

United States
Department of
Agriculture

Animal and
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Cooperating State
Departments of
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Revised
April 22, 2010

2010 Taxonomic Guidelines, Screening & Identification Aid for the European Grapevine Moth Survey

Lobesia botrana (Denis & Schiff.)
Tortricidae



**Guidelines for Handling Survey Samples for Identification of *Lobesia botrana*, the
European Grapevine Moth (EGVM) for States (except CA)**
Joel Floyd, PPQ Domestic Diagnostics Coordinator

Several states in 2010 are conducting surveys for the European Grapevine Moth (EGVM) , *Lobesia botrana*, under CAPS and Farm Bill funding. These guidelines are for state and PPQ personnel servicing traps, for screeners, and for taxonomists involved with the survey.

All traps that are approved for the EGVM survey are sticky traps must be sorted and screened for suspect adult moths in the family Tortricidae. (Larvae should not be surveyed for and will not be identified as part of this survey.) Proper sorting and screening will limit the material forwarded to the taxonomic specialists in your state capable of identifying suspects of this species. Because the adults are small and have somewhat variable wing patterns, morphological screening can be difficult. The often obscured condition of specimens captured in sticky traps further compounds the difficulty in recognizing suspects of the target species.

Fortunately traps baited with the EGVM pheromone lure are thought to be more specific than the Light brown apple moth pheromone, but will likely still capture other moth species including other tortricids. Some of these non-target, endemic species may be similar to EGVM in appearance. Consequently, most screening for this survey will occur by someone familiar with characteristics of tortricids and identifications of suspects by a taxonomist familiar with the characteristics described in the “Screening and Identification Aid for the EGVM” by Todd Gilligan, Steven Passoa, and Marc Epstein, seen below.

Traps should be serviced at regular intervals according the guidance provided by the program. If traps are stored for more than a few days after collection, they should be kept in a cool location such as a refrigerator until they can be examined and processed. This will prevent deterioration of the specimens. Do not freeze traps except to kill the adults still alive in traps, which is necessary. And do not send live moths in traps through the mail to other locations.

Sorting of Traps

Sorting can be performed at the field level to only select traps with Lepidoptera of the appropriate shape and size according to the screening aid guidance.

1st Level Screening of Traps

1st Level Screening is defined as recognizing moths that appear to be in the family Tortricidae. This will require examination through a good hand-lens or preferably through a dissecting microscope and should be performed by a screener with experience or training in recognizing characteristics of Lepidoptera families. Traps containing suspect tortricid moths, or if there is any doubt, are to be sent to a diagnostic laboratory or taxonomist in the state experienced with adult Lepidoptera identification.

2nd Level Screening of Traps

2nd Level Screening is defined as selection of suspect EGVM based on external wing pattern characteristics and dissection of the male's abdomen to inspect the genitalia. This should be performed by a taxonomist familiar with Lepidoptera dissection techniques. When forwarding dissected specimens, the genitalia from these specimens should not be mounted on slides. The genitalia should be sent forward in alcohol in a vial (in microvials with glycerin if available) with the whole adult moth specimen for final identification.

The routing and confirmation of the EGVM from either screening level will depend on the stated taxonomic ability of a state/PPQ taxonomic infrastructure. State departments of agriculture, universities, or PPQ in a state with the taxonomic ability and capacity to make determinations to the 2nd level screening will first forward any suspect EGVM specimens to one of the PPQ-designated Lepidoptera Identifiers listed below. If confirmed as suspect EGVM that potentially represent new state or county records, the specimen(s) will be forwarded to John Brown with the USDA-ARS Systematic Entomology Laboratory in Washington, DC for final confirmation.

States that do not have 1st and/or 2nd level screening ability can forward specimens or sorted whole traps to one of the PPQ-designated Lepidoptera Identifiers. Prior approval and notification for doing this must go through the PPQ Regional Survey Coordinators informing them of the need, and the number of traps anticipated.

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PPQ-designated Lepidoptera Identifiers.

