A SYSTEM OF CAGING *THrips palmi* FOR LABORATORY BIOASSAY OF PATHOGENS

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*Thrips palmi* Karny (Thysanoptera: Thripidae) is an Asian species considered native to the Malaysian-Indonesian region. It is now found throughout Asia, Africa, Central and South America and the Caribbean. *T. palmi* has been present in the Caribbean since 1985 (Anonymous 1989) and is a serious threat to vegetable production in the region. It attacks over fifty plant species including eggplant, sweet peppers, cucumbers, melons, pumpkin, squash and beans as well as other commercially important crops (Wang & Chu 1986). Reports from Puerto Rico, Guadeloupe and Trinidad and Tobago indicate that *T. palmi* caused losses ranging from 50-90% in vegetable plantings (Franqui et al. 1991, Etienne and van Watermuren 1991, Jones 1990). Chemical control of *T. palmi* has not been successful due to resistance to a range of insecticides (Guyot 1988, Jones 1990). Consequently, we are currently evaluating pathogens for the control of *T. palmi*. So far, a new species of *Hirsutella* Patouillard has been discovered attacking *T. palmi* in Trinidad (Hall, 1992) and other fungi such as *Verticillium lecanii* (Zimm.) Viégas and * Beauveria bassiana* (Bals.) Vuill. cause disease in related *Thrips* species (Binns et al. 1982, Gillespie. 1980). In order to simply, reliably and accurately bioassay the abilities of such pathogens to infect *T. palmi*, a new caging system has been developed and is described in this note.

In order that a caging system for pathogen bioassay be of value, the following criteria must be met: (i) the insects must be individually contained to eliminate the risk of cross-contamination; (ii) the insect should be able to move freely within the chamber; (iii) the system should permit clear observation of the insects at all times; (iv) there should be sufficient air exchange to supply the needs of the insect and leaf tissue and to prevent condensation (in which small insects such as thrips may drown) on the walls of the chamber or on the leaf discs; (v) the substrate, normally leaf tissue, must be maintained in a palatable form for the duration of the bioassay; (vi) there should be minimal handling of the insect; (vii) the control mortality must be within acceptable limits (below 10%); (viii) the cages must be escape-proof and; (ix) the system must be reasonably simple to set up so that large numbers of assays may be performed without undue difficulty. Meeting these criteria is especially important when working with a small, highly mobile insect such as *T. palmi*.

There is a variety of caging systems for thrips. Most of these have been developed to assay insecticides, to study life-cycles or rear thrips. These range from group rearing on whole leaves in mesh and plastic clip or sandwich cages to individual rearing in glass vials such as the Plaster of Paris block cage (Lewis 1973). The latter cage contains pieces of leaf or grass spikelets kept in glass tubes with one end embedded vertically in plaster and the other plugged with cotton wool wrapped in gauze to prevent the thrips from becoming entangled in the fibres. This is an excellent technique, as are those described by Morse et al. (1986) and Teulon (1992) but all these methods have some disadvantages such as becoming unwieldy when dealing with large numbers of
insects during routine bioassays or thrips not being easily visible. Gillespie (1985) developed a system to bioassay fungal pathogens of *T. tabaci* Lind. This method used leaf discs supported on water agar in individual compartments of a 10 x 10 x 2 cm compartmented square petri dish, covered in “clingfilm” with a small perforation for gaseous exchange. This system was unsuitable for *T. palmi*, a much more mobile pest, over 90% escaping from the dish or becoming trapped in the moisture on the surface of the agar. In addition, problems with saprophytic bacteria or fungi growing on the water agar and the leaf discs were encountered.

