (and resistance) was associated with amount of radiation at wavelengths of 575 and 700 millimicrons. Increased light at 575 m μ increased solidness; increased light at 700 m μ decreased solidness.

c. Fall Armyworm and Corn Earworm. In preliminary sorghum insects resistance tests, 355 entries from India were evaluated for resistance with laboratory reared larvae. Ninety-three were resistant to the fall armyworm and 75 to the corn earworm.

d. Rice Insects. At Crowley, La., over 1000 varieties, including entries of the Uniform Yield Nursery, Arkansas Disease Nursery, New International Blast Nursery, and World Collection of Rices were screened for the host plant resistance to the rice water weevil and stem borers. Twenty-seven of these varieties apparently had some degree of resistance to the rice water weevil.

e. Cereal Leaf Beetle and Hessian Fly. New and superior sources of resistance to the cereal leaf beetle in spring wheats (C.I.11490, C.I.9321, and C.I.9294) and winter wheat (C.I.8519) are now being utilized to develop a new series of backcrosses with commercial spring and winter varieties. Resistant F₃ and F₄ lines from crosses of both winter and spring varieties with cereal leaf beetle resistant lines have been developed at Brookings, S. Dak., and tested at Galien, Mich.

At East Lansing, Mich., adequate sources of germ plasm with resistance to the cereal leaf beetle have been found in wheat. Research has been initiated at Brookings, S. Dak., to incorporate resistance to the cereal leaf beetle in both hard red spring and winter varieties of wheat. Successful crosses were made between lines rated resistant to the cereal leaf beetle and the spring varieties Chris and Crim and the winter varieties Hume, Winalta, and Pawnee. More than 200 F₄ winter wheat progenies from the crosses of resistant lines to Hume (38), Winalta (129), and Pawnee (44) have been rated resistant.

The higher level of resistance recently identified in laboratory tests in the spring wheat lines C.I.11490, C.I.9294, and the winter wheat line C.I. 8519 has been incorporated into the breeding program.

Spring wheat resistant lines, on which young larvae have not been able to survive, have been crossed with Justin, Crim, Chris, and several other advanced hard red spring wheat lines.

At Lafayette, Ind., Purdue wheat breeders have used pubescent spring and winter wheats, Agropyron, and Triticum persicum fuliginosum as resistant parents crossed with well adapted Hessian fly resistant wheats. Second and third generation hybrids are now ready for selection.

No highly resistant oats have been found. Crosses that were made with lines showing promise of resistance earlier have not shown resistance in field tests. Many of these earlier lines with potential, C.I. 4042, C.I. 4051, C.I. 4706, and C.I. 7495, may have escaped infestation.

Three or four lines in barley appear to have some resistance to the cereal leaf beetle. C.I. 6469 appears to be the most resistant. It has been crossed

Forty entries of unknown resistance from Tennessee were selected from the breeding nursery and evaluated to identify their resistant genes. All resistant entries had the H₃ resistant gene.

Thirty-five foreign wheat entries being used as parents in the hybrid wheat program were evaluated for Hessian fly resistance. One entry was resistant to Races A and B, indicating that it has either the H₅, the H₆, or an unknown resistant gene.

One hundred and seventy-two new wheat introductions were tested to Races A and B. Nine entries reacted resistant to Race A, seven of which also reacted resistant to Race B. These wheats will be added to the gene pool of germ plasm resistant to the Hessian fly.

Eight hundred and twenty-five entries of wheat crosses having agrotriticum parentage were evaluated for resistance to Race D. One hundred and sixteen reacted resistant and will be added to the resistant germ plasm gene pool.

At Manhattan, Kans., approximately 2,100 lines were tested to the Great Plains race in the greenhouse. Entries from the Kansas intra-state nursery contained selections have the Parker, Pawnee, and Warrior types of resistance combined with wheat streak mosaic resistance. The Kansas observation nursery contained entries having the Parker, Ottawa, and Pawnee types of resistance combined with Bison, giving good wheat streak mosaic resistance. The Kansas breeding nursery consisted of the wheat crosses Shawnee X Apache, Apache X Parker, and Scout X 12855.

Parker, a Hessian fly resistant wheat variety having Marquillo resistance, was released.

Two hundred entries with Monon (H₃), Benhur (H₆), and Stadler resistance were evaluated against the Great Plains race; 91 entries reacted resistant.

Three new Hessian fly resistant wheat varieties were released in 1967. Riley 67 and Arthur are Indiana releases and have the H₃ resistant gene. Parker, a Kansas release, has the Marquillo resistant gene.

Genetic studies with F₂ and backcross generations indicate that the ability of Race A and Race E to infest varieties resistant to the Great Plains race is simply inherited, probably controlled by one or two recessive genes.

Retardation of cruxin activity is believed to be associated with plant stunting observed in plants attacked by Hessian fly larvae.

Studies conducted on the possible relationship between growth regulators and Hessian fly resistance in wheat showed that all the wheats tested infested or non-infested (Turkey, Monon, Knox 62, and Ribeiro) possessed growth regulators. Ribeiro was resistant to all races, and had fewer auxins than did the other varieties. The Hessian fly larvae removed more auxins

with the variety Dickson, and the progeny is in the F₃ generation. Resistant lines have also been crossed with the varieties Dickson, Parkland, and Trophy. Resistance to the cereal leaf beetle in wheats is related to the amount and type of leaf pubescence. Pubescence has a threefold effect on the beetle, acting as a deterrent to oviposition, promoting desiccation of eggs, and inhibiting first instar larval feeding.

Wheat breeding material from five State experiment stations in the eastern soft wheat region and three State experiment station in the hard red winter wheat region were evaluated at Lafayette, Ind., for Hessian fly and/or cereal leaf beetle resistance. Approximately eleven thousand lines, hybrids, varieties, and selections were evaluated for Hessian fly and/or cereal leaf beetle resistance.

Non-preference by cereal leaf beetle adults for oviposition on pubescent leaf surfaces continues to be the major mechanism of resistance in wheats. Antibiosis also occurred in the form of egg desiccation when eggs were placed on pubescent leaf surfaces, and very little larval weight gain occurred when larvae fed on pubescent leaves.

f. Hessian Fly. Thirty-six entries from Georgia Agricultural Experiment Station containing crosses between H₇ resistant Georgia 1123 and H₆ resistant Benhur and Knox 62 were evaluated for resistance to Races A and B. Many reacted resistant to Race A, but only one entry reacted resistant to Race B.

Approximately 6,000 head selections, hybrids, and lines from the Purdue regular fly nursery, preliminary yield nursery, advanced yield nursery, fly stem rust nursery, and Septoria nursery were evaluated for resistance to Races B and D in replicated tests. All wheats have one or more of the H₃, H₅, H₆, or Marquillo resistant genes in their parentage. Many of the F₂ and backcross lines containing the H₅ gene in combination with one or more of the H₃ or H₆ genes were saved, vernalized to break the winter habit, and utilized in the crossing program in the greenhouse. In the fall nursery at New Carlisle, Ind., approximately 4000 entries of small grains were evaluated for cereal leaf beetle resistance. Entries included Purdue breeding material with Agropyron parentage, pubescent wheats, and F₂ and F₃ space planted wheats having pubescent Triticum persicum fuliginosum and pubescent spring and winter common wheats as resistant parents.

Arthur, a Hessian fly resistant wheat variety, was released by the Indiana experiment station in cooperation with the Entomology and Crops Research Divisions of the U. S. Department of Agriculture. Arthur is a very high yielding wheat having the H₃ gene for resistance to Races A, C, and F.

Other material evaluated were as follows: One hundred and fifty advanced wheats from Ohio with Hessian fly resistance in their parentage were tested to Races A and B. Many resistant types occurred, all with the H₃ gene involved.

from plants that were susceptible to the race than those that were resistant. Some growth inhibitors were identified: IBA (indolebutyric acid) was the major auxin removed by the larvae of Races A, B, C, and D; however, some IAA (indoleacetic acid) was also removed.

Hessian fly extracts of Race B and Race D larvae prevented or reduced IBA (synthetic growth producing auxin) and IAA (natural growth producing hormone) activity on wheat and oat coleoptiles, when tested in artificial media. Coleoptiles did not elongate as much as when the growth hormone was used alone.

e. Miscellaneous Insects. Studies conducted in India on insect pests of sorghum and millet under PL 480 indicate that lines are being found with resistance to insects that can be used to develop varieties in India with resistance to several sorghum and millet insect pests.

G. Insect Vectors of Diseases (3.6 SMY)

1. Corn. A non-persistent virus found infecting field corn was proven to have physical-chemical and host range characteristics similar to those of bromegrass mosaic virus. Seedlings mechanically inoculated develop local lesions on inoculated leaves followed by systemic invasion, whorl necrosis and is ultimately lethal. Transmission trials with English grain aphid, green peach aphid, corn leaf aphid, oat-bird cherry aphid, greenbug, and by soil inoculations have been negative. Transmission has been successful with rootworm larvae and adults of D. virgifera and D. undecimpunctata howardi.

Trapping studies conducted under a grant at the University of Missouri, on insect transmission of viruses causing stunting of corn showed that at least 36 aphid species have been found in corn fields in the Missouri Area. Three known vectors have been trapped - corn leaf aphid, greenbug (maize dwarf mosaic), and brick-red sowthistle aphid (sugarcane mosaic virus).

Tests conducted under a cooperative agreement at the Ohio Agricultural Research and Development Center on the role of mites as vectors of corn viruses have produced some interesting data on aphid movement of mites.

Large numbers of eriophyid mites were found on grease slide traps at the time of seedling emergence (June 1). Numbers of mites moving in the air began to drop off July 3 when mites first began to appear on corn (Oh 28, B-37) inbreds. A steady drop in mite movement occurred from July 3 to August 21 while mite numbers of corn (especially B-37) increased.

2. Small Grain. At Brookings, S. Dak., recovery of BYDV from field populations of Macrosiphum avenae, Rhopalosiphum maidis, R. padi, R. rufiabdominalis, and Schizaphis graminum showed that in years when aphid populations and incidence of BYD were low, viruliferous aphid populations were also low. Virus was also recovered from trapped migrating R. maidis alates.

Studies conducted at Brookings, S. Dak., also indicated that plant age at time of attack by barley yellow virus, influence the plant reaction obtained. When Selkirk (hard red spring) and Langdon (durum wheat) were infested at four different plant ages with 0, 2, 4, and 8 viruliferous aphids for three days highly significant differences resulted from infection with virus for components of yield including heads per plant, plant height, heading date, kernels per head, 1000-kernel weight, and weight of kernels per head. Highly significant differences due to age of plant at time of infection were recorded for tillering, heads per plant, height at 21 days and maturity, and heading data. Varieties responded differently for plant height, heading date, kernels per head, and 1000-kernel weight. Differences due to different number of viruliferous aphids were non-significant.

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AREA NO. 7 - COTTON INSECTS

USDA and Cooperative Programs

Location of Intramural Work	Scientist Man Years F. Y. 1968		
	Research Problem Area		
	207	701	Total
Arizona	7.0		7.0
Louisiana	3.0		3.0
Mississippi	14.0		14.0
South Carolina	5.0		5.0
Texas	19.0	0.2	19.2
Maryland (Beltsville)		0.1	0.1
<u>Total</u>	<u>48.0</u>	<u>0.3</u>	<u>48.3</u>

Intramural program is supplemented by extramural support representing (a) 2.8 SMY's at State Agricultural Experiment Stations 1/, and (b) 1.4 SMY's at other U.S. Institutions. 2/

1/ RPA 207-2.8

2/ RPA 207-1.4

Problems and Objectives

The control of insects continues to be a major cost in the production of the cotton crop. Although current insecticides used for control of cotton pests are effective, improvements in the use of insecticides are needed to insure their safety and efficiency, and to reduce undesirable residues on subsequent as well as on adjacent crops or forage areas and in cottonseed. Since 1947 when organic insecticides began to have wide usage on cotton, more than twenty species of cotton pests have developed resistance to certain insecticides emphasizing the need for developing basic information to solve or to avoid the problem and to develop other methods of control that are more effective, economical, or desirable.

Major objectives of the research are to develop methods for control of cotton insects by use of:

1. The sterile insect release technique.
2. Parasites, predators, pathogens, or other biological agents.
3. Insect resistant varieties of cotton.
4. Attractants (including sex), repellants, feeding stimulants and other biologically active compounds.
5. More effective conventional and systemic insecticides that will not result in residues in cottonseed.

Progress - USDA and Cooperative Program

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

A. Basic Biology, Physiology, and Nutrition (10.5 SMY)

1. Cotton

a. Boll Weevils. In 1968, spring woods-trash examination for boll weevils were made in Central Texas, Northeast Louisiana, Delta, and Hill sections of Mississippi, and in four areas in the Carolinas. Boll weevil survival in the spring of 1968 was lower in all areas than in 1967. In Central Texas the spring survival was lower than for any year since the survey began in 1959. In Madison Parish, Louisiana, where similar records have been kept for the past 32 years, the survival in 1968 was 19% compared with an average of 43%. There have been only 8 years when the survival percentage was as low or lower than in the spring of 1968. In Mississippi the average survival of 8.6% was lower than in any year since 1963. Survival was generally lower in the Carolinas with an average of 1022 weevils per acre surviving in Florence County, S.C. for a percentage of 18.8. This

is the lowest survival on record except for 1963, 1961, and 1958.

In studies at Tucson, Ariz. hibernating boll weevil complex adults emerged in markedly fewer numbers from bolls buried 7.5 cm or deeper. None emerged from bolls buried 30 cm. High mortality occurred during the winter among weevils released into leafy trash in the fall.

Emergence of hibernating boll weevil complex adults from bolls was higher from the bolls with higher moisture content. Increased mortality occurred among hibernating weevils freed into simulated ground trash when trash moisture was below 0.44 grams of water/gram of trash.

Fifteen live and 75 dead boll weevils were found in 26 pounds of bolls collected from approximately 1 acre of a 30-acre field of standing stalks near Waco, Texas in March 1968. Two of the live weevils were removed from pupal cells and 13 apparently had not left the bolls to enter usual hibernation sites.

At Waco light boll weevil survival in 1968 occurred in green bolls collected before a killing frost in 1967 and in bolls collected from standing stalks in March. Two boll weevils emerged from 3 cages containing 27 gallons of green bolls collected and installed on the soil surface in November. Four weevils emerged from 3 cages containing 45 pounds of cotton bolls collected from standing stalks and placed on the soil surface on March 7 and 12 weevils from 3 cages containing 45 pounds of this material placed on screens 6" above the soil surface.

Thirteen weevils were collected in May and June, 1968 on 12 3x5 ft flight screens spaced 32' apart at Waco along the edge of a field that had been planted to cotton in the previous year and bordered by a wooded area 5' to 15' deep. The first weevil was collected on May 6 and the last on June 14.

In studies at Waco, there were 69.3 acres of cotton in 4 fields and 12.6 acres in hibernation sites in the vicinity of a boll weevil sex attractant test. Other cotton was approximately one mile distant. The cotton fields were sampled 3 times with a machine that collected 1237 weevils. In addition 904 weevils were caught in the boll weevil sex attractant test for a total of 2,141. Thus 170 weevils were collected per acre of hibernation area, or 31 per acre of cotton.

In the spring of 1968, traps baited with 5 male boll weevils in separate compartments and coated with a sticky substance to collect responding weevils, were placed near hibernation sites at various locations across the Cotton Belt to survey overwintered boll weevil

populations. Some of the results of collections were as follows: 1106 overwintered boll weevils were collected in 2 fields in Darlington and in 1 field in Florence Counties, South Carolina from May 7 to 21; 3287 at State College, Miss. from April 15 to June 21; 41 at Stoneville, Miss. from May 1 to June 28; and 663 from May 1 to June 21 in Kent, Dickens, and Crosby Counties, Texas, in the 1967 boll weevil reproduction-diapause population control program. In the same time 5494 overwintered boll weevils were collected in Stonewall and Glasscock Counties, Texas outside the treated area. Slightly more males than females were collected at all locations. Cotton had not been planted when the traps were installed and condition of the crop in most areas ranged from "up to a stand" to "2-to 6-leaf stage" in the latter part of the reporting period. The value of the male baited traps as a boll weevil detection tool was demonstrated on May 22 when a boll weevil was collected in a trap baited with sterile males in a field west of El Paso, Texas. The El Paso area has had incipient infestations of the boll weevil in recent years. However, infestations, when found, were usually in the months of September and October after light population build-up had occurred.

At Waco, traps baited with male boll weevils in separate compartments containing fresh squares and traps containing 5 males in a single compartment with squaring cotton plants caught significantly more weevils than traps containing 1 or 5 males on artificial diet, 1 male on squaring cotton plants, or squaring plants alone. One male on squaring cotton plants and 5 males on artificial diet were more effective than 1 male on artificial diet, or than squaring cotton plants alone. A total of 904 weevils was caught in the traps between May 1 and June 21.

In a test at Tallulah, La., results were similar except that significantly more weevils were caught in traps baited with male boll weevils in separate compartments and traps baited with 5 males on artificial diet. There was little difference in the number of weevils collected in traps baited with one male on artificial diet, one male on squaring cotton plant, or 5 males on squaring cotton plants. The fewest number was collected on squaring cotton plants alone. A total of 1410 boll weevils was collected in the traps. Slightly more males than females were collected at both locations.

At Tucson, Ariz. immature stages of the boll weevil complex on moist medium or on dry or moist filter paper survived 30 hours of intermittent exposure to soil temperatures of 60° C in ambient air temperatures of 32° C. When the ambient air temperature was raised to approximately 40° C, 100% mortality resulted from 25 hours intermittent exposure at soil temperatures of 60° C but not at 54° C.

When the humidity of the ambient air was raised or when exposure was on wet rather than on dry soil, survival was not improved.

Studies at Tucson indicated that the boll weevil may be readily moved in air currents. Vertical air velocities of 2.5 to 5.5 meters per second will suspend passive boll weevils. Winds and turbulences in dust devils and wind currents at high altitudes reach these velocities. Velocities in excess of 31 meters per second were required to free certain weevils from their foothold on cotton leaves. However, irregularities in the air streams enhance the possibilities of weevils being dislodged from their footholds.

At Tucson, exposure for 25 hours on hot soil at 140° F resulted in total mortality of immature boll weevils in cotton squares. Five exposures of five hours each gave 95% mortality at 130° F if the squares remained dry, and total mortality if the exposure was on wet soil and high humidity was maintained in the ambient air. Some mortality occurred with similar exposure at 110° and 120° F compared with a check at 75° F. Reduction of the ambient air temperature to 90° F markedly reduced the mortality at all exposure temperatures.

At Tucson, thurberia weevils required 14 days less developmental time at 59° F than boll weevils from cultivated cotton. At 95° the thurberia weevils required about 1.4 more days for development from egg to adult. Developmental periods for male weevils from thurberia, and males from the Buckelew, Stanfield, and Santa Cruz strains from cultivated cotton were 72.4, 87.2, 89.8, and 89.2 days at 59° F; 34.0, 34.6, 35.4, and 34.2 days at 68° F; and 21.3, 20.9, 21.3, and 21.1 days at 77°; 17.1, 17.0, 17.7 and 16.5 days at 86° F; and 19.0, 17.0, 17.9 and 17.5 days at 95° F respectively. Data on female development was similar.

At Baton Rouge, La., thurberia weevils from Arizona lived longer than boll weevils from Louisiana under conditions of starvation when both were reared and held at the same temperatures. The median life-span of adult thurberia weevils reared at 22° C on a larval diet containing 8% sucrose was about 4 months when held in capsules without food at 27° C. The ability of thurberia weevils to withstand starvation merits study because it may be related to the high survival of thurberia weevils following gamma irradiation.

Seasonal changes in the cotton plant significantly influenced oviposition and feeding of the boll weevil in controlled laboratory tests at State College, Miss. Development time and weight of weevils were not affected significantly by maturation of cotton.

Incompatibility was found among hybrids from crosses of boll weevils and thurberia weevils in studies at Baton Rouge. The F₁ females of the cross laid less than one-third as many eggs as those of the pure boll weevil strain. Most females behaved as if they were ineffectively inseminated and laid many of their eggs outside the cotton bud. A high percentage of eggs obtained from females that were not ovipositing normally were found to be infertile. When these hybrid females were allowed to mate with males from the boll weevil parental strain, they began to oviposit normally, indicating that they were now effectively inseminated. There, also, was evidence of some incompatibility between individuals from the two parental strains. This incompatibility is such that a weevil control program based on the large-scale release of thurberia weevils would not appear to be particularly effective.

In laboratory tests at Florence, S. C. the effects of 10, 25, and 50 mg of melatonin per 100 gms of adult laboratory diet were studied. The 50 mg level decreased egg production during the 20 days of the test.

Serotonin and reserpine were fed to weevils at 50 mg per 100 g of laboratory diet at Florence. Egg production between controls and treated weevils was compared after adults fed for 0 to 5 days and 6 to 10 days. Reduction in egg production was most marked at the 6 to 10 day interval with serotonin causing a 73% decrease and reserpine a 94% decrease in eggs per female per day. Mortality with reserpine was 71% compared with 21% in the control.

In studies at Baton Rouge, diapausing boll weevils began to lay eggs within 1 to 2 weeks following treatment with a synthetic juvenile hormone-like material. This accelerated breaking of diapause was accomplished by topical applications of from 50 to 100 µg of the methyl ester of epoxyfarnesenic acid. Treatment of newly emerged weevils that were preconditioned towards diapause with 50 µg of this material greatly reduced the incidence of diapause but did not eliminate it altogether. Treated pupae had difficulty shedding the pupal integument, but those that did molt successfully showed no juvenilized features.

Boll weevils remained alive for several weeks without food or water when they were held individually in gelatin capsules at 21° C in the laboratory at Baton Rouge. Thurberia weevils were even better adapted to survival under these conditions, 50 percent of the adults were alive after 14 weeks. These weevils may be considered to be in diapause even though they have not had the opportunity to feed as adults.

In studies at State College, Miss., 23 amino acids were identified by column and thin-layer chromatography in Delta Pineland Smooth Leaf cotton squares. Five of the amino acids had not previously been

reported in cotton squares. They were beta-alanine, cysteic, ethanolamine, glucosamine, and taurine. Protein and non-protein amino acids in nine hosts and non-hosts of the boll weevil also were determined.