PPQ Eastern Region

1. Julieta Brambila
USDA, APHIS, PPQ
CAPS Program
For regular mail: P.O. Box 147100
For overnight carriers: 1911 SW 34 Street
Gainesville, FL 32608

Phone: (352) 372-3505 ext. 438 or 464

E-mail: julieta.brambila@aphis.usda.gov

2. Richard Brown
Mississippi Entomological Museum
For Regular Mail: P.O. Drawer 9775
For Overnight Carriers: 100 Twelve Land
Mississippi State, MS 39762

Phone: (662) 325-2990

E-mail: RBrown@entomology.msstate.edu

PPQ Western Region

3. Kira Zhaurova
USDA, APHIS, PPQ
Minnie Belle Heep 216D
2475 TAMU
College Station, TX 77843

Phone : 979-450-5472

E-mail : kira.zhaurova@aphis.usda.gov

4. Eric LaGasa
Pest Program / Plant Protection Division
Washington State Department of Agriculture
For regular mail: P.O. Box 42560
For overnight carriers: 1111 Washington Street, 2nd Floor NRB
Olympia, WA 98504-2560

Phone: Desk phone: 360 902 2063

Email: elagasa@agr.wa.gov

States handling their own EGVM taxonomy will follow state guidance for the documentation, preparation, and selection of suspects for state identifiers. State identifiers or PPQ taxonomists sending suspect EGVM for preliminary confirmation by a PPQ-designated Lepidoptera identifier must include a completed PPQ form 391. Each PPQ form 391 forwarded will need to have minimally the following minimum information:

1. Trap number
2. Collection number with two letter state abbreviation
3. Collector name, affiliation, phone number, and e-mail address
4. Location (address, coordinates, and State and County)
5. Nearby host plant genus and species (if applicable)
6. Number of suspect specimens in sample
7. Trap service dates:
 - a. Date last serviced with lure change
 - b. Date trap or trap insert pulled for sample submission

A fillable PPQ form 391 is found at:

http://cals-cf.calsnet.arizona.edu/azpdn/labs/submission/PPQ_Form_391.pdf

The state taxonomist should write their tentative determination in line 23 of the PPQ form 391, with his/her name as determiner. Label each trap and/or specimens with the trap

number and collection number. Mark the outside of the package with the words: “EGVM Survey.”

Suspect EGVM adult specimens are to be sent dry with the dissected genitalia not mounted on slides but placed in alcohol in a small vial (in microvials with glycerin if available) accompanying the adult. Be sure specimens are well protected with cushioning when shipping to prevent damage during transport (follow the instructions listed in the “Screening and Identification Aid for the EGVM”).

Communications of Results

Not all of the moths in the trap will be identified by PPQ-designated Lepidoptera taxonomists to the species level if not suspect EGVM. Negative results mean EGVM is not in the sample. All negative EGVM determinations made by PPQ-designated Lepidoptera identifiers will be communicated by them by e-mail back to the SPHD, SPRO, regional program managers, and submitter in the state of origin. These can be accumulated and sent summarized in a spreadsheet at the end of the week.

Any positive suspect EGVM identification that are not new state or county records will be also communicated directly, but daily as they occur, to the SPRO, SPHD, and submitter and the EGVM regional survey coordinators.

When PPQ-designated Identifiers are forwarding a suspect new state or county EGVM records, notification that specimens are being forwarded to SEL will be sent by e-mail to ppq.nis.urgents@aphis.usda.gov e-mail address. A copy of the PPQ form 391 with the tentative determination must be e-mailed or faxed to the NIS urgent team (PPQ National Identification Service, fax number: 301-734-5276). The NIS urgent team will have the PPQ form 391 information entered in the PestID system and notify SEL that specimen(s) are being forwarded for final confirmation. NIS will send an e-mail alert to the headquarters PPQ Emergency and Domestic Programs staff that suspect EGVM specimens from a new state or county are being forwarded to SEL for confirmation. They will in turn notify the SPHD and SPRO in the state of origin, the Regional Program Managers.

Once SEL makes a final determination, positive or negative, the notification will be sent to the PPQ Emergency and Domestic Programs staff in Riverdale, MD who will forward the confirmations to the national and regional program managers, SPHD and SPRO, who in turn will notify the appropriate state level personnel including the submitter.

TORTRICIDAE

Screening and Identification Aid

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

T. M. Gilligan¹, S. C. Passoa², M. E. Epstein³

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The European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller), is one of the most destructive grape pests in the Palearctic. *L. botrana* larvae cause damage to grapes by feeding on fruit, resulting in direct damage and secondary infection of feeding sites by botrytis bunch rot (gray mold; Fungi). *L. botrana* was discovered infesting grapes in Napa Valley, California in 2009; it has not been reported from North America outside of California.

