NOTE

Technique for the Collection of Adult Parasitoids and Detection of Parasitoids in Fourth-Instar Bandedwinged Whitefly (Homoptera: Aleyrodidae)[1]

Jason B. Oliver[2] and Michael J. Gaylor

Department of Entomology and Alabama Experiment Station, Auburn University, AL 36849-5413 USA

J. Entomol. Sci. 36(1): 97-100 (January 2001)

Key Words Aleyrodidae, Bandedwinged whitefly, *Trialeurodes abutilonea,* dissection, parasitoid, *Eretmocerus, Encarsia*

Parasitoids that attack whiteflies (Homoptera: Aleyrodidae) frequently are collected and held for emergence by placing infested leaves in some type of container (Dysart, 1966, Ann. Entomol. Soc. Amer. 59:28-33; Noyes, 1982, J. Nat. Hist. 16: 315-334). However, specimens may be damaged by storage pests (e.g., psocids) or mold growth, or adult eclosion may be reduced by leaf desiccation (Rose, 1990, pp. 229-234, *In* D. Rosen [ed.], Armored scale insects their biology, natural enemies and control Vol. 4B., Elsevier, NY). When large volumes of lightly-infested leaf material are collected, emerged parasitoids may be difficult to locate among the leaves. Additionally, if multiple whitefly species or cryptic insects (e.g., agromyzids) are present, parasitoid host origin is uncertain (Noyes 1982, Rose 1990).

Regardless of how whitefly nymphs are held, some parasitoids may die within the host. Thus, a method of detecting unemerged parasitoids is needed for accurate estimates of percentage parasitism or for determination of species importance. The most direct way of detecting unemerged parasitoids is visual examination of the host. In the bandedwinged whitefly, *Trialeurodes abutilonea* (Haldeman), the color of the nymphal case and abdominal fatbodies and the position of compound or ocellar eyes within the nymphal case have been used to visually determine parasitism (Watve, 1971, The biology and control of the banded-wing whitefly, *Trialeurodes abutilonea* (Haldeman), on cotton in Louisiana. Louisiana State Univ. Ph.D. Diss. 137 pp.). However, specimen color also may be affected by the host plant of the parasitized whitefly (David and Ananthakrishnan, 1976, Curr. Sci. 45: 223-225; Liu and Oetting, 1993. Res. Bull. Num. 412, Georgia Agric. Exper. Stn.) by stage-specific variation of the whitefly or parasitoid (Gerling, 1966a, Can. Entomol. 98: 707-724; Gerling, 1996b, Can. Entomol. 98: 1316-1329) and by specimen desiccation. Parasitoid compound

[1]Received 24 January 2000; accepted for publication 26 April 2000.

[2]Nursery Crop Research Station, Tennessee State University, 472 Cadillac Lane, McMinnville, TN 37110 USA. To whom offprint requests are to be addressed (email: jasoliver@blomand.net).

eye position cannot be used to detect larval parasitoids or to make genus-level separations. Dissection of whitefly nymphs is slow, difficult with early-instar parasitoids, and unsuitable for desiccated specimens. Immunological and molecular techniques have been used to detect parasitoids within whitefly nymphs (Guirao et al., 1994, Boletin de Sanidad Vegetal, Plagas. 20: 757-764; Wool et al., 1984, J. Appl. Entomol. 98: 276-279), but these frequently require considerable development time and may be expensive. The technique reported here was developed after techniques typically used for mounting whiteflies were inadequate for *T. abutilonea* parasitoid detection.

Adult parasitoid collection. Fourth-instar *T. abutilonea* were randomly removed from host plants using forceps to separate a small section of leaf tissue with one or more nymphs. The leaf material with associated whitefly nymphs was glued to the base of a disposable plastic Petri dish (100 × 15 mm). The Petri dish was closed and sealed with Parafilm® M (American National Can, Greenwich, CT) after 2 to 3 d to allow excess moisture to escape. Moisture removal could be improved by using a screened Petri dish. Collection data were recorded on the Petri dish with a felt tip marker. Containers were held at room temperature.