Laboratory studies at Baton Rouge showed that galactose is metabolized to galactitol in starved boll weevils. This is the first time that this metabolic process has been detected in an insect. Galactitol is not metabolized although it appears in the hemolymph of galactitol fed weevils. Galactose is present in the blood of galactose fed weevils. Even though feeding this sugar produces elevated trehalose and glycogen levels, the precursor probably is glucose rather than galactose because commercially available galactose is contaminated with 3.9% or more of glucose. It is likely that, except for conversion to galactitol, galactose is quite poorly metabolized by the boll weevil.

Differences in carbohydrate and lipid reserves did not explain why thurberia weevils lived longer than boll weevils when held without food in gelatin capsules. Lipid levels in newly emerged individuals were about the same and the titer of trehalose was actually lower in thurberia weevils than in boll weevils. Water content also probably is not a factor since moisture content of the thurberia weevils is no greater than that of the boll weevil. When the insects are held on moistened cellucotton, thurberia and boll weevils have approximately the same average life span although thurberia weevils live much longer in capsules than boll weevils. The rates of utilization of lipids and of trehalose being roughly the same for both thurberia and boll weevils held on moistened cellucotton indicate that, under these conditions, metabolic rates are similar. Since starved weevils in gelatin capsules lived longer than starved individuals given water, results suggested that either crowding or availability of water shortened the life span of unfed, newly emerged weevils by increasing their metabolic rate.

In studies at State College, glycine C¹⁴ was absorbed initially from the gut in large quantities in the boll weevil. When the haemolymph was examined after this amino acid was fed to weevils, the initial rate of absorption was fast, but decreased rapidly and leveled off. Comparison of absorption with injected glycine indicated that the apparent rapid decrease was not due to metabolic activity.

Indole acetic acid (IAA) was found in both larval and adult weevils and appeared to be transferred to the square in feeding. When labeled IAA was injected into weevil larvae and adults, analysis of the media on which the insects fed showed that the labeled IAA and other as yet unidentified metabolites had been transferred from the insects to the media.

Free indole acetic acid (IAA) was present in the older leaf blades of inactive cotton plants. Free IAA has not been detected in seedlings, flower buds, or expanding leaves. The lack of IAA in growing parts may be due to conjugation with protein or to consumption during the growth process.

Studies conducted under contract by the Texas Agricultural Experiment Station in trap crop plantings of cotton near College Station showed that peak emergence of weevils occurred in the first week of June. Beginning in the last week in June, migrating summer generation weevils were collected from the plots. Incidence of diapause in the population was recorded in July with the peak occurring in mid-August. The percentages of diapausing weevils in the population decreased thereafter.

b. Bollworms. At Brownsville, Texas, rearing efficiency of Heliothis spp. was improved with the use of honeycomb-like hexcells consisting of cardboard coated with fiber-glass instead of plastic cups to hold larvae on the rearing medium. Treatment of eggs with both heat and sodium hypochlorite to reduce contamination reduced hatch. Use of proflo (defatted cottonseed meal) to replace casein in the diet retarded larval development.

At Tallulah, La., when cages containing virgin female tobacco budworm moths were attached to 15-watt blacklight traps in a cotton field, 92.3 percent of the tobacco budworm moths caught were males compared with 84.9 percent in traps without virgin females. A similar effect was noted in the collection of H. zea. The ratio of H. zea to H. virescens adults caught in a blacklight trap was 73:1 while that of the larvae collected from cotton was 13:1. Of 1033 moths collected in light traps from July 1 to Sept. 30, only 14 were H. virescens. Of a total of 600 bollworm larvae collected periodically from cotton 44 were H. virescens.

In studies at Stoneville, Miss. about 12.5% of overwintering Heliothis zea survived the winter of 1966-67. Since this was an unusually mild winter, the survival rate would probably be lower in most years.

More larvae of Heliothis virescens than H. zea were collected in central Texas cotton fields in 1967. Of 3,232 larvae collected periodically throughout the season, 52% were the tobacco budworm. This compares with 47% in 1966, 53% in 1965, 7.4% in 1964, and 46% in 1963.

At Waco, 4 tobacco budworm larvae were collected in May and 9 in June, all on Texas Star. In March, 3 bollworm larvae were collected from 1 host plant, 131 in April from 13 different host plants, 134 in May from 10 host plants, and 53 in June on 3 host plants.

In studies at Florence, S. C. significant differences were found in the fatty acid composition of larvae of the bollworm and tobacco budworm. Tobacco budworm larvae contained more C16:1 and C18:1 and less C18:0 and C18:2 than bollworm larvae. In general, the differences in the fatty acids in the bodies of the two species were similar to those in the bodies of insecticide-resistant and susceptible boll weevils.

In studies at Florence, fatty acids from the nervous system and body of H. zea were analyzed by gas liquid chromatography. The nervous system has more stearic acid (16.3% vs. 9.7%) and less oleic acid (19.9% vs 37.8%) than the body. Thin layer chromatography of a total lipid extract of H. zea showed 37% phospholipid, 13.5% partial glycerides, 49% triglycerides, and 0.4% cholesterol esters.

In studies at Baton Rouge, trehalose was present in Heliothis zea adults. There was considerable variation between individuals, but the titer of trehalose appeared to be at a maximum after moths fed for a single day on a 10% sucrose solution. After the first day, trehalose levels tended to return to approximately the same level as in unfed moths less than 24 hours old.

At Florence, hourly light trap catches of H. zea indicated that the moths are active throughout the night with maximum catches in the period from 1 to 2 a.m. EDST. H. zea were taken in the trap beginning on April 6 and continuing through September. Maximum catches were taken in the first and last weeks of August and the last week of September.

At Waco, Texas in 1967 a total of 4,666 bollworm moths were collected in a blacklight trap compared with 1,859 and 4,105 in 1966 and 1965, respectively. However, the 1967 number was lower than in any year from 1956 to 1964. 5.9% were tobacco budworm moths in 1967, compared with 6.5% in 1966, 15.6% in 1965 and 1.1% in 1964.

At Waco, Heliothis zea (Boddie) larvae were collected on 9 hosts (alfalfa, corn, cotton, maize, soybeans, sorgo, wild petunia, wild morning glory, and tomato) of 32 inspected in Falls and McLennan Counties, Texas. Heliothis virescens (F.) larvae were collected only on cotton and wild petunia.

Studies at Stoneville, Miss. indicated that young bollworm moths may fly higher than older moths. A greater percentage of female moths caught in a light trap 35 feet above ground level were unmated than those caught in light traps about 6 feet above the ground. Other physiological conditions also indicated that the high-flying moths were younger than those flying near the ground.

Bollworm larvae enter diapause about the middle of September in the Mississippi Delta at Stoneville. Only 1 adult emerged from pupae by the end of December from larvae collected after September 13, 1967. In 1966 no adults emerged from pupae in the fall from larvae collected after September 19.

Heliothis zea moths did not become active until the compound eyes had dark-adapted (secondary iris pigments migrated to dark adapted position) in laboratory studies of their normal behavior at Florence. Generally, moths mated when some or all of the following conditions occurred: (1) moths were 30 to 60 hrs old; (2) light level was less than 0.015 uw/cm^2 ; (3) secondary iris pigments of compound eye migrated from green to brown-gold position (dark adapted); (4) there was slight breeze or movement of air (5) there was no recent contact with other sex (not caged together for long periods); (6) the relative humidity was 50 to 100%; (7) the temperature was 70 to 82° F; (8) 0.5 cubic feet space per 10 moths was provided; and (9) lush growing cotton was available.

At Brownsville, all larval instars of the bollworm and tobacco budworm were given a choice of various cotton plant parts for feeding. Bollworm larvae preferred squares over bolls until the 4th instar, whereas tobacco budworm larvae preferred squares until the 5th instar.

At Brownsville, a positive correlation was found between the number of bollworm eggs found in cotton fields and the number of bollworm moths collected in light traps during the preceding week.

Results of laboratory tests at Brownsville indicated that the braconid parasite Apanteles margniventris (Cresson) might have potential in an inundative release program for control of bollworm and tobacco budworm populations. The female parasite has an average longevity of 5.1 days and will sting approximately 125 host larvae. Preliminary data indicated that the parasite can be easily mass reared.

Results of a statewide survey in Arkansas conducted in August, September, and October, 1967, under a grant with the Arkansas Agricultural Experiment Station showed that diapausing bollworm populations occurred on cotton, grain sorghum, and late planted corn. Bollworm larvae have been found in considerable numbers on pigweed. Several plants have been caged to determine spring emergence dates of adults from pupae that may have developed on pigweed. Similar studies are in progress on cotton and grain sorghum. Studies continued with plant growth substances in artificial medium to determine their effect on larval diapause response curves.

c. Pink Bollworm. At Brownsville, the use of quart and gallon size fiber cartons instead of 9-ounce paper cups for larval medium increased efficiency in rearing the pink bollworm. The gallon carton was superior in larval yield and required less time for loading. A problem still exists in the use of eggs versus first instar larvae implanted on the medium. Although treatment of eggs with 0.2% sodium hypochlorite solution had little effect on hatch, the eggs placed in rearing containers continued to give a poor larval yield compared with that obtained from first-instar larvae implanted on the medium.

In studies at Brownsville, late fourth-instar larvae exposed to light at different wave lengths were repelled by near ultraviolet and attracted by a passband in the violet region of the visible spectrum.

At Waco, a total of 10,113 pink bollworm moths was collected in a blacklight trap in 1967, which is the highest number for any year since the trap has been in operation, beginning in 1953. The previous high, 7,552, was in 1958. The high numbers collected this year resulted from a buildup following a very dry, mild winter.

In the laboratory at Brownsville, when casein and wheat germ were deleted from the usual pink bollworm diet and cottonseed was added, best results were obtained at a protein concentration of 8% from glanded flaked meats. Addition of Brewer's yeast to cottonseed diets containing 6% protein resulted in an increase in larval weights.

d. Other Insects. In studies at Tucson, attraction to and survival of lygus bugs on cotton were dependent on the availability of sugars in the plant secretions, particularly nectar from the extra-floral nectaries. Thus lygus bug populations generally do not develop on cotton until the leaf nectaries begin secreting nectar. Adults and late instar lygus nymphs are most responsive to the sugar attraction.

At Tucson, lygus bugs were the most injurious mirid on young cotton in Arizona. Their feeding is responsible for the blasting of "pinhead" squares and a serious systemic reaction on the young plants. Psallus sp. may cause similar damage, but is rarely abundant enough to do so in Arizona. Spanogonicus sp. and Rhinacloa sp. may also cause this typical damage but only when populations reach high levels rarely attained in the field.

A new host of Alabama argillacea has been found by Brownsville Laboratory personnel. The cotton leafworm was found on Hampea sp. in Veracruz, Mexico, during each of several visits in the area.

Studies at Stoneville indicated that spider mite infestations are most detrimental to cotton production when the earliest bolls are developing. In a field cage test, plots of cotton infested after first bloom and before the first bolls were 3/4-inch in diameter produced significantly less seed cotton than plots infested after the first bolls were of this size. Plots infested before blooming began had a reduced yield but did not differ significantly from the treated check.

A unisexual strain of the salt-marsh caterpillar has been maintained for more than 12 generations in the laboratory at Baton Rouge. The "male-lethal factor" present in females of this strain was inactive when injected into another arctiid moth (Diacrisia virginica). However, the blood-borne factor appeared to be slightly toxic to the male progeny from another more closely related species (Estigmene congrua).

A diet suitable for rearing Chrysopa carnea was developed at College Station, Texas. Nutritional requirements for this predator appear to be similar to that of most other insects.

Studies at Tucson show that populations of lygus bugs increase at a logarithmic rate. Standard sweep nets were used to sample adult lygus bug populations in Eloy and Pinal Counties, Arizona, in several crops in 1967. There was a logarithmic rate of increase that permitted analysis by regression equations. Populations decreased naturally in mid to late July in both alfalfa and cotton. After these trends were determined, a cursory examination of reports in the Tucson laboratory files from 1936 to 1939 indicated similar fluctuations in populations in cotton and alfalfa in the Salt River Valley of Arizona.

In studies at Tucson, development time of Eucelatoria armigera in Heliothis zea ranged from 49.4 ± 2.4 days at 50° F to 10.7 ± 0.7 days at 86° F. 90° F rearing temperatures extended the period to 11.5 ± 0.9 days. No live flies were produced at 95° F. Higher pupal survival occurred in the flies reared at low temperatures in H. zea than in H. virescens. The converse was true at higher temperatures.

Longevity of female Lespesia archippivora was inversely related to temperature. Mean life span varied from 49 days at 59° F to 12 days at 95° F. Egg production was highly erratic but low temperatures inhibited oviposition more markedly than high temperatures. Optimum egg production occurred at 86° F.

Cotton insect populations were surveyed in 1967 at nine separate locations in a study conducted by the Arizona Agricultural Experiment Station under a cooperative agreement. One hundred sample points

randomly selected in each field were inspected for the populations of insects. Each of the sample points consisted of a one-plant, five-plant, and ten-plant unit. Data was collected at intervals throughout the growing season in Arizona for the bollworm, beet armyworm, salt-marsh caterpillar, cabbage looper, lygus bugs, and pink bollworm, and for the boll weevil at Waco, Texas, Florence, S. C., and Tallulah, La. Data are being analyzed.

B. Insecticidal and Cultural Control (10.5 SMY)

1. Cotton

a. Boll Weevil. In field tests at Florence, S. C., State College and Stoneville, Miss., Tallulah, and Waco, Texas, ultra-low volume applications of azinphosmethyl at 0.125 and 0.25 pound and malathion at 0.25 and 0.5 pound were as effective against the boll weevil as azinphosmethyl at 0.25 pound per acre applied in a conventional spray.

In a field experiment at State College, azinphosmethyl applied as an ultra-low volume spray at .125 pound per acre at 4- or 5-day intervals in droplet sizes of 140, 200, and 300 microns gave boll weevil control equal to that applied at 0.25 lb per acre in conventional spray. While there was no statistical difference between the treatments, the 200 micron droplets consistently gave slightly more effective control than the 300 micron droplets.

In field experiments at Tallulah, toxaphene plus DDT plus methyl parathion gave slightly better boll weevil control than methyl parathion, toxaphene plus DDT, DDT plus methyl parathion, or toxaphene plus methyl parathion. The materials were applied as emulsifiable concentrates with a ground spray machine.

In field tests at Stoneville six experimental insecticides applied as sprays gave control of light boll weevil infestations equal to that of the standard.

In replicated small-plot field tests at Florence, two experimental insecticides were as effective against the boll weevil as the treated control of toxaphene at 2 pound plus DDT at 1.0 pound plus methyl parathion at 0.5 pound per acre.

Toxicity values for the boll weevil were determined for 18 experimental materials with the topical application technique at Florence, S. C. The toxicities of two were about equal to that of methyl parathion.

In field experiments at State College, azinphosmethyl applied as an ultra-low volume (ULV) spray at .25 lb/acre was slightly more effective than malathion applied as a ULV spray at 1.25 lb/acre in a boll weevil population reproduction-diapause control experiment. There was no difference in boll weevil control between treatments during the reproduction control phase in September. However, azinphosmethyl was slightly more effective than malathion in the diapause control phase in October.

In a replicated test at Florence, sidedress applications of Union Carbide UC-21149 to cotton at and after application of a defoliant in October significantly reduced number of weevils that survived the winter.

Side-dressings of UC-21149 granules to cotton plants or General Chemical GC-6506 applied to main stems showed promise for suppressing boll weevil infestations in field experiments at Stoneville. Side-dress applications of UC-21149 at 2 and 4 pounds per acre applied when plants began to square held the boll weevil infestation below that of an untreated check for 6 to 8 weeks. Similarly, GC-6506 at 0.25 and 0.5 pound per acre applied to cotton stems reduced the weevil infestation below that of an untreated check for 6 to 8 weeks.

In field experiments at Waco, overwintered boll weevil populations were reduced 50% in plots sidedressed with 1.0 to 2.0 pounds per acre of UC-21149 when plants began to square; 90% in plots sidedressed with 4.0 pound per acre, and 100% in plots treated conventionally twice with azinphosmethyl spray at 0.25 pound per acre. In another experiment UC-21149 at 1.0 and 2.0 pound per acre applied as a side-dressing when plants began to square gave control of a heavy overwintered boll weevil population equal to that obtained with 2 ultra-low volume spray applications of malathion at 1.0 pound per acre or azinphosmethyl at 0.3 pound per acre. Significantly better control was obtained with all treatments than with azinphosmethyl at 0.25 pound per acre applied in a conventional spray.

A program to control late-season reproductive and diapausing populations of boll weevils reduced overwintering populations to low levels in the Presidio, Texas-Ojinaga, Chihuahua area. Eight applications of malathion in 1965 and 1966 and eight of azinphosmethyl in 1967 applied as ultra-low volume sprays beginning in early September of each year protected the top crop as well. Although the program drastically reduced populations it did not achieve eradication.

Field studies were conducted by the Texas Agricultural Experiment Station under a cooperative agreement of the total aggregate acreage sprayed in the 1967 reproduction-diapause boll weevil population control program on the High Plains of Texas of 505,962 as compared with

1,020,225 acres sprayed in 1966. Most of the treatment was concentrated in Dickens and Kent Counties where fields received 5 to 7 applications. Records indicated that the spray program reduced the population by 86 percent. Results of an experiment conducted in the control zone and in Stonewall County indicated that ultra-low volume applications of azinphosmethyl at 16 fluid ounces per acre was considerably more effective in reducing diapausing boll weevil populations than malathion applied at 12 to 16 fluid ounces per acre. Bollworm and tobacco budworm populations were greater in treated than in untreated areas.

Chemical and biological assays at College Station, Texas indicated that UC-21149 granules made with a petroleum charcoal base possessed controlled release properties. When corn cob and petroleum charcoal granules were compared for rate of release of UC-21149 into moist sand over a 14 day period, rate of release was considerably slower from the petroleum charcoal granules than from corn cob granules. Rate of release appears to be related to mesh size. In both petroleum charcoal and calcium sulfate systems, the rate of release decreased as the size of the granules increased.

At College Station, results indicated that UC-21149 in lanolin applied to stems at the rate of 1 lb of toxicant per acre was almost as effective as 4 lbs of toxicant per acre applied to the soil. In field tests, UC-21149 applied as a sidedressing to cotton in 5-acre plots when plants began to square controlled boll weevils for 2 to 5 weeks.

Metabolism studies of trichlorfon at College Station showed the presence of potentially toxic conjugates. The glycosidic conjugates are formed in both plants and animals and are not detected by currently used residue methods.

b. Bollworms. In field experiments at Waco, bollworm infestations increased sharply in a field plot experiment 3 weeks after plants were sidedressed with UC-21149 at 1.0, 2.0, and 4.0 pounds per acre. There, also, was an increase in plots treated conventionally with azinphosmethyl. Bollworm infestations also were heavier in the UC-21149 treated plots than in untreated plots at College Station.

In a field plot experiment at Waco, Texas, poor control of a heavy bollworm infestation (73% tobacco budworm) was obtained with 3 experimental materials.

In field tests at Tallulah, GC 6506 applied at 1.0 pound per acre compared favorably with 2 pounds of toxaphene plus 1.0 pound of DDT plus 0.5 pound of methyl parathion in the control of the bollworm.

Methyl parathion applied in an ultra-low volume spray was highly effective against heavy infestations of bollworms and tobacco budworms in field experiments at Brownsville.

In field tests at Stoneville, ultra-low volume spray applications of 0.25 pound of azinphosmethyl plus 1.0 pound of TDE per acre and 0.5 pound of malathion plus one pound of DDT per acre gave bollworm control equal to the same rates of the same materials applied in conventional sprays. Other materials applied as ultra low volume treatments that gave good bollworm control were Azodrin at 0.6 pound, Bay 41831 at 0.5 pound, and toxaphene at 2 pounds plus DDT at 1 pound per acre.

At Florence, S. C., 4 candidate materials applied conventionally in small cage-field tests showed promise against the tobacco budworm but one was phytotoxic.

Although applications of GC-6506 to cotton stems for control of bollworms greatly increased yields over the check at College Station, Texas, yields were greater in plots receiving a standard spray, or 3 applications of nuclear polyhedral virus per week.

c. Pink Bollworms. In aerial spray tests against the pink bollworm at Deer Valley near Phoenix, azinphosmethyl or Azodrin at 1 pound per acre in 2 gallons of water gave the best control. Bay 41831 at 1 pound and carbaryl at 2.5 pounds per acre in 2 or 5 gallons of water gave about the same control but were less effective than azinphosmethyl or Azodrin. Malathion at 1.25 pounds per acre was the least effective. The test was conducted by the Plant Pest Control Division.

In tests at Phoenix, rototilling of heavily infested cotton fields after harvest was less effective than plowing or disking as a cultural control practice against the pink bollworm. About twice as many moths emerged in the spring and early summer in a rototilled plot and over a longer period than in the plowed or disked plots.

d. Other Insects. Four experimental materials showed ovicidal action against eggs of the two-spotted mite in tests at Stoneville, Miss.

In field tests at Stoneville, applications of undiluted dicofol at 1 quart; Azodrin at 1/6 gallon and Geigy GS-13005 at 1 quart per acre applied with a mist blower gave good control of spider mites. Ethion at 1 quart per acre was less effective.

At College Station, Texas, laboratory studies with sprayed caged plants indicated that malathion applied as an ultra-low volume spray at 1.25 pounds was more injurious to green lacewing (Chrysopa larvae) and big-eyed bugs (Geocoris) than azinphosmethyl at 0.25 pounds per acre.

In a small plot field test at Waco, Azodrin at 0.25 lb/A and GC-6506 at 0.125 and 0.25 lb/A applied to mainstems of cotton plants gave control of a heavy cotton fleahopper infestation equal to that obtained with toxaphene at 1.5 pound plus DDT at 0.75 lb/acre applied conventionally.

C. Biological Control. (7 SMY)

1. Cotton

- a. Boll Weevil. At State College, Miss., Heterolaccus grandis reproduced throughout the season in one of three 1/10 acre cotton plots in which 55 mated females were released and parasitized 31% of a heavy weevil population by October 4. Bracon kirkpatricki and B. mellitor reproduced very poorly after being released early in the season, suggesting that they may be better suited to parasitizing weevils later in the season. The native Aliolus curculionis populations increased to the extent that they parasitized 50% of boll weevil larvae in July and early August but disappeared thereafter.

