Executive Summary

INTEGRATING BIOLOGICALLY-BASED STRATEGIES FOR TURFGRASS PEST MANAGEMENT PHASE II

University of Georgia

S. Kristine Braman

The encompassing objective is to develop and refine best environmentally-oriented management practices for long-term maintenance on golf courses. The potential for compatibility among two biologically-based management strategies: host plant resistance and biological control will be evaluated in this three year project.

In Phase I, genotypes were screened for potential resistance to a guild of insect pests that limit turfgrass growth, establishment or appearance. During Phase II we propose to take the next step and develop strategy that will allow enhancement of biological control with parasitoids and predators by using grasses with partial, yet incomplete resistance to selected turfgrass pests. Using turfgrasses with intermediate resistance introduces sustainability into the system by reducing the potential for insect pests to overcome the plant's defenses. By combining plant resistance to insects with enhanced activity of natural enemies, a more stable system may result with greatly reduced need for chemical intervention. A series of laboratory and field experiments will examine this relationship.

Field evaluation of interaction between resistant plants and natural control of fall armyworm

Six zoysiagrass, paspalum, and bermudagrass cultivars representing a range of resistance to fall armyworms as previously determined among ca. 100 turfgrass selections that we have recently evaluated will be used for the proposed field and lab studies. They include 'Palisades' and 'Cavalier' zoysiagrasses; 'Tifsport' and 'Tifeagle' bermudagrasses; 561-79 and Sea Isle 1 paspalumgrasses. These grasses have been planted in the field in a randomized complete block design with six replications. Plots (each 25 m^2)are located at the Georgia Station in Griffin.

A new turfgrass research area in the Research and Education Garden was developed during 2000. Irrigation was installed during May and research plots sprigged with appropriate grasses on May 22. Cover was nearly 100% in all plots by October 2000. Field sampling will be initiated summer 2001.

Laboratory evaluation of influence of pest-resistant plants on fall armyworm susceptibility to natural enemies.

Prey acceptability studies were conducted during summer 2000 for the big eyed bug, *Geocoris uliginosus*, a very common predator in turfgrass. Turfgrasses included Cavalier, Palisades, 9601, Diamond and Royal zoysiagrasses; Tulsa tall fescue, Dawson E+, Tifsport and Tifeagle bermudagrasses and 561-79 and Sea Isle 1 seashore paspalums. The predator/prey profile developed indicated that big eyed bugs will feed on fall armyworms fed either susceptible or resistant grasses. Furthermore, while fall armyworm larvae reared on susceptible grasses were suitable prey for adult big eyed bugs only from day 1 through day 6, those larvae reared on more resistant grasses remain smaller and were acceptable prey for big eyed bugs for a much longer period. This expanded "window of vulnerability" is a positive indication of compatibility among two biologically-based management strategies.

Field evaluation of interaction between resistant plants and control of fall armyworm with standard chemical, microbial and alternative products.

Field plots will be treated with bacterial products (*Bacillus thuringiensis),* Spinosad=Conserve, and Dursban to determine whether grasses displaying partial resistance to fall armyworms also render the pest more susceptible to standard chemical, microbial and

alternative products.

UNIVERSITY OF GEORGIA

INTEGRATING BIOLOGICALLY-BASED STRATEGIES FOR TURFGRASS PEST MANAGEMENT PHASE II

2000-2003 Research Grant
(First Year of Support)
2000 Annual Report

Dr. Kris Braman PI

Assessment of potential synergy between pest-resistant plants and control by natural enemies

The encompassing objective is to develop and refine best environmentally-oriented management practices for long-term maintenance on golf courses. The potential for compatibility among two biologically-based management strategies: host plant resistance and biological control will be evaluated in this three year project.