Specimens were stored within the Petri dishes for at least 2 yr before slide mounting, and longer storage is probably feasible. Advantages of this holding method included convenient long-term storage and protection from destructive insects, minimal mold damage with adequate leaf-drying time, certainty of parasitoid-host-origin, facilitation of adult parasitoid location in holding containers, and potential to further process uneclosed specimens for parasitism estimates (as described below). The method was particularly valuable for organizing specimen collections and collecting from lightly-infested host plants. Problems with this method were its limitation to small sample sizes, potential for parasitoid escape from containers before Parafilm sealing, and mold damage when leaf tissue was not sufficiently dry. Using this method, we were able to plate a total of 13,696 fourth-instar *T. abutilonea* during two summer field seasons. Only 1,083 (7.9%) adult parasitoids eclosed. Although the fourth instar of the host is the final nymphal stage, many nymphs were still too immature to complete development (i.e., initial feeding portion of the stadium, characterized by a flattened-body shape). Therefore, a method for determining parasitism of the remaining 92.1% of the sample is desirable and is described below.

Detection of uneclosed parasitoids. Desiccated fourth-instar *T. abutilonea* were carefully removed from the leaf tissue within Petri dishes by pushing a forceps tip, moistened in 70% ethyl alcohol, beneath the specimen. Specimens were transferred to 70% ethyl alcohol in Pyrex® (Corning Inc., Corning, NY) glass test tubes (No. 9825). Test tubes were modified by removal of the base with a glass cutter and by insertion of a rubber stopper (size 00 covered with a piece of plastic sandwich bag for protection of rubber from chemical treatments) into the cut opening. Specimens were introduced into the threaded end of the vial, a piece of cloth was placed over the opening, and a vial cap with a hole drilled in the center was screwed over the cloth. The alcohol was removed through the cloth with a non-needled glass syringe. Technical grade hexane was introduced through the cloth with a spray bottle. The tube was shaken vigorously for ~20 s. Hexane was removed through the cloth with a non-needled glass syringe. Hexane residues were allowed to evaporate for a minimum of 10 h. Then, specimens were removed from the cloth and from the plastic covers on each end of the vial by scrapping with forceps. A nylon screen was placed over the threaded end of the vial, the vial cap was replaced, and the remaining specimens

were back-rinsed onto the screen with tap water introduced from the opposite end. Specimens were transferred to wells in porcelain plates containing a solution (5:1) of lactophenol and acetocarmine dye (Nur, 1990, pp. 367-370, *In* D. Rosen (ed.), Armored scale insects their biology, natural enemies and control Vol. 4A., Elsevier, NY). Specimens were soaked in the solution for ~15 h, and subsequently slide-mounted in polyvinyl alcohol (PVA). With adequate stocks of lactophenol-acetocarmine dye, this step could be efficiently completed in the Pyrex test tubes following hexane evaporation. Slides that were to be permanently kept were ringed with clear fingernail polish after ~1 mo of drying. Microscopic analysis was made using Nomarski differential interference lighting system at 10X to 40X magnifications.

Proper wax-removal and specimen clearing was necessary to adequately view developing parasitoids. Hexane and lactophenol were the most effective wax-removing and clearing agents, respectively. Tetrahydrofuran and xylene were used for wax removal on other whitefly species (Garrido et al., 1980, Boletin del Servicio de Defensa Contra Plagas e Inspeccion Fitopatologica. 4: 32-41; Pizza and Porcelli, 1993, Bollettino della Societa Entomologica Italiana 125: 3-5), but we did not test tetrahydrofuran, and xylene was not effective. Other wax-removing solvents that we tested and were unsatisfactory were ammonia, carnoy (chloroform:absolute alcohol-:glacial acetic acid, 4:3:1), chloroform, charcoal lighter fluid, ether, ethyl acetate, methanol, 2-propanol, and unleaded gasoline. Remnants of the lateral palisade wax layer frequently remained on specimens after hexane treatments, but ventral and dorsal areas usually were unobstructed by wax. Potassium hydroxide (KOH) and glacial acetic acid have been used for clearing aleyrodid specimens (Bink, 1979, Entomol. Berichten, Deel. 39: 158-160; Gerling 1966a; Pizza and Porcelli 1993). However, in our study, specimens with larval parasitoids detected before treatment with KOH were generally undetectable after KOH treatment.

