Designation: E2198 – 10

Standard Specification for *Phytoseiulus persimilis* Athias-Henriot (*Acarina:Phytoseiidae*)[1]

This standard is issued under the fixed designation E2198; 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 method determining whether the number of *P. persimilis* in the shipment and the purity of shipments meet the quantity specification.

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. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *bulk carrier*—includes vermiculite, corn grits, or wood shavings.

2.1.2 *carrier*—vermiculite, corn grits, wood shavings, or bean leaves.

2.1.3 *critical value*—a number that the test statistics is compared to in order to determine whether the quantity 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.

2.1.4 *life stage when shipped*—adults and immature.

2.1.5 *name of product—Phytoseiulus persimilis* Athias-Henriot

2.1.6 *package claim*—the number of adults expected in each container.

2.1.7 *preferred host prey*—two-spotted spider mite, *Tetranychus urticae* Koch

2.1.8 *test statistics*—the average number of adults in a sample.

3. Classification

3.1 *Phylum*—Arthropoda.

3.2 *Class*—Arachnida.

3.3 *Order*—Acarina.

3.4 *Family*—Phytoseiidae.

3.5 *Genus*—Phytoseiulus.

3.6 *Species*—persimilis.

Test Method for Determining the Number of Live *P. persimilis* per Shipment Supplied on a Bulk Carrier and Assessment of Purity of Shipments

4. Scope

4.1 The test describes the method for determining if the number of the predatory mites conforms to the advertised standard and the method for assessment of purity of shipments.

5. Summary of Test Method

5.1 The carrier with the predatory mites is mixed thoroughly and a few random samples are taken, one per container. Measurements may be done either by weighing, or by measuring volume. The size of each sample is scaled to maintain the same proportion (2 %) of the sample to the total. The predatory mites running out of or staying in the carrier are counted and recorded. Results are used to calculate the mean number of mites running out of the carrier. The mean is the known attribute of the assessment method and is used to judge whether the specification is met. In each container, possible live contaminants will be identified and recorded.

6. Significance and Use

6.1 This method was developed to determine that the number of *P. persimilis* supplied in the shipment meets the package claim. The application of this method will ensure a standardized evaluation of the product and a judicious decision about product compliance to the package claim.

7. Materials

7.1 Non-toxic white glue or dish detergent, light source, scale (0.01 gr minimum), mixing container, and magnifier (10×).

8. Test Unit

8.1 A single shipment with *P. persimilis* is considered a test unit.

[1] This specification is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and are the direct responsibility of Subcommittee E35.30 on Natural Multi-Cellular (Metazoan) Biological Control Organisms.

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9. Pre-Test Conditions

9.1 Store the containers with *P. persimilis* between 10 and 12°C, RH 60 ± 5 %, for a minimum of 1 h and maximum of 24 h prior to examination.

10. Sampling

10.1 Select four or more samples (bottles) per shipment.

11. Mixing and Weight Recording

11.1 Record the weight of the container with carrier and predators. Rotate the contents of the container gently until all predators are removed from the container wall and mixed well with carrier. Pour half of the carrier with predators into a mixing container. Mixing container should be approximately four times larger than container with *P. persimilis*. Mix the remaining contents for a few seconds and empty into the mixing container. Rotate carrier with predators gently for approximately 15 s so that carrier always touches container walls. Record the weight of empty container and determine total weight of carrier and predators. Take one sample per bottle each consisting of 2 % of carrier weight or volume for 200 to 500 mL containers, or minimum 5 % of carrier weight or volume for <200 mL containers.

12. Counting Arena and Test Conditions

12.1 Pour a thin line of a white glue or liquid detergent to form a circle on a white sheet of paper and spread each sample inside the circle. This will prevent predators from escaping during counting. Place the sample directly under a warm bulb (75 W), positioned about 27 cm above table. The test is typically carried out at the temperature 25 ± 2°C.

13. Counting Procedure

13.1 Count and record all predators running out of the carrier. Record and identify any other live organisms in each sample. Examine the content of the carrier within the circle of glue and record the presence of live insects or mites other than *P. persimilis*.

14. Properties of the Test

14.1 The test assumption is that a shipment meets the package claim. The test statistics and known properties of the counts of predators on a carrier are used to judge whether the package claim is met. This is done by comparing the test statistics to a critical value (CV) that is tied to a probability of incorrectly declaring a shipment to be substandard and to the number of samples tested. The CV for four sample sizes and different probabilities of error are presented in Table 1. A CV is determined by selecting a sample size (the number of bottles assessed) and a probability of error. For example, if four samples were assessed and a probability of error of 0.05 was chosen, CV = 32.

14.2 Each probability of error in Table 1 is a measure of the likelihood of wrongly categorizing the package to be deficient when in fact it has the specified number of predators. More precisely, these are the probabilities of obtaining a test statistics that is less than or equal to the CV when the package claim is exactly met.

TABLE 1 Critical Values for the Three Sample Sizes (4, 6, and 10 bottles) and Corresponding Probabilities of Error

True Mean /n	Probability of Error for Sample Size		
	4	6	10
30	0.02	0.01	0.00
31	0.04	0.01	0.00
32	0.05	0.03	0.01
34	0.12	0.07	0.03
36	0.21	0.16	0.10
38	0.34	0.31	0.26
40	0.50	0.50	0.50
42	0.34	0.31	0.26
44	0.21	0.16	0.10
46	0.12	0.07	0.03
48	0..05	0.03	0.01
50	0.02	0.01	0.00
52	0.01	0.00	0.00

14.3 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 10.0. This model was found to well describe counts of predators on a carrier. Additional details on this statistical model are provided in the Appendix.

15. Interpretation of Results

15.1 The quantity of *P. persimilis* in the shipment will be considered below the package claim when the test statistics is smaller than the selected critical value (CV). If an acceptable error rate is 0.05, then the CV for a sample size of 4 is 32 (Table 1). For the example presented in the Table 2, the test statistic 40 is larger than 32; therefore, the shipment would be classified as meeting the package claim.

16. Precision and Bias

16.1 The probabilities of error in Table 1 reflect the precision of the test. There is no consistent bias in the assessment of quantity of *P. persimilis*.

17. Keywords

17.1 *Phytoseiulus persimilis*; predator; quantity; spider mites

TABLE 2 Example: Test Statistic = 40

NOTE 1—Based on four random samples take from 500 mL containers with 2000 predators. The four 2 % samples contain an average of 40 mites.

Sample Number	No. of Mites that Left the Carrier
1	38
2	42
3	45
4	35
Average 160/4 = 40	

APPENDIX

(Nonmandatory Information)

X1. STATISTICAL BACKGROUND

X1.1 The test assumes that a shipment meets the requirement that the number of mite predators in a container meet the package claim. Hence the null hypothesis is:

$$H_o = \mu \geq 100 \quad \text{(X1.1)}$$

where:

μ = mean number of predators in a sample.

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 (for example, ≤ 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:

$$Pr\{\bar{x} \leq CV \mid \mu \geq 100\} = 0.05 \quad \text{(X1.2)}$$

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 40 and determine CV so that:

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

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.

BIBLIOGRAPHY

(1) van Lenteren , et al., "Guidelines for Quality Control of Commercially Produced Natural Enemies," *Quality Control and Production for Biological Control Agents—Theory and Testing Procedures*, J.C. van Lenteren, Ed., CABI Publishing, 2003, pp. 278 –279.

(2) ASTM E2200 Specification for Information Included with Packaging of Multi-Cellular Biological Control Organisms