In Phase I, genotypes were screened for potential resistance to a guild of insect pests that limit turfgrass growth, establishment or appearance. During Phase II we propose to take the next step and develop strategy that will allow enhancement of biological control with parasitoids and predators by using grasses with partial, yet incomplete resistance to selected turfgrass pests. Using turfgrasses with intermediate resistance introduces sustainability into the system by reducing the potential for insect pests to overcome the plant's defenses. By combining plant resistance to insects with enhanced activity of natural enemies, a more stable system may result with greatly reduced need for chemical intervention. A series of laboratory and field experiments will examine this relationship. The studies included in this project are:

Study 1. Field evaluation of interaction between resistant plants and natural control of fall armyworm

Field Experiment 1 (years 1-3). Six zoysiagrass, paspalum, and bermudagrass cultivars representing a range of resistance to fall armyworms as previously determined among ca. 100 turfgrass selections that we have recently evaluated will be used for the proposed field and lab studies. They include 'Palisades' and 'Cavalier' zoysiagrasses; 'Tifsport' and 'Tifeagle' bermudagrasses; 561-79 and Sea Isle 1 paspalumgrasses. These grasses have been planted in the field in a randomized complete block design with six replications. Plots (each 25 m^2)are located at the Georgia Station in Griffin.

Once grasses are established, newly hatched fall armyworms will be released monthly into each plot (20,000 per plot). Larvae will be recovered 1, 4, 7 and 10-days post-release using a Vortis vacuum sampler to collect live larvae from a known area. Larvae recovered will be placed on artificial diet and held at 24°C to observe any parasitoid (primarily small braconid wasps but also tachinid flies in older larvae) emergence.

A new turfgrass research area in the Research and Education Garden was developed during 2000. Irrigation was installed during May and research plots sprigged with appropriate grasses on May 22. Cover was nearly 100% in all plots by October 2000. Field sampling will be initiated summer 2001.

Study 2. Laboratory evaluation of influence of pest-resistant plants on fall armyworm susceptibility to natural enemies.

Lab Experiments (years 1-3). The same turfgrasses examined in the field experiment and additional selections will be used in lab studies to determine length of time fall armyworm larvae are susceptible to parasitism by the parasitoid *Cotesia marginiventris* and predation by the predators *Geocoris uliginosus* and common ant species. This parasitic wasp, the predaceous big-eyed bug and ants are common natural enemies of fall armyworms and other caterpillars.

Ten larvae per plant type will be exposed to each natural enemy daily during the length of the larval stage which may extend from 18 to as many as 50 days. Prey acceptability will be noted daily for the different predator/prey/host plant combinations. This experiment is designed to help us understand the mechanism of observed differences in rate of natural enemy -induced mortality of armyworms among grass types.

Prey acceptability studies were conducted during summer 2000 for the big eyed bug, *Geocoris uliginosus*, a very common predator in turfgrass. Turfgrasses included Cavalier, Palisades, 9601, Diamond and Royal zoysiagrasses; Tulsa tall fescue, Dawson E+, Tifsport and Tifeagle bermudagrasses and 561-79 and Sea Isle 1 seashore paspalums. The predator/prey profile developed indicated that big eyed bugs will feed on fall armyworms fed either susceptible or resistant grasses. Furthermore, while fall armyworm larvae reared on susceptible grasses were suitable prey for adult big eyed bugs only from day 1 through day 6, those larvae reared on more resistant grasses remain smaller and were acceptable prey for big eyed bugs for a much longer period. This expanded "window of vulnerability" is a positive indication of compatibility among two biologically-based management strategies.

<u>Study 3.</u> Field evaluation of interaction between resistant plants and control of fall armyworm with standard chemical, microbial and alternative products.

Field Experiment 2 (year 3) . Field plots will be infested as above during year 3 with fall armyworms. Plots will be treated with bacterial products (*Bacillus thuringiensis),* Spinosad=Conserve, and Dursban to determine whether grasses displaying partial resistance to fall armyworms also render the pest more susceptible to standard chemical, microbial and alternative products.

Proposed Research Schedule for 2001

Field assessment of differential fall armyworm survival, predation and parasitism rates will be conducted monthly during spring, summer and fall, 2001. Ongoing laboratory studies continue to develop predator or parasitoid/prey profiles to establish effects of pest-resistant grasses on the next trophic level.

Final Report
to
The United States Golf Association

A Multigene-Transfer Strategy to Control Pathogens and Enhance Environmental Stress Tolerance in Creeping Bentgrass

From

Mariam Sticklen
Professor
Crop & Soil Sciences Dept.
102 CIPS
Michigan State University
East Lansing, MI 48824-1311
Tel: (517) 353-9140
FAX: (517) 353-1698

Joseph Vargas
Professor
Bot. & Plant Pathol. Dept.
206 CIPS
Michigan State University
East Lansing, MI 48824-1311
Tel: (517) 353-9430
FAX: (517) 353-5598

Note: Dr. James Baird was one of the Co-PIs of the proposal. However, he has not been involved with this final report because he is no more at MSU.

