N O T E

Standardized Rearing Method for Establishing and Maintaining Colonies of *Bradysia impatiens* (Diptera: Sciaridae)[1]

Milton D. Taylor,[2] Richard D. Willey,[3] and Raymond Noblet[4]

Clemson University, Clemson, SC 29634 USA

J. Entomol. Sci. 37(1): 130-133 (January 2002)

Smith-Stocking (1936. Genetics 21: 421-436) was the first to use an agar culture method to rear *Bradysia coprophila* Lintner. She found that the bottom layer of nonnutritive agar provided a consistently moist substrate for pair matings or mass cultures. She used sterilized ground straw on the solidified agar for oviposition and a mixture of equal parts of animal-poultry yeast, powdered mushroom, and straw as the larval food. Subsequent researchers, including Steffan (1966. Univ. Calif. Publ. Entomol. 44: 1-77), Kennedy (1973. Ann. Entomol. Soc. Am. 66: 1163-1164), and Harris (1995. Ph.D. Diss., The Univ. Georgia, Athens 59 pp.) used modifications of the Smith-Stocking procedure. Steffan (1966) employed glass shell vials and a 2:1 agar substrate mixture of bacto-agar and bacto-corn meal agar with a larval food composed of chopped straw, grass compost, and brewers' yeast. He also used slant agar cultures to increase the surface area for adults and larvae. Kennedy (1973. Ann. Entomol. Soc. Am. 66: 1163-1164) proposed a method for rearing *B. impatiens* Johannsen in shell vials on nonnutritive bacto-agar slants. Unlike Steffan (1966), Kennedy (1973) completely sterilized grass clippings to eliminate contamination by mites or other organisms. Later, Harris (1995) found that the addition of lactic acid (0.1%) to the nonnutritive agar (2%) reduced bacterial growth.

Standardized rearing methods are important to improve reproducibility of results across laboratories. Using easily obtained standard ingredients of consistent quality, we developed the following method for establishing and maintaining a colony of *B. impatiens* with minimal cost in equipment, space, and time by simple modifications of the methods of Smith-Stocking (1936), Steffan (1966), and Kennedy (1973).

Ten ml of a 3.25% bacto-agar (Difco Laboratories, Detroit, MI) suspension was pipetted into 25 × 95 mm shell vials, stoppered with a large cotton ball, and placed upright in metal racks. After autoclaving batches of culture vials at 121°C and 861.8 kPa for 20 min and allowing them to cool in the autoclave to 50°C, vials were placed in storage racks (standard arthropod collection type), angled to approximately 20°,

[1]Received 06 November 2000; accepted for publication 15 January 2001.

[2]Department of Environmental Toxicology, Clemson University, Pendleton, SC 29670 USA and to whom offprint requests are to be addressed (e-mail: taylor@clemson.edu).

[3]Family and Youth Development, Clemson University, Clemson, SC 29634 USA.

[4]Department of Entomology, The University of Georgia, Athens, GA 30602 USA.

until the agar solidified. Cooled culture vials were used immediately, stored in racks at room temperature (for up to 14 d), or stored under refrigeration at 4°C until needed.

Dehydrated alfalfa hay (Purina Mills Inc., St. Louis, MO) was sifted through an 850 micron US Standard Sieve (Dual Manufacturing Co., Chicago, IL) and autoclaved at 121°C and 851.8 kPa for 30 min. Cooled alfalfa meal was placed in screw-top plastic jars holding approximately 750 ml and stored at −20°C until needed. To maintain weekly supplies of food at room temperature, alfalfa meal was stored in capped 50-ml centrifuge tubes in the rearing room.

Sterilized alfalfa meal was used as both the larval food source and oviposition substrate. Female flies oviposited in cracks and crevices in the agar substrate or on and under the alfalfa particles. We also found females often would oviposit on agar slants without a food substrate, but oviposition was delayed 1 to 2 d.

Adult *B. impatiens* were collected from a commercial greenhouse range located in Easley, SC, with an aspirator (Hausherr's Machine Works, Toms River, NJ). Approximately 75 to 100 adult flies—roughly equal numbers of males and females—were collected in each aspiration chamber. Flies were allowed to mate in the aspiration chambers while returning to the laboratory. Identification was confirmed by W. A. Steffan at The Burpee Museum, Batavia, IL, from a representative sample of adult flies.

