

Standard Specification for Eretmocerus eremicus Rose and Zolnerowich (Hymenoptera: Aphelinidae)¹

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1. Scope

- 1.1 This specification describes a method for determining whether the quantity and quality of adult *Eretmocerus eremicus* in a shipment adhere to quantity and quality specifications. The test also allows the purity of shipments to be determined. Included are referenced documents, a description of standard terminology, specifications, and the test method.
- 1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

2. Referenced Documents

2.1 ASTM Standards:²

E2200 Specification for Information Included with Packaging of Multi-Cellular Biological Control Organisms (Withdrawn 2010)³

3. Terminology

- 3.1 name of product—Eretmocerus eremicus Gahan.
- 3.2 preferred hosts and prey—Greenhouse Whitefly, Trialeurodes vaporariorum (Westwood), Silverleaf Whitefly, Bemisia argentifolii Bellows and Perring and Tobacco Whitefly, Bemisia tabaci (Gennadius).
- 3.3 *life stage shipped*—pupa within the host insect, *T. vaporariorum*.
- 3.4 *card*—a sample unit. Pupae of *Eretmocerus eremicus* are mounted on cardstock strips that are subdivided into "cards".
- 3.5 package claim—the number of adults expected to emerge from and leave each card.
- ¹ This specification is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.30 on Natural Multi-Cellular (Metazoan) Biological Control Organisms.
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- ² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.
- ³ The last approved version of this historical standard is referenced on www.astm.org.

- 3.6 *test statistic*—the average number of wasps caught during the flight test expressed as a percentage of the package claim.
- 3.7 critical value—a number that the test statistic is compared to in order to determine whether the quantity and quality requirement has been met. Critical values depend on the probability of error that can be tolerated and on the number of samples used in the test.

4. Classification

- 4.1 Phylum—Arthropoda.
- 4.2 Class—Insecta.
- 4.3 Order—Hymenoptera.
- 4.4 Family—Aphelinidae.
- 4.5 Genus—Eretmocerus.
- 4.6 Species—eremicus.

5. Summary of Test Method (Determining the Number of *E. eremicus* Released from a Shipment and its Conformity to the Standard)

- 5.1 The test describes methods for determining whether the number of adult *E. eremicus* that are capable of flight meets or exceeds the package claim. A method of assessing the purity of a shipment is included.
- 5.2 The quantity of *E. eremicus* per card and their flight ability is determined by counting the number of wasps caught on a sticky trap suspended in a test chamber. Three or more test chambers are used per shipment, each chamber contains one randomly chosen card with *E. eremicus* pupae. The numbers of wasps caught on each sticky card are counted and results are used to calculate the average number of wasps per card. This average is used in combination with the known sampling distribution and variation in counts to judge whether specifications are met. Live contaminants are identified and recorded.
- 5.3 Quantity and Quality Specification—The test described in this document is used for both the quantity and quality assessment. To meet the quantity and quality specification defined by the "package claim", 100 % of the number of adults should be capable of flight as measured by the flight test described herein.

5.4 *Purity*—The purity specification is that shipments, and hence samples, do not contain species other than *Eretmocerus eremicus*.

6. Significance and Use

6.1 This method was developed to determine that the numbers of *E. eremicus* supplied in a shipment meet the package claim and that wasps at receipt have good flight capability. The application of this method will ensure a standardized evaluation of the product and judicious decisions about product compliance to the package claim.

7. Materials

- 7.1 Test chamber $(6.5 \times 8 \text{ cm acrylic tube})$.
- 7.2 Support wire.
- 7.3 Double-sided dry-stick yellow sticky trap $(2.5 \times 4 \text{ cm})$.
- 7.4 Nylon screen (100 micron mesh).
- 7.5 Clear plastic cling-film (see Fig. 1).
- 7.6 Headband magnifier (7 to 10×) is optional.

8. Test Unit

8.1 A single shipment of *E. eremicus* is considered a test unit.

9. Pre-Test Conditions

9.1 If required, a shipment can be stored for a maximum 24 h at 10 to 15°C, relative humidity (RH) 60 to 90 %.

10. Sample Size

10.1 Choose three or more sample cards per shipment (see the interpretation of results to decide on the sample size).

11. Sampling and Testing

11.1 Place one card on the floor of each chamber. Position the yellow sticky trap inside the chamber on the support wire. Seal the top of the chamber with plastic wrap. Place the chamber within the crop, away from the heating pipes and out of direct sunlight. Test conditions should be within 20 to 25° C and 60 to 90 % RH.

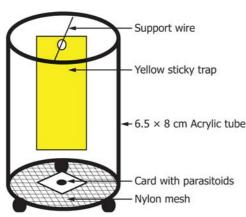


FIG. 1 Test Chamber

12. Counting Procedure and the Assessment of Purity

- 12.1 After two weeks, count the number of wasps caught on the sticky trap and calculate the average for all chambers used. Express the average as a percentage of the number expected (the package claim) from the card. This value is called the test statistic.
- 12.2 Examine the parasitized scales and the yellow sticky trap and record the presence of other live insects or mites appearing among the parasitoids.

