AN AUTOMATED SYSTEM FOR COLLECTION AND COUNTING OF PARASITIZED LEAFMINER (DIPTERA: AGROMYZIDAE) LARVAE

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The leafminers *Liriomyza sativae* Blanchard and *L. trifolii* (Burgess) are serious pests of a wide variety of vegetable and ornamental crops in the United States and many other countries (Minkenberg & van Lenteren 1986, Spencer & Steyskal 1986). The braconid larval-pupal parasitoid, *Opius dissitus* Muesebeck, parasitizes both *L. sativae and L. trifolii* (Coly 1984, Lauridsen et al. 1993, Petitt 1993) and occurs commonly in Florida (Schuster & Wharton 1993, Schuster et al. 1991). *Opius dissitus* has been reared and released for augmentative biological control of *L. sativae* in greenhouses in The Land at Epcot, Lake Buena Vista, FL since the mid 1980's (Petitt 1993).

In The Land's insectary, *O. dissitus* is reared using *L. sativae* as the host on bush lima bean (*Phaseolus lunatus* L. cv. Henderson) primary leaves. Leafminer larvae in which *O. dissitus* has oviposited complete their larval development, cut a hole in the upper leaf surface, crawl out, and drop from the leaves. As larvae drop from the leaves, they are collected in a small vial at the base of a large funnel. The parasitized leafminers remain in vials in the insectary until just before eclosion of the adult parasitoid, at which time the vials are transported to the release site.

Research on the life history and biological characteristics of both the host (Petitt & Wietlisbach 1992, 1994) and parasitoid (Petitt & Wietlisbach 1993) has made rearing more cost effective by reducing the amount of time and plant material required per parasitoid produced. Decreased production costs have enabled use of the parasitoid to be expanded to include biological control of *L. trifolii* in over 100 flower beds (approximately 200,000 susceptible plants per year) in the landscape throughout the Walt Disney World Resort.

Mortality problems were encountered in rearing as production expanded and larger numbers of parasitoids were collected per vial. In some instances, eclosing adult wasps could not free themselves from the interior of large masses of puparia which were stuck together. Changing the collection vial several times per day to avoid mortality from overcrowding was inefficient. An automated system was sought to count the larvae and change vials as their capacity was reached. Accurate counts of parasitoids would also facilitate evaluation of augmentative releases.

The Electronic Grain Probe Insect Counter (EGPIC) system which was designed to monitor insect infestations in stored product facilities (Shuman et al. 1996) was evaluated as a means of counting *L. sativae* larvae. The EGPIC system consists of an infrared beam sensor head through which the insects fall, infrared beam generation and detection circuitry, data storage and a user interface. The objectives of this study were 1) to determine whether, and how accurately, the EGPIC system could count *L. sativae* larvae dropping through the sensor head and 2) to modify the system so that when a parasitoid collection vial is filled to its designated capacity the system responds by placing an empty vial into position at the base of the funnel.

Preliminary studies conducted with the EGPIC system showed that it could accurately count leafminer larvae as they dropped through the sensor head. System modifications were made and software was written to integrate the EGPIC sensor head (described in detail by Shuman et al. 1996) into a leafminer collection system (Fig. 1). The system counts parasitized larvae as they drop through a funnel into one of a ring of collection vials mounted on a turntable with a registered advance. A detection/interface circuit connects the sensor head output to a personal computer which directs

