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Standard Specification for *Amblyseius cucumeris* (= *Neoseiulus*) *Oudemans* (Acarina: Phytoseiidae)[1]

This standard is issued under the fixed designation E2646; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This specification includes standard terminology, classification, and referenced documents as well as description of the test methods for determining the number of *Amblyseius cucumeris* Oudemans supplied on the bulk carrier or in slow release sachets. Description of the method for assessing the purity of shipments is also included.

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E2200 Specification for Information Included with Packaging of Multi-Cellular Biological Control Organisms (Withdrawn 2010)[3]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—Amblyseius cucumeris* Oudemans.

3.2 *preferred host prey*—Western flower thrips (*Franklinella occidentalis*).

3.3 *onion thrips (Thrips tabaci)*—also cyclamen and broad mites.

3.4 *life stage when shipped*—all stages, eggs, nymphs, adults.

4. Classification

4.1 *Phylum*—Arthropoda.

4.2 *Class*—Arachnida.

4.3 *Order*—Acarina.

4.4 *Family*—Phytoseiidae.

4.5 *Genus*—Amblyseius = Neoseiulus.

4.6 *Species*—cucumeris.

5. Summary of Test Method (Determining the Number of *N. cucumeris* Released from a Slow Release Sachet)

5.1 The test describes methods for *(1)* estimation of the number of *A. cucumeris* released from sachet based on examining a few randomly selected sachets per shipment, and *(2)* assessing the purity of shipment.

5.2 A few sachets are chosen randomly from a shipment and each sachet is suspended from a wire hanger and placed on a sticky trap surface (“Catch it,” Silvandersson Sweden AB). Mites are allowed to disperse from sachets, and each week, mites caught on the sticky trap are counted and recorded.

6. Significance and Use

6.1 The biological control of flower thrips by *N. cucumeris* is, in part, dependent on accurate release rates and consistent release pattern of *N. cucumeris*. Supplying fewer predatory mites than indicated on the label or restricting duration of release may upset host-predator balance and could lead to the failure of biological control. This test was developed for the benefit of biocontrol producers and IPM practitioners.

7. Materials

7.1 Yellow sticky traps with a grid and coated with dry glue.

7.2 A 20-cm high wire hanger.

8. Test Unit

8.1 A shipment of *A. cucumeris* is considered a test unit.

9. Pre-Test Conditions

9.1 Samples could be held in a cool place at 10 to 20°C out of direct sunlight for a maximum of 24 h before testing.

10. Sample Size

10.1 Choose four or more sachets from a shipment (needs to be determined).

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[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.

11. Testing and Calculations

11.1 Suspend each sachet on a wire hanger and place on a sticky trap surface, one per trap (Fig. 1). Laboratory evaluation should be carried out under 16:8 D:N photoperiod 18 to 21°C and 75 to 80 % RH (relative humidity). When testing is carried out in the greenhouse, place the sticky traps and sachet inside a small cage (45 by 30 by 27 cm) made of a white breathable fabric (Fig. 2). Position the cage within the crop but away from the heating pipes.

11.2 Allow mites to disperse until from the sachet until no more mites are released; that is, six or more weeks. Change the sticky traps weekly and count and record the number of *N. cucumeris* caught on the trap. Calculate the average number of the predatory mites released per sachet

12. Results

12.1 The average number of *A. cucumeris* released per sachet should be equal to or greater than the number specified on the package.

13. Summary of Test Method (Determining the Purity and Number of Live Adults and Nymphs in 1.5-L Containers with *Amblyseius cucumeris*)

13.1 This test describes a method of estimating the number of *A. cucumeris* in a standard 1.5-L package with bran carrier, based on a random sampling and counts of live nymph and adult predators.

14. Significance and Use

14.1 The biological control of flower thrips by *A. cucumeris* depends on accurate release numbers of predators and absence of other harmful insects and mites. This test will be typically employed by producers and users of the product to assess the number of live biological control agents delivered.

15. Materials

15.1 A mixing container larger than the product volume.

15.2 1-mL measuring spoon.

15.3 8-cm petri dishes.

15.4 Sixteen dram vials.

15.5 70 % alcohol in a wash bottle.