13. Properties of the Test

13.1 In the absence of other data, the quantity and quality of wasps in shipments are assumed to meet or exceed package claims. The test statistic and known properties of the counts of wasps on sticky cards are used to judge whether the package claim is met. This is done by comparing the test statistic to a critical value (CV) that is tied to a probability of incorrectly declaring a shipment to be substandard and to the number of cards tested. The CVs for three sample sizes and different probabilities of error are presented in Table 2. A CV is determined by selecting a sample size (the number of cards assessed) and a probability of error. For example, if 3 cards were assessed and a probability of error of 0.05 was chosen, CV = 90.

13.2 Each probability of error in Table 2 is a measure of the likelihood of wrongly categorizing the package to be deficient when it in fact has the specified number of healthy, flight capable insects. More precisely, these are the probabilities of obtaining a test statistic that is less than or equal to the CV when the package claim is exactly met. Note that for a particular CV, the probability of error declines with increasing sample size reflecting the fact that the precision of the sample information increases as the number of samples increase. The probabilities of error were calculated using a normal distribution model with a standard deviation of 10.60. This model was found to well describe counts of wasps caught in the flight test chamber. Additional details for this statistical model are provided in the appendix.

14. Interpretation of Results

14.1 The quantity and quality of wasps in a shipment will be considered below the package claim when the test statistic is smaller than the selected critical value. If an acceptable error rate is 0.05, then the CV for a sample size of 3 is 82 % (Table 2). For the example presented in the Table 1, the test statistic (90 %) is larger than 82 %; therefore, the shipment would be classified as meeting the package claim. If 5 samples were

TABLE 1 Example—Based on 3 Random Samples and an Expectation of 60 Wasps/Card (the Package Claim)

Sample Number	Wasps on Yellow Card		
1	57		
2	54		
3	51		
Average 162/3 = 54			
Observed/Expected · 100	Observed/Expected · 100 = 90%		
Example: Test statistic = 90			

TABLE 2 Critical Values for the 3 Sample Sizes (3, 5 and 10 Cards) and Corresponding Probabilities of Error

	-		
	Probability of Error for Sample Size of:		
Count as % of standard	3	5	10
84	0.00	0.00	0.00
85	0.01	0.00	0.00
86	0.01	0.00	0.00
87	0.02	0.00	0.00
88	0.02	0.01	0.00
89	0.04	0.01	0.00
90	0.05	0.02	0.00
91	0.07	0.03	0.00
92	0.10	0.05	0.01
93	0.13	0.07	0.02
94	0.16	0.10	0.04
95	0.21	0.15	0.07
96	0.26	0.20	0.12

used, the CV with an error rate of 0.05 is 86 % (Table 2) so the shipment would again be classified as meeting the package claim. However, if 10 samples were used, the CV for an error rate of 0.05 is approximately 91 %, so now the shipment would be classified as not meeting the package claim.

15. Precision and Bias

15.1 The probabilities of error shown in Table 2 reflect the precision of the test. The bias for *E. eremicus* has not been assessed.

16. Keywords

16.1 Bemisia argentifolii; Bemisia tabaci; Eretmocerus eremicus; Greenhouse Whitefly; purity; quantity; Silverleaf Whitefly; Tobacco Whitefly; Trialeurodes vaporariorum; whitefly parasite

APPENDIX

(Nonmandatory Information)

X1. STATISTICAL BACKGROUND

X1.1 The test assumes that a shipment meets the requirement that the number of wasps that will emerge and fly is at least 100 % of the package claim. Hence the null hypothesis is:

$$H_a: \mu \ge 100 \tag{X1.1}$$

where:

 μ = the scaled mean number of wasps in a shipment that are capable of flight.

X1.2 We wish to determine a critical value (CV) with which to compare a sample mean to so that the probability of incorrectly declaring a shipment substandard is acceptable (e.g., ≤ 0.05). This CV will depend on the number of samples

taken from a shipment and will increase as the number of samples increase. Thus, we seek to find a CV such that:

$$Pr\{\bar{x} \le CV \mid \mu \ge 100\} = 0.05$$
 (X1.2)

X1.3 This is not possible unless μ is fixed. Because this probability will decrease for a fixed value of CV and increasing values of μ , we can set μ to 100 and determine CV so that:

$$Pr\{\bar{x} \le CV \mid \mu = 100\} = 0.05$$
 (X1.3)

X1.4 If sample counts (x) are distributed as normal random variables with known standard deviation, appropriate values of CV can be determined using a normal cumulative distribution function.

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(2) Luczynski, A., et al, "Influence of Cold Storage on Pupal Development and Mortality During Storage and on Post-Storage Performance of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae)," *Biological Control*, Vol 40, 2007, pp. 107–117.



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