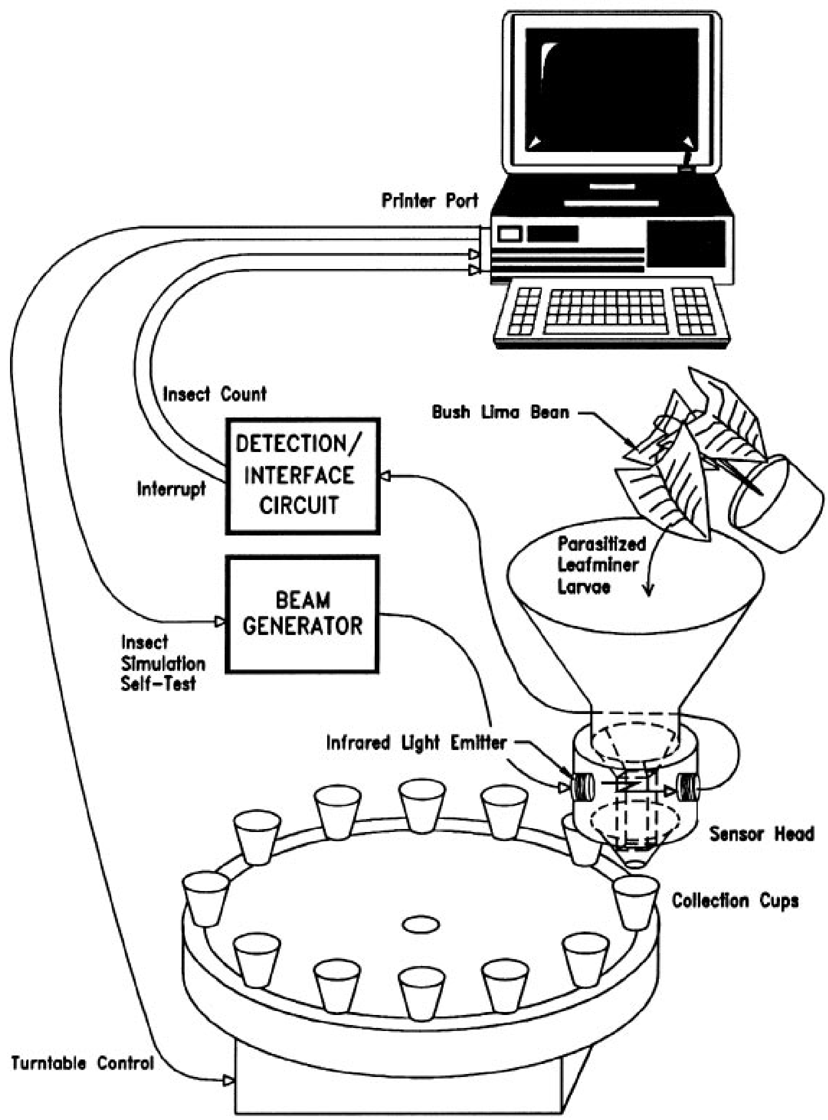

Fig. 1. Diagram of the automated counting and collection system for parasitized leafminer larvae dropping from plant foliage.

system operation. When a vial has received the designated number of larvae, the computer rotates the turntable to start filling the next vial. The system also creates a database of time-stamped insect counts.

Description of Leafminer Collection System Components

The sensor head uses an infrared light emitting diode to produce a beam that is sensed by a phototransistor. The beam traverses a rectangular channel through the sensor head. The fall of an insect through the channel partially masks the beam and results in a signal on the phototransistor output. The channel is 45-mm long; a plastic insert that spans its entire length provides for surface smoothness and minimizes the possibility of insects getting caught in the channel or producing multiple counts. The cross section of the channel (11×3 mm) is narrower than the sensor elements' diameters (5 mm) to obscure the weaker edges of the beam, and thus, enhance the uniformity of detection sensitivity across the channel. The strength of the beam and the quiescent operation point of the phototransistor are set to optimize performance for this particular larval size. The detection/interface circuitry responds to phototransistor output signals above a threshold level (user adjustable and employing hysteresis for noise immunity) by generating a (digital) count pulse (on a line dedicated to that sensor head) read by the computer.

In its current implementation, the computer's printer (parallel) port is configured by software for system digital signal reads and writes. In this way, the computer can service up to seven sensor head/turntable units. For larger implementations, a commercial, digital I/O board would have to be installed in the computer. The number of units that may be serviced by a single computer with a commercial digital I/O board is dependent upon the rate insects are falling and the speed of the computer, but these factors were not addressed in this study.