L. botrana is a member of the Tortricidae, a large family of moths (Lepidoptera) that includes many pest species. In North America there are approximately 1200 species of tortricids, which are often referred to as “leafrollers” because the larvae of some species feed inside a rolled leaf. Most tortricid moths are small and brown with a wingspan of approximately 10-30 mm. *L. botrana* can be distinguished from many other tortricids by its wing pattern and small size; however some species are superficially similar to *L. botrana*. Other species of *Lobesia* and related *Paralobesia* (both subfamily Olethreutinae) are present in North America, and a genitalic dissection is necessary to separate these species from *L. botrana* in most cases.

This aid is designed to assist in the screening and identification of *L. botrana* adults collected from sticky traps in the United States. It covers basic sorting of traps, first and second level screening, and identification based on morphological characters. Basic knowledge of Lepidoptera morphology is necessary to screen for *L. botrana*. Basic knowledge of Tortricidae and dissection techniques are necessary to identify suspect *L. botrana* specimens.



Figure 1: Adult *L. botrana*. Wing pattern is very consistent with no obvious difference between males and females.



Figure 2: Adult *L. botrana* showing color variation.



Figure 3: *L. botrana* larva feeding on grapes.

Sorting

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

Lobesia botrana pheromone traps should be sorted initially for the presence of moths of the appropriate size, color, and shape. Traps that contain moths meeting all of the following requirements should be moved to level 1 screening:

- 1) Moths are approximately 4-7 mm ($\frac{1}{4}$ inch) long (forewing length) (Figs. 5 & 6)
- 2) Moths have an overall shape that is similar to the outline depicted in Fig. 4. Note that moths caught on their side or back may have a different outline (Fig. 6).
- 3) Moths appear superficially similar to those depicted in Figs. 1-2 & 6-7.

The appearance of moths caught in sticky traps can vary substantially depending on the amount of sticky glue on the moth. For this reason, any small, tortricid-like moth should be sent forward to level 1 screening.



Figure 4: Outline of *L. botrana* in resting position.



Figure 5: Actual size (4-7 mm forewing length) of a *L. botrana* adult.