In studies at State College, the first of several non-inclusion body insect viruses, Tipula iridescent virus (TIV) was tested for infectivity to the boll weevil. TIV infected larval and adult boll weevils through injection and feeding. High incidence of infection per os occurred. The virus was persistent in the feeding-stimulant bait formulation exposed to sunlight during May for up to 7 days, and some virus was still infective after 14 days exposure.

- b. Bollworm. In studies of Heliothis populations on wild tobacco in the lower Rio Grande Valley of Texas, maximum estimates of Heliothis spp. eggs and larvae on Nicotiana repanda in a 3360 acre area during February to April, 1967, were 9300 and 7100, respectively. Parasitism of medium sized larvae averaged 30.1%. Campoletis perdistinctus was the most common parasite.

In studies at Brownsville, the ichneumonid parasite, C. perdistinctus Viereck, preferred the bollworm, fall armyworm, southern armyworm, and tobacco budworm over the cabbage looper when confined with larvae of all five species on 10-inch cotton terminals in the laboratory. Exposure of host larvae to various parasite densities resulted in more or less parasitism of hosts but did not change the host preference of the parasite. In field cage tests bollworms and tobacco budworms were preferred over the other three hosts.

At Tucson, parasitization of Heliothis zea and H. virescens with Lespesia archippivora was highly successful in cage tests early in the season but effective parasitism declined as the season

progressed. Increased mortality of the parasitic females due to higher temperatures is believed to be responsible for the decrease in effectiveness.

In a small-plot field experiment at Waco, there were significantly fewer Heliothis spp. predators and parasites in untreated 321 OP variety of cotton than in untreated Coker 413, untreated Lankart 57, and Lankart 57 treated with toxaphene plus DDT.

The most important parasites of Heliothis larvae in the Stoneville area in 1967 were Microplitis croceipes and Cardiochiles nigriceps. Both of these parasites attack H. zea and H. virescens. The egg parasite, Trichogramma minutum, was present in very few numbers in 1967, particularly after mid July.

At College Station, Texas, releases of Chrysopa larvae in field experiments gave highly effective control of bollworms and a 3-fold increase in yield of cotton.

At Brownsville, a commercial virus preparation was exposed to various temperatures for 15 and 30 minutes and 1 and 2 hours. No virus activity was lost after 2 hours at 60°C. However, virus activity decreased after a 15 minute exposure at 71.1° C. These data indicate that pasteurization is inadequate to destroy polyhedral suspensions of the nuclear polyhedrosis virus. Assays of cotton foliage immediately and at intervals after field application of virus indicated that activity was lost 1 day after application.

In studies at Brownsville, paraffinic oil that was compatible with the nuclear-polyhedral virus was tested with an ultraviolet (UV)-absorbing agent, 4% CAB-O-SIL, and 1.5% polyethylene in virus formulations and their effect on virus residue was determined. These formulations were ineffective as virus extenders. The formulation with the UV-absorbing agent did not give improved results over a formulation containing one-fifth the amount of the material.

In laboratory tests at Brownsville, Heliothis nuclear polyhedral virus formulated with lactose and standardized at 1.75×10^9 polyhedral inclusion bodies/gram retained its infectivity to tobacco budworms and bollworms for 10 years when stored at -25°C. However, when it was stored at room temperature most of the virus activity was lost. Virus applied on leaves and exposed to sunlight for 6 hours showed an 80% loss in activity.

At College Station, results of studies of feeding and searching behavior of green lacewing (Chrysopa) larvae provided useful information to support an inundative release program for control of bollworms. Lacewing larvae killed much greater numbers of small

bollworm than large larvae. However, third instar lacewing larvae killed bollworm larvae weighing in excess of 30 mg. Distance that lacewing larvae moved down the row was only slightly affected by size of cotton. Lacewing larvae are apparently most active at about 80°F.

Preliminary biological and searching studies of an Arizona strain of Trichogramma minutum at Tucson indicated that the egg parasite may not be efficient enough for use in field releases. Longevity and ovipositional periods of Trichogramma minutum were short and the average number of eggs (9.0) per female was low. The parasite showed preferences for eggs of the bollworm, tobacco budworm, cabbage looper, and beet armyworm. It parasitized eggs of the salt-marsh caterpillar only when forced to do so. Searching behavior was erratic.

In studies at Tucson, third instar larvae of Chrysopa carnea consumed 44 bollworm eggs in 24 hours. First instar larvae consumed an average of 4.3 and second instar larvae 14.2 bollworm eggs per larva. Developmental times for C. carnea from eggs to pupae ranged from 20.2 days at 68° F to 10.4 days at 86°F.

Life history studies of 4 species of spiders occurring in cotton in San Joaquin, California cotton fields were conducted under a grant to the California Agricultural Experiment Station, at Davis. Erigone dentosa developed through 6 stages with an average life span of approximately 99 days. The spiders spent 8 to 11 days in the egg case after hatching. Females averaged 14 egg cases with 13 eggs per case. Dictynia reticulata spent 17 to 20 days in the egg case. They averaged 7 molts with about one third molting 8 times. Developmental time was the same for both sexes. In the adult stage, females lived 146 days and males 61 days. A high percentage of Paradosa raniulosa males reached the adult stage after the eighth molt and females after the ninth. The developmental period from egg hatch to adult was 274 days for females and 221 days for males. The females tear open the egg case to permit emergence of spiderlings that immediately climb on the back of the mother where they remain for 5 to 9 days, leaving only to take water. Molts in Misumenaps deserti males ranges from 6 to 9 and 8 to 12 in females. This species is long lived and rearing studies are incomplete. Males appear to live about one year and females one and one half years.

Results of laboratory studies conducted under a cooperative agreement by the Texas Agricultural Experiment Station indicated that wind had little effect on virus residue and further supports the hypothesis

that solar radiation is a major factor in the rapid loss of viral activity on cotton foliage. Residue studies of the virus applied to plants in the field indicated an 80 percent or greater loss of activity after 1 day.

A caged field test was conducted under a grant by the Arkansas Agricultural Experiment Station to determine the efficiency of Coleomegilla maculata as a predator of bollworm eggs and first instar larvae on frego bract and normal cotton. The bollworm eggs were placed on the inside and on the outside of the bracts of squares. In four of six experiments higher percentages of eggs were destroyed on frego cotton. The beetle was reared on corn pollen for 3 generations and on cotton pollen for 2 generations.

c. Pink Bollworm. In laboratory tests at Brownsville, first- and last-instar pink bollworm larvae were exposed to Bacillus thuringiensis (Thuricide) incorporated into the diet at 1:500, 1:1000, 1:2000, and 1:4000 ppm. Thuricide prevented pupation in all pink bollworms treated as first or last-instar larvae.

In studies at Brownsville cytoplasmic polyhedrosis adversely affected pink bollworm development. Ten-day-old diseased larvae weighed an average of 1.4 mg compared with 6.3 mg for untreated larvae. Infected male and female pupal weights averaged 17.3 and 19.9 mg, compared with 19.9 and 24.2, respectively, for the uninfected pupae.

A new type dry preparation of Bacillus thuringiensis was made by a new procedure in the laboratory at Brownsville. The dry preparation of the Bacillus thuringiensis spore-crystal complex was extremely toxic to first-instar pink bollworm larvae when incorporated in an artificial diet.

d. Other Insects. Control of cabbage loopers, salt-marsh caterpillars, and beet armyworms with Lespesia archippivora was promising in cage tests at Tucson. Early in the season the tachinid parasitized 86 percent of the salt-marsh caterpillar larvae. Some preference for bollworms in the presence of cabbage loopers was evident. High temperatures late in the season in the cages caused heavy mortality of the female flies.

In laboratory and small cage tests at Tucson, adult Nabis alternatus were fairly effective against lygus and bollworms. Similar tests indicated Collops vittatus was ineffective against lepidopterous larvae. Geocoris punctipes was ineffective against lygus, poor against bollworm but fairly effective against cabbage loopers. Orius sp. was fairly effective against 1st instar beet armyworms, and Sinea confusa showed promise as a predator of bollworms.

The lygus bug egg parasite, Anaphes ovijentatus was found often in alfalfa fields near Tucson, Arizona but only once on cotton. The parasite readily parasitizes eggs of Lygus hesperus, Nabis alternatus, and Spissistilus festinus and is easily reared in the laboratory. Anaphes ovijentatus readily oviposited in lygus eggs 2 to 7 days old. However, declining percentages of eggs were parasitized as the age of the eggs increased.

Preliminary laboratory tests at Tucson indicated that large numbers of Trichogramma sp. must be present to maintain high intensity searching capability. The Trichogramma sp. studied searched rapidly and oviposited freely in cabbage looper eggs in a simple searching arena or on artificial plants under favorable conditions. However, their behavior frequently was erratic and complete failures occurred in some tests.

Results of studies under a grant to the Alabama agricultural Experiment Station showed that a pathogenic fungus Entomophthora fresenii Nowakski attacked the two-spotted and carmine spider mites on cotton at several locations in Alabama. Surveys made during epizootics showed that as high as 80% of the mite population was infected by this pathogen. Several stages in the life cycle were observed and the secondary spores appear to be the infective stages of the organism.

D. Insect Sterility, Attractants and Other New Approaches to Control (9.5 SMY)

1. Cotton

a. Boll Weevil. At Tallulah, Louisiana, over 50 percent of boll weevils that were collected from ground trash adjacent to cotton that was treated with an attractant bait containing a red dye was visibly marked. The bait was applied to the cotton each week from July 1 to September 30. Ten square yards of ground trash were collected on September 22 and 28 and a total of 27 weevils were recovered with 15 or 55% being marked.

At State College, Mississippi, essential oils in concentration of 50 ppm from steam distillation of whole cotton plants were rich in boll weevil attractant. Seven thousand two hundred and ninety kg of whole cotton plants were steam distilled and 345 grams of oil that was attractive to weevils was obtained.

At State College, response of boll weevils late in the season was more than 20 times greater to male weevils than to cotton plants even where large numbers of squaring plants were grouped together. Weevils entering the woods in September and October continued to respond to the male pheromone. Many male weevils, also, responded to traps baited with males.

The longevity of boll weevils sterilized with irradiation was increased when they were treated in an atmosphere of nitrogen in the laboratory at Baton Rouge. The protective effect was most noticeable with dosages of 8,000 r or less. However, nitrogen also had a beneficial effect at a dose of 12,000 r. High percentages of dominant lethals were produced in mature sperm but there was some recovery of fertility after 10 days indicating that treatment with nitrogen gave some protection to spermatogonia or spermatocytes. Results of preliminary studies of mating competitiveness are promising.

In studies at Baton Rouge, thurberia weevils reared under diapause-inducing conditions tolerated large doses of gamma radiation. The median lifespan of thurberia weevils irradiated at the age of 1 day with 12,000 r was about 19 days with some individuals living more than 30 days. In contrast, boll weevils from Louisiana had a median life-span of only 13 days with the same treatment. Dissections of several individuals indicated that many weevils from both groups were in diapause. The weevils were reluctant to mate, and would therefore not be effective in a sterile male-release program. However, reproducing thurberia weevils may also tolerate gamma irradiation better than reproducing boll weevils.

In studies at Tucson, boll weevils irradiated with neutrons showed a decline in reproductive performance but the dose used was not high enough to cause complete sterility. Mortality of the irradiated adults was low.

Both gamma irradiation and apholate inhibited the synthesis of nucleic acids in eggs of the boll weevil, in studies at State College, Mississippi. When developing boll weevil eggs were irradiated at levels up to 10,000 r the amounts of RNA and DNA were less than that in untreated eggs. Similarly both nucleic acids decreased in eggs treated with a 19.25 mg.ml solution of apholate.

Feeding of boll weevils was reduced 60 percent on squares treated with a 50 percent concentration of tung oil emulsion in water compared with feeding on untreated squares, at State College. Little or no reduction in feeding was noted on squares treated with concentrations less than 50 percent. The same numbers of eggs were laid in treated and untreated squares, and there was no difference in percentages of hatch.

Results of studies under contract with Southern Research Institute, Birmingham, Alabama, showed that 149 of 391 dosages of chemosterilants that were screened were toxic to boll weevils. Fifty non-toxic compounds reduced oviposition to 1 egg or less per female. Five non-toxic compounds prevented oviposition. Nine compounds reduced egg hatchability to 10 percent or less. Oviposition was reduced to less than 1 egg per female by eight of the compounds. ENT 52514, 60242, 52553, 61182, and 25012 greatly reduced fecundity and eggs that were laid failed to hatch. ENT

61285 and 61177 reduced egg hatch and larvae from eggs that hatched failed to develop to adults.

b. Bollworm. Crude cottonseed oil and water extracts of cotton seeds and flakes had equal feeding stimulant activity to bollworm larvae in studies at Brownsville. Comparable feeding stimulation was obtained in Heliiothis spp. with crude cottonseed oil and the water extracts prepared with glanded cottonseed meats (dehulled cottonseed) and flakes (dehulled cottonseed after oil extraction).

Results of laboratory tests conducted under a cooperative agreement with the Texas Agricultural Experiment Station indicated the presence of an ovipositional stimulus in a chloroform extract of tobacco leaves. Results of field studies in small plots and large cages indicated that the tobacco budworm prefers to oviposit on tobacco plants, especially flowering plants, over cotton plants. Cotton yields in cage experiments ranged from 425 pounds of seed cotton per acre in cages having no tobacco plants to 1292 and 1300 pounds, respectively, in cages having 8 and 16 tobacco plants. The increased yields resulting from the trapping of the budworm on tobacco plants amounted to almost 900 pounds of seed cotton per acre. Moths were released into the cages at the rate of 2000 per week. The data indicates that from 1600 to 3200 tobacco plants per acre of cotton should trap more than 95% of the oviposition of the tobacco budworm. However, tobacco plantings would have to be timed so that flowering plants would be available at all times to budworm moths.

c. Pink bollworm. Studies at Brownsville indicated that more than a 19:1 sterile:normal male ratio would have to be maintained to cause a decline in a pink bollworm population. In a laboratory experiment, adult males were sterilized with 40 kr of gamma radiation and caged with normal moths so that the sterile:normal male ratio was 4:1, 9:1, and 19:1. The reduction in reproductive potential with the above ratios was 62.5%, 70.3%. and 80.0% respectively.

At Brownsville, female moths were exposed to 40 kr of gamma radiation after mating for 5 days. No hatch of 1767 eggs collected from 114 irradiated females occurred. Sixty-two percent of the eggs from untreated females hatched.

Ethanol extracts of cotton and wild tobacco were attractive to female pink bollworm moths. Screening tests indicated that the odor produced by ethanol extracts of NR-293 cotton squares and flower petals, Pima glanded flowers, and wild tobacco flower petals were attractive to female pink bollworm moths.

In studies at Tucson, under conditions of complete isolation, neutron-irradiated pink bollworms showed no obvious decline in life-span compared with unirradiated moths. Females lived about 4 days longer than males.

Neutron irradiated male pink bollworms transferred spermatophores to untreated mates but this apparently failed to initiate normal egg production in the female. Preliminary results indicated that females produced more eggs when an excess of males was present.

Preliminary radiation experiments with the pink bollworm indicated that this moth has diffuse centromeres as expected. Mating studies indicate some reduction in egg production from irradiated females but rearing losses preclude any conclusions on developmental effects.

In studies at Phoenix, open container type sex lure traps were more effective than conventional traps in trapping male pink bollworm moths. A sex lure trap made from an open container, such as a tin can with a wire across it near the top for attaching the lure, loop for suspending the trap, and detergent and water as a trapping fluid, was as effective as the conventional pink bollworm sex lure trap.

In field tests conducted for 5 weeks at Phoenix, traps baited with 60 mg. of ENT 33478 (cis-7-hexadecene-1-ol acetate) per trap consistently collected more male pink bollworm moths than traps baited with 5, 15, or 25 female equivalents of natural lure. However, in most tests the traps baited with ENT 33478 caught fewer males than traps baited with 50 equivalents of natural lure.

d. Other Insects. Preliminary tests at Stoneville with caged tarnished plant bugs in the field indicate that virgin females are very attractive to males. There was almost no response to caged males.

Apholate gave temporary sterility of the tarnished plant bug at Stoneville. Preliminary laboratory tests indicate that apholate is effective as a temporary chemosterilant but ENT 51909a was almost ineffective at dosages tested.

E. Evaluation of Equipment for Insect Control and Detection (2 SMY)

1. Cotton

a. Boll Weevil. At State College, Mississippi, a modified insect collecting machine was more efficient than the original machine in determining field populations of boll weevils. An average increase of 64 percent in efficiency was noted for the modified machine over the original "bug catcher". Although results obtained with the "bug catcher" were not consistent, when time required to check a given plot and

number of weevils caught were compared, the machine was almost 17 times more efficient than a crew of men in collecting weevils.

At Florence, a ULV ground sprayer that retains the air blast principle but with air outlets close to the plants to reduce drift has been developed and tested. A large fan assembly is used as a source of auxiliary air that forces the air through the boom to direct the atomized material down into the plant. The materials are atomized with Fischer solid-cone, internal-mix pneumatic nozzles and sprayed into the auxiliary air stream. The insecticide is then picked up by the 65 mph air stream and carried down into the plants. Preliminary performance data indicate that more material is deposited on the target area than with a mist blower.

At Stoneville when the same dosages of an insecticide were applied as ultra-low volume sprays with a spinning disc, Hahn, or a John Blue mist blower, all gave effective control of boll weevils and bollworms with no appreciable differences in control among the three types of spray equipment.

In a study under a grant with the Georgia Agricultural Experiment Station an actual count of the size and number of aerial spray droplets on bollworms and boll weevils killed by a contact spray of methyl parathion was made. The maximum size of droplets on 541 bollworm larvae was 115 microns in diameter. On 139 weevils the maximum size of a droplet was 63 microns. The data showed that droplets larger than 50 microns in diameter were of no major significance in the kill of these cotton insects in their natural environment by a contact insecticide. The upper limit of biological activity of contact sprays in cotton in July-August in Tifton, Georgia was obtained with droplets 100 to 115 microns in diameter. The upper limit of significant biological activity was obtained with droplets up to 50 microns in diameter.

b. Bollworms. At Florence, an air whistle (Feller Jet Resonator) was found to have a higher sound level output at 20 KHz than any other sound source tested for the acoustic response of H. zea. The tympanic organ of the H. zea moth detected the sound output from the whistle at 350 feet. Only a slight reduction in H. zea hourly catch in a blacklight trap was noted when the air whistle was operated in competition with the trap for 6 nights. The major drawback of the whistle was that its output could not be easily chopped into short duration pulses to be alternated with periods of silence which apparently are necessary to elicit evasive behavior in the free flying H. zea moths.

At Florence, characteristics of the ultrasound produced by the arcing of an electric grid were determined from tape recordings. Arcing of electric grids used in lighttraps to kill insects produced pulses lasting 0.6 to 1.2 msec with major ultrasound frequencies of 40 to 45 KHz and a pulse

rate of approximately 125 per second when the grid was adjusted to give a staccato pattern of arcing. The tympanic organ of the H. zea moth detected the arcing at 288 feet. Hourly lighttrap catches of H. zea were slightly reduced when the arcing grid was operated.

Yield data in field plot tests conducted under a cooperative agreement with Texas Agricultural Experiment Station showed that rotary brush applicators or soil injectors operated on both sides of the plants at a depth of five inches caused no mechanical damage to the cotton plants. Yields were reduced when soil injectors were operated at a depth of 8 inches on both sides of the plant and 5 inches from the drill.

c. Pink Bollworm. Pink bollworm larvae survived in lint ginned in a roller cotton gin. Highest survival was in the newer high-capacity roller gin. Use of lint cleaners reduced survival. It is suspected that many of the surviving larvae were in cottonseed remaining in the lint samples.

F. Varietal Evaluation for Insect Resistance (3 SMY)

1. Cotton

a. Boll Weevil. At State College, Mississippi, preference and anti-biosis studies indicates that the male Hampea sp. plant is as attractive as cotton to boll weevils, but produces a significantly smaller weevil. Female plants are non-preferred and a high degree of antibiosis in the buds to weevil larvae probably explains why the female tree is not attacked in nature.

In a genetic study in cotton at State College, the linkage value for the glandless (gl_2) and nectariless (ne_1) genes in the A genome was shown to be $32.79 + 1.30\%$ cross over units and the linkage value for glandless (gl_3) and nectariless (ne_2) in the D genome was $38.89 + 1.10\%$ cross over units. The establishment of these linkages further marks these two chromosomes and now three-point linkage tests may be conducted using the duplicate genes for glandless, nectariless, and withering bract to establish gene order. Both glandless genes and both nectariless genes showed independence with the Sm gene for D_2 smoothness.

b. Bollworm. Bollworm infestations were heavier in glandless than in glanded cotton strains at Waco, Texas. Inspections of glanded and glandless cottons were made on 2 seed farms in Central Texas. On each farm planting dates, insect control, fertilization, and irrigation were the same for the glanded and glandless cottons. In both locations there were more bollworm injured squares and bolls in the glandless than in the glanded cotton. However, under conditions of heavy insecticidal treatment for bollworm control, differences were not great.

Bollworm infestations differed in small fields in McNair glanded and glandless cotton strains in North Carolina. In an unreplicated study, the bollworm damage in a 5-acre field of glandless cotton was 4 times as great as that in a 30-acre field of glanded cotton. Both strains received the same insecticidal treatment.

Several G. hirsutum races showed antibiosis promise against bollworms at Brownsville, Texas. Growth and survival of bollworm and tobacco budworm larvae were reduced when reared in the laboratory on squares from several races of G. hirsutum grown in Tampico, Mexico.