The system is interrupt driven so the computer is relatively idle (taking care of clocks, other overhead functions, and available for other chores, such as user utilities) until a falling insect causes an interrupt. The interrupt driven design (as opposed to a polling design) also allows the computer to respond immediately to a falling insect so that a quick succession of counts can be acquired without error. The interface circuitry generates an interrupt pulse (on a common line for all sensor heads) to notify the computer when any sensor head output is detected. The computer then checks all the lines dedicated to individual sensor heads for a count pulse so that the computer can then determine and record through which sensor head an insect dropped. In addition, the computer periodically initiates a test of system operability by momentarily reducing the output of the infrared beam generator to simulate the fall of insects simultaneously through all sensor heads, and then checks for appropriate interface circuitry outputs.

The computer's functions are supervisory as well as data acquisition, storage, and presentation. A configuration screen allows the user to change various parameters affecting data acquisition. For example, the 'Retrigger Threshold' is the amount of time that must pass between two consecutive insect counts from the same sensor head in order for the second count to be registered. The purpose of this threshold is to prevent one insect from being counted twice, and thus, it also determines the maximum insect count rate. The default count rate is 10 insects per second. The 'Turntable Rotation Control Signal' can be specified to accommodate different vial sizes and the 'Insects Per Vial' parameter can be set to any value desired.

During data acquisition, a user interface/status screen displays operational data in real-time and allows for data file annotations. The resulting data file contains time-stamped insect counts, and all other system events, such as starting acquisition, selftesting, retriggers, insect counts during turntable rotation, and data backup to hard disk. Additional user options include data backup time interval, auto archiving to any drive, and an audible indication of insect counts. The information in a data file can be presented in many ways, such as summarized, ordered by time or sensor head, and stripped of system events, and it can be exported to a printer or a spreadsheet program.

System Verification and Operation

A leafminer collection system with two sensor head/turntable units was initially tested by setting the 'Insects per Vial' parameter at 20 insects and running the system for eight days, during which time 53 and 50 vials were collected on units 1 and 2, respectively. The mean number of larvae collected per vial (±S.E.M.) was 19.5 (±0.2) for unit 1 and 19.8 (±0.2) for unit 2. The number collected on each turntable unit ranged from 17 to 23 insects. Given that these results were considered acceptable for the desired purpose, this two-unit system was installed at The Land at Epcot on February 17, 1994 and the system is still operating at present.

Three further sets of counts were conducted at five, 13, and 19 months after installation at The Land. In each case, counts were made of actual numbers of pupae per cup for ten vials that were filled when the 'Insects per Vial' parameter was set at 200 insects. The mean (±S.E.M.) of the ten counts were 201.2 (±2.6), 197.6 (±2.8), and 200.6 (±2.6) insects. Counts ranged from 184 to 214 insects per vial for the 30 vials. Overall, the accuracy was well within what would be acceptable for this purpose.

The system has been very reliable. It has been used for the past 20 months to count and collect about 13,000 parasitoids per week into vials containing 200 each. Only three operational problems have occurred during this entire period. In each case, the rectangular channel became obstructed (once by a group of larvae, once by a piece of bean leaf, and once by soil from the potted lima beans). Of course, there is an upper limit to the number of larvae that can pass through the channel per unit of time without it being obstructed; however, no attempt was made to quantify that upper limit.

The system has facilitated wider scale and more efficient use of the parasitoid because of easier and quicker distribution and has greatly improved our ability to evaluate parasitoid releases.

Summary

Efficiency of augmentative biological control of *Liriomyza* leafminers has been improved by development of an automated system for collection and counting parasitized larvae as they drop from plant foliage to pupate. The system uses an infrared beam to count larvae as they drop through a funnel into one of several collection vials on a turntable. When a vial has received the designated number of larvae, the turntable rotates so that the next vial will begin to fill. Operation and tests of the system for over two years have shown that it is very reliable and accurate.

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