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Standard Specification for *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae)[1]

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1. Scope

1.1 This specification covers information on and the test method for determining purity, sex ratio, and number of adults in shipments of the predatory beetle *Cryptolaemus montrouzieri*.

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E2200 Specification for Information Included with Packaging of Multi-Cellular Biological Control Organisms (Withdrawn 2010)[3]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 *Definitions of Terms Specific to This Standard:*

3.1.1 *carrier*—shredded paper.

3.1.2 *life stage when shipped*—adult.

3.1.3 *name of product—Cryptolaemus montrouzieri* Mulsant.

3.1.4 *preferred host prey*—mealybugs.

4. Classification

4.1 *Phylum*—Arthropoda.

4.2 *Class*—Insecta.

4.3 *Order*—Coleoptera.

4.4 *Family*—Coccinellidae.

4.5 *Genus*—Cryptolaemus.

4.6 *Species*—montrouzieri.

TEST METHOD—DETERMINING THE PURITY, SEX RATIO, AND NUMBER OF ADULTS IN SHIPMENTS OF *C. montrouzieri*

5. Summary of Test Method

5.1 This test method describes a method of counting the number of *C. montrouzieri* beetles packaged in a carrier.

5.2 The number of *C. montrouzieri* will be determined by examining a minimum of three units and determining the mean value. In each container, live contaminants will be identified and recorded.

6. Significance and Use

6.1 The efficacy of mealybug control by *C. montrouzieri* depends on accurate release numbers of beetles, presence of male and female beetles, and absence of live product contaminants. Accurate packaging and maintenance of purity and viability of *C. montrouzieri* shipments is, therefore, essential for the effective management of the target pest. This test method is intended for use by producers and end-users of the specified biological control agent. It is complementary to the quality guidelines for *Cryptolaemus montrouzieri*[4].

7. Materials

7.1 Dissecting microscope or headband magnifier (7 to 10×).

7.2 30 DR snap-top plastic vial.

7.3 Pharmaceutical pill counting tray.

7.4 Fine-pointed No. 5/0 paint brush.

8. Test Unit

8.1 A single container with *C. montrouzieri* is considered a test unit. A minimum of three containers per shipment will be randomly selected from the shipping box.

[1] This specification is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agentsand 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.

[4] Developed by the International Organization of Biological Control and published in Lenteren, J. C. van (ed.), 2003.

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9. Pre-Test Conditions

9.1 If samples must be held before testing, keep at 15°C, relative humidity 60 to 90 %, for a maximum of 24 h.

9.2 Chill beetles at 10°C for one hour prior to counting.

10. Sampling

10.1 Specify the number of adults that are expected in each test unit as indicated on the package before commencing test.

11. Counting Procedure and Sex Ratio Determination

11.1 Carefully remove any packing material by hand, allowing the beetles to drop back into container. If beetles have been shipped in a large container, it is advisable to transfer the beetles to a snap-top vial or bottle for ease in handling. Gently tap a portion of the beetles onto the counting tray and immediately start counting beetles. Record the count, then brush the counted beetles into the pill tray reservoir. Store the counted beetles in a second container with a snap-on lid. Repeat the process until all the beetles are counted. While counting the beetles, record the presence of any live arthropods other than adult *Cryptolaemus* beetles. Since the beetles start to warm during the counting process, the sample container must be repeatedly tapped on a hard surface to cause the beetles to drop to the bottom of container to prevent escape. For quantities greater than 500 beetles, sub-sampling 10 % by weight or volume is recommended. For determining sex ratio, beetles can be examined chilled or dead. The ventral surface of each beetle is examined and the sex recorded. The sex is determined by the color of the anterior femur: orange for males and black for females. Count number of females and males in a minimum sample size of 100 beetles.

12. Calculations and Interpretation of Results

12.1 The following formula is used to estimate the mean number of adults per test unit:

12.1.1 Estimation of mean number of adults released per test unit:

$$x = \frac{\Sigma}{n}$$

where:

x = mean number of adults produced per test unit,
Σ = sum of counts of each test unit, and
n = total number of samples examined.

12.2 *Interpretation of Results*—The mean of the three test units should be equal to or greater than the number specified on the package. Sex ratio should be equal to or greater than 40 % females. No contaminants should be present except acceptable food source such as sterile or frozen moth eggs.

13. Precision and Bias

13.1 The precision and bias of this test method has not been determined.

14. Keywords

14.1 *Cryptolaemus montrouzieri*; mealybugs; predatory beetle

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