

## INSECT REMOVAL FROM STICKY TRAPS USING A CITRUS OIL SOLVENT

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**ABSTRACT:** A new procedure using citrus oil was developed for removing both heavily sclerotized and soft-bodied insect specimens from sticky traps. The scales of adult Lepidoptera are usually left intact. Procedures for using standard techniques such as pinning, slide preparation, and genitalic dissection are also discussed.

Although sticky traps are used extensively for studying and monitoring insect populations (Peterson 1964; Murphy 1985), removal and identification of trapped specimens is difficult (Lindgren *et al.* 1983; Murphy 1985; Knodel and Agnello 1990). The sticky material in the traps, usually polyisobutylene (PIB), often obscures or distorts critical characters needed for accurate determination. Murphy (1985) tested the use of various solvents to remove sclerotized insects, such as Coleoptera and Hymenoptera, from sticky traps. He found that polar solvents are unsuitable and suggested several alternative nonpolar solvents including toluene, heptane, hexane, and xylene. Ethyl acetate, methychloroform, petroleum spirits, gasoline, and kerosene proved less effective. Because all these solvents are, to some extent, toxic to humans and flammable, they must be used under a fume hood in the laboratory away from flames or electric equipment. Besides the laboratory hazards, these solvents are ineffective for extracting adult Lepidoptera and soft-bodied insects (Murphy 1985).

Because some of the targets of the USDA exotic pest detection program are microlepidoptera, we sought alternative solvents and procedures for sticky trap insect identification. One potential alternative solvent is citrus oil, which has the initial advantage that it is on the GRAS (Generally Regarded As Safe) list of the Food and Drug Administration.

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## METHODS

We tested citrus oil marketed by three sources for effectiveness in the extraction of various orders of insects imbedded in PIB. Formulations included Durkee® lemon extract used as a food additive and purchased at a local supermarket; a histological clearing agent marketed as Histo-Clear® by National Diagnostics; and Livos® thinning agent #7222 sold by Livos Plant Chemistry Inc.\* Insect specimens used in the tests were those submitted on delta, Rebell®, and wing traps to the USDA Pest Identification Laboratory in Reynoldsburg, Ohio. The effectiveness of the formulations was observed for most orders of insects.

## RESULTS

All citrus oil formulations examined were viable alternatives to other solvents listed by Murphy (1985), although cost per unit volume varies widely. Our tests of various handling techniques suggest the following procedures for removing and identifying specimens from PIB.

**Screening Samples.** Sticky trap bottoms are most effectively screened with an illuminated magnifying glass mounted on a stable base. It is also advantageous to cover the work area with scraps of cardboard to protect the work surface from being fouled with PIB or scratched with a scalpel blade. If a stereomicroscope is used, protect the objective lens with a neutral density or polarizing filter to prevent contact with PIB.

**Insect Removal.** If the specimen is fresh and heavily sclerotized, for example a beetle, it may be lifted directly from the trap bottom and placed into solvent. A few drops of citrus oil on the trap bottom will loosen the specimen and ease removal. However, if the specimen is dry and brittle or soft bodied, it should be left untouched on the trap. The extraneous portion of the trap (and any excess PIB) surrounding the insect should be cut away with a scalpel before it is placed in the solvent bath. Movement should be minimized, because any distortion of the PIB will probably damage the specimen by pulling it apart. The volume of the solvent bath should be at least sufficient to cover the specimen. After a few hours the insect will float clear of the trap and PIB. Most specimens can be left in the solvent overnight until any residual PIB has dissolved. To prevent saturation of the citrus oil, the cut portion of the trap bottom should be removed after the insect has been freed. The length of time

\*FOOTNOTE. Mention of commercial products in this paper does not constitute a recommendation by the United States Department of Agriculture.



required in the solvent varies with the amount of PIB to be dissolved and the condition of the solvent. Solvent effectiveness will eventually decline when it becomes saturated and, consequently, the time required to remove the PIB will increase. Glassware (e.g., petri dishes) must be used because citrus oil will react with plastic.

One way to speed removal of the PIB is to use an ultrasonic cleaner. The insect is put in a small vial filled with solvent and placed in the ultrasonic cleaner with water. The vial is required for two reasons. It dampens the sound waves protecting fragile insects from excessive movement and potential damage, and it conserves solvent because it is not necessary to fill the whole tank. Most hard-bodied insects will be cleaned in 5-10 seconds. Wings of Lepidoptera can also be descaled in this manner for morphometric and venational studies.