PPA 701 Insure Food Products Free of Toxic Residues from Agricultural Sources

A. Insecticide residue determinations (0.3 SMY)

1. Cotton. In 1966 plots of cotton were treated with Union Carbide UC-21149 in a 10 percent granular formulation (Temik at rates from 1 to 4 lb/acre (1.12 to 4.48 kg/hectare) of active ingredient. In 1967 similar treatments ranging from 1 to 6 lb/acre were made. Cotton picked from the plots at intervals of 60 to 183 days after treatment was ginned and the seed was sampled. Neither UC-21149 nor UC-21149 sulfoxide residues were found in any of the 1966 or 1967 samples. UC-21149 sulfone residues were not found in the 1966 samples but were detected in two 1967 samples. Seed from a plot treated with 2 lb/acre and sampled 137 days after treatment contained 0.015 ppm of the sulfone; seed from a plot treatment with two 1 lb/acre applications separated by 46 days and sampled 137 days after the last treatment contained 0.008 ppm of sulfone.

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AREA NO. 8 TOBACCO INSECTS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years FY 1968		Total
	207	701	
Florida	1.9	.1	2.0
Maryland (Beltsville)	.3		0.3
North Carolina	4.0		4.0
South Carolina	.9	.1	1.0
St. Croix, V.I.	1.0		1.0
Total	8.1	.2	8.3

Intramural program is supplemented by extramural support representing 1.6 SMY's at State Agricultural Experiment Stations^{1/}.

1/ RPA 207 - 1.6

Problems and Objectives

Insecticides used for tobacco insect control may cause undesirable residues and off-flavors in cured tobacco. Residues may remain in or on the leaf during commercial processing and some have been found in the mainstream of smoke from commercial cigarettes. In addition, many of the insects previously controlled with chemicals have become resistant to all currently registered materials. Noninsecticidal methods for controlling tobacco pests are urgently needed.

The objectives of the research are to develop and utilize for the control of tobacco insects:

1. Selective, nonpersistent chemicals.
2. Insect resistant tobacco germ plasm.
3. New approaches such as sterility, attractants, and biotic agents.
4. Integrated control programs using methods such as chemicals, light traps, sterility, parasites, predators, and pathogens.

Progress - USDA and Cooperative Programs

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

A. Basic Biology, Physiology and Nutrition (1.2 SMY)

1. Tobacco Hornworm. At Oxford, N.C., successful laboratory mating of Apanteles congregatus, a braconid parasite of the tobacco hornworm, was obtained through control of ambient light and provision of adequate horizontal resting surfaces. Mating occurred primarily in the afternoon and oviposition in the morning. First and second instar hornworm larvae were preferred for oviposition. A complete life cycle was obtained for the first time in the laboratory.

In field-collected tobacco hornworm larvae at Oxford, 95% of the Tachinid parasite eggs were laid on the first 3 segments; however, in the laboratory on hornworms on artificial diet, 81% of the eggs were laid on segments VII to XI. Field-collected larvae averaged 24 eggs and lab-reared only 9.

At Oxford, artificial leaves used as oviposition sites for the tobacco hornworm gave consistently good results. Crude extracts of tomato or of tobacco leaves sprayed on artificial leaves stimulated oviposition, and in several trials artificial plants were more effective in inducing oviposition than the natural hosts. During oviposition experiments, the surrogates were as effective as the NC 95 tobacco variety, and were more effective than other varieties. Tobacco plants grown in greenhouses were seldom as effective as the artificial plants.

In experiments to determine factors influencing the selection of oviposition sites by tobacco hornworm females, a combination of aroma and moisture (leaves moistened with crude tobacco extract) was found to be the most effective oviposition stimulus.

Gravid female hornworms exposed to artificial oviposition sites under low light levels (0.1 ft-c) selected sites on the basis of visual stimulation, alone. Females visited untreated artificial leaves, sheathed in black or white felt to simulate hairy surfaces of natural host plants. A few eggs were deposited on untreated leaves, indicating that tarsal contact, alone, is sufficient stimulus for some moths. White leaves were preferred to black leaves, suggesting that reflectance may be important in the selection of oviposition sites. The use of both treated and untreated, camouflaged, artificial leaves in several experiments indicated that the female navigates to specific leaf sites primarily by vision, although a short-range olfactory stimulus is necessary for landing. The females also responded to shadows cast by the overlying leaves on an underlying white felt surface. Visiting females approached these shadows in the same manner as approaching host plant leaves. Several eggs were deposited around the edges of the shadows.

Of more than 50 dyes screened at Oxford for staining of the tobacco hornworm spermatophore in vivo, only Rhodamine B, pararosaniline (basic, acetate, and HCl forms), acid fuchsin, and methylene blue chloride were effective. No toxic effects resulted from 0.1% concentrations of these dyes. Although all of the first 3 dyes stained the spermatophore red, Rhodamine B was distinguishable by its fluorescence under ultraviolet light.

At Oxford, a new technique for mass rearing the tobacco hornworm on artificial diet showed promise of greatly increasing efficiency through the exclusion of contaminants, and avoiding the need for diet changes and handling of the larvae. In preliminary trials prepupal yields ranged from 70 to 90% of 200 larvae transferred compared with 50% or less with the previous method.

In studies at St. Croix, V.I., tobacco hornworm eggs were held at 60° F for 8 days without appreciably reducing their viability. When offered a choice, adult tobacco hornworm moths consumed twice as much of a 25% honey-water solution as a 25% dextrose-water solution, and seven times as much honey solution as beer.

An extensive search on St. Croix for wild host plants of the tobacco hornworm revealed it to be feeding on Solanum caribaeum and Solanum torvum.

A time interval trap operated on St. Croix showed that maximum activity of the tobacco hornworm occurred between 8:00 p.m. and 1:00 a.m.; 68.6% of the moths were caught between these hours.

In releases of 674 marked male moths over a 12-day period on St. Croix, blacklight traps baited with virgin females captured 151, or 22% of the

released moths. Moths dispersed widely from each release site. The releases demonstrated that use of the sterile male method would require no more than 3 release sites for the entire 84-mile² island and that baited traps were comparatively efficient in capturing male moths.

2. Cabbage Looper. At Quincy, Fla., increasing the wheat germ and substituting Torula for Brewer's yeast in Shorey's bean diet for the cabbage looper reduced the cost from \$1.29 per gallon to \$0.85, in addition to improving yield, size, and rate of development of the looper larvae. Further savings are anticipated from the substitution of Gelcarin for the standard agar. Facilities now permit rearing of 50,000 cabbage loopers weekly.

B. Insecticidal and Cultural Control (.6 SMY)

1. Wireworms. At Florence, S.C., new equipment was developed for applying insecticides to control wireworms in tobacco soil. A tractor equipped with one distributor spreads the insecticide granules on the soil and covers them to a depth of 3 to 4 inches by use of two sets of disk hillers and two large sweeps, operated behind the rear wheels of the tractor.

In field experiments, Union Carbide UC-21149, Bay-37289, Dyfonate, Mocap, carbofuran, and Dasanit gave consistently effective wireworm control when applied as granules.

2. Flea Beetles, Budworm, Hornworm. At Florence, the most effective insecticide for the control of foliage feeding tobacco insects was carbofuran. Applied to the soil in granules, it gave good control of tobacco flea beetles, budworms, and hornworms for several weeks. Granular disulfoton applied to the soil also gave effective control of the tobacco flea beetle and the green peach aphid.

Field plot experiments at Florence indicated good control of tobacco hornworm, tobacco flea beetle, and green peach aphid with dust or spray applications of a combination insecticide mixture leaving little residue on the treated plants. The insecticide mixture contained Mobam, rotenone, and parathion.

C. Biological Control (.8 SMY)

1. Tobacco Budworm and Cabbage Looper. At Quincy, Fla., parasitism of *Heliothis* eggs by a local species of *Trichogramma* (*T. prediosum*) showed 9% on tobacco and 37% on corn in a 7-acre cage. Weekly aerial applications of 2 quarts of Thuricide (*Bacillus thuringiensis*) per acre applied to cigar wrapper tobacco was as effective as the conventional insecticide treatment in controlling tobacco budworms and cabbage loopers.

D. Insect Sterility, Attractants, and Other New Approaches to Control (1.7 SMY)

1. Tobacco Hornworm and Budworm. At Oxford, N.C., 8,990 sterile male hornworms were released in an 80-mile² area encircled with a 5-mile-wide band of 3 blacklight traps per mile². After the first brood, male and female moth captures in the test area were 56 and 52% lower, respectively, than outside. Tomato hornworms, probably affected by sterile males, showed reductions of only 25% for males and 12% for females.

In tests of blacklight trap installation height ranging from ground level to 98 feet, catches of tomato and tobacco hornworms were high in the ground level trap. Indications were that such an installation effectively captures moths falling to the ground after they are attracted to traps located at higher levels.

Use of a mark-release-recapture method in a 19-mile² area of Johnston County, N.C., during the period June 29-September 1, 1965, provided estimates of approximately 23,630 male and 22,590 female tobacco hornworm moths in the area. During 1967, there was an estimated 28,546 pairs of moths in 98,470 acres of land or 9.35 per acre of tobacco from June 2 to September 1.

In Oxford tests, the tobacco hornworm moths marked with fluorescent paint applied as an aerosol were visible at night 100 ft from a blacklight lamp emitting in the range of 3200 - 3800 Å.

During 1962 and 1967 in two areas near Oxford, movement and survival of tobacco hornworm moths were compared with use of a mark-release-recapture method. Of 13,824 moths released, 260 (1.9 percent) were recaptured. The maximum distance of assumed natural flight was 6.8 miles; the mean distance traveled was 1.7 miles. The limited data available indicate that female moths disperse as widely as males. The dispersal pattern was about the same for laboratory reared moths as for wild moths, which were trapped, marked, released, and recaptured. In one night moths became well distributed over the area out to 3 miles from the release site.

At Oxford, blacklight traps spaced at 1-mile intervals in a 16-mile² area and baited with two virgin female hornworm moths caught four times as many males as unbaited blacklight traps. The ratio of male to female moths was greater in low population areas.

At Florence, S.C., extracts of the abdominal tips from virgin female tobacco hornworms were attractive to males in field studies.

On St. Croix, after 2 years of trapping with 3 blacklight traps per mile² over the entire island, tobacco hornworm populations were approximately 30% as high as recorded during the year prior to the trapping program.

On St. Croix, 2 virgin female hornworms placed at a blacklight trap increased the male tobacco hornworm collection by 7 times and 4 virgin females increased the collection by 10 times. Blacklight traps baited with virgin females and placed 10 feet high collected 1.7 times more male moths

than when placed 5 feet high, and 2.3 times more than those placed 1 foot high. Crude extracts of the virgin females were ineffective in increasing male captures. A comparison of a North Carolina and St. Croix strain revealed that, although virgin females of both strains effectively increased captures of male hornworm moths, the St. Croix strain was the most effective.

2. Heliothis sp. At Quincy, Fla., Heliothis zea and H. virescens moths were released in a 7-acre cage containing 5 evenly-spaced standard blacklight traps equipped with chemosterilant feeders (tepa-sucrose solution). After 3 days, the percent reduction in egg hatch compared with a 1-acre control cage was 70% for H. zea eggs collected from corn and 9% from H. virescens eggs collected from tobacco. Eggs from moths of both species collected from the 7-acre cage 3 days after release showed a reduction in hatch of 39% for H. virescens and 91% for H. zea.

In an area of approximately 1/2-mile², 26 blacklight traps each baited with 10 virgin female H. virescens moths showed a substantial increase in male catch over unbaited blacklight traps.

3. Cabbage Looper. At Quincy, Fla., populations of female cabbage looper moths were higher inside a 1-mile² area containing 60 blacklight traps, each baited with .1 gram of synthetic sex pheromone, than in outside areas with installations of 3 unbaited blacklight traps per mile². Spermatophore counts showed that 30% of the females collected from traps inside the 1-mile² area had mated while 52% of the females taken from traps outside were mated. Total mating within the 1-mile² area was 26% less than in outside areas. However, there was no difference in egg and larval counts or in percent egg hatch within and outside the area.

4. Miscellaneous Tobacco Insects. At Quincy, Fla., during the 1965, 1966, and 1967 growing seasons, integrated insect control programs were conducted using disulfoton, light traps, the pathogen Bacillus thuringiensis, and nonpersistent insecticide treatments as needed. Compared to conventional programs, the integrated programs reduced the total number of seasonal insecticide applications on cigar wrapper tobacco by 80, 76, and 43 percent, respectively. There was no significant difference in plant damage to tobacco between the integrated programs and the conventional insecticide program. In 1967, local cooperators grew 71 acres of tobacco under the integrated program and showed a saving of approximately \$26.00 per acre compared with the regular insecticide program.

Blacklight traps were spaced around the perimeter of a cigar wrapper tobacco field in conjunction with weekly aerial applications of the pathogen Bacillus thuringiensis beginning 2 weeks after transplanting. The mean number of plants damaged by tobacco budworms and cabbage loopers was .88 plants per 100 plants examined as compared with 5.5 plants damaged in fields under conventional insecticide programs where insecticide was applied once or twice per week. When the treatment in conjunction with the blacklight traps consisted of weekly applications of 1% parathion and

10% DDT, the mean number of plants damaged was .13 compared with 6.0 under the conventional insecticide program.

E. Evaluation of Equipment for Insect Detection and Control (.1 SMY)

At Florence, S.C., soil samples indicated higher larval populations of the southern potato wireworm near blacklight traps than in areas not associated with traps. Apparently a significant portion of the adults attracted are not trapped.

F. Varietal Evaluation for Insect Control (.2 SMY)

At Oxford, N.C., approximately 80 species of Nicotiana and 160 varieties of N. tabacum were tested to determine potential resistance to tobacco hornworms and budworms.

RPA 701 - INSURE FOOD PRODUCTS FREE OF TOXIC RESIDUES FROM
AGRICULTURAL SOURCES

A. Insecticide Residue Determinations (.1 SMY)

At Florence, S.C., in cooperation with industry, no off-taste and flavor was shown in cured tobacco samples after soil treatments for wireworms with diazinon wettable powder, and granular formulations of diazinon, Bay-37289, Dasanit, Union Carbide UC-21149, Mocap, or carbofuran.

Publications - USDA and Cooperative Programs

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

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Varietal Evaluation for Insect Control

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AREA NO. 9 - SUGARCANE AND SUGARBEET INSECTS

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-Years FY 1968
Idaho	Sugarbeets	1.8
Washington	Sugarbeets	3.3
Louisiana	Sugarcane	3.0
Florida	Sugarcane	1.0
Total		9.1

Intramural program is supplemented by extramural support representing 3.1 SMY at State Agricultural Experiment Stations and PL 480 funds in one country totalling about \$20,000.

Problems and Objectives

Many species of insects cause losses amounting to millions of dollars annually to sugarcane and sugarbeets through direct feeding damage and/or damage from diseases spread by insects. Surveys show that the sugarcane borer alone causes an annual loss exceeding \$5 million. Comparable losses in sugarbeets can be attributed to sugarbeet root maggot, beet leafhopper, and beet webworm. Sugarcane mosaic continues to cause losses amounting to millions of dollars annually. Beet yellows and associated western yellows virus disease of sugarbeets transmitted by insects continue to threaten the sugarbeet industry. Emergency control methods for vectors of the viruses of these diseases are urgently needed. Reducing crop losses in sugarcane and sugarbeets due to insect damage and diseases they cause could result in annual net benefits of at least \$20 million.

Major objectives of the research are to develop and utilize:

1. Knowledge of the biology and ecology of insects.
2. Varieties resistant to insect pests.
3. New approaches to insect control.
4. Safe insecticides that will control insects and insect vectors of diseases, but do not leave harmful residues.
5. Methods to eliminate the weed reservoirs of insect vectors.

A. Basic Biology, Physiology, and Nutrition (2.3 SMY)

1. Sugarbeet

a. Sugarbeet root maggot. At Paul, Idaho, the sugarbeet root maggot overwintered in the larval stage, with 55% found in the 6 to 9-inch layer of soil, 30% at 3 to 6 inches, and the remainder below 9 inches. The maggots began an upward movement in mid March and 68% were found in the upper three inches of soil by April 30, 93% of which were in the pupal stage. The first fly emergence was noted on April 28, and the peak was reached on May 29. Raw beet juice was the most attractive to adults of several materials tested, and fermenting juice is more efficient than fresh juice.

The diapause chilling requirement of the root maggot can be met by a 2-month cold-storage treatment. Good egg production and viability has been obtained by feeding the adult flies a diet of Brewer's yeast, sugar, and water.

b. Aphids. A mechanical trap utilizing yellow baffleplates for impaction surfaces and bottles of a dilute formaldehyde preservative was developed at Yakima, Wash., for determining hourly, as well as longer, periods of flight activity of the green peach aphid. Most flights occurred during daylight and twilight hours of the day. Temperature was a lesser deterrent than wind to aphid flight.

2. Sugarcane

a. Sugarcane Borer. Survival in Louisiana for the winter 1966-67 was 81.5% compared to 11% for 1965-66. First generation counts in May-June showed an estimated average of 2297 borer-killed plants per acre compared to 1390 in 1966.

The harvesttime infestation averaged 16% of the joints bored. Infestations during five previous years were 14% in 1966, 18 in 1965, 11 in 1964, 12 in 1963, and only 6 in 1962 following one of the coldest winters in this century in Louisiana. Percentage of joints bored for 1935-66 averaged 16%. The 4400 stalks examined in 1967 harvesttime survey had 55,793 joints compared to 51,819 in 1966 when an early freeze necessitated lower than normal topping of the crop. Estimated crop loss resulting from borer injury was 12% in 1967.

The Florida sugarcane borer harvesttime survey indicated an overall average of infestation (joints bored) of 6.5%. This compares with 2.4% for 1966-67, 4.0% for 1965-66, and 6.4% for 1964-65. This represents a loss or reduction in production of some 49,853 tons of raw sugar for the 1967-68 season. Populations of the "cane fly" or West Indian sugarcane delphacid reached a high population level on approximately 100,000 acres in January and February, some stunting of younger cane resulted. Wireworms continue to be a problem in Florida.

Losses to the sugarcane crop in Florida due to borer injury were studied on 105 split-plots of sugarcane grown under two controlled levels of borer infestation (high and low). The high level had an average bored-joint infestation of 62% and the low level of 40%, a difference of 22%. This difference caused a total loss of 1869 pounds of sugar per acre. Seventy-four percent of this total (1381 pounds) was lost in the field while only 26% (488 pounds) was lost in the factory. Of the 74% field loss, 54% (1015 pounds) was due to lighter-weight stalks and 20% (366 pounds) to fewer stalks per acre. From the 26% loss in the factory, 14% (260 pounds) was due to lower sucrose, 3% (56 pounds) to lower purity, and 9% (172 pounds) to reduced extraction.

b. Yellow Sugarcane Aphid. Infestations of the yellow sugarcane aphid, Sipha flava, increased enormously in all fields in Louisiana where carbaryl was applied for borer control. A grass worm, Cisseps fulvicollis, caused economic injury to young sugarcane in spring of 1966 for first time in Louisiana.

B. Insecticidal and Cultural Control (3.2 SMY)

1. Sugarbeet

a. Sugarbeet Root Maggot. At Rupert, Idaho, granular formulations of Bay 37289, Bay 25141, diazinon, Niagara NIA-10242, phorate, Stauffer N-2790,

and Union Carbide UC-21149 applied to sugarbeets in a 6-inch band at planting time gave better than 95% control of sugarbeet root maggot.

Sprays of azinphosmethyl, Bay 39007, diazinon, and dimethoate applied by ground equipment to sugarbeets when the sugarbeet root maggot flies were emerging gave good control of the maggots and yield increases.

Three airplane applications of ultra-low-volume malathion at the rate of eight ounces per acre to an isolated 584 acres of sugarbeets reduced adult fly population 97% and gave a 3-ton increase in yield in 1967. In 1968, 200 acres near Minidoka, Idaho, were sprayed the same way. However, unfavorable weather retarded beet germination, and the beets were in the cotyledon stage when the flies emerged. With such a scarcity of foliage, very poor control was obtained. Ultra-low-volume malathion dissipates very rapidly when there is not much foliage present.

b. Aphids. At Yakima, Wash., greenhouse tests simulating early spring conditions during which seeding and sidedressing of sugarbeets with systemic insecticides for aphid control may start with soil temperatures near 40° F showed that the insecticide dissipated during the 42-day germination period and failed to prevent establishment of colonies of the green peach aphid, Myzus persicae. However, when the soil temperature was raised to 50° F at seeding and sidedressing, 100% control was obtained for about three weeks after plant emergence.

Foliage applications of phorate controlled the green peach aphid for nine weeks and UC-21149 for 16 weeks; phorate controlled the potato aphid for ten weeks, and UC-21149 for 12 weeks.

c. Leaf Miner. At Yakima injury to sugarbeet leaves from a leaf miner was reduced materially by one pound of either phorate 86%, Azodrin 83%, demeton 80%, or carbophenothion 76% in 24 gallons of water per acre.

Gravid females of one leaf miner species were not repelled whereas gravid females of another leaf miner species were repelled for nearly two weeks by seven different organophosphorous sprays.

2. Sugarcane

a. Sugarcane Borer. Thuricide at 1-1/2 quarts per acre gave good sugarcane borer control, with 2.3% joints bored; Azodrin at .5 pound per acre had 3.7% joints bored; endrin at .3 pounds per acre 4.4% bored joints; check plots had 6.2% bored joints.

Average net tons per acre of sugarcane harvested from plots treated with insecticides at time of planting for control of wireworms, Melanotus communis, in heavily infested muck soil are as follows: check (no treatment) 9.1 tons; Chevron RE-5353, 2.7 pounds actual per acre in granules (G), 17.8 tons; Ortho 9006, 2.7 pounds (G), 25.1 tons; parathion, 4.6

pounds (G), 32.8 tons; Mocap 1.6 pounds (G), 33.9 tons; diazinon 4.0 pounds in emulsifiable concentrate (EC), 40.0 tons; diazinon 4.0 pounds (EC), 40.6 tons; Akton, 2.0 pounds (EC), 41.9 tons; Dasanit 3.8 pounds (G), 43.1 tons; carbofuran 3.8 pounds (G), 43.2 tons; Dyfonate 3.8 pounds (G), 47.9 tons.

Eighteen chemicals were evaluated against the sugarcane borer in three small plot and four airplane experiments. Percentages of controls, based on bored joint (internode) infestation, ranged from 5 to 94. Abate at one pound per acre in an airplane experiment gave 80% control compared to 81% for Azodrin and 69% for azinphosmethyl.

