## 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

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## 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.