Consequently, a new caging method had to be developed which met the specified criteria: Plaster of Paris was used as the matrix for supporting the leaf discs. Plaster was added to 50ml of water, stirred for 15-30 seconds and poured immediately into the cover of a compartmented square petri dish. Pumpkin leaf discs (1.2 cm in diameter) were then quickly aligned to the compartments (one disc per compartment, ventral side facing up) in the petri dish base and gently pressed onto the wet Plaster of Paris.
forming a good seal between the edges of the leaf discs and the plaster. The compartmented base was inverted above the discs and pressed into the wet Plaster of Paris (Fig. 1). It is important that the plaster be sufficiently wet to form a complete seal around each leaf disc as well as around each compartment to prevent any chance of thrips becoming hidden from view during the assay or escaping. The precise quantity of Plaster of Paris cannot be stated since the requisite amount varied both between and within batches of plaster; a useful guideline is to add just sufficient plaster to absorb all the visible water. The leaf discs should be taken from mature leaves since expansion of younger leaf material continues in the Plaster of Paris matrix, causing buckling of the disc and thereby destroying the seal. After the plaster has set, the cover of the dish (Fig. 1) is gently prised off. The dish now consists of 25 tightly-sealed compartments on a solid plaster tile. After the initial condensate (formed from the setting of the plaster) has dried, the thrips are introduced on to the discs (Fig. 2) in the compartments, using a pig’s bristle paintbrush (trimmed to a few hairs) through holes (6mm in diameter) drilled into the corner of each compartment. Beforehand, these holes must be covered with “3M Medical Micropore Tape” (3M Centre, St. Paul, Mn.). The inner surface of the tape must be lightly dusted with talcum powder before the dish is sealed into the Plaster of Paris since this prevents the insects from becoming trapped on the adhesive material. After introducing the insects, the hole is resealed with the tape. Since the holes and tape occupy only a small proportion of each individual chamber, the thrips can still easily be viewed. The whole tile is placed on moist cotton wool to hydrate the plaster. This system has met all our requirements. Thrips are easily handled and observed, they cannot escape and the method is not time-consuming. Also, the leaf discs remain in good condition for up to 10 days (25°C, 100%RH) and do not become overgrown by saprophytes. Furthermore, in our conditions, condensation does not form after introducing the insects.

Fig. 2 Cross-section through individual cell of compartmented container for T. palmi:
(a) micropore tape; (b) hole in corner of compartment; (c) compartment wall embedded in plaster; (d) insect on leaf disc; (e) pumpkin leaf disc; (f) plaster tile; (g) moist cotton wool; (h) plastic tray.
We believe that this is the first relatively simple system developed for satisfactorily caging *T. palmi* so that microbial agents may be bioassayed. So far, in 6-day assays, control mortalities of either adults or larvae have been reduced from 90% with the simple systems developed for other thrips species, to less than 15% in our system. In addition, we observed that this 15% mortality was due to fungal infection probably brought in from the field. To avoid this problem, we have developed a laboratory mass-rearing technique (to be described elsewhere) to supply clean, uncontaminated insects for bioassay. Maximum control mortalities are now 8%. This technique may be used for studies on the life history of *T. palmi* or modified to bioassay chemicals or pathogens of other, small highly mobile leaf-feeding arthropods such as mites, aphids, other thrips and leafhoppers. For sessile insects, e.g. whitefly immatures, the system may be simplified further; an ordinary circular petri dish containing as many leaf discs on plaster as desired may be used.

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DIFFERENTIATION OF NYMPHAL INSTARS IN SCHISTOCERCA AMERICANA (ORTHOPTERA: ACRIDIDAE)

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American grasshopper, Schistocerca americana (Drury), the most destructive of Florida's grasshoppers, only occasionally reaches outbreak status. Because of the infrequency of crop loss, it is poorly studied. During 1991, American grasshopper caused significant loss to citrus and ornamental plants in west-central Florida (Pasco, Polk, Sumter and Hernando Counties) and limited damage to field crops and ornamentals in north-central Florida (Marion, Levy, Gilchrist and Alachua Counties). As area-wide suppression efforts were initiated it became apparent that the dearth of past research had resulted in some inadequacies in fundamental information about American grasshopper. For example, the number of nymphal instars has been reported as 5 (Griffiths & Thompson 1952) and 6 (Kuittet & Connin 1952). In the more detailed latter study, the authors acknowledged that time did not permit rearing and that their report was not definitive. Here I report a more detailed determination of instar number and provide a key for their determination.

Laboratory rearings were conducted with eggs produced by adults collected at Dade City (Pasco Co.), Florida, in June 1991. Upon hatching, some hoppers were reared individually (N = 20) in 250 ml vented plastic containers; each contained Romaine lettuce (Lactuca sativa), bahiagrass (Paspalum notatum), and dry diet mix consisting of wheat bran (50%), whole wheat flour (25%) and soy flour (25%). Two groups of about 25 nymphs were also reared as groups in 15 x 15 x 15 cm screen cages and fed lettuce, grass, and dry diet. Hoppers were maintained at 30-32°C and a photoperiod of 16:8 (L:D). Cages were checked daily to ascertain whether grasshoppers had molted; instars were simply recorded for nymphs reared individually, but newly molted nymphs were transferred to other cages for group-reared hoppers. In the latter case, these transfers allowed separation of instars but maintenance of density effects.

Field collections were made in June, July, August, and September of 1991 and frozen for later examination. About 100 nymphs from each collection were sorted and compared to instars determined from laboratory rearings.