Low volume concentrates of Azodrin and azinphosmethyl at .75 pound per acre in an airplane experiment gave controls of 69% and 77% compared to 85% and 84% for the respective insecticides conventionally applied and 83% for azinphosmethyl in granular formulation at one pound per acre.

Maximum borer mortality in leaf sheaths, attained one week after insecticidal application, were 98% for Azodrin, 96% for azinphosmethyl, and 74% for carbaryl. Live borer counts were high in all treatments three weeks after application.

Azodrin and azinphosmethyl in herbicidal-combinations of 2,4-D and Silvex gave high effective borer and tie vine controls. Borer control obtained with the two 2,4-D-insecticidal treatments was slightly higher than the corresponding insecticidal treatment alone.

C. Biological Control (1.6 SMY)

1. Sugarbeet

The Pacific brown lacewing was more abundant than predaceous Typhlodromus mites on sugarbeets infested with the two-spotted spider mite at Yakima, Wash. It seldom visits sugarbeets until mite colonies have been established and then deposits one or more glistening, white eggs within or near the webbed-in mite colony. Infested leaves have often been freed of mite infestations by this agile searcher.

2. Sugarcane

a. Sugarcane Borer. Laboratory tests at Canal Point, Fla., showed that virgin females laid an average of 192 eggs and mated females laid an average of 331 eggs. The sterile eggs were unsuitable for Trichogramma development. Eggs sterilized by heat were suitable for Trichogramma development in the laboratory but apparently were not parasitized in the field.

Studies conducted in 1967 to determine if effectiveness of natural Trichogramma populations could be substantially increased by adding to the

the environment large numbers of fresh sterile sugarcane borer eggs on a sustained basis showed no significant differences between the arbitrarily selected high and low egg density levels for either percent parasitism or borer infestation. Parasitism in the high host egg density level did not increase as the experiment progressed and at the conclusion of the experiment was only 3%.

Studies conducted at West Palm Beach, Fla., to determine the degree of parasitism by Lixophaga diatraea, in relation to borer population showed that the release of five female parasites per acre in a field infested with 100 borers produced 21% parasitism; 5 to 500 borers, 6%; 25 to 100 borers 14%, and 25 to 500 borers, 3%. Parasitism in check plots (no release) of 100 borers, 10%, and 500 borers, 2%.

Additional studies using higher release levels in young (24 inches high), first year ratoon cane, increased percent parasitism as follows: 50 parasites (25 female) per acre with 100 borers 85% parasitized; 50 parasites with 500 borers 70%; 250 parasites with 100 borers 94%; and 250 parasites with 500 borers 86%. One non-release check plot had 32% parasitized borers, but non-release 100 and 500 host borer plots each yielded 0% parasitized borers.

The parasites Apanteles flavipes and Agathis stigmaterus are now established over the sugarcane growing area of Florida. Both are most abundant on the eastern and western fringes of the production area. Trichogramma sp., an egg parasite is established on the sandland areas. Levels of parasitism ranged from 2% - 20% for Agathis with an average of near 14% for the area. Apanteles parasitism ranged between 3% in the central growing area to 10% on the eastern sandlands to 98% in one small field southwest of the main growing area.

Introduced parasites which have been successfully reared on D. saccharalis in the laboratory were Paratheresia claripalpis and Plapozenillia sp. (Bolivian strain).

D. Varietal Evaluation for Insect Resistance (0.9 SMY)

1. Sugarcane

a. Sugarcane Borer. In the 1967 preliminary screening tests at Hcuma, La., for borer resistance were conducted on 186 sugarcane varieties in single field plots under conditions of artificial infestation. Three of these, H. 61-55, H. 61-207, and H. 61-577, showed superior borer resistance and sugar production.

In advances replicated field tests 27 unreleased sugarcane varieties and one control variety were artificially infested in 1967. Results of this test showed three varieties, CP. 65-433, CP. 66-491, and L. 61-43 to be outstanding in both borer resistance and sugar production. Of these three

varieties CP. 66-491 has the best record, and has been consistently good in both borer resistance and sugar production in all tests conducted over a three-year period.

Progeny from basic crosses and backcrosses involving wild (Saccharum spontaneum and S. robustum), cultivated canes, and basic crosses from the Sugarcane Breeding Institute at Coimbatore, India, were grown and evaluated for borer resistance at Houma, La., under conditions of artificial infestation. Three varieties from Canal Point, Fla., and 18 from India with low infestation readings were saved for retest as promising borer resistant varieties.

E. Insect Vectors of Disease (1.1 SMY)

1. Sugarbeet

a. Beet Leafhoppers. Losses of sugarbeets from curly top, transmitted by the beet leafhopper were more severe in eastern Washington in 1966 and 1967 than for many years. Although only resistant sugarbeets were grown, the resistance was not sufficient to cope with unusually large populations of leafhoppers and an extremely virulent strain of the virus.

Replacing the Russian-thistle with crested wheatgrass or rangeland in the Magic Valley of Idaho has reduced the population of beet leafhoppers to one of unimportance in recent years.

In western Idaho and eastern Oregon where large acreages of sagebush have been sprayed and seeded to grass by the Bureau of Land Management, germination has been poor. Russian-thistle has taken over these areas and large populations of leafhoppers were produced last year. The sugarbeets in this area are showing a very high incidence of severe curly top. Some fields may lose as much as 25% of the plants to curly top.

b. Aphids. Fifteen lots of sugarbeet roots from plants heavily infected with beet western yellows examined at Yakima, Wash., contained approximately the same percentage of sucrose. The sucrose content in noninfected beets ranged from 14.1 to 17.7% (average 15.3%) and infected beets from 12.6 to 17% (average 15.1%).

2. Sugarcane

A new strain of sugarcane mosaic virus has spread into the important commercial varieties. In some fields almost 100% of the plants are infected. Chemical control studies conducted on insect vectors of sugarcane mosaic virus and of preferred wild host plants of some of the more common vectors indicate that seven applications of diazinon and TDE both alone and with a spreader-sticker, reduced vector populations by 43 to 100% but had no effect on mosaic spread. Four applications of 2 pounds DDT plus 4 pounds toxaphene with a spreader-sticker and of demeton gave 81

and 84% reductions in vector populations, respectively, and reduced mosaic infection by 22%. The DDT-toxaphene spray alone gave a 91% reduction in vector populations and a 78% reduction in mosaic infection. The rusty plum aphid, Hysteroneura setariae, the only vector species for which sugarcane is a natural host, was controlled 100% with all insecticides.

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AREA NO. 10 ORNAMENTAL, SHRUB, FLOWER, AND TURF INSECTS

RPA 906 - CULTURE AND PROTECTION OF ORNAMENTALS AND TURF

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968
New Jersey	3.8
New York	2.0
Maryland (Beltsville)	.7
Washington	1.0
Total	7.5

Problems and Objectives

Annual losses to outdoor ornamental plants by aphids, borers, cutworms, mealybugs, scales, spider mites, and other pests are estimated to be nearly three-quarters of a billion dollars. These losses result from feeding damage and/or diseases which are insect transmitted. In addition, these insects cause an estimated \$80 million damage to horticultural specialty crops grown commercially in greenhouses and nurseries. Insect and mite resistance to pesticides has resulted in increased damage in recent years. The practice of florists depending on a few establishments for cuttings of young plants, such as chrysanthemums, carnations, geraniums, and roses, often results in the inadvertent distribution of pests to many areas and excessive losses before control measures can be applied.

Major objectives of the research on methods to control ornamental and turf insects are to utilize:

1. Information on the biology, ecology, and population dynamics in developing control practices.
2. New and selective insecticides.
3. Resistant varieties.
4. New approaches such as sterility, attractants, repellents, and biotic agents.

Progress - USDA and Cooperative Programs

A. Basic Biology, Physiology and Nutrition (1.3 SMY)

1. Ornamentals

a. Greenhouse whitefly. At Beltsville, Md., the developmental life cycle from egg to adult eclosion in growth chambers was determined to be 36 days at 60° F, 23 days at 80° F, and 31 days under greenhouse conditions of 65° night and 85° day temperatures. Eggs hatched in 6 days at 80° F, 12 days at 60° F in growth chambers, and 10-11 days in the greenhouse. Young nymphs and newly emerged adults are more susceptible than other stages to insecticides.

b. Flower thrips. The migration peaks occurred at Ft. Valley, Ga., from May 24 to 29; at Beltsville, Md., from June 22 to July 3; and at Farmingdale, N. Y., during the second week of July. Populations were highest at Ft. Valley, intermediate at Beltsville, and lowest at Farmingdale.

c. Miscellaneous insect pests. At Beltsville, Md., new insect pest distribution and new host damage were recorded as follows:

A whitefly, probably A. spiraeoides, known previously only from Florida, was abundant on bearded iris in Washington, D.C.

The strawberry whitefly, Trialeurodes packardii, considered a minor pest of strawberry and other hosts, severely damaged mockorange and honeysuckle in Elkland, Pa.

The apple maggot, Rhagoletis pomonella, reared from berries of Pyracantha lalandi from Mickleton, N.J., has been previously found damaging this host only from Texas.

The Florida flower thrips, Frankliniella bispinosa, known previously only from Florida and coastal areas in Alabama and South Carolina, was collected on sticky cards at Fort Valley, Ga.

At the University of Georgia under a research grant, bagworm eggs collected in November 1966 and stored at 8° C for various periods up to 11 months and 9 days continued to hatch but none hatched after longer storage. Eggs collected in March 1967 failed to hatch after a cold storage of more than 7 months.

Number of bags on 2 trees increased from 34 in 1966 to 1542 in 1967 or at a ratio of 45:1. An increase of 20 to 1 was found in sampling a larger infestation. Current year's bags contained dead larvae or pupae due to parasites or disease so the expected increase in 1968 would be much less than 20 to 1.

2. Lawns and Turf

a. Japanese beetle. In studies at Moorestown, N.J., 14 to 37% of Japanese beetle eggs hatched and larvae reached the pupal stage after incubation in grass-seeded plastic pans containing soil mixtures of top soil, peat, sand, and vermiculite. Mature 3rd-instar larvae were obtained in 82-100 days. Continuing progress has been made at Moorestown, using a larval diet based on an acetone powder of grass. When 3rd-instar larvae were allowed to feed on the diet, 61% prepupated and 39% became adults. Incubation of Japanese beetle ova at Moorestown, in a 2:1 mixture of Michigan peat and sassafras sandy loam soil containing 1% redtop-white clover for food yielded 59% 3rd-instars and 16% adults. This media is currently being used in a large-scale rearing program designed to furnish 25 to 30,000 3rd-instar larvae.

b. European chafer. Adult European chafers in Geneva, N.Y., emerged from soil when light intensities at dusk had dropped to 30 foot candles and settled in trees when intensities had fallen to 1.25-1.0 foot candles.

B. Insecticidal and Cultural Control (2.3 SMY)

1. Ornamentals

a. Aphids and mites. At Sumner, Wash., 1% lindane dust at 4 pounds active ingredients per acre in the furrow effectively controlled tulip

bulb aphid on stored iris bulbs 74 days after harvest. Complete kill of tulip bulb aphids on stored iris bulbs was obtained in 1 week in a growth retarding chamber when resin strips (10 x 2½ in) containing 20% dichlorvos were distributed at the rate of 1 or 2 per 1000 cubic feet. Tulip bulb aphids were also controlled on dormant iris bulbs by exposure to 95° F for 10 days or 100° F for 7 hours without damage to the iris bulbs.

In decreasing order of effectiveness Union Carbide UC 21149 applied as Temik, disulfoton, phorate, Niagara NIA-10242, and oxydemetonmethyl controlled crescent marked lily aphids on greenhouse-forced Easter lilies when applied at 4 pounds active ingredient per acre of granules to the soil surface of potted lily plants 3 to 5 inches high.

At Farmingdale, N.Y., a species of Rhizoglyphus mite was found feeding on bruised Easter lily bulb scales and on roots where bulb rots were present. Extensive feeding also occurred on apparently healthy roots and bulbs. Root tip injury was reduced by soil applications of demeton and UC-21149 applied as Temik at rates of 20 lb active/acre, but 7 other materials were less effective.

At Farmingdale, UC-21149 applied to the soil of potted rose plants killed aphids and spider mites for 2 months or longer.

Dimethoate, carbofuran, phorate, and UC-21149 applied to the soil were tolerated by poinsettia cultivars but only UC-21149 controlled a recently recognized highly resistant strain of spider mites. Azodrin was nontoxic to foliage but disrupted color development in the bracts. Formetanate and Dowco-213 caused no injury to foliage and applied as foliage sprays controlled the new resistant mites.

b. Narcissus bulb fly. At Sumner, Wash., 10% phorate granules at 2 pounds active ingredient per acre in furrow application was as effective in controlling narcissus bulb fly as the standard 10 minute heptachlor soak.

c. Vegetable leafminer. At Beltsville, Md., dichlorvos impregnated corn cobs released vapors which were toxic to vegetable leafminer adults for 12 days. Residues in plants exposed to vapors were toxic to adults for 2 hours after removal but not for 24 hours. Leafminer eggs and larvae were more readily killed by dichlorvos in resistant chrysanthemum cultivars than in susceptible ones.

d. Omnivorous leaf roller. At Beltsville, Md., methomyl and Azodrin effectively controlled omnivorous leaf roller larvae on greenhouse roses. Slightly less effective but equal to Zectran were Matacil, trichlorfon, and Bacillus thuringiensis. Of 9 insecticides tested Zectran, carbaryl, Matacil, Azodrin, and Gardona were still highly toxic 3 and 7 days after treatment.

e. Scale insects. At Beltsville, Md., carbofuran, Bidrin, and azinphos-methyl gave effective control of 2nd- and 3rd-instar Florida wax scale. Summer oil-ethion mixture also gave good control but caused defoliation.

At Beltsville, Md., applications of dimethoate or ethion-summer oil sprays in August against nearly mature scales or in early spring against younger scales gave effective control of tulip tree scale.

2. Lawns and Turf

a. Japanese beetle. In laboratory tests at Moorestown, N.J., azinphos-methyl, carbaryl-Pinogel, dimethoate, naled, carbaryl, and trichlorfon gave 90-100% kill of Japanese beetle adults with a dosage of less than 1 mg. In field-weathering tests, carbaryl at 8 oz/acre was the most persistent on foliage, giving 90-100% control for 10 days after application.

b. European chafer. Applications of several dosage levels of finely divided carbaryl and DDT dust were made in surrogate transportation vehicles in Geneva, N.Y. DDT and carbaryl at 1 g/1000 ft³ were deemed most practical. Both materials were toxic to both species within 2 hours and DDT caused the deaths of all Japanese beetle and European chafer adults in 24 and 48+ hours, respectively, after treatment. Carbaryl was also effective against adults of both species, but required longer than 48 hours for complete kill.

A number of materials were studied at Geneva, N.Y., in an effort to find a substitute for chlorinated hydrocarbon insecticides for grub control. Two of these experimental materials satisfactorily controlled first-instar European chafer larvae for 9-12 weeks. Control was not satisfactory with either material after 15 months.

C. Insecticide Sterility and Persistence (.1 SMY)

1. Ornamentals. At Beltsville, Md., dichlorvos impregnated on granules of corn cobs or vermiculite in 1962 remained toxic to insects for at least 6 years. Chemical analysis indicated that 2.2 to 9% of the dichlorvos was retained at the end of this period.

2. Lawns and Turf. Residues of chlordane and DDT from Japanese beetle control soil treatments applied in 1948 at Moorestown, N.J., were determined by gas chromatography. No residues of chlordane were detected from plots treated with 11 or 19 lb/acre. Where DDT was applied at 25 and 60 lb/acre, however, residues of 6.6 and 12.7 ppm were obtained. These amounts are about 1/4 and 1/3, respectively, of the amounts determined by analysis 2 years after application.

D. Biological Control (1.0 SMY)

1. Lawns and Turf.

a. Japanese beetle. Spores of Bacillus popilliae produced on artificial medium by the Northern Utilization laboratory at Peoria, Illinois, were fed to Japanese beetle larvae in tests at Moorestown, N.J. When fed a dose containing 2.8×10^5 spores obtained from diseased grubs, 62% of the test larvae became infected. No larvae became infected when fed a similar dose of spores produced on artificial medium.

E. Insect Sterility, Attractants, and Other New Approaches to Control
(2.5 SMY)

1. Ornamentals

a. Bagworm. In studies under a grant to the University of Georgia, the pheromone produced by female bagworm moths was attractive to males in field tests. Males were trapped in a pheromone baited trap in preference to unbaited traps.

2. Lawns and Turf.

a. Japanese beetle. In replicated field tests conducted by personnel of the Moorestown, N.J., laboratory at Lumpkin County, Ga., with 88 candidate lures, ENT-14189 + eugenol, 9:1, captured 2.5 times as many beetles as the standard (phenylethyl butyrate + eugenol, 9:1). In field tests carried out on Nantucket Island, Mass., by personnel of the Moorestown station, traps baited with phenylethyl butyrate mixed with an equal amount of Solvasol 35 caught 35 beetles/trap as compared to 18 beetles/trap captured with phenylethyl butyrate + eugenol (9:1) the standard bait.

In tests at Moorestown, N.J., ova were collected from females confined in 125 ft³ cages with different ratios of tepa-sterilized to fertile male beetles. The percent sterility of these ova generally tended to reflect the proportional numbers of sterile to fertile males in the cages, thus indicating equal mating competitiveness of the treated males.

b. European chafer. Of 235 lures screened at Geneva, N.Y., for attractancy to the European chafer, ENT-26,035 and ENT-33,181, were 3.0 and 1.8 times more attractive, respectively, than the standard, butyl sorbate.

The attractancy of virgin female European chafer adults was demonstrated for the first time at Geneva, N.Y. In preliminary tests, the virgin females were slightly more attractive than the standard bait when exposed in black chafer bucket traps.

F. Evaluation of Equipment for Insect Detection and Control (0.1 SMY)

1. Lawns and Turf. In tests conducted at Geneva, N.Y., baited red plastic survey traps captured 1.2 - 15 times more European chafers than did baited black standard traps.

G. Insect Vectors of Diseases (.2 SMY)

1. Ornamentals. In tests at Beltsville, Md., the green peach aphid transmitted tomato aspermy virus (TAV) and alfalfa mosaic virus (AMV) to Tephrosia and Vernonia and cucumber mosaic virus (CMV) to Vernonia and Limnanthes. These viruses were also transmitted mechanically to the same hosts. CMV and bean yellow mosaic virus (BYMV) were transmitted mechanically to kenaf and Limnanthes. All of these viruses produced systemic infections in their respective hosts. Only local infection followed mechanical inoculations of TAV to kenaf and Limnanthes. No infections resulted from aphid or mechanical inoculations of potato virus Y (PVY) in any of the four hosts or of BYMV in kenaf, Limnanthes or Vernonia. The results indicate that virus infection by the common and widespread viruses CMV, TAV, AMV, and BYMV are a greater threat to Tephrosia and Vernonia crops than to kenaf or Limnanthes. None of these crops appear susceptible to PVY. Kenaf is a new source of paper pulp; Tephrosia contains rotenone; Limnanthes and Vernonia seed contain oils used in plasticizers.

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AREA NO. 11 - LIVESTOCK INSECTS AND OTHER ARTHROPODS

USDA and Cooperative Programs

Location of Intramural Work	Scientist Man-Years F.Y. 1968		Total
	210	701	
Maryland (Beltsville)	2.0	0.2	2.2
California	2.8		2.8
Colorado	1.0		1.0
Florida	2.7		2.7
Louisiana	4.0		4.0
Mississippi	1.4		1.4
Nebraska	2.0		2.0
Oregon	2.2		2.2
Texas	15.9	3.4	19.3
	<u>34.0</u>	<u>3.6</u>	<u>37.6</u>

Intramural program is supplemented by extramural support representing (a) 0.7 SMY's at State Agricultural Experiment Stations 1/, (b) 0.3 SMY at other U.S. institutions 2/, and (c) PL-480 funds in 1 country representing 55,306 U.S. dollars equivalent.

1/ RPA 210 -- 0.7

2/ RPA 210 -- 0.3

Problems and Objectives

Insects and other arthropods attacking various livestock classes cause estimated annual losses of \$878 million. Losses are direct in the reduction in weight gains, milk and egg production, and depressed value of products, such as meat, hides, and wool. Indirect losses are through diseases spread by the insects. Practical, but not fully adequate, control methods have been developed for many livestock pests, but for some pests, no adequate control methods have been found. Insecticide residues in livestock and livestock products and byproducts and the development of insect resistance to insecticides are continuing problems involved in use of conventional insecticides.

The objectives of the research are to develop and evaluate new or improved methods to control livestock insects through:

1. Research on their biology, ecology, and habits.
2. Insecticides that will not create residue problems.
3. Alternate methods such as attractants, repellents, and sterilization techniques.
4. Bioenvironmental management practices.
5. Parasites, predators, and pathogens.

Progress - USDA and Cooperative Programs

RPA 210 - CONTROL OF INSECT PESTS OF LIVESTOCK AND POULTRY

A. Basic Biology, Physiology, and Nutrition (7.5 SMY)

1. Poultry

a. Mites - Research on biology of the turkey mite, Neoschongastia americana continues under a cooperative agreement with the University of Georgia. The mites appear in the vicinity of Athens, Ga. in late May and remain until about mid-September. Heaviest infestations occur in pine, as opposed to hardwood shade. A lesion begun by one mite attracts other mites. The first mite is in the center of the lesion, the latter mites attach in a ring around the first mite. About 2 weeks is required for engorgement, after which the mites drop off. Mites appear in greater numbers following a rain. Red soils support more mites than gray soils. As a lesion becomes old it becomes incapable of supporting mites; eventually the lesion dries up, falls from the bird and leaves no scar. The mites showed no preference as to age or sex of the turkeys.

At Kerrville, Tex. attempts to establish a laboratory colony of the turkey chigger, Neoschongastia americana were unsuccessful.

b. Lesser mealworm - A cooperative agreement supported by the Entomology and Market Quality Research Divisions was initiated with the University of Maryland in the biology, ecology, and control of the lesser mealworm, Alphitobius diaperinus, suspected vector of Marek's disease or avian

leukosis. Two laboratory colonies of the insect were established and a satisfactory diet was developed for rearing larvae and maintaining adults. Temperature was found to be the primary environmental factor controlling the incubation period and percent hatch of the eggs. The majority of the pupae recovered were in the upper inch of soil, but pupation occurred also in litter without soil in laboratory jars. The sexes can be separated in pupal and adult stages through characters found in the research. A survey of 54 broiler houses on the Del-Mar-Va peninsula showed that 38.8% had infestations of the insect. When a population of adult beetles was placed in litter of an unheated henhouse (except for infrared brooder bulbs), larvae appeared in a few weeks. Apparently the lesser mealworm reproduces without difficulty the year around in Maryland. Leukosis is now so prevalent in Maryland broiler houses that a proposed study comparing incidence of the disease with presence of the insect has been cancelled.