Occasionally, a film of dried PIB will adhere to the specimen when it is removed from spent solvent. This residue can usually be removed by rinsing the insect in xylene and/or absolute ethanol. Leaving material overnight in fresh citrus oil is another option. Although specimens may be left in the citrus oil for extended periods without apparent damage, they do become more brittle after 24 hours.

**Pinning.** After allowing the specimen to air-dry for a few minutes, it may be pinned. If the insect must be relaxed before pinning, it can be immersed in water for a few hours (or in subboiling water for a few minutes).

**Alcoholic Specimens.** Insects which normally are stored in alcohol may be rehydrated by placing them in subboiling water for a few minutes before permanent preservation in 80% alcohol.

**Slide-mounted Specimens.** Very small specimens, e.g., springtails, thrips, mites, scale crawlers, some nematoceran Diptera, and Hymenoptera for which the preparation of slide mounts may be necessary, can be transferred directly from PIB into Euparal. Specimens that need to be cleared before mounting should be handled as in the above section, thus significantly reducing clearing time in KOH. Warming the Euparal prior to mounting is helpful.

**Preparation of Genitalia.** The following procedure can be used to prepare genitalia of moths trapped in PIB. Standard techniques discussed by Holloway *et al.* (1987) have been modified and shortened to save time in screening large samples.

1. Pull the abdomen from the trap substrate.
2. Immerse in citrus oil to clean specimen.
3. If still not free of PIB, return specimen to solvent for another 12 hours.



4. When cleaned of PIB and if time permits, place abdomen in 10% potassium or sodium hydroxide (KOH or NaOH) for 12-24 hours at room temperature. Alternatively, wear safety goggles and boil the abdomen in hot hydroxide until it is soft.

5. Wash abdomen in water, or preferably, a 5% solution of glacial acetic acid and water to neutralize the KOH or NaOH.

6. Place abdomen in 50% alcohol and mechanically brush scales from it.

7. Stain with mercurochrome or chlorozal E black if desired.

8. Either place specimen in vial of 70% alcohol, mount in Hoyer's solution, or clear and dehydrate the specimen for mounting in a resin such as Euparal or Canada balsam.

The time required to prepare lepidopteran genitalia varies extensively. Large moths generally require a longer KOH or NaOH bath than smaller moths. Typically, a large moth may require almost a day at room temperature, whereas smaller moths may need only a few hours. Therefore, we recommend monitoring the progress of maceration. For those unfamiliar with this technique, we suggest making trial runs with moths of various sizes before attempting to use this technique on actual unknowns. Specimens left in citrus oil too long will be brittle, while those left in KOH too long will be over-cleared and difficult to see.

In many cases where quick determination is required, "valve-ripping" may be utilized. In this procedure the genitalic valva is grasped at the base, pulled off the abdomen, placed in citrus oil, and then cleaned in alcohol. Identification of many genera of Tortricidae and Noctuidae can be confirmed by examining only the shape of the valve.

## CONCLUSION

The success of each preparation depends largely on the condition of the specimen when it is removed from the trap bottom. Fresh specimens that are shallowly embedded in the PIB provide the best results, but even those totally immersed can usually be recovered if they have not decomposed. Larger insects usually fare better, because small insects are more likely to become immersed and decay. Extensive struggle by the insect on the trap after capture often results in loss of setae and scales. Additionally, scales, setae, and wings are often dislodged if the specimens are manipulated before removal of the polyisobutylene. Specimens removed with citrus oil using the above procedures have been maintained for more than two years with no adverse effects.

Citrus oil offers distinct advantages over previously used solvents for removing PIB, and most orders of insects have been extracted suc-



cessfully from sticky traps using this procedure. Generally, the technique works well for all taxa tested, but a higher percentage of success occurs in fresh and more sclerotized specimens. Unlike solvents listed by Murphy (1985), citrus oil does not leave specimens unduly brittle, and subsequent laboratory and curatorial techniques can be easily accomplished after removal. Solvent toxicity is reduced or absent, although a fume hood is still recommended to avoid breathing the fumes. Most important, soft-bodied insects and Lepidoptera can be treated without damage, if properly handled. Several problems remain: citrus oil is flammable, the process remains time consuming, all specimens are not recoverable, trap bottoms are often not reusable, and no specimens are perfect "display quality." Other dry-trapping methods with screens to remove unwanted nontargets are recommended if specimen quality is critical.

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