

## A cold-tolerant strain of *Ormia depleta*

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**Introduction.** *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. Three classical biological control agents were introduced from South America, and released in Florida in the 1980s against these pest mole crickets. 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 which will provide permanent free area-wide biological control of the target pest. The cost of classical biological control is all subsumed under the heading "research."

A tropical stock of *Ormia depleta* was imported from Piracicaba, Brazil (23°S) in 1987 and first released in Alachua County, Florida, in April 1988, by the University of Florida mole cricket research program (at that time funded by a State of Florida appropriation). In 1989-1991, further laboratory rearing of the fly was supported by 28 Florida golf courses (sponsored by the Florida TurfGrass Association). In that project, about 10,000 flies were reared and released in all areas of Florida. In summer 1992, a survey showed that the fly had established populations widely in central and southern Florida. By the end of 1994, the fly was found to occupy 38 counties of peninsular Florida, as far north as Alachua County. It contributed to control of pest mole crickets on Florida golf courses (see Florida Turf Digest 11(1): 21-24). Research on the fly then almost ceased for lack of funding. Nevertheless, "starter stocks" of the fly were provided to and released by collaborators in Alabama, Georgia, and North Carolina, but there was no evidence these became established.

Fig. 1. Adult *Ormia depleta* established in central and southern Florida as a classical biological control agent of pest mole crickets.

**Evidence for effect of *Ormia depleta*.** Evidence presented (of suppression of pest mole cricket populations by *Ormia depleta*) in Florida Turf Digest 11(1): 21-24 is statistically significant for golf courses. Furthermore, each female fly is capable of producing several hundred larvae, each of which is capable of killing a mole cricket. Thus, the 1400-1800 adult female flies trapped each year at the University of Florida Gulf Coast Research Station (Bradenton) represent tremendous local killing power. If such numbers of flies are widespread in numerous localities in central and southern Florida, then very large numbers of mole crickets must die as a consequence. Unfortunately, the University of Florida mole cricket research program now has no other permanent trapping stations in central and southern Florida because operators relocated. We can, however, use temporary traps to show presence of *Ormia depleta* on any preselected night. Golf course managers in general have been less than enthusiastic about *Ormia depleta*: they cannot see it because it is nocturnal, and they cannot readily assess the benefit it provides. Only one Florida golf course superintendent (Steve Kuhn, when he was at Doral), was provided with traps for *Ormia depleta*, and he became convinced of the fly's efficacy.

**Advantages of *Ormia depleta*.** This fly seems to be totally host-specific, harmless to non-target organisms. Furthermore, it seems to be self-sustaining: it was introduced in 1988 and remains

established in the area of peninsular Florida to which it is adapted, so there is no point in releasing more flies in that area.

**Hypothesis.** The current northern limit of *Ormia depleta* is in Alachua County, and the fly has been shown to be present in all the other counties at the same latitude and farther south, but no farther north. Furthermore, occupation of this northern layer of counties seems to be seasonal -- in the fall -- requiring recolonization each year from farther south.

Fig. 2. Current distribution of the mole-cricket-killing fly *Ormia depleta* in Florida.

The stock of the fly that was released came from Piracicaba (at 23°S) which is in a tropical zone, whereas Alachua County is at 29°30'N, which is in a temperate zone. Thus, a cold-hardy strain of the fly, from farther south in South America (cooler climate), might be better adapted to persist in northern Florida and states to the north in the winter.

**Basis for the hypothesis.** Fly larvae develop in pest mole crickets, and develop more slowly at cooler temperatures. Fly pupae develop in the soil and develop more slowly at cooler temperatures. Fly pupae may even be able to spend the entire winter in a dormant state (called diapause) in the soil. Diapause is known in many insect species, but perhaps a more familiar example is from vertebrate animals: in the northern USA, bears hibernate. Perhaps in cooler parts of South America the fly may have populations that can diapause. So, let us look at the capabilities of *Ormia depleta* flies from at least 29°S in South America

**The search in the far south of Brazil.** Armed with the thoughts above, practical knowledge of mole crickets, and funds from USGA, Will Hudson (University of Georgia) and I flew to Porto Alegre, Brazil, then journeyed by road to Pelotas (almost 32°S), in November 1998. We had the necessary importation permit for *Ormia depleta* from USDA/APHIS and we were finally able to obtain a Brazilian collection and export permit. Our Brazilian host, entomologist Luiz Salles, made everything possible. Our first 2 weeks were largely spent trying to collect enough mole crickets to act as hosts for the flies we hoped to collect. Most sites we visited yielded very few mole crickets. The lucky discovery of some sand pits with many mole crickets (Fig. 3) made possible the collection of over 200, which we housed and fed in plastic buckets in Luiz' laboratory. From the beginning of our stay, we tried to catch *Ormia depleta* at night with two sound-emitters (which emulate *Scapteriscus* song and attract gravid female flies), but we were totally unsuccessful in the first 2 weeks, whereupon Will returned to Georgia.

Fig. 3. Howard Frank excavating mole crickets from sand pits near Pelotas, Brazil, November 1998.