2. Beef

a. Horse flies and deer flies - Research was concluded under a grant to the University of Wyoming on the biology and behavior of tabanid flies. Additional data was received on the behavior of Tabanus reinwardtii and Chrysops fulvaster. For males of the former, morning activity period averaged 88 minutes; for those of the latter, 93 minutes. The inactive periods for T. reinwardtii were spent resting head downward near the base of willow shoots along streams. During their activity period, the males of reinwardtii swarmed around a metal drainage tube or metal drums in full sunlight; those of fulvaster congregated in groups at various locations along a stream.

3. Beef and Dairy

a. Mosquitoes - At Lake Charles, La. studies were continued on the biology of salt-marsh and rice field mosquitoes. In tests with screened and un-screened enclosures, small numbers of salt-marsh mosquito larvae still appeared in two plots 3 years after screening. Egg hatches derived from early spring and late fall females of Aedes sollicitans, A. taeniorhynchus, Psorophora confinnis, and P. ferox showed a varied hatching response. Certain females produced egg batches in which some or all eggs were in diapause. The trait was most pronounced in fall batches as opposed to spring batches. It was very evident in P. confinnis, less so in A. taeniorhynchus, and present only to a minor degree in P. ferox and A. sollicitans. Eighty-eight water samples analyzed from tree holes above or adjacent to the ground that were producing mosquito larvae, showed that Orthopodomyia signifera, Toxorhynchites rutilus septentrionalis, Anopheles barberi and the non-biting midge Corethrella appendiculatus were generally restricted to habitats characterized by high pH, very high levels of potassium, and high levels of carbonates and bicarbonates. Aedes triseriatus were usually found in habitats with chemical characteristics similar to but with lower concentrations than for these other

species. Soil samples from 30 breeding sites that produced A. sollicitans, A. taeniorhynchus, and/or P. confinnis showed little or no difference in pH, carbonates, bicarbonates, or sulfates. Nineteen oviposition preference tests showed a relationship to concentrations of inorganic salts.

At Corvallis, Oreg., studies continued on species composition and biology of the Culicoides gnat fauna of Benton County. A total of 17 species, of which 8 may be undescribed, were identified in the 1966 collections.

Indications were found at Corvallis, Oreg. that Aedes dorsalis may be a facultative ecological homologue of Aedes melanimon. Mosquito larvae collected near Chemult, Oreg. appeared to fit the description of Aedes communis nevadensis rather than typical A. communis communis. Orthopodomyia californica was found in Oregon for the first time. Culex tarsalis reared at 25° C were stronger and more fertile than those reared at 30°C.

b. Horn fly - Under a cooperative agreement at Mississippi State at State College, Miss. field studies on the incidence of diapause in the horn fly (with field-caged dung) indicated the earliest date for diapause was September 15 and the latest was November 17 in 1967. Flies developing after November 17 did not survive the winter. Spring emergence was first noted in March. Emergence in the spring occurred when the soil temperature of 60°F was recorded at a depth of 1 inch.

At Kerrville, Tex. no difference in feeding activity rate was noted between virgin females, mated females, and male horn flies in "bitometer" tests. Tests indicated that female horn flies mate only once, male horn flies begin to mate when 2 days old (36% mated), increase their mating activity on the 3rd and 4th days (60% and 82% mating, respectively). Photoperiod is apparently not important in the development of colony horn flies at a temperature of 27°C. Growth and metamorphosis occur at 10°C to 32°C, but adult flies do not oviposit unless transferred to a temperature of 30°C. Progeny of these adults developed without interruption when returned to lower temperatures. The cow manure diet was superior to a fish meal diet at 10°, 16°, and 22°, but equal to the fish meal diet at 27° and 32°C. Pupae obtained by rearing at lower temperatures were smaller than those reared at higher temperatures. Activity tests also indicated that adult horn flies consume 4.5 to 13.2/μl of blood per fly per day. In a qualitative and quantitative investigation of the fatty acids present in laboratory-reared horn fly pupae, tentative identifications were made of 15 acids.

c. Stable fly - At Kerrville, Tex. an apparatus (termed the "aktagraph") was designed and constructed with the cooperation of the Agricultural Engineering Research Division to record stable fly activity. First tests show a higher level of activity in light than in the dark for both males and females, and males are more active than females regardless of light

conditions. Flies fed at 0900 were less active than flies fed at 1400, indicating the possible presence of an endogenous activity rhythm. In both sexes there appears to be a peak of flight activity at about 16-18 hours after feeding, followed by a gradual decrease in activity. However, there was no difference in the average number of flight per fly per hour in the apparatus over the range of 1 to 10 male flies.

At Lincoln, Neb. freezing temperatures were lethal to exposed adult stable flies, but after freezing weather, stable fly larvae in various development stages and pupae can be found that will complete development at higher temperatures, and an extended period of freezing weather is needed to bring complete relief from stable flies. In the vicinity of Lincoln, no viable pupae could be found after January 4, but live adults were found as early as March 8th the following spring.

d. Horse flies - At Stoneville, Miss. horse flies were collected landing and feeding on 2 steers tethered 300 feet apart for a 3-week period. A total of 1406 tabanids were collected as soon as they landed on an animal without waiting to see if they would feed. Another 495 were collected after they fed. Tabanus abdominalis were primarily active from 0430 to 1230; T. subsimilis reached its peak between 0930 and 1030. Eight other species were also collected. Statistical analysis is underway with the hope that time of day and other environmental factors can be correlated with the activity periods of horse flies.

Under a grant at the University of Utah, the most promising survey method for adults in marsh lands along the eastern shore of Great Salt Lake was trapping by means of carbon dioxide-baited adhesive traps and carbon dioxide-baited plastic cone traps. Surveys were also continued in 2 areas using counts of pupal skins adhering to the surface of the soil after adult emergence, to locate emergence sites and determine fly abundance as associated with emergence factors. In impoundments where water is maintained at a constant or near constant level, the larvae are forced to migrate to the moist soil of adjacent, slightly elevated areas where pupation and emergence occurs.

4. Beef, Dairy, and Sheep

a. Screw-worm - Research continued at Mission, Tex. on the biology of the screw-worm fly. Over 8,000 sterile released flies were recovered from 7 liver-baited traps placed in a deep narrow canyon in Sonora, Mexico, apparently from releases made over the mountains at least 5 miles on either side, or up and down the canyon for at least 10 miles. In the laboratory, Mexico strain flies could not survive exposure at 0-5° F for 10 minutes, but 20% survived 10-15° F for 15 minutes. These males were capable of inseminating only 24% of available females as compared with 82% insemination by control males. Even a 10-minute exposure reduced the insemination rate to 50% of the available females. Increased

mortality following such exposures would be a factor in high-altitude releases during the winter months.

A critical age for achieving capacity to pupate was 84 to 94 hours after hatching, and the critical larval weight required was from 18 to 22 mg. Mating capability in males increased as a function of larval weight up to 60-69 mg. No differences in capabilities attributable to larval weight or adult size were noted among males from larvae weighing more than 60 mg.

Male puparia had larger diameters than female puparia within a single rearing group, but there were also differences of diameter between rearing groups. Dry whole egg in the rearing medium promoted maximum growth. Increasing the suckle concentration to 6% was beneficial in both starting and finishing media. Larvae reared on a new hydroponic medium were equivalent in size or even greater in size than those reared on the standard fresh ground meat and blood medium.

In the Mexico strains of flies, differences in mating frequency between wound-reared and meat-reared flies could not be attributed to environmental influences in the larval stage. After 5 generations, mating was as frequent in one strain as in the other, but about the 22nd generation, there was an increase in mating frequency with the Mexican substrain reared on wounds. Variations in the number of eggs per egg mass were smaller in the wound-reared flies than with either the Florida strain or the Mexican strain reared on meat.

The relationship between body weight or primiparous females and the number of eggs laid was linear. Maternal and not paternal age adversely influenced normal fertility. The typical life cycle of flies in January at Cotaxtla, Veracruz, Mexico was substantially the same as that recorded for the Mexican strain at Mission, Tex. However, fluctuating temperatures at Cotaxtla caused most mating to occur 9 days after emergence. While oviposition occurred over a wide range of temperatures, the preferred range was 91-93° F.

b. Grubs and bots - At Kerrville, Tex. a large number of larval Hypoderma bovis were manually expressed from the backs of cattle in the government-owned herd at Camp Stanley. The presence of this, the northern cattle grub, in native (unshipped) cattle is unusual. An attempt is being made to eradicate this infestation by hand-removal of all grubs periodically. The first 4 grubs (2nd instar larvae) were taken from these cattle on January 19, 1967, and 16 more (then 3rd instar larvae) were squeezed out February 24, 1967. The peak, 541 3rd instar and 7 2nd instar larvae were taken on March 28, 1967. By April 27th, the grubs were nearly gone and only 8 3rd instar larvae were taken. None were found on January 4, 1968, but 15 2nd instar and 6 3rd instar grubs were taken February 1. On March 7, 1968, 46 3rd instar grubs and only 2 2nd instar grubs were recovered, a great reduction over the March 1967 counts.

In a study at Kerrville, first instar larvae of cattle grubs were transplanted from the submucosal lining of cattle gullets to incisions made in shaved white mice and the incisions sutured or clamped. The larvae migrated from the implant site to the thoracic and abdominal cavities, causing many host deaths from traumatic lesions as they moved. Once situated in the two cavities, however, the larvae survived without growth for at least 286 days and no more mice died.

5. Cross Species

a. Mosquitoes - At Fresno, Calif. surveys indicated that the major problem species will probably be 3 Aedes, 4 Culex, and 1 Anopheles.

Under a grant to Louisiana State University 12 species of mosquitoes from 7 areas of salt marsh were identified. Total number of blood meals examined was 3,526. Of these, 80% had fed on beef cattle, 12% on rabbits, 5% on horses, and only 0.4% on humans. Very few mosquitoes had fed on more than one kind of host. In a companion study, 12 species of mosquitoes were identified from 2 swamp areas. Of a total of 84 blood meals identified, 60% were from horses, 15% from rabbits, 12% from beef cattle, and 6% from humans. No amphibian or reptile hosts were identified from mosquitoes of either salt-marsh or swamp areas.

b. Little house fly - Research was concluded on the little house fly at Corvallis, Oreg. Several years ago it was noted that a more efficient colony resulted from introducing larvae, as opposed to eggs, in the rearing medium. Rearing modifications since then made a re-evaluation advisable, and a 72% yield can now be obtained by seeding the medium with eggs. An average of 1451.8 adults was obtained from 72 grams of eggs.

c. House fly - At Corvallis, Oreg. research was concluded on the genetics of house flies. The inheritance of resistance was determined by crossing strains according to standard genetic procedures. Tests confirmed that resistance to pyrethrins was linked to hydrocarbon insecticide resistance in the kdr-0 strain.

The tin-R gene confers resistance to organotin insecticides and intensified resistance to other insecticides by reducing absorption of the insecticides. Piperonyl butoxide synergized DDT in a strain for which resistance depends upon conversion to polar metabolites; the DDT analogue Dimite synergized DDT in a strain for which resistance depends upon the enzyme DDT-dehydrochlorinase.

d. Sand flies - Research has continued under a grant to Virginia Polytechnic Institute on the biology of Culicoides gnats. Results from light trap studies indicated that the two major species were Culicoides sanguisuga and C. guttipennis. The former fed on all hosts in significantly higher numbers at dusk while the latter did not feed during daylight at all but fed equally at dusk and in darkness. Under these

experimental conditions, the site of the host--mammals and birds--is the major factor in attracting most of the collected species of Culicoides. Both species are now being reared in the laboratory.

e. Horse flies - Research continued under a grant at Louisiana State University on the determination of host animals of tabanids in certain areas of Louisiana. Eleven species were collected in 6 Louisiana parishes. Of 273 tabanids examined, 70% had fed on beef cattle, 25% on horses, 0.3% on deer, 0.3% on rabbits, and 4% had fed on 2 hosts, cattle and horses.

f. Ticks - Research continued at Kerrville, Tex. on the biology of ticks. In Amblyomma maculatum held between $27^{\circ} \pm 0.5^{\circ}$ C at 80% relative humidity, the length of photoperiod determined the time of ovipositional peak. The peak occurred immediately after onset of oviposition in ticks subjected to an 8-hour photoperiod. There was delayed oviposition when 15-minute, 12-hour, and 16-hour photoperiods were employed. At a given photoperiod, temperature and humidity were also important. With a photoperiod of 12 hours, constant temperature and constant relative humidity gave more efficient egg production than fluctuation temperature and humidity. There was no difference between males and females in length of nymphal quiescence, a term defined as the period between descent of the engorged nymph and the time of moulting to adult. However, the duration of nymphal quiescence in males of Amblyomma americanum was significantly shorter than in females and the reverse was true of Dermacentor variabilis.

Also at Kerrville, Anocentor nitens produced engorged females in 24 days after placement of the immature ticks on cattle in stalls. In similar studies with Dermacentor albipictus, engorged females were obtained in 22 days. Photoperiod studies were also conducted with this tick. At a constant temperature of 27° C and 80% relative humidity, engorged females laid eggs that hatched into larvae whose willingness to feed depended upon photoperiod to which the adults and eggs had been held. With an 8-hour photophase, the larvae attached and fed at 4 weeks of age, on both a cow and guinea pigs and continued willing to feed for 12 weeks. Larvae from the 12-hour photophase treatment began to attach at 10 weeks. Only after 12 weeks did larvae from the 16-hour photophase treatment begin to attach. A photophase longer than 4 hours does not break the larval quiescence as efficiently.

g. Sheep and goat lice - At Kerrville, Tex. survival of Angora goat lice (Bovicola limbata) in the laboratory on scrapings of Angora goat skin suggested that the species could be colonized in the laboratory. After some preliminary tests, a standardized diet was obtained and the lice have now been colonized for 6 generations. Females could be mated at a relatively early age. Examination of the seminal vesicles proved a satisfactory means of determining whether mating had occurred. At a constant 33° C in constant relative humidities of 61%, 68%, 72%, 77%, and 82%, better than 70% hatch of eggs was obtained. A peak of moulting from the first to the second instar occurred on the 6th day of nymphal life (range 5 to 9 days). Five days later a peak of moulting to the third nymphal

instar occurred (range 4 to 9 days). The peak of moulting from the third nymphal instar to adult occurred on the 6th day of the third instar in 2 lots of lice and between the 7th and 8th day in another lot. The males moulted as early as the 5th day of the nymphal instar and the females as late as the 14th day. Another goat louse, Bovicola crassipes, was also studied in the laboratory. The skin scraping diet proved adequate and it, too, has been colonized for 6 generations. A 75% or better hatch of eggs was obtained at 33° C in constant relative humidities of 61%, 68%, 72%, 77%, and 80%.

h. Imported fire ant - Cooperative agreements to study different phases of the biology of the imported fire ant were initiated with Mississippi State University, the University of Georgia, the University of Florida, and Louisiana State University

Research has continued under a PL-480 grant in Uruguay on biology of the imported fire ant. In mating, the males flew above the ground in a cone "circling" flight pattern; they left this cone to seize a flying new queen and returned to the cone after mating. Female and attendant male fell to the ground where actual mating took place. After mating, the queen departed on an extended flight which therefore became a migration or distribution flight. There has long been a question as to whether certain apterous females resembling queens could serve as queens. When 40 such females were placed in artificial nests with workers, all but one female oviposited and the workers cared for the eggs and ensuing larvae.

B. Insecticidal and Sanitation Control (8.6 SMY)

1. Poultry

a. Poultry mites - Research was initiated at Kerrville, Tex. on the control of turkey chiggers, *Neoschongastia americana*. Several insecticides showed promise in preliminary laboratory tests: Dursban, diazinon, dichlorvos, Geigy 13005, and carbaryl. In small plot tests, diazinon and ethion appeared promising and Dursban provided good control for 7 weeks after application. To allow time for healing of lesions on the birds, at least 4 weeks effectiveness is needed. Some research was also done with the northern fowl mite, Ornithonyssus sylviarum. Shell SD-8447 applied to poultry house litter as a 3% powder and to dust boxes as a dry 75% wetttable powder controlled the mite. Control was faster with the litter treatment than with the dust box treatment.

b. Poultry lice - Research continued at Kerrville, Tex. on control of poultry lice. Shell SD-8447 applied to poultry house litter as a 3% powder or to dust boxes as a dry 75% wetttable powder controlled lice but gave faster control applied to the litter than to the dust boxes. Dichlorvos-impregnated wing and leg bands gave good control. However, placement of the treated bands on the cages was just as good. Lice could also be controlled by treating only 50% of the birds when the birds were free on the litter.

2. Poultry and Dairy

a. House flies - At Gainesville, Fla., the search for new insecticides effective in controlling house flies in poultry establishments and dairy barns was continued. In laboratory tests, 18 candidate insecticides were equal to or more effective than the ronnel standard against susceptible and resistant house flies in spray tests. In sugar bait tests, 15 out of 73 tested were more effective than the trichlorfon standard. As residues on plywood panels, 8 of 43 compounds tested were effective for 24 or more weeks against both susceptible and resistant strains of flies. In wind-tunnel tests, the F₁₉₉ generation of the Cradson-P colony (resistant) showed more than 12-fold resistance to parathion, more than 23- to 44-fold resistance to malathion, diazinon, and ronnel.

DDT resistance could not be measured and is virtually complete. This colony also showed at least 2-fold cross-resistance to all of the new materials tested in sugar bait tests except naled.

In field tests near Gainesville, Fla., residues of candidate insecticides were tested against natural populations of house flies. Dimethoate residues were effective for at least 7 days and usually 9 to 11 days. Dimethoate, ENT-25841, and ENT-27334 remained effective as larvicides in manure under caged poultry for about a week, giving 75% mortality or more. Sixteen other materials tested were ineffective.

3. Sheep

a. Sheep nose bot - Research was terminated under a grant to the University of Kentucky on basic studies on the mode of action of systemic insecticides applied to sheep for sheep nose bot control. Sixteen different anti-helminthic compounds were ineffective. Of 6 different organic phosphate insecticides tested, only fenthion and famphur gave 100% control of the three instars of the sheep nose bot, Oestrus ovis, at 3 and 10 mg/kg daily, respectively. A coumaphos-fenthion water treatment controlled first instar larvae and gave considerable control of second instar larvae, but permitted survival of third instar larvae.

4. Beef and Dairy

a. Mosquitoes - Field tests near Corvallis, Oreg. with commercially-formulated emulsions of Abate, Dursban, and fenthion involved natural populations of snow-water Aedes (fitchii, hexodontus, cinereus, communis, and increpitus). The temperature fell below freezing at night and heavy rains fell after treatment. Control in three plots treated with 0.05 lb of fenthion per acre averaged 99%. In 2 plots treated with Dursban at 0.025 lb/acre control averaged 99.5%. Control was 99% and 100% in 2 plots treated with Abate at 0.025 lb/acre, but only 10% in a third plot and no control was obtained in a fourth plot. The relative tolerance of "non-diapausing" larvae of Mansonia perturbans to insecticides was

checked. Tolerance to DDT and malathion was essentially the same as that found previously with "diapausing" larvae of this species.

Seventy-two compounds were tested as repellents in spot tests on cattle at Corvallis, Oreg. against Aedes aegypti in the laboratory and in the field against A. dorsalis. Only three of the compounds provided repellency: ENT-28740, ENT-33516, and ENT-33518. They gave excellent repellency for 2 days at 1,000 mg/ft² but little or none at lower dosages.

Studies continued at Corvallis on the attractiveness of log pond waters to ovipositing Culex pipiens quinquefasciatus. The previously-tested chloroform extracts proved to be attractive even after evaporation to dryness; however, when the amount of dry extract increased above the equivalent of 20 microliters of chloroform extract, the attractiveness decreased. At the equivalent of 80 μ l the attractiveness was the same as distilled water and at the equivalent of 160 μ l attractiveness was less than distilled water.

Under a contract with the University of California, good mosquito control was obtained in rice fields and pastures treated with low-volume sprays by helicopter in the Colusa area, including the Colusa Wildlife Refuge. Dursban was applied at 0.05, 0.025, and 0.0125 lb/acre and fenthion at 0.1 lb/acre. The swath width was 250 feet and a volume of 6.1 fl oz/acre was applied. So far, during studies of possible effects on bees and wildlife, no damage has been noted. In cooperation with the U.S. Navy and the California Bureau of Vector Control, studies were made in Kern County to determine whether low-volume applications could be successfully made at altitudes of 1,000 and 2,000 feet. Both larvae and adult mosquitoes were killed from altitudes up to 2,000 feet over swaths extending to 1/2 mile wide at dosage rates less than 8 fluid ounces per acre.

b. Horn fly - At Kerrville, Tex. a technique for determining susceptibility of horn flies to insecticide treated cloths was developed. LC-50 values of 0.0003%, 0.0004%, 0.004%, and 0.004% were obtained for coumaphos, Ciodrin, methoxychlor, and ronnel, respectively. The following concentrations and insecticides gave adequate horn fly control on dairy cattle, using the ultra-low volume automatic sprayer on a regular daily basis: carbaryl, 1%; malathion, 1%; methoxychlor, 1%; Ciodrin, 0.5%; coumaphos, 0.25%; and Compound 4072, 0.1%. The estimated cost of certain commercial insecticides at recommended rates was from 4 to 50 times more expensive than ultra-low volume applications of the same insecticides. In field tests, 1 ml of 0.5% Ciodrin per animal, applied twice daily provided adequate protection from horn flies in a dairy herd.