Executive Summary

Previously, Sticklen's research team developed creeping bentgrass clones that contain the glufosinate ammonia (Liberty or FinaleTM) resistant herbicide, a chitinae gene, a proteinase inhibitor gene, and a drought and salt tolerance mannitol dehydrogenase (*mt1D*) gene. Studies have shown that the chitinase genes can make transgenic plants resistant to pathogenic fungi such as *R. solani*, etc..

Our research team confirmed that glofosinate ammonia has fungicidal as well as herbicidal properties. Therefore, we have been able to simultaneously control weeds as well as turfgrass pathogens (mainly *Sclerotinia ulnocarpal* and *Rhizoctonia solani*) by spaying this herbicide on transgenic creeping bentgrass expressing the bar gene under greenhouse conditions.

Research by Dr. Vargas laboratory has shown two out of 44 independently transformed lines of our transgenic creeping bentgrass, transcribing the elm chitinase gene, has improved resistance of plants to R. *solani* under controlled environmental conditions.

Experiments performed by Dr. Baird's laboratory has shown no translation of our transcribed *mtlD* gene in certain lines of transgenic turfgrass. Of course, none of these transgenic lines tested for western blotting also showed accumulation of mannitol, as a sign for drought and/or salt tolerance. With no surprise, none of these transgenic lines tested showed any drought and/or salt tolerance either. Since we made synthetic peptides and produced extra antibodies to the MTLD protein, we plan to test the rest of *mtlD*-transcribed transgenic lines for expression of MTLD protein by western blotting. If positive, then once again we will perform mannitol test and will also test plants for drought and or salt tolerance. Should MSU have a new turfgrass physiologist in place soon. this work will be performed with collaboration of the new physiologist.

Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a desirable species for use on golf courses throughout most of the United States due to its tolerance of low mowing heights, density, and other turf quality characteristics that enhance the game of golf. Whether or not it is grown within or beyond its zone of adaptation, creeping bentgrass is limited by environmental stresses associated with drought and temperature extremes, and by pathogenic diseases that prey on stressed turf.

The most promising approach to combating the major biotic and abiotic stresses associated with creeping bentgrass and other turfgrasses is through the development of transgenic plants. These plants are created by the introduction of genes (fundamental units of heredity) into existing deoxyribonucleic acid (DNA), the primary carrier of genetic information. Thus, it would be advantageous to insert genes into creeping

bentgrass that express greater resistance to stresses induced by extreme environmental conditions and pathogens.

Multi-Gene Transformation Studies

Initially, Dr. Sticklen's laboratory developed a genetic engineering system for creeping bentgrass using a marker (*gus*) gene to determine success of gene incorporation (Zhong et al., 1991; Zhong et al., 1993) into turfgrass. Then, under the financial support of the United States Golf Association, her team successfully incorporated a gene for resistance to glufosinate (FinaleTM), a non-selective herbicide (Liu, 1996). Additional research conducted showed that glufosinate has fungicidal, in addition to herbicidal, properties. As a result, we have been able to simultaneously control weeds and diseases caused by the pathogenic fungi *Rhizoctonia solani* (brown patch) and *Sclerotinia homoeocarpa* (dollar spot) by spraying the herbicide on transgenic creeping bentgrass expressing this gene (Liu et al., 1998).

Our next challenge was to insert a chitinase gene cloned and characterized in Sticklen's laboratory into creeping bentgrass. Chitinases are enzymes (proteins) that degrade chitin, a structural polysaccharide of fungal cell walls and insect exoskeletons. Since fungi cause the major pathogenic diseases of turfgrasses, expression of the chitinase gene in creeping bentgrass is expected to promote disease control via chitin degradation. Studies have shown that chitinase genes can make transgenic plants resistant to pathogenic fungi such as R. solani (Graham and Sticklen, 1994). The Sticklen laboratory team cloned and characterized a full-length chitinase gene which contains the necessary chitin-binding domain from American elm (*Ulmus americana*) (Hajela and Sticklen, 1993; Sticklen et al., 1993; Hajela et al., 1993). Then our team constructed a plasmid containing this chitinase gene regulated by the 35S promoter, and successfully inserted this chitinase gene into creeping bentgrass (Chai, 1997). The, collaboration was made with Dr. Vargas for laboratory and greenhouse levels inoculation studies of transgenic plants. These studies showed that two out of 44 independently transgenic turfgrass genetic lines were resistant to *R. solani*. The studies were repeated at the laboratory and the greenhouse levels, and results were submitted to Phytopathology as two out of 44 transgenic lines showed 3 to 5 fold resistance to *R. solani* (Chai et al, 2000; Green et al, 2000). A more detailed report of the screening of transgenic plants is shown below.