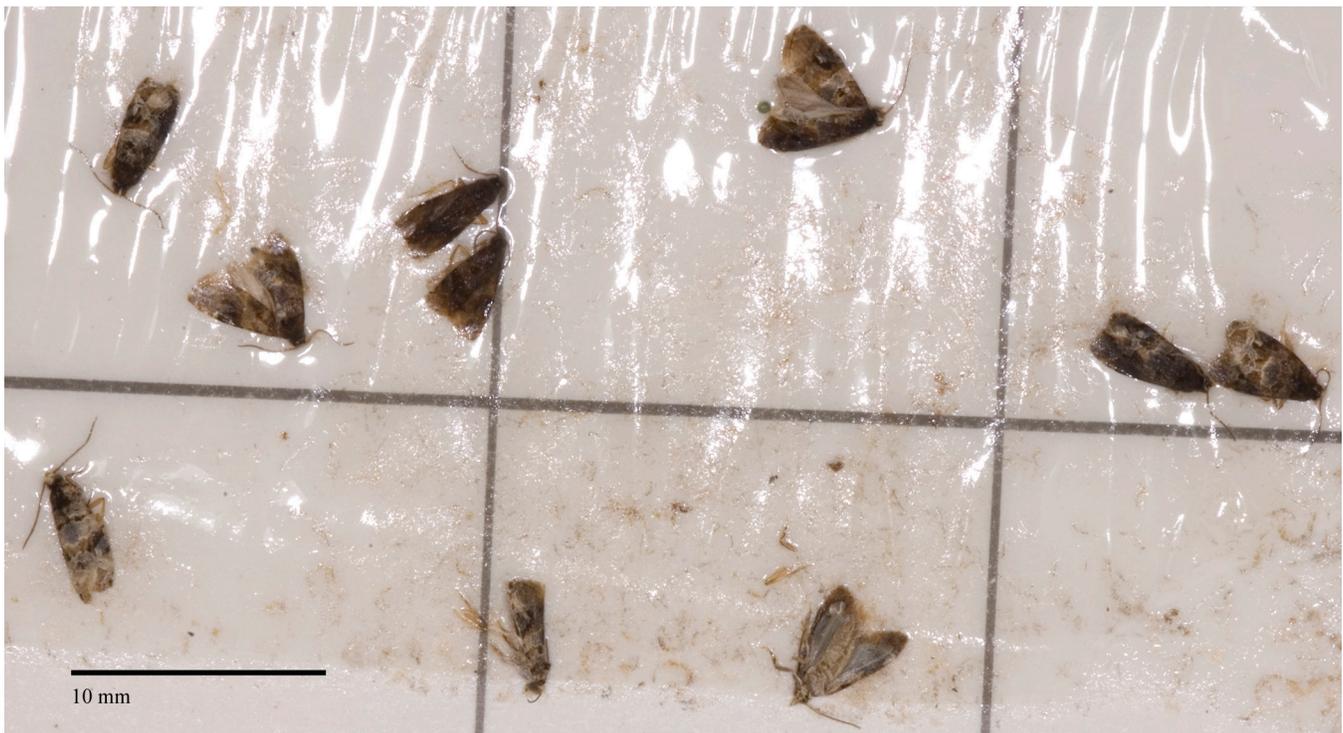


Figure 6: Examples of *L. botrana* adults caught in a sticky trap.

Level 1 Screening

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

Moths that meet the sorting requirements should be screened for suspect tortricids. Level 1 screening is difficult for small moths and may need to be performed by a trained Lepidopterist. **When in doubt distinguishing first level screening characters, forward traps that have passed the sorting requirements to a trained taxonomist.** Suspect tortricids in traps should not be manipulated or removed for screening unless expertise is available.

Tortricid moths can be identified by the following combination of characters:

- 1) Antennae simple, thread-like, and never pectinate (feathery).
- 2) Tympanum absent. Members of the Pyraloidea may appear similar but have a tympanum at the base of the abdomen.
- 3) Labial palpi pointed and project forward. Some families have labial palpi that curve upwards.
- 4) Maxillary palpi are very reduced and not visible in tortricids. Maxillary palpi may be visible under magnification in some commonly intercepted pyralid species.
- 4) Proboscis (tongue) unscaled. Members of the Gelechioidea and Pyraloidea have a scaled proboscis.
- 5) Chaetosema (patch of bristle-like setae) present above the compound eye. (Chaetosema may be difficult to see without a high-quality microscope.)

Moths meeting the above criteria should be sent to level 2 screening. Traps to be forwarded to level 2 should be carefully packed following the steps outlined in Fig. 8. Traps can be folded, with stickum-glue on the inside, making sure the two halves are not touching, secured loosely with a rubber band, and placed in a plastic bag for shipment. Insert 2-3 styrofoam packing peanuts on trap surfaces without moths to cushion and prevent the two sticky surfaces from sticking during shipment to taxonomists. **DO NOT** simply fold traps flat or cover traps with transparent wrap (or other material), as this will guarantee specimens will be seriously damaged or pulled apart – making identification difficult or impossible.



Figure 7: Male *L. botrana* in a sticky trap.

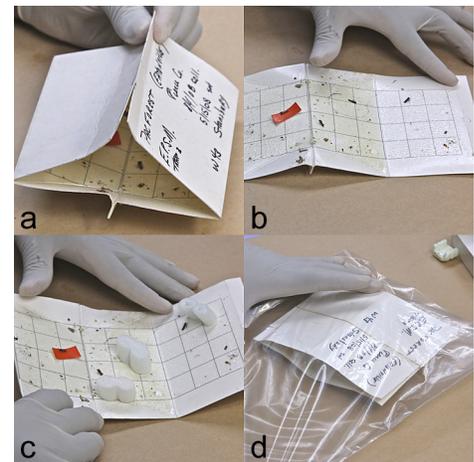


Figure 8: Recommended packing method for shipment of sticky traps: a & b) open and unfold trap; c) place 2-3 packing peanuts in areas of trap with no moths; d) fold trap, secure with rubber band, and place in plastic bag.