Experimental compound ENT-25682 in feed of cattle at 5 mg/kg daily completely eliminated horn fly larvae and partially eliminated house fly larvae as well from the manure of cattle so treated. Menazon at the same dosage was ineffective. Range cubes containing Bacillus thuringiensis exotoxin were shown in laboratory tests to be capable of

inhibiting the development of horn fly larvae when 1 pound of treated cubes was consumed per animal per day. In field tests, pastured cattle were fed 1 lb/animal daily for a treatment period of about 3 weeks followed by 2 lb/animal daily for a period of 4 weeks. Though 100% inhibition of horn fly breeding was not obtained, there was a reduction in numbers of horn fly adults on the cattle.

c. Stable fly - At Kerrville, Tex. 67 compounds were evaluated in spot-tests on cattle as toxicants and repellents for stable flies. Four compounds, ENT-27339, ENT-27453, ENT-27465, and ENT-27474, were Class IV toxicants at 0.5%; ENT-27474 was also Class IV as a repellent at 5% and 0.5%. One lot of ENT-27339 gave such fast knockdown that the flies were not able to feed on the cattle. In field tests, naled and dichlorvos applied as mists with two types of mist blowers were effective in reducing the numbers of flies for 1 to 2 days after application, but not as long as a week. Ciodrin (0.5%) at 1 to 2 qt/head and methoxychlor (0.5%) at 1 qt/head reduced stable fly numbers from 20-25/leg to 10/leg or less for 4 days, but not for 7 days. Ronnel (0.5%) at 1 qt/head decreased stable flies from 20-25/leg to 5/leg 1 day after treatment but gave no decrease 4 days after treatment. Shell SD-8447 (0.25%) at 1/2 to 1 qt/head did not reduce the numbers of flies. Diazinon, dimethoate, fenthion, and Shell SD-8447 were effective in controlling stable flies when applied to resting surfaces; only diazinon failed to give control for more than 3 weeks.

d. Horse flies - A field test indicated that a combination of carbaryl (1%), pyrethrins (0.1%), and piperonyl butoxide (1%) was effective for at least 24 hours against horse flies. In this field test, 75 Guernsey heifers were sprayed; counts averaged 28 horse flies per head. Twenty-four hours after spraying, only 8 flies per head were observed, with an average of 1 per head feeding.

e. House fly - At Lincoln, Neb. diazinon, dimethoate, and fenthion applied as residual sprays to stable fly resting places reduced house fly numbers for 2 weeks. The fenthion residue was the most effective. Dichlorvos mist sprays applied in buildings infested with house flies reduced the number of flies for several days and naled for a week.

f. Cattle grubs - At Corvallis, Oreg. research was completed on control of cattle grubs. Inadequate control was obtained with systemic insecticide dosages below the recommended amounts with famphur, Ruelene, and fenthion. Imidan pour-on treatments at 7.1 and 4.7 g/head provided 100% control of grubs. Stauffer R-3828 at 20 g/head resulted in 93% control. Ruelene gave 100% control and coumaphos gave 99% in one test and 100% in another.

g. Boophilus ticks - Kerrville, Tex. entomologists continued to evaluate toxicants for control of Boophilus spp. at Nuevo Laredo, Mexico. These ticks have been eradicated from the United States several times but remain

a threat with cattle being shipped across the Mexican border. Of 75 insecticides screened against Boophilus, only 2 compounds rated Class 4, ENT-27562 and ENT-27399. Experimental synergists ENT-28708 and ENT-28709 did not increase the effectiveness of carbaryl against Boophilus when used in the form of emulsions and gave only slight indications of synergism when wettable powder suspensions were used.

In field tests at Nuevo Laredo, Dursban in a dipping vat was highly effective at concentrations ranging from 0.006% to 0.05%, but did not consistently give 100% control at any of these concentrations, even in freshly charged vats. Complete mortality is needed for border quarantine work. In an un-recharged vat, 0.012% Dursban gave 99% control of Boophilus annulatus, but only 94% control of B. microplus. In a vat with moderate use, aged 7 weeks, 0.185% Dursban gave 100% effectiveness against annulatus and 99.98% control of microplus. In a similar vat aged 13 weeks, 0.0134% Dursban gave only 98% control of annulatus, but was still 99.9% effective against microplus. A 0.5% toxaphene spray afforded 99+% control of both species; 0.05% dioxathion spray gave less control of microplus than annulatus, but the reverse was true of 0.03% lindane spray. Sprays of ENT-27043, ENT-27314, and ENT-27019 afforded greater than 99% control of both species of ticks. Both 0.06% Imidan spray and 0.1% Azodrin spray gave more than 99% control of annulatus and less than 99% control of microplus.

h. Miscellaneous flies - At Stoneville, Miss. daily applications of synergized pyrethrins in a water-based spray did not protect beef cattle adequately from stable flies, horse flies, and horn flies and weight gains were less than in unsprayed control animals. However, a group of steers pen-sprayed every 3 weeks with 0.5% methoxychlor averaged higher gains than pasture mates that were not sprayed. Only 0.25% of Shell SD-8447 in the ultra-low volume automatic sprayer was needed to obtain horn fly control.

5. Beef, Dairy, and Sheep

a. Screw-worm - Research continued at Mission, Tex. to develop better screw-worm larvicides. Of 19 promising materials tested, four were effective after 24 hours at a concentration of 1 ppm (ENT-numbers 27474, 27481, 27483, and 27490). Dursban applied as a spray at concentrations of 0.025, 0.05, and 0.1% were not as effective for the control of 3- and 4-day old screw-worm larvae as a standard application of 0.25% coumaphos. New and 1-year old 5% coumaphos powders killed all larvae in wounds of sheep in less than 3 hours.

b. Grubs and bots - Research continued at Kerrville, Tex. to find satisfactory control measures for bots and grubs of livestock. In the screening program, 21 of 44 selected compounds evaluated were systemically active when tested in guinea pigs. Of 77 systemic treatments administered to cattle for control of cattle grubs, 14 gave greater than 90% control. Effective were sprays containing 0.05% fenthion, 0.25% Imidan

(1 or 2 sprayings) and 0.25% menazon. As pour-on treatments, the following were effective: 2%, 3%, and 4% Imidan pour-ons at 1 fluid ounce per hundredweight (CWT) and 4% Imidan at 1/2 fl oz/CWT. Effective in capsule were ENT-27072 and ENT-27330, both at 10 mg/kg. Effective when administered in feed for 10 days were menazon at 1 mg/kg daily, and ENT-25682 at 5 mg/kg daily. When calves were treated with recommended pour-on treatments in May, June, July, and August, the time of treatment did not prove to be an important factor in control, but a number of grubs were found in the backs of certain individual cattle and thus the overall average control ranged from a low of 81.2% in July to a high of 90.2% in May, with the other months giving intermediate results.

6. Cross Species

a. House fly - At the University of Georgia, research was initiated under a grant on the synthesis of fluorescent insecticides and their reaction with enzyme systems. Three new unique carbamates for in vivo tracer studies having significant insecticidal activity were prepared and characterized. They contained iron, pi-bonded on a quasi-aromatic system and hence are stable and suitable for use with electron microscopy. Unfortunately these carbamates have no topical toxicity when applied to house flies, apparently because they are ionic in character. Similar molecules with greater lipophilic character will be synthesized. Their non-fluorescence is apparently a function of quenching by the iron atom. A new fluorescent phosphonate was synthesized and is being tested for insecticidal activity.

b. Ticks - Research continued at Kerrville, Tex. on control of ticks of livestock. Of 21 compounds screened for effectiveness against Anocentor nitens, 13 showed promise. LC-50's were determined for 37 compounds, based on inability of exposed ticks to lay viable eggs as a mortality concept (Reproductive Index). Of 59 insecticides screened for control of Dermacentor albipictus, 4 were placed in Class 4 (effective at 0.01%). In a field test, control was obtained with 0.5% toxaphene, 0.25% Imidan, 0.25% bromophos-ethyl, 0.25% Shell SD-8447, and 0.05% Dursban. In laboratory tests with Amblyomma americanum, LC-50's determined for malathion, toxaphene, DDT, fenthion, and dioxathion were 0.1-0.2, 0.03-0.1, 0.001-0.003, 0.0006-0.001, and 0.00006-0.0001%, respectively. In field tests on cattle, coumaphos was as effective as the toxaphene standard. A pour-on of 4% coumaphos was effective but pour-ons of 8% Ruelene and trichlorfon exhibited only low activity against this tick. Under a PL-480 to the Ministry of Agriculture at Beit Dagan, Israel, the life cycle of Rhipicephalus sanguineus has been worked out using a gerbil and a rabbit as hosts. This tick proved more vigorous and vital than R. secundus. Pyrethrum extract still had acaricidal properties at concentrations as low as 0.0008% and 0.00165% for Hyalomma excavatum larvae and nymphs, respectively, whereas R. sanguineus nymphs were found to be 1.5 to 2 times more sensitive to pyrethrum than excavatum nymphs. Adult

female gerbils attracted more excavatum larvae and nymphs than adult male gerbils. Similarly, engorged larvae and nymphs were found to drop from the gerbil at a definite circadian cycle; most of the nymphs were collected between 2200 and 0400 hrs.

c. Imported fire ant - At Gainesville, Fla. the addition of water glass (sodium silicate) to the attractant-mirex bait formulation for the imported fire ant increased the amount of oil that could be incorporated in the bait and also increased the retention of the oil. Baits treated in this manner are more effective and longer-lasting.

C. Biological Control (6.8 SMY)

1. Poultry

a. Poultry lice - At Kerrville, Tex. research was conducted to find biological control agents for poultry lice. Two new samples of a dust containing the bacterium Bacillus thuringiensis were applied to white leghorn layers infested with the chicken body louse, according to the standard method used to apply insecticide dusts to individual birds. Excellent control was obtained with both samples, 100% by the 19th day with one sample and by the 21st day with the other. In another test, both samples produced complete control by the 21st day; attempts to reinfest the birds with a hundred or more lice per bird were unsuccessful on the 38th, 45th, and 50th days. Application of B. thuringiensis exotoxin to the vent areas only resulted in 100% control in 29 days.

b. House fly - At Gainesville, Fla. a fungus, Entomophthora muscae, from field-collected house flies from a poultry farm produced high mortality in laboratory-type insecticide screening tests. Larvae reared in standard medium plus the pathogen resulted in 100% kill of emerging flies. Histopathology studies showed that all body tissues were affected except the chorion of developing eggs. However, preliminary tests in screened cages showed no mortality of flies under field conditions. Laboratory tests conducted to determine the effect of Bacillus thuringiensis exotoxin mixed with the larval medium showed high effectiveness against flies of both the regular, insecticide-susceptible strain and the resistant Cradson-P strain

c. Miscellaneous flies - Research was concluded under grant to the University of California at Berkeley on the effect of predaceous mites in reducing fly production from poultry droppings.

Fuscuropoda mites (presumed competitive rather than predaceous) and Macrocheles mites (predaceous on flies) were most abundant in the older, unremoved manure, as were predaceous beetles, both adults and larvae. The fly Ophyra became densest in new manure 2 or 3 weeks after removal of the old manure, as did Fannia. The common practice in California of laying down a bed of wood shavings after manure removal appeared to

increase the density of staphylinid beetles, Fannia femoralis, and Stomoxys calcitrans (stable fly), but did not significantly affect the densities of Musca domestica, Ophyra leucostoma, Fuscuropoda sp., or Macrocheles muscaedomestica. Application of 0.01% Bay 39007 provided high mite mortality but low fly mortality. Both the Bay 39007 and an 8.3% ronnel application reduced F. femoralis populations up to 22 days. Mites in untreated plots were also affected by the Bay 39007 suggesting great mobility of the Macrocheles mites. Fuscuropoda mites consumed no more than 1 fly per mite. However, Glyphtholaspis confusa (mite), Macrocheles muscaedomestica (mite), and Philonthus spp. (staphylinid beetle) consumed about 10 flies apiece, and Margarinatus merdarius and Carcinaps pumilio (histerid beetles) consumed about 30 prey apiece, in laboratory studies. This is the first evidence of predation by the beetles.

2. Beef and Dairy

a. Mosquitoes, sand flies, and gants - At Lake Charles, La. an effort is being made to explore the protozoan Thelohania in detail. Patent infections of Thelohania were observed in larvae of 4 species of Aedes, 4 of Anopheles, 3 of Culex, Culiseta inornata, Orthopodomyia signifera, Psorophora confinnis, and the non-biting competitive midge, Corethrella brakeleyi. Peroral transmission of Thelohania inimita in C. inornata was observed on several occasions in the field. Stempellia magma was found in larvae of Culex restuans and Psorophora ferox; another Stempellia sp. was found in larvae of Toxorhynchites rutilus septentrionalis and the sand fly, Culicoides nanus. Plistophora spp. were found infecting larvae of O. signifera, C. territans, C. inornata, and the sand flies, Culicoides arvicola, C. nanus, and in Corethrella brakeleyi. Plistophora caecorum was found infecting larvae of C. inornata. In laboratory tests, Aedes aegypti appeared the most resistant to Plistophora culicis infection, whereas C. inornata was the most susceptible of 7 species of mosquitoes in 4 genera tested. The host species was found to affect size variation in the spores of this protozoan.

Fungi infecting mosquitoes also continued to be studied at Lake Charles. Field infections of Coelomomyces spp. were found in Aedes sollicitans, A. vexans, Anopheles crucians, A. quadrimaculatus, Culiseta inornata, Psorophora ciliata, and P. howardii. From 3% to 76% of the A. crucians populations in a pond near Chloe, La. were infected with Coelomomyces punctatus, with an average infection of 32%. Infections of this fungus were acquired by first-instar larvae of A. bradleyi, A. crucians, and A. quadrimaculatus in 24 of 49 screened plastic containers that were placed in the pond, but tests with Anopheles punctipennis were negative. Coelomomyces quadrangulatus was found infecting small numbers of larvae of A. crucians and A. quadrimaculatus. The basidiomycete commonly found in female Aedes sollicitans was isolated a number of times from a yellow exudate that is often seen on the salt-marsh grass Spartina spartinae.

In research at Lake Charles the mosquito iridescent virus (MIV), was found in field collections of Aedes fulvus pallens, A. taeniorhynchus, A. vexans, and Psorophora ferox, and a possible infection in Aedes thibaulti. A single specimen of A. taeniorhynchus with the blue type of MIV was also collected. Additional infections with an iridescent virus (CuIV) were observed in larvae of Culicoides arbuticola in a tree hole at West Bay, La. Over 60% of the larvae were infected. Transmission per os was demonstrated with this virus in this species of sand fly. The level of infection of CuIV in Culicoides spp. from two tree holes ranged from 31% to 50% and averaged 37%. Forty serial passages of the regular MIV in A. taeniorhynchus to colony taeniorhynchus produced a mean transmission rate of 16%. Forty-one serial passages of the blue MIV produced a mean transmission rate of 17%. When females of taeniorhynchus were injected with regular MIV, about 45% of their progeny developed patent infections; when the females were permitted to feed on a 2% sucrose-MIV suspension, a small percentage of their larval progeny developed patent infections. Tests also showed that live, frankly infected larvae could release sufficient virus in a container to produce frank infections in uninfected larvae. Adult females were inoculated with the blue MIV and some of their larval progeny developed patent infections in the 4th instar. Adult taeniorhynchus in a cage were sprayed with MIV and the virus was transmitted transovarially to a small number of their larval progeny. The survivors were permitted to pupate and emerge; the resulting adults produced larvae (F₂) with a 38.4% level of infection. Transovarian transmission was demonstrated for the first time both with the blue MIV of taeniorhynchus to colony taeniorhynchus and the blue MIV of P. ferox to colony P. ferox.

At Lake Charles nematodes were found for the first time in Aedes atlanticus, A. mitchellae, Psorophora ciliata, P. discolor, and Uranotaenia lowii. Romanomermis nematodes were found infecting larvae of A. atlanticus, A. mitchellae, A. vexans, Anopheles crucians, A. punctipennis, A. quadrimaculatus, Culex erraticus, C. restuans, Psorophora ciliata, P. confinnis, Uranotaenia lowii, and U. sapphirina. This nematode exhibits very little host specificity and has been observed to penetrate and develop in 23 species of mosquito larvae, but not in Aedes triseriatus, Culex territans, or Psorophora ferox. Eggs of Romanomermis hatch in 7-10 days and the pre-parasitic juvenile enters the host larva through the cuticle. Maturation to the postparasitic stage takes 7-9 days; molting to adult, copulating, and laying eggs requires 11-15 days.

Romanomermis is now being maintained at Lake Charles in the laboratory. It most readily invades the second instar of the laboratory host, Culex pipiens quinquefasciatus. Third instar larvae were slightly less susceptible than first instar. Some parasitism occurred in fourth instar larvae but all parasitized fourth stage larvae failed to pupate. About 34% of the parasitism took place in the first 5 hours of exposure. The nematode has little tolerance to salinity; there was a sharp drop in parasitism between 0.015N and 0.03 N Sodium chloride and no parasitism in

water above 0.04N. Volume of water in the test container had little effect on the ultimate degree of infection. Gastromermis sp. nematodes in larvae of A. crucians and A. quadrimaculatus. The juveniles hatch 10-16 days after oviposition, maturation to postparasitic stage takes 6-7 days after onset of parasitism.

The first mermithid nematode infection in any Culicoides sand fly was found in Culicoides nanus. Romanomermis nematodes were found in C. nanus and C. arbicola. Nematodes of the family Tetradonematidae were recovered from larvae of C. arbicola and the midge Corethrella brakeleyi.

The mermithid nematode, Agamomermis culicis, was found infecting about 20% of almost 5,000 female Aedes sollicitans collected from 24 different localities in Louisiana during warm weather, and 5% of 1256 adults from 9 different localities in Louisiana during warm weather. Over 95% of some adult sollicitans populations were infected; parasitism in individual collections ranged from 0 to 100%. This pathogen may be the prime factor in preventing outbreaks of sollicitans in Louisiana and Texas, during normal years.

At Corvallis, Oreg., microsporidiosis in Culiseta incidens collected from Philomath log ponds ranged from 0 to 80%. The infectivity of Thelohania campbelli was increased by the presence of certain bacteria in the medium in laboratory studies.

At Riverside, Calif., under a research contract with the University of California, four isolants of Christoliphoris bacillus, each from different field collected mosquitoes, are being investigated; a large percentage of field populations are infected and mortality was high. A metabolite produced by an Aspergillus was found to be highly toxic to mosquito larvae. Purification and identification of this in additional metabolites is continuing, as are infectivity tests against other species of mosquitoes. Five isolates of field-collected green and blue-green algae produced metabolites toxic to mosquito larvae. Field observations that suggested a close correlation between the presence of certain algae and absence of mosquito breeding were confirmed by tests giving 98-100% mortality of third stage C. tarsalis and C.p. quinquefasciatus larvae placed in filtered water from pools containing the algae.

Research continued under contract with McNeese State College at Lake Charles, La., on predators and parasites of mosquito larvae. Most emphasis was placed on three common minnows, Cyprinodon variegatus, Gambusia affinis, and Molliensia latipinna. In laboratory tests to determine relative rate of mosquito larvae consumption, Gambusia scored highest on basis of number of larvae eaten per fish per day and also on the ratio of weight of larvae consumed per fish per day through the average body weight of the fish tested. All three species fed actively enough on mosquito larvae to justify field experiments to test their usefulness.

b. Face fly - Research continued under a grant to the Virginia Polytechnic Institute on the effect of native parasites on natural regulations of face fly populations and other dung-breeding Diptera. The wasp Muscidifurax raptor, known to parasitize house flies and certain other flies, was found parasitizing face fly pupae, but only about 5% succeeded in emerging from the face fly puparia. Mechanically cracking the puparia enables the parasite to emerge and this method has been used for over a year to build up a successful colony.

Face fly larvae protected from birds but not from insects suffer about 54.6% mortality. Field-reared larvae exposed in trays of sand in the field suffer about 71% mortality, indicating that native parasites and predators take a heavy toll. About 10% of wild-caught face flies contained nematode parasites. Aphaereta pallipes was the most extensively collected parasite on dung-breeding diptera, but even with the method of cracking the face fly puparia, not enough were reared to maintain a colony. Eucoila sp. and Xyalophora quinquelineata rearing attempts were also unsuccessful. All of these species, plus Phaenocarpa sp. and Spalangia nigra were able to parasitize face fly puparia. Percent parasitism of dung-breeding Diptera collected by Berlese funnel and a sandbox near the ground supplied with manure were by the two methods, respectively: Ravinia assidua 82.6 and 55.4%; R. pectinata 31.2 and 30.7%; R. querula 26.9 and 69.5%; Orthelia caesarion 6.1 and 16.1%; and Musca autumnalis (face fly) 3.1 and 8.3%.

Face fly populations near Lincoln, Neb., were lower than in previous years. The counts did not exceed 5 flies/face until after August 10 and about 32% of all flies examined were infected with the nematode Heterotylenchus autumnalis. An investigation was made of high populations of face flies reported in Montana, Idaho, and Oregon. As high as 48 flies/face were found in one Montana herd. Infection by H. autumnalis was much lower than in Nebraska, only 0.7%. During the end of the 1967 face fly season, however, the incidence of H. autumnalis increased in western Montana and northern Idaho, 38.5% and 15.1%, respectively. In laboratory studies, parasitism of the male face fly by autumnalis did not interfere with mating, but females were unable to reproduce. Male flies did not disseminate the parasite. Infective autumnalis do not parasitize face fly eggs, but are highly infective to day-old larvae and less infective to older larvae. Infection in the colony seldom exceeded 24% until a method was developed to produce 75% infection. No evidence was obtained to indicate that Heterotylenchus might parasitize the closely related house fly.

Approximately 25,000 laboratory-reared staphylinid predator-parasites of the face fly were released near Hamilton, Montana. This staphylinid beetle, Aleochara tristis, disappears shortly after release in the field. The significance of this disappearance is not yet understood.