Comparisons of disease resistance in different independent lines of transgenic creeping bentgrass transcribing the chitinase gene

Seven transgenic lines (711, 7204, 7205, 7208, 815-1, 815-7, 9106, 910-10, 9601, 9603, & 9606) of *A. palustris* carrying a selectable marker for bialophos resistance (*bar* gene), and the class I basic elm chitinase (pHS2) were screened for resistance to *Rhizoctonia solani* AG 1-Ia (casual agent of brown patch) and *Sclerotinia homoeocarpa* (casual agent of dollar spot) under controlled environmental conditions. Parental cultivars of *A. palustris*, Penncross and Putter, and a transgenic line of each parental cultivar containing only the *bar* gene were included as controls. Growth, color, and turfgrass quality varied within the eleven transgenic creeping bentgrass lines examined. Only lines 711, 815-1,

9603, 9606, and 9604 were equivalent in turfgrass quality compared to their parental cultivars. Coarse texture or low shoot density resulted in poor turfgrass quality in the other transgenic lines.

Brown patch screen. Controlled environment experiments were conducted 15 May through 27 July, 1998 to assess levels of *R. solani* resistance among the transgenic lines and their parental cultivars as discussed by (Green et al., 2000). To summarize our findings, two transgenic lines 711 and 9603 had significantly ($P \leq 0.01$) improved resistance to *R. solani* as compared to their parental cultivar, Penncross, and the Penncross derived *bar*-only line 9604. Transgenic lines 711 and 9603 were found to have approximately a 3- and 1-fold improved level of resistance to *R. solani* AG 1-Ia, respectively, when compared to Penncross. Other transgenic lines derived from the creeping bentgrass cultivars Penncross and Putter did not provide significantly improved resistance to *R. solani*.

Dollar spot screen. Controlled environment experiments were conducted 12 February through 15 May 1999 to assess levels of *S. homoeocarpa* resistance among the transgenic creeping bentgrass lines and their parental cultivars. None of the transgenic lines containing the chitinase gene showed improved levels of resistance to the dollar spot pathogen as compared to their parental cultivars. Whereas, four of the transgenic lines carrying the chitinase gene (910-12, 9601, 9603, 9606) showed reduced levels of resistance to *S. homoeocarpa* as compared to their parental cultivar. Failure of the pHS2 chitinase gene to provide improve resistance to this ascomycete fungus, agrees with other literature where differences in fungal cell wall composition appear to inhibit the effective suppression of some fungal pathogens by the chitinase enzyme . These reports suggest that variation in carbohydrates and proteins on the fungal cell wall surface protect the chitin in the cell wall from the chitinase enzyme.

Testing drought tolerance of transgenic creeping bentgrass

In addition to our herbicide and pathogen resistance transformation studies, we successfully incorporated one drought and salt resistance gene [mannitol 1-phophate dehydrogenase (*mtlD*)] regulated by a monocot specific promoter and intron (*Act1* promoter) into creeping bentgrass (Chai,1997). This portion of the project was in collaboration with Dr. James Baird. the former faculty member of the Department of Crop and Soil Sciences at Michigan State University. The *mtlD* gene is associated with drought and salinity tolerance in plants (Tarczynski et al., 1992, 1993). Dr. Sticklen's laboratory also confirmed that this gene (*mtlD*) too was incorporated and transcribed in transgenic creeping bentgrass. Since Dr. Wu laboratory at Cornell University transferred this *mtlD* gene regulated by the same promoter (rice *Act*1) into rice and confirmed that transgenic rice had become tolerant to both drought and salt (Xu et al., 1996), we conducted studies to see whether our transgenic turfgrass transcribing the mtlD gene were tolerant to drought or salt. In our studies, Dr. James Baird's laboratory tested several of our transgenic lines, and found no resistance of these lines to drought or salt. We also