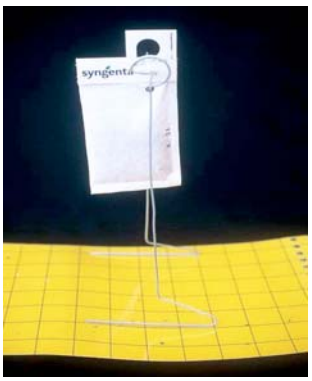

FIG. 1 Sticky Trap with Sachet Suspended from Wire Hanger

FIG. 2 Light Fabric Cage Containing Sticky Trap with Sachet Suspended from Wire Hanger

15.6 Three sieves made from 150-mL wide mouth plastic jars the following mesh sizes:

15.6.1 *Top*—Mesh pore size = 1200 microns (standard window screen).

15.6.2 *Middle*—Mesh pore size = 700 microns.

15.6.3 *Bottom*—Mesh pore size = 75 microns.

15.7 0.5 % dish-washing detergent solution in a wash bottle.

15.8 9-cm diameter petri dishes.

15.9 Mite-counting grid attached to a 9-cm petri dish bottom.

15.10 Wide field 16× binocular microscope.

15.11 Hand counter with three counters.

16. Test Unit

16.1 A single container of *A. cucumeris* is considered a test unit.

17. Pre-Test and Test Conditions

17.1 A container could be held in a cool place at 10 to 20°C out of direct sunlight for a maximum of 24 hours before testing.

18. Sample Preparation and Treatment

18.1 Pour the contents of one shipping container into a bucket larger than the sample and mix by stirring the bran mixture thoroughly.

18.2 Immediately collect a 2 mL sample with the measuring spoon and place into a vial with 10 mL or sufficient alcohol to completely wet the sample. Repeat mixing and sampling for the desired number of samples (3 minimum).

18.3 For each sample collected:

18.3.1 Thoroughly rinse the sample through the series of sieves underneath a gentle flow of hot water (30°C) for at least one minute (the screens should be held slightly apart to obtain the best water flow and prevent overflowing and loss of sample). Remove the top sieve and rinse the remaining two

screens for an additional 20 seconds and then remove the second (middle) sieve.

18.3.2 Using the soap solution, rinse the contents of the bottom sieve into the edge by holding the sieve on an angle, then rinse the entire contents into a petri dish base by inverting the sieve over the dish while rinsing with the soap solution. Repeat this twice to be sure to remove the entire sample from the sieve. Use the minimum amount of soap solution to avoid over filling the dish.

18.3.3 If the sample is to be counted immediately, soap bubbles may be removed by dripping a small amount of 95 % alcohol into the dish.

18.3.4 Place the sample dish onto the counting grid and swirl it to evenly distribute the material in an even layer in the dish.

18.3.5 For each 2 mL (cc) sample count the *A. cucumeris* adults and nymphs under 16× magnification in all black segments (pies) which form half of the area of the counting grid. This count gives you the number per mL or cc of bran mixture.

NOTE 1—Mites that were dead prior to sampling appear a darker color and flattened, with their legs curled inward. Live mites are rounder or fuller in appearance with legs spread outward.

18.3.6 Calculate the average number of live mites per millilitre for all samples taken and multiply this number by the total volume in millilitre of the sampled container to determine the total number of live mites present.

18.4 To assess purity, examine the inside of the lid, container, and surface of bran mixture when opened and also identify and record any unusual insect or mite found during the quantity test above.

NOTE 2—*A. cucumeris* is cultured on a smaller grain mite, *Acarus siro* or *Tyrophagus spp.*. These will appear as a contaminant but are necessary as food for the *A. cucumeris* and not usually a problem under most use conditions.

19. Interpretation of Data

19.1 The total number of nymphs plus adult *A. cucumeris* should be equal to or greater than the label content number specified on the package.

19.2 No live organisms other than *A. cucumeris* and the host grain mites should be found in this test.

19.3 If any of the above conditions are not met, the shipment is considered below standard.

20. Precision and Bias

20.1 The precision and bias of these methods have not been determined.

21. Keywords

21.1 *Acarus siro*; *Amblyseius cucumeris*; *Neoseiulus cucumeris*; purity; quantity; *Tyrophagus spp.*

BIBLIOGRAPHY

(1) van Lenteren, et al, "Guidelines for Quality Control of Commercially Produced Natural Enemies," *Quality Control and Production of Biological Control Agents—Theory and Testing Procedures*, J. C. van Lenteren, Ed., CABI Publishing, 2003, pp. 292–294.