Presence of *T. abutilonea* parasitoids was determined by one or more characters. Adult and pupal parasitoids usually were obvious, because of their darkened compound eyes and well-defined antennae and legs. The sex and genera of parasitoids generally could be determined by using the shape and segmentation of antennae or by genitalia (Gerling 1966a,b). Larval parasitoids were detected by the presence of a larval-body-outline, mandibles, spiracles, or crystalline objects possibly urate in origin (Gerling 1966b). The larval-body outline of older *Eretmocerus* larvae and pre-pupae were generally distinct. Specimens with poorly-contrasting body outlines (e.g., some early instars) could frequently be found using associated characters, such as the larval entry scar (Rose and Zolnerowich, 1997, The genus *Eretmocerus* [Hymenoptera: Aphelinidae]: parasites of whitefly [Homoptera: Aleyrodidae]. Texas A&M Univ.). The *Eretmocerus*-larval-entry-hole in the venter of *Bemisia tabaci* (Gennadius) retained its dimensions during parasitoid development (Gerling et al., 1990, Archives Insect Biochem. Physiol. 13: 247-253). During the early stadia, *Eretmocerus* larvae almost always were adjacent to the larval entry scar. A translucent zone and a capsule containing cells of unknown origin always surrounded *Eretmocerus* developing inside *B. tabaci,* generally through most of the second stadium (Gerling et al. 1990). The capsule apparently separates the parasitoid from the hemolymph of the whitefly and gradually disintegrates as the parasite ages. Some *Eretmocerus* specimens within *T. abutilonea* had a translucent zone between the parasitoid larva and the apparent outer capsule edge. Only fourth-instar *T. abutilonea* were examined in our study. Consequently, most *Eretmocerus* larvae probably were old enough that the capsule had begun to dissolve or had completed disintegration. Parasitism by an

Eretmocerus larva was indicated by the presence of a semi-circular or shield-shaped larva, by hook-like mandibles (Gerling 1966b), or sometimes by a larval entry scar. Mandibles were apparent only during the first stadium, possibly because the head and mouthparts are recessed during the second and third stadia (Gerling et al. 1990). Parasitism by an *Encarsia* larva was determined by the presence of a vermiform larva or by sickle-shaped mandibles (Gerling 1966a).

The larval clearing technique was advantageous when processing samples ≥30 specimens. Using this method, we were able to slide mount a total of 8,120 fourth-instar *T. abutilonea* during two summer field seasons. A total of 1,971 nymphs (24.3%) were determined to have unemerged adult or larval parasitoids. Although total processing time required 2 to 3 d, specimens were not handled during most of that period. Unemerged adult whiteflies and parasitoids often could be detected before chemical treatments, but processing extra specimens required little additional time and facilitated parasitoid identifications to genus. The ability to process dry nymphs allowed parasitism estimates to be made with either living or dead specimens. The clearing method also permitted detection of parasitoids with a small body size, such as first instars, that might be difficult to locate by dissection. Additionally, slide-mounting created a permanent record. Frequently, a white, "fat-like" material was visible in parasitized specimens after placement in PVA. The white material permitted easy detection of parasitism, but was only briefly visible (i.e., ~ ≤5 min). A problem with this technique was the poor larval-body-contrast with some *Eretmocerus* and most *Encarsia* specimens. Usually, it was easier to detect *Encarsia* mandibles than to discern the outline of the encarsian-larval body.

We thank M. Cupp (Auburn University) for her assistance with microscopy and photography, and M. Rose (Montana State University) and M. Williams (Auburn University) for their assistance with this research.