designed synthetic peptides and produced antibodies against the MTLD protein. Dr. Baird's student conducted few western blots from our transgenic lines in which tall showed not translation of our transcribed *mtlD* gene in transgenic turfgrass. Interestingly, none of these transgenic lines tested for western blotting showed accumulation of mannitol, as a sign for drought and/or salt tolerance. Since our northern blots clearly show the transcription of *mtlD* gene in several of our transgenic lines, we plan to use the rest of antibodies and test other transgenic clones that have transcribed the *mtlD* gene to see whether any of the other lines show the translation of the *mtlD* gene.

Improving the Expression of Genes in Creeping Bentgrass

We constructed a plasmid containing the chitinase gene and the rice *Act1* promoter and intron. The objective of this part of the proposal was to see whether we could improve the level of expression chitinase gene in turfgrass, as compared to the level of its expression under control of the 35S promoter. Then, we genetically engineered creeping bentgrass with the plasmid containing the rice *Act1* promoter. To date, we have not found a clear result showing that there are much expression level differences between these two promoters in creeping bentgrass. Of course, we have produced and tested a small number of transgenic turfgrasses with the plasmid containing the rice *Act1* promoter.

Cross Breeding of Certain Lines of Transgenic Turfgrass

Michigan State University and Pure Seed Testing, Inc. entered into a license agreement whereby the Oregon Research Corporation conducted further testing of transgenic plants developed at Michigan State University. This private sector also cross bred our transgenic lines with their inbred lines and produced hybrid seeds. The Pure Seed Testing, Inc. also studied the distance needed to be kept between transgenic and the non-transformed turfgrass fields in order to avoid transfer of transgenic pollen grains to the surrounding fields. It was unfortunate that anti-biotechnologists destroyed the Pure Seed Testing, Inc. transgenic fields.

Personnel Trained Under the Financial Support of the USGA

1. Dr. David Green	Postdoctoral Associate	Dr. Vargas's Lab.
2. Ms. Susan Redwine	MS Student	Dr. Baird's Lab.
3. Dr. Chien-An Liu	Ph. D. Student	Dr. Sticklen's lab.
4. Dr. Benli Chai	Ph. D. Student	Dr. Sticklen's Lab.
5. Mrs. Robabn Sabzikar	Technician	Dr. Sticklen's Lab.

Please note that partial salaries of the above personnel were paid through the USGA grant.

Patents or Provisional Patent Applications Filed

1. U.S. Patent in progress: MSU 4.1-153, Serial # 08/036,056, March 23, 1993.Method for isolating a grass plant with foreign DNA. On appeal.

2. U.S. Patent in progress: MSU 4.1-315, Serial # 60/015,485, Apr. 15, 1996.Simultaneous control of weeds and turfgrass diseases with spray of Bialaphos on genetically engineered turfgrass. Provisional filed on Apr. 15, 1997.

3. U.S. Patent in progress: MSU 4.1-395, ID 98-031, May 26, 1998.Disease resistant transgenic turfgrass containing a chitinase gene. Filed in 1998.

Conclusions

At conclusion, we developed a very reliable system of genetic engineering for turfgrass, transferred multi-genes in plants, and tested transgenic plants for herbicide, disease and drought tolerance. Cross breeding was performed by a private sector that confirmed the integration and expression of transgenes into the company's commercial lines.

It is ashamed that biotechnology researchers and biotechnology private sectors have been disrespectfully blamed for their research programs. We believe that it is only a matter of time to educate public in understanding and appreciating the applications of biotechnology, especially the application of biotechnology to improve a non-food/non-feed crop such as turfgrass.

Acknowledgement

At the end, we would like to sincerely appreciate the financial and moral supports of the USGA. This support resulted in training of several graduate students, technicians, and postdoctoral associates at Michigan State University.

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ANNUAL REPORT TO THE US GOLF ASSOCIATION, OCTOBER 2000

A parasitic fly that kills mole crickets: its use in states north of Florida

J. Howard Frank
Entomology & Nematology Dept.
University of Florida

Start Date: 1998
Number of Years: 3
Total Funding: $26,680

Objectives:

1. To explore farther south in South America (colder climate) to obtain stocks of the fly *Ormia depleta*, a natural enemy of *Scapteriscus* (pest) mole crickets.