Level 2 Screening

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

Suspect tortricids should be cleaned to identify suspect *L. botrana* specimens. Details on cleaning specimens caught in sticky traps can be found on the following website:

<http://keys.lucidcentral.org/keys/v3/LBAM/dissections.html>

Cleaned specimens should be pinned and labeled. Initial screening is based on wing pattern, which varies little between individuals. *L. botrana* and close relatives can be separated from most other Nearctic tortricids by a combination of the following wing characters (depicted in red in Figs. 9 & 10); note that male *L. botrana* lack a forewing costal fold:

Wing Pattern

- 1) All *L. botrana* individuals have a prominent leaden-gray bar that runs across the middle of the forewing (Fig. 9). Often this bar is outlined in white. In a resting individual these markings form a complete, transverse, gray bar across the middle of the specimen (Fig. 10).
- 2) Many *L. botrana* individuals have a prominent inverted “Y” in the outer half of the forewing. This “Y” is formed from leaden-gray scales outlined in white (Figs. 9 & 10). This feature may not be evident in some specimens, especially those that are worn; however close examination should reveal some remnants of this pattern element.

Specimens that pass initial wing pattern screening most likely belong to the genera *Lobesia* or *Paralobesia*. These specimens should be dissected for further determination. Standard dissection procedures for Lepidoptera are outlined in Clarke (1951) and Robinson (1976). Separated abdomens and genitalia preparations should be carefully labeled to remain associated with pinned specimens. Specimens may need to be sent to a tortricid specialist for positive identification.

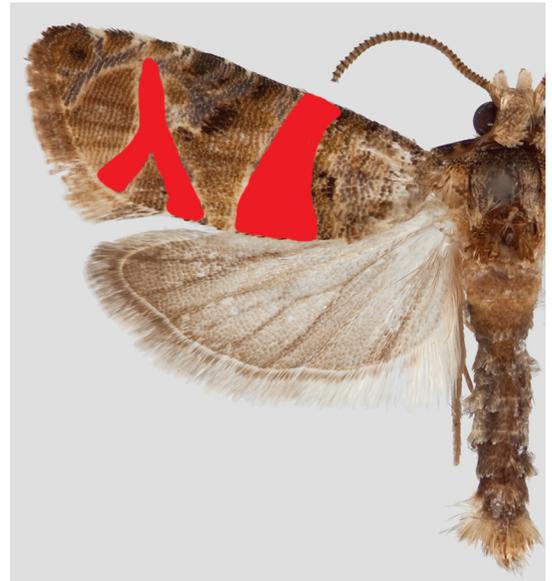


Figure 9: Diagnostic *L. botrana* forewing pattern elements (highlighted in red): prominent leaden-gray bar across the middle of the forewing and an inverted “Y” on the outer half of the forewing. Closely related *Paralobesia* share these same markings and cannot be separated by wing pattern alone.

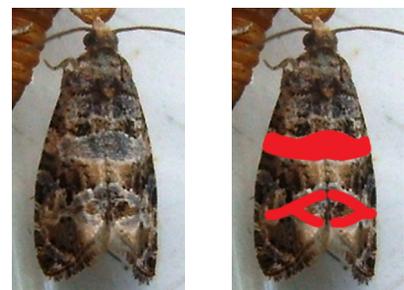


Figure 10: Diagnostic *L. botrana* wing pattern markings (highlighted in red) in a resting individual.

Identification

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

Dissected *L. botrana* can be separated from Nearctic *Paralobesia* based on the following genitalic characters:

Males

- 1) Valvae long and narrow with a row of spines on the ventral margin (Fig. 11). Cucullus separated from the sacculus by a distinct notch where spines are absent.

Females

- 1) Ductus bursae long, slender, and undifferentiated from the corpus bursae which is gradually enlarged anteriorly (Fig. 12).
- 2) A long, feather-shaped signum is present (Fig. 12).

Male Nearctic *Paralobesia* have a cluster of long setae (the saccular tuft) that extends from the ventral margin of the valva that is not present in *L. botrana*. Most female Nearctic *Paralobesia* lack a signum.

Paralobesia viteana is a native pest of grapes in eastern North America. This species is easily separated from *L. botrana* by genitalic characters: male *P. viteana* have a prominent lobe projecting from the valva that is absent in *L. botrana*, and female *P. viteana* lack a signum. The only common native *Lobesia* species in eastern North America, *L. carduana*, is not likely to be associated with grapes. Gilligan et al. (2008) should be consulted for identification of olethreutines in eastern North America. This volume contains detailed descriptions and illustrations of many species of *Paralobesia* that appear superficially similar to *L. botrana*.