I remained for a further 2 weeks. In a rented car, I drove the southeastern part of the state of Rio Grande do Sul and, with dusk approaching, I set the sound emitters by the side of some river or other likely spot on some lonely country road. I caught no *Ormia depleta* until 4 nights before I was due to leave Brazil. That night, on the road to Capão de Canoa, north of Osório, I caught 3 flies. In the morning, I hurried back to Luiz' laboratory, more than 4 hours to the south, and we dissected the flies and inoculated the larvae they contained onto the stockpiled mole crickets. I still had a few days in Brazil, so 2 nights later I was back in the same spot, this

time to my chagrin catching only one fly. Its progeny were inoculated onto mole crickets in Luiz' laboratory late the next day. My time was up, but I had no permit to export mole crickets from Brazil -- only flies. So the infected mole crickets were left in charge of Luiz, who promised to ship to me (in Florida) any immature flies (they would be pupae) that emerged. I left Brazil, and I left the sound-emitters in charge of Luiz who said he would run them some more in hope of catching more flies.

After much discussion by e-mail, Luiz shipped the fly pupae he had obtained to me. They came by post office express and were due to arrive before Christmas. I visited the Gainesville post office every day and learned where I could (at back of the building) obtain fastest delivery of parcels. Two days after Christmas, the package arrived. There were only 48 fly pupae in it, because (for unknown reasons) vastly fewer fly larvae had emerged from the more than 160 inoculated mole crickets than we had expected. This was bad, because these flies, as adults in a cage, won't participate in sex unless it is group sex, and to them 50 or more is a group. In quarantine in Gainesville, the adult flies began to emerge from the pupae into a cage. But only 25 adult flies emerged -- the rest had died as pupae. There was no sign of mating. No progeny were produced!

I had left 2 sound-emitters with Luiz. In the latter part of January he sent me another e-mail message to say that he had stayed at a country hotel near Mariluz, east of Osório, and taken the sound-emitters with him. He ran them in the grounds of the hotel and, in one night caught 8 flies. If he could catch 8 flies in one night, then how many flies could I catch in a week? Could I catch flies alive and bring them directly to Florida? (Forget about dissecting them and inoculating their larvae onto mole crickets). So, a week later I arrived again in Porto Alegre, whereupon Luiz drove me to the hotel and left me. I had built some portable traps to surround the sound-emitters, in attempt to catch flies alive (those Luiz had caught died within a few hours because of the method of trapping them in a sticky substance). My return flight was 7 days after my arrival, and the last night I would have to spend in Porto Alegre to catch the early morning flight. For the first 3 nights, using the same places Luiz did, and using 3 sound emitters now, I caught no flies. This was surely a cruel joke! But the 4th, 5th and 6th nights I caught flies and tried to keep them all alive in a cage in my hotel room. Not an easy task when the hotel management was urging me to use an electrical insecticide dispenser to kill mosquitoes that were entering the room through louvre windows! By the morning of the 7th day I had 7 living flies in the cage (2 had died). All 7 made it alive, on ice, on the long journey back to Florida (including bus journey in Brazil, overnight in Porto Alegre, layover in São Paulo, arrival in Orlando, and car journey to Gainesville). They were dissected in Florida, the following morning, and the larvae they contained were inoculated onto about 200 mole crickets that had been made ready from a stock in my laboratory. Will Hudson drove from Tifton, Georgia, to help in the dissection and inoculation.

**The new stock of flies.** Let's call this the Osório stock because Mariluz does not show on the best road map I have of Brazil. Osório is at nearly 30°S latitude. The original stock imported into Florida was from Piracicaba at 23°S -- that latitude is well over 500 miles north of the latitude of Osório. Development and survival of the fly larvae in mole crickets in my laboratory was excellent -- almost 600 adult flies were obtained. Then came the new problem: only four of the roughly 300 female flies became gravid (pregnant). The others died without progeny. We had used the same methods for them that we had used successfully over more than 10 years for the earlier (Piracicaba) stock. How could we ever provide enough gravid flies for releases in other

southern states when we could (only just) obtain enough gravid female flies to continue the laboratory colony? This new (Osório) stock was surely different, but not in a way we had foreseen.

As we reared the Osório stock, generation after generation, the proportion of gravid females gradually increased. After all, any female that did not mate failed to pass on its genes to the next generation, so, there was strong selection pressure. After a few generations, we even were able to supply a few token gravid female flies to Will Hudson (Georgia) and Seth Johnson (Louisiana) for releases in those states, but we are still very far short of being able to provide 100 gravid female flies per generation for release in one of the other southern states. We are slowly getting there, but the cost is high. At each generation of flies, we use about 200 laboratory-reared mole crickets, at a cost of roughly \$1000 in labor. Thus far, we have reared 8 generations of flies. We are now getting about 10% gravid female flies per generation, far better than the initial 1.3%, but yet not nearly high enough.

**Summary and Plans.** We have a new stock of *Ormia depleta* flies in culture from 30°S in southern Brazil. We call it the Osório stock. Established in February 1999, it has been through 8 laboratory generations. We now have permits for release of the Osório stock of flies in southern states, so the flies are no longer in quarantine and their care is easier. A student (Hector Cabrera) working with us has begun a behavioral comparison of the Osório stock with the old (Piracicaba) stock (which we no longer routinely maintain in culture). Release of the Osório stock in other southern states has been delayed because of the low proportion of the Osório stock becoming gravid in each laboratory generation. The proportion of flies becoming gravid in each generation has increased eightfold since the initial importation. However, if we cannot increase it substantially more, we will resort to the method that we used in 1987-1992 for release of the original stock of flies in Florida: this was to place fly pupae (not adults) in the field, and let the adult flies emerge and find mates in the field (instead of releasing gravid female flies ready to attack mole crickets). After all, the original method was successful in establishing populations in Florida.