2. To culture the captured South American flies in our laboratory and supply them to collaborators in other states for release.

Scapteriscus mole crickets, all of South American origin, are the most damaging insect pests of southern turf. Because of the economic importance of turf in the South, they are the most important pests of turf in the USA. In the 1980s, three classical biological control agents were introduced from South America into Florida to control mole cricket pests. One of these agents is the parasitic fly *Ormia depleta*. Classical biological control does not aim to produce a marketable product -- instead, it aims to introduce and release a biological control agent that will provide permanent free area-wide biological control of the target pest. The cost of biological control is all subsumed under the heading "research."

Ormia depleta is a parasitoid ("parasitic") fly whose adults are free-living and whose larvae develop in and kill mole crickets. A tropical stock of *Ormia depleta* from 23°S in Brazil (Piracicaba stock) was brought to Florida in 1987. Rearing methods were developed at the University of Florida. Investigation showed that gravid (pregnant) female flies detected their mole cricket hosts by sound (of singing male mole crickets) at night. Only tawny (*Scapteriscus vicinus*) and southern (*S. borellii*) mole crickets produced song attractive to the flies, and non-pest native mole crickets did not. Nevertheless, female mole crickets in close proximity to singing males also were parasitized in the laboratory and field. In the laboratory, adult flies did not survive long and did not reproduce unless fed artificial nectar. They would mate only under fading natural light at sunset. About 10,000 of laboratory-reared progeny of this stock were released in 1988-1992, funded by the Florida Turfgrass Association, in all regions of Florida. By the end of 1994, a population of this fly was found by surveys to have colonized 38 counties of peninsular Florida to 29°N. Surveys farther north failed to show northward extension of the population. Small starter stocks of the fly later supplied to collaborators in Alabama, Georgia, and North Carolina apparently failed to establish populations in those states.

The research program maintains year-round trapping stations for mole crickets at Gainesville (two stations) and Bradenton, Florida. Before the South American biocontrol agents (*Ormia depleta*, *Larra bicolor*, and *Steinernema scapterisci*) were released in or near Gainesville (only *Ormia depleta* was released at Bradenton), numbers of mole crickets trapped varied from year to year, but showed no trend upward or downward for the years 1979-1988. After 1992, numbers showed a downward trend that continues through 1999-2000, A difficulty was in separating the effects of the three biological control agents that had established populations. The trapping stations use artificial song of mole cricket males as "bait" for flying female mole crickets. Few mole crickets trapped at the stations were found to be parasitized by *Ormia depleta* larvae. However, the fly larvae burrow into, feed inside, and kill mole crickets, so it would not be surprising that mole crickets infected by these larvae are soon rendered incapable of flight.

In an extensive survey in most peninsular Florida counties in 1992, trap records showed that numbers of adult flies varied enormously from place to place. The numbers of flies must depend in part on number of mole crickets present in a local area, because mole crickets are the only hosts used by the fly larvae. But surely the number must also depend upon energy sources (nectars) available. Unfortunately, it was difficult to detect the energy sources used in nature because the adult flies are nocturnal, and they can fly rapidly. Attempts to observe adult flies feeding on plant nectars at night in nature were unsuccessful. Attempts to find pollen grains on the body surfaces of field-collected adult flies (this might indicate their feeding on floral nectaries) were only marginally successful -- few pollen grains were ever found, and these were difficult to identify to species level.

Hypothesis A. The Piracicaba stock of the fly has not extended its population north of 29°N because it is from 23°S, and is a tropical strain that fares poorly in colder winters. A stock from farther south (colder winters) in South America might be better adapted to survival farther north (colder winters) in North America.

Hypothesis B. Energy sources (the food of adult flies) undoubtedly vary from place to place in the field, and these may limit the local abundance of fly populations even when mole crickets (the food of the fly larvae) are abundant.

The New Stock of Flies. In 1999, funded by the USGA, a stock of the flies (Osorio stock) was brought from nearly 30°S, near the town of Osorio in the Brazilian state of Rio Grande do Sul, to Florida. This was the farthest south that *Ormia depleta* was detected in southern Brazil in a survey in November-December 1998. To avoid any possibility of confusion, the old (Piracicaba) stock was evicted from the laboratory before the new (Osorio) stock was imported; progeny of the old stock may be trapped in the field in Florida when necessary for later comparisons. Rearing methods that had been developed for the old stock were now applied to the new stock.