In western North America, only two species of *Paralobesia* have been recorded. Neither are likely to be associated with grapes and both can be separated from *L. botrana* by genitalic characters. Powell and Opler (2009) should be consulted for identification of olethreutines in western North America.

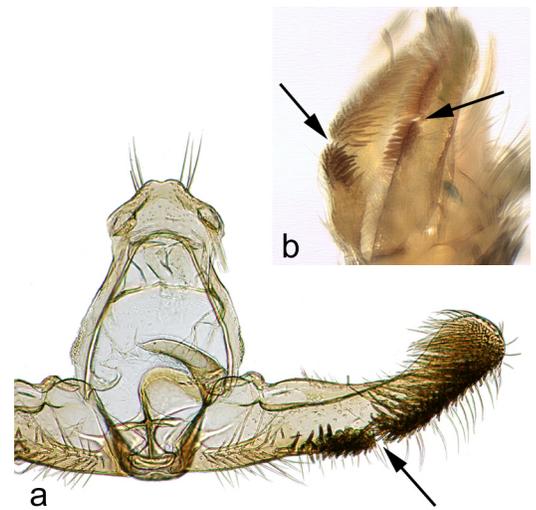


Figure 11: *L. botrana* male genitalia; a) slide mounted, b) unmounted. Arrows denote diagnostic notch in valvae.



Figure 12: *L. botrana* female genitalia. Arrow denotes female signum.

Key and References

European Grapevine Moth

Lobesia botrana (Denis & Schiff.)

Key to Screen and Identify *L. botrana* Suspects in the United States

The following key covers screening and identification of suspect *L. botrana*. Sticky traps normally capture only males moths, although female moths have been captured in California. Both sexes of moths are included; figures on previous pages should be consulted for specific characters.

- 1. Abdominal or thoracic tympanum present; antenna pectinate; labial palpi upturned; proboscis scaled or absent; moths not about 4-7 mm long..... **Not *L. botrana***
- 1'. Abdominal and thoracic tympanum absent; antenna simple; labial palpi projecting forward; proboscis not scaled; moths about 4-7 mm long **2**
- 2. Forewings with prominent median gray bar and outer inverted "Y," both outlined in white, or forewing pattern unknown..... **3**
- 2'. Forewings without prominent median gray bar and outer inverted "Y" **Not *L. botrana***
- 3. Males..... **4**
- 3'. Females **5**
- 4. Male valva with distinctive notch between cucullus and sacculus..... ***L. botrana suspect***
- 4'. Male valva without distinctive notch between cucullus and sacculus..... **Not *L. botrana***
- 5. Female with long narrow ductus bursae gradually expanding into corpus bursae, and a large, single signum ***L. botrana suspect***
- 5'. Female without a long narrow ductus bursae gradually expanding into corpus bursae, and signum absent or two signa present..... **Not *L. botrana***

References

Eastern & Midwestern North America non-targets:

Gilligan, T. M., D. J. Wright and L. D. Gibson. 2008. Olethreutine moths of the midwestern United States, an identification guide. Ohio Biological Survey, Columbus, Ohio. 334 pp.

Western North America non-targets:

Powell, J. A. and P. A. Opler. 2009. Moths of western North America. University of California Press, Berkeley. 369 pp.

Genitalia Dissections:

Clarke, J. F. G. 1941. The preparation of slides of the genitalia of Lepidoptera. Bulletin of the Brooklyn Entomological Society, 36:149-161.

Robinson, G. S. 1976. The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. Entomologist's Gazette. 27:127-132.

Acknowledgements

The authors would like to thank Joel Floyd for prompting the creation of this document. We thank Roberto Gonzalez, Universidad de Chile, for specimens, information on *L. botrana* in Chile, and use of Figs. 3 & 10. We thank Eric LaGasa, Washington State Department of Agriculture, Jerry Powell, University of California Berkeley, and Richard Worth, Oregon Department of Agriculture, for providing details on West Coast *Paralobesia*. Julieta Brambila, USDA/APHIS/PPQ, John Brown, USDA/SEL, Richard Brown, Mississippi Entomological Museum, and Eric LaGasa provided helpful comments.