3. Cross Species

a. Imported fire ant - Under a PL-480 grant in Uruguay progress is being made on methods of introducing Solenopsis (Labauchena) daguerri into an imported fire ant colony with associated workers of the latter ant. Immobilization by cold (9-11°C) was found less hazardous to the ants than carbon dioxide anesthesia which killed some of the younger ants. A hypothesis on the mechanism of parasitization in the field has been advanced: Parasitization may be established when a daguerri ant queen (there are no workers) meets an imported fire ant founder queen in the process of founding a new colony.

b. House flies - Research under a PL-480 grant continued in Korea on the possible effects of dung beetles on house fly control. In additional tests to determine the ability of dung beetles to disperse manure with 7 species of beetles of various sizes, it was found that the size of the beetle was correlated with the amount of dung dispersed. Scarabaeus and Coprisochus were the most effective with Onthophagus lenbii running a close third. Onthophagus was found to be attracted to one-day-old droppings. Four other species were also attracted to day-old droppings, but lenbii was found in greater numbers than any other species. Scarabaeus affinis and Gymnopleurus mopsis appear to have good potential usefulness and additional rearing work on these two is being undertaken.

D. Insect Sterility and Other New Approaches to Control (7.5 SMY)

1. Poultry

a. Mites - Under a grant to the University of Mississippi apholate showed most promise of 5 compounds tested as chemosterilants via tarsal contact with young adult northern fowl mites. The internal anatomy of the female mite has been studied with no evidence for "ringed tubes" that have been reported for other mites.

b. House fly - At Gainesville, Fla., 61 of 575 candidate chemicals screened caused complete sterility in adult house flies. Untreated male flies exposed to topically chemosterilant-treated male or female flies became sterilized from 60% to 80% of the time. House flies forced to migrate through expanded polystyrene foam strands treated with 5% tepa were sterilized. Only 10 centimeters of depth was needed to accomplish sterility. Male flies were completely competitive with untreated males, but 10 days after treatment there was 7% more mortality in the treated males. Field females were less receptive sexually to laboratory-reared sterile males than to fertile field males. The maximum uptake of tepa recovered in flies that had migrated through the strands was 21.1 µg for the integument, 24.9 µg for the homogenate, and 57.8 µg for environmental contamination (feces and vomitus) per fly.

A chemosterilant bait containing 1% of hempa applied on droppings in a poultry house at half-weekly intervals at the rate of 5 g/m² produced a 75.0 to 93.6% reduction in the adult fly population for 9 weeks. In another field test, the natural population of house flies was suppressed with insecticide before and during release of insecticide resistant sterile flies. Under this integrated test there was a marked reduction in flies and the ratio of released to wild flies increased from 3:31 to 6:1. This change in ratio was accompanied by an expected gradual increase in the sterility of the wild population of house flies.

At Corvallis, Oreg., research was concluded on new methods of house fly control. A mathematical model was prepared that suggests that population control of the house fly could be obtained through use of conditioned genetic lethals. A conditioned lethal is one not lethal in the laboratory, but lethal under field conditions. At least 17 such conditional lethals are being colonized at one laboratory or another in the world.

c. Poultry lice - At Kerrville, Tex., research was conducted with Shell SD-8447 as a possible systemic for control of poultry lice. Given in the feed of chickens at 100, 200, and 400 ppm, this insecticide failed to give control of the lice.

2. Beef and Dairy

a. House fly - Investigations of physical methods for control of flies were continued at Beltsville, Md., in cooperation with the Animal Husbandry and Agricultural Engineering Research Divisions. Flies were more attracted to light radiation when thirsty than when water was available. They responded in greater numbers to radiation directed horizontally than to radiation directed either up or down, and response was greater to linear fluorescent lamps placed horizontally than vertically. Increasing the length a horizontal lamp caused a greater proportional increase in attraction than did a similar increase in length of a vertical lamp. Multiple lamp arrangements increased the number of flies attracted, but the increased attraction was less than the increase in energy input. Additional tests tended to confirm effects of temperature, sex, and height of trap on the response of house flies to particular wavelengths, but results were inconsistent. For example, orange radiation was more attractive than ultraviolet at cool temperatures in laboratory tests, but orange proved ineffective in barn tests during cool fall weather. A trap was designed to capture the flies alive in order to eliminate any bias that might be caused by odors from charred and dead insects. In studies to investigate components of attractant-toxicant devices, total surface area of the toxicant covering was directly related to effectiveness and gauze was superior to metal screen in retaining the insecticide. Of available blacklight attractants tested, conventional phosphor lamps proved superior to Philips' phosphor and BLB.

b. Stable fly - Research continued under a grant to Oklahoma State University on inhibition of arthropod reproduction by organometallic compounds. Cadmium acetate, chloride, iodide, and succinate fed in blood at levels below those producing lethal results, reduced egg laying by stable flies. In the rearing medium, they retarded larval development and altered the appearance and functioning of ovaries of surviving flies emerging from the medium. The results were, in general, correlated with the amount of cadmium in the compound. Viability of the eggs was not reduced and no repellency to cadmium salts was noted. Malformations of the female reproductive system of two other kinds of insects (Periplaneta americana and Culex pipiens quinquefasciatus) were also noted, but no such malformations occurred with zinc or indium compounds, though possible malformations occurred with tin compounds.

At Kerrville, Tex., experimental chemosterilant ENT-26316 was still effective against stable flies after storage for 6 years at room temperature when exposed as a film deposited in open glass jars. Topical applications of apholate previous to administration of tritiated thymidine prevented the incorporation of the thymidine into the DNA of nurse cells and follicular cells of the ovaries of stable flies.

c. Horn fly - At Kerrville, Tex., a sterile-male release study was made with an "isolated" herd of Angus cattle. Males were sterilized on the day of emergence by gamma irradiation. There was some reduction of horn flies in the herd, but the ratio of sterile to wild flies was never as high as desired. Releases of marked flies showed that an isolation of 2 miles from the nearest cattle was not sufficient; marked flies flew the 2 miles in no more than 12 hours. In laboratory studies, sterile males were competitive with normal males, but the total hatch was even less than expected and there was a drop in number of eggs laid by any group having sterile members, as compared with the controls.

d. Tsetse flies - Under a PASA agreement with AID, research is continuing in Salisbury, Rhodesia in cooperation with the University College of Rhodesia on the feasibility of the sterile male technique for the control of tsetse flies. Productivity of the Glossina morsitans II heavyweight colony fell to a mean of 700 pupae per month, due mainly to the weakened condition of the host guinea pigs which underwent an epidemic of brucellosis. A third Glossina pallidipes colony was initiated but pupal production was insufficient for sustained colonization and the colony was discontinued in the 29th week. Tests conducted in Bristol, England with Dr. Nash's colony of G. morsitans suggested that "lop-eared" rabbits are good hosts. Pupal production per female fly was more than twice that obtained in Salisbury; good results were also obtained with goats as hosts. These studies suggest that efficient indoor colonies of morsitans can be obtained. Further flotation technique studies have not produced a means of separating morsitans pupae by sex or age. Such a

technique would permit employment of field-collected pupae in eradication programs without reliance on known age which is the only present method of separating the sexes. Exposure of adults to carbon dioxide for anesthetic purposes reduced feeding on day of emergence, reduced the longevity of flies exposed when 2 or more days old, and temporarily inhibited feeding by freshly-emerged flies.

Ultraviolet light was attractive to adults of both morsitans and pallidipes under controlled conditions in the laboratory and under ambient conditions in a small outdoor cage between 1600 hrs and 0800 hrs. Under laboratory conditions it inhibited the normal negative response of morsitans males to high temperatures. High mortality resulted since the exposed flies failed to retreat to available areas of optimum temperature. Indoors, blue light elicited a positive response from males and females of morsitans, red and white light were more attractive to females than males, and yellow light was least attractive. Glossina pallidipes' response to blue or red light was erratic and yellow light was less effective. Blue and red light were ineffective in outdoor small cage tests. In field tests in the natural habitat of morsitans and pallidipes, ultraviolet light was ineffective. Laboratory and outdoor cage tests revealed no obvious selection or preference for various colored surfaces by either species. Thus, it appears that the sole use of light or color will not effectively attract these species in the field, but color or light, alone or combined, are not ruled out as possible adjuncts for other possible attractants under field conditions.

Sterile males were released on the main island of the Sampakaruma group at about 3,800 per month for the first 4 months, 1,900 per month for the next 3 months, and 900 per month during the eighth month. As a result, male collections remained high even when collections in the control areas were low. The percentage of females in the total collection dropped well below that of the control areas during the eighth month, suggesting that the release program may be effective. However, final evaluation of the release program must await a further period of release and observation.

3. Beef, Dairy, and Sheep

a. Screw-worm - Research continued at Mission, Tex., on the sterile male technique for control of screw-worms. Percentages of eclosion ranged from 97 to 98% among pupae irradiated with doses of gamma radiation up to 17,000 rads, but results were inconclusive on the effects of short exposure to high intensity radiation (15,000 to 60,000 R/minute) on longevity and sexual activity in screw-worm flies.

Chemosterilants continued to receive emphasis. Thirty-six of 345 promising chemicals tested produced some sterility, but 22 of the compounds sterilized only after multiple oral treatment of both sexes for 5 days.

Candidate chemosterilant ENT-50838 sterilized males first mated at 5 days of age. Those first mated at 12 days of age remained sterile through 3 additional matings. Males sterilized with this chemosterilant effected more matings (5.7) than males treated with the solvent alone (2.5). Males treated topically during the afternoon were at least twice as susceptible to sterilizing action as males treated at midday or during the forenoon. Mature sperm from males treated intrathoracically with this material were not further affected by storage in females for 7 days. Candidate chemosterilant ENT-51799 was completely effective when both sexes were treated topically or orally. When both survival and sterility are considered, this material is a very efficient chemosterilant for screw-worms. Males treated orally with it were equally aggressive in comparison with untreated males, and competed sexually about as well as untreated males. Candidate chemosterilant ENT-52218 sterilized flies when both sexes were topically treated.

b. Ticks - At Kerrville, Tex., studies continued on the possible use of insect hormones in tick control. Results of studies on hormonal termination of diapause in Dermacentor albipictus indicated that 1.0 $\mu\text{g}/\mu\text{l}$ of 5 β -cholest-7-ene, 2 β ,3 β ,14-alpha-triol-6-one was as effective as the previously tried dosage of 10 $\mu\text{g}/\mu\text{l}$ in terminating diapause. Though 2-hydroxyecdysone at 10 $\mu\text{g}/\mu\text{l}$ was effective, neither this compound nor any methyl ester compounds of 10,11-epoxy-farnesenic acid were effective at 1.0 $\mu\text{g}/\mu\text{l}$.

Research was initiated under PL-480 research contract with the Israel Institute for Biological Research, Ness Ziona, Israel, on the development of sterility methods for population control of some soft ticks.

E. Attractants (3.6 SMY)

1. Poultry and Dairy

a. House fly - At Gainesville, Fla., over 450 cyanides, thiocyanates, and isothiocyanates were tested as arrestants for house flies. About 10% of the cyanides were more effective than the sucrose standard. The best was N,N-bis(2-cyanoethyl)-m-toluamide. Tests with over 50 toluamide and benzamide derivatives suggested that the cyanoethyl moiety may be responsible for the arrestant activity of this material. Three of a series of straight-chain aliphatic nitriles--acetonitrile, propionitrile, and butyronitrile--were strong arrestants, with propionitrile the best. Activity decreased abruptly with chain lengths greater than 4 carbon atoms. It is hypothesized that these chemicals act at the site of tarsal receptors to stimulate a sweet taste. Olfaction could influence results of such tests, but olfactory tests revealed no attractive qualities with 52 nitriles.

2. Beef and Dairy

a. Horn fly - Research continued under grant support at the New Mexico State University, Las Cruces, N. M. on the response of horn flies to extracts of animal tissues and to putrefaction products. Unfed 16-hr old adult flies showed an 87% greater response to an airstream when it was pulled over the back of a cow. Carbon dioxide at 4 concentrations above ambient air concentration invoked negative responses. Known repellents caused a significant oviposition depression in the olfactometer. Twelve other compounds were also tested, but showed no significant increase in oviposition response.

b. Stable fly - At Lincoln, Neb., studies were initiated on attractants for the stable fly. CSMA medium aged for 4 to 10 days was more attractive than that aged either less than 4 days or more than 13 days.

3. Poultry, Beef, and Dairy

a. House fly - Efforts to purify, characterize, and identify the attractive principle in extracts of female flies have continued at Beltsville, Md. At Corvallis, Oreg., 14 samples prepared by the chemists were evaluated, bringing the total number of extract-derived samples tested up to 270. Benzene extracts have never shown strike responses on the part of the male flies. No evidence was found in two series of tests that would support the hypothesis that benzene extracts contained a masking agent. Rather, lack of male strike response was due to lack of pheromone in the extracts.

4. Beef, Dairy, and Sheep

a. Screw-worm - At Mission, Tex., odors from screw-worm infested sheep were drawn from around the sheep through a tube containing activated charcoal, alumina, and fluorosil. Hexane and ethyl ether extracts of this substrate were tested in the olfactometer and 81% of the screw-worm flies captured were males. Flies captured in a trap attached to a wounded sheep in the same room were 94% female.

Females of the Mexican screw-worm fly strain did not respond to male pheromones of either their own strain or of the Florida strain, but Florida strain females responded to pheromones of both strains. After the Mexican strain had been bred in the laboratory for a few generations, some females began to respond to the male pheromone of the Mexican strain. A selected strain of responding females of the Mexican strain is being established.

Limited field tests with screw-worm infestation odors indicate that such an odor may be attractive when associated with a live animal. A simulated sheep attracted no flies, but a trap on a live sheep (unwounded, but baited with infestation odor) did. Preliminary oviposition tests showed that a larger number of flies oviposited when light and moisture were

present in addition to oviposition medium. When guinea pigs were used as an attractant, the number of flies ovipositing increased as the infested wound aged. Starvation of the flies for 18 to 24 hours prior to testing increased the number of flies ovipositing in each test group.

Research also continued under cooperative agreement with the Instituto Nacional de Investigaciones Agricolas in Mexico. Early data suggested that the Villahermosa-Teapa, Tabasco, area had a greater abundance of screw-worms than the State of Veracruz and that a peak of activity occurred in September and October. However, central Veracruz had about 4 times as dense a population in the winter of 1967-68 as in the previous winter and in the Villahermosa area the 1967-68 winter population was about 50% lower than in the previous winter. In retrospect, it appeared that there were two periods of high incidence in Veracruz, one in April, May, and June, and another in December, January, and February. Incidence in Villahermosa was apparently relatively uniform throughout the year.

Traps placed in sunlight captured from 3 to 6 times as many adults as traps partially or completely shaded. Flies were captured with the standard liver attractant, but not with 19 other materials tested. In a comparison of horse-meat reared and hydroponic reared flies, two groups each containing 100,000 flies, sterilized at Animal Health Division's facility at Mission, Tex., were dyed and released near Juan de Alfaro. Only 17 of the flies reared on horse meat were recovered and none reared by hydroponic medium.

F. Insect Vectors of Animal Disease (3.2 SMY)

1. Beef, Dairy, and Sheep

a. Bluetongue - Research continued at Denver, Colo. on bluetongue disease of sheep and cattle, in cooperation with veterinarians of the Animal Disease and Parasite Research Division. A new rearing medium which is more easily standardized than manure was developed for Culicoides vectors of the virus. Preliminary data indicate that the threshold of bluetongue virus in Culicoides is concrete and limited. In a bluetongue epidemic in 1966 near Billings, Mont., attempts to propagate the virus in the laboratory failed when macerated Culicoides, Simulium, stable fly, and mosquito supernatant was inoculated into embryonating chicken eggs. However, susceptible sheep placed in the pasture where the epidemic occurred were infected with bluetongue, strain BT-ox-254. This strain does not readily propagate in chicken embryos, which probably accounts for failure to propagate the virus with macerated insects in those studies.

2. Beef, Dairy, and Other (Horses)

a. Anaplasmosis - Research continued at Stoneville, Miss., on the transmission of bovine anaplasmosis by mosquitoes, gnats, and horse flies.

Anaplasmosis transmission was at a low level in Mississippi during this year's study. One animal in the 6-week group of the third replicate and one in the 2-week group of the first replicate contracted the disease. The data obtained supported those obtained in 1965 and 1966, indicating that mosquitoes do not have a role in transmission in Mississippi. The data further indicate that Tabanidae (horse or deer flies) may have a role, and suggest that eye gnats of the genus Hippelates, feeding at punctures made by horse flies, also have a role.

In studies at Beltsville, Md., in cooperation with the Animal Disease and Parasite Research Division, colonies of Dermacentor albipictus (the winter tick) and Anopheles quadrimaculatus (the common malaria mosquito) were enlarged in preparation for transmission trials. Attempts have been made, using fluorescent antibody techniques, to confirm the previous reports that Anaplasma marginale may be found reproducing in the Malpighian tubules of Dermacentor andersoni nymphs. No such reproduction was found at Beltsville. Some success has been obtained towards achieving a tissue culture from D. andersoni nymphs, but extended growth of cells has not been obtained. Some success has also been obtained in inducing expulsion of oral secretions of the ticks into capillary tubes, offering the possibility of isolating A. marginale from the oral secretions of infected ticks.

b. Equine piroplasmiasis - Research at Beltsville, Md., on transmission of equine piroplasmiasis continued in cooperation with the Animal Disease and Parasite Research Division. Studies showed alternation of generation for Babesia caballi in Dermacentor nitens. Two types of asexual development occurred. Small, spherical parasites were first observed in the gut contents of the tick, and apparently these developed into larger, clavate (club-shaped) forms that rounded up and divided by multiple fission to produce motile, club-shaped forms. These forms invaded cells of the hemolymph, Malpighian tubules, and ovaries, where they underwent a second asexual cycle. Forms arising from this second cycle were also club-shaped; they either reinvaded these tissues, or entered those of the salivary glands. Development in the salivary glands produced small, pyriform bodies by multiple fission. These are probably the infective forms for the vertebrate host, passed during feeding of the tick.

RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC RESIDUES FROM
AGRICULTURAL SERVICES

A. Insecticide Residue Determinations (3.6 SMY)

1. Poultry. A dust containing 3% of Shell SD-8447 was used to treat litter in several chicken houses. From the 1st to the 5th week after treatment, residues ranging from zero to 0.058 ppm were found in the skin of the back. In the fat, residues from the 1st to the 4th week ranged from zero to 0.048. No residues were detected in the fat after 4 weeks or in the skin after 5 weeks. Residues ranging from 0.003 to 0.022 ppm

appeared in the eggs until 2 weeks after treatment when no more residues were found.

2. Beef and Dairy. Research continued at Kerrville, Tex. to determine whether certain insecticides and candidate insecticides would cause residues to appear in livestock treated with these materials. Past tests have shown high residues of Stauffer R-3828 and its oxygen analog in the fat of cattle treated with this material. An 8-week test was conducted with two levels (5 mg/kg/day and 10 mg/kg/day) given orally to 6 animals at each level of treatment. At the end of the treatment period, R-3828 in the omental fat was 109 ppm at the low dosage and 302 ppm at the higher dosage. These residues dropped rapidly (47.7 and 97.1 ppm, respectively 4 weeks after treatment stopped) but were not entirely eliminated after 14 weeks (1.66 ppm at the 5 mg dosage and 1.44 ppm at the 10 mg dosage). The oxygen analog was about 2 percent of that of the parent material during the treatment period but rapidly decreased to zero after the treatment stopped. A test was run to determine whether the insecticide would be transferred from the body fat to the wax of the hair of the body. A yearling calf was given an oral dose of 100 mg/kg and hair samples analyzed periodically. Residues found in the hair 3 days after treatment showed 1.22 ppm, equivalent to 17.4 calculated in the wax and 1.01 ppm of the oxygen analog, calculated to be equivalent to 1.44 ppm in the wax. The amount of R-3828 in the wax approached the level in the body fat 21 days after treatment. The 21-day levels in the wax were 62.6 ppm of R-3828 and 6.29 ppm of the oxygen analog. In a steer treated orally with C¹⁴-labeled R-3828, 70-85% of the radioactive material appeared in the urine as an unknown compound or compounds that probably are conjugates with glucuronic acid or other biochemicals. The principal extractable compound in the blood was the oxygen analog; the predominant compound in the feces was the parent compound.

A clean vat was charged with 0.22% As₂O₃ (from a standard, commercially available arsenic dip product). Eight animals (1 Jersey milk cow, 1 thin 550-lb calf, and 6 Angus 700-lb calves) were dipped twice, 1 week apart. Milk samples taken 1, 2, 3, 5, 7, 10, and 14 days after the second dip showed arsenic residues in the milk at all except the 10-day sample. The lower limit of sensitivity in the milk for the method of analysis was 0.01 ppm; this was exceeded (0.02 ppm) at 1 day, 2 days, and 5 days after treatment. The average residue level in tissues of the 6 Angus calves 48 hours after the second treatment was 0.13 ppm in muscle, 0.06 in heart, 0.16 in kidney, 0.07 in spleen, 0.14 in liver, and 0.05 in brain. Results in the thin calf were comparable, slightly lower in muscle, kidney, and liver, but higher in heart, spleen, and brain. The arsenic residues continued to appear 14 days after treatment in the Angus calves, present in all of the tissues but highest (0.10 ppm) in the liver.

A series of tests were run with the ultra-low-volume automatic sprayer which applies 1 ml of spray to each animal twice each day. One dairy cow was treated with 0.5% Shell SD-8447 daily for 21 days and another

with a 1.0% spray in the same manner. No residues appeared in the milk analysis by gas chromatography, sensitive to 0.001 ppm. No Compound 4072 was detected in the milk of 2 cows treated with 0.25% and 0.5% ULV sprays and a sensitivity level of 0.002 ppm. No residues were detected in the milk of 2 dairy cows ULV-sprayed with 1% and 0.5% coumaphos. Residues ranging from 1 to 6 ppb were found in the milk of a cow sprayed with 1% methoxychlor and from 4 to 10 ppb in milk of another cow treated with 2% methoxychlor.

In other studies at Kerrville, Tex. no residues were detected in the milk of cows exposed to backrubbers treated with either 1% or 2% coumaphos, even after 28 days of self-treatment. Four steers sprayed with either 1.0% or 0.5% of carbaryl were slaughtered at 1 or 8 days after treatment. Only one tissue sample taken 1 day after treatment with 1.0% carbaryl showed evidence of residues. The method of analysis was cholinesterase inhibition, giving an equivalent of 0.02 ppm of carbaryl.

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RPA 210 - CONTROL OF INSECT PESTS OF LIVESTOCK AND POULTRY

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RPA 701 - INSURE FOOD PRODUCTS FREE FROM TOXIC RESIDUES
FROM AGRICULTURAL SOURCES

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