The main initial problem with the Osorio stock of *Ormia depleta* was that few female flies became gravid at each generation. For some reason, the rearing conditions were not ideal. Over 8 generations of flies were reared in the laboratory until the percentage becoming gravid had risen from 1.3% to about 10%, which still was far from ideal. During this time, there was strong selection pressure for ability to reproduce under the laboratory conditions, because those flies that did not reproduce failed to pass on their genes. There was no simple alternative to this selection pressure even though it may have eliminated genetic variability in desirable traits. By the fall of 1999, the improved ability to rear the Osorio stock allowed shipment of token numbers of adult flies to collaborators in Louisiana and Georgia.

Subsequently, in early summer 2000, shipments of adult flies (gravid females) were made to collaborators in North Carolina and Texas, and to Georgia and Louisiana again. A week or so following each shipment of adult flies, approximately 200 fly puparia were shipped to each of these collaborators. Adult flies were shipped overnight by Federal Express, and puparia by 2-day Federal Express, all in chilled containers. Adults were released on the evening of receipt. Collaborators set up puparia in containers, held them for emergence of adult flies, and released the adults into the field. The collaborators selected the sites of release. Unfortunately, for family reasons, our South Carolina collaborator could not participate. The delay in making these shipments and releases (from 1999 to 2000) makes it better to begin monitoring for establishment of populations of flies (by attempting to trap, and thus killing, adult females) in 2001 rather than 2000. We are prepared to continue the shipments in 2001 if this proves necessary.

New Findings. If the Osorio stock of *Ormia depleta* is avoid the effects of colder winters, one method it might have would be to spend the winter months underground in diapause (hibernation) in the pupal stage. The stimulus for induction of diapause varies among insect species. One of the methods used by insects is that declining daylight hours in the fall induce diapause, and increasing daylight hours in spring bring the insects out of diapause. A 7-week experiment was designed to test this possibility. In mid-summer in northern Florida there are approximately 14 hours of daylight (10 of darkness) and in mid-winter the reverse of this. Therefore, beginning in July 2000, cages of newly-emerged adult flies were exposed in a window (at room temperature) to normal daylength. A control set was exposed to 10-hours of daylength, by covering cages for 4 hours (until 10:30 am, based upon time of local sunset and sunrise) in the early morning with double, large, black, plastic bags. When female flies in the cages became gravid, their larvae were extracted "by Caesarian section" and "inoculated" onto mole crickets. The "inoculated" mole crickets were immediately exposed to the same light regimes, as were the fly pupae that were produced. The duration in days of each fly pupa was recorded. The test was of whether the fly pupae exposed (their parents were exposed, they themselves were exposed as larvae inside mole crickets, and they themselves were exposed as pupae) to 10-hour daylength would spend substantially

more time in the pupal stage than those exposed to 14-hour daylength. The answer was negative. Therefore, it seems that if diapause occurs in these fly pupae, it is not initiated or terminated by daylength alone.

Graduate student Hector Cabrera is investigating effects of temperature on development of *Ormia depleta* and has a lot of preliminary data. He will investigate combined effects of temperature and daylength to expand the results shown above.

Graduate student Craig Welch is investigating energy sources used by adult *Ormia depleta*. He has a lot of preliminary data.

New Equipment. Electronic devices that produce artificial mole cricket song were conceptualized in the early days of the University of Florida's mole cricket research program. Initially they were used as the "bait" for mole cricket traps. Later they were found to be the only practical method for attracting gravid female *Ormia depleta*, and are essential to this program. Various models were developed and/or produced by four Florida-based electronics specialists during the University of Florida's program. Unfortunately, not one of these electronics specialists manufactured them to meet our schedule: orders placed with time-limited funding available were very rarely filled on time, and were usually months or years late, or were never filled. This played havoc with our research plans and available funding. Our efforts also affected collaborators in other states who needed these devices. There is new hope: a company plans to produce such devices as a part of a control method for mole crickets (rather than as a research method). If all goes well, this should result in a commercial supply of the devices. More information will be provided after production begins.