Culture vials were prepared by dusting the agar surface with alfalfa meal and tapping the inverted shell vial to remove any excess. Adult flies were anesthetized by passing a gentle stream of CO_2 (13.79 to 27.58 kPa) through the aspiration chamber for approximately 20 s. Approximately 6 to 12 flies—roughly equal numbers of males and females—were transferred from the aspiration chamber to each culture vial by gently tapping the aspiration chamber against the lip of the culture vial. Because most *Bradysia* spp., including *B. impatiens*, are monogenic (Metz, 1925. Chromosomes and sex in *Sciara*. Science 61: 212-214; Metz and Moses, 1928. Proc. Nat. Acad. Sci. Wash. 14: 930-932), several females were included in each culture vial to increase the odds of both sexes being present among emerging adults. However, culture vials of a single sex could be produced by placing a single fertilized female in each vial. A cotton stopper was placed in the culture vial before flies began to recover from anesthesia. Culture vials were labeled with rearing information and placed in tissue culture racks (Limited Plastics Inc., Lemon Cove, CA) holding 10 to 19 culture vials slated laterally at a 35° angle. Stock cultures were maintained at 25°C ± 1°C and 60 to 75% relative humidity under fluorescent lighting with a photoperiod of 12:12 (L:D) h.

Adults were collected daily from culture vials containing emerging adults of the same generation with an aspirator and allowed to mate in the aspiration chamber for 30 min before anesthetizing them with CO_2. Approximately 10 to 15 flies were transferred to each prepared culture vial. Typically, 10 to 19 culture vials were prepared each weekday once adults began emerging in the original culture vials. Oviposition—as individual eggs to groups of 10 or more—started within hours and extended over 2 to 4 d. Adults survived in culture vials for no more than 5 d, resulting in relatively synchronous development and emergence of adult offspring. Removal of any remaining live adult female flies from culture vials after 24 h resulted in greater synchronicity of larval development. This became standard procedure whenever insects were required in large numbers for testing the following week. Synchronous development was defined as a vial on d 7 that contained no more than 5 first and third instars.

First instars hatched on d 3.5 to 4, molted to second instars on d 6 to 7, to third

instars on d 7 to 8, to fourth instars on d 9 to 10, and pupated on d 12 to 15. Generally, adults began to emerge on d 15 for males and d 16 for females. By d 7 or 8, larvae had consumed the dusting of alfalfa meal, and larval food was added sparingly to cover the agar surface. Additional food was added every 2 d or as needed, depending upon the number of larvae in the culture vial, until about d 11.

When adults ceased emerging (approximately d 30), vials were autoclaved for 10 min at 121°C and 851.8 kPa. The liquified agar was poured off and shell vials washed with Alconox® (Alconox Inc., New York, NY) detergent, allowing multiple rearings from the same shell vials.

Differences in fecundity for male-producing and female-producing "virgin females"—defined as females from culture vials started with a single female—and like producing females from mixed-sex culture vials was determined using Student's *t*-Test.

Using this rearing method, we found mean (±SD) fecundity of 140 ± 34.4 eggs for male-producing "virgin" females, N = 4, and 149 ± 21.2 eggs for female-producing "virgin" females, N = 9. For females from mixed-sex culture vials—females isolated within 1 d after emergence—mean (±SD) fecundity was 89 ± 23.3 eggs for male-producing females, N = 10 and 102 ± 27.5 eggs for female-producing females, N = 11. The significantly lower number of eggs produced by both male- and female-producing females from mixed-sex vials ($P < 0.01$ in both cases) probably reflects mated females laying a portion of their eggs before removal and isolation. The fecundity we observed was consistent with the report of Carson (1946. Genetics 31: 95-113) who reported an average of 111 eggs for female-producing *B. impatiens* and 153 eggs for male-producing females and Kennedy (1974. Ann. Entomol. Soc. Am. 67: 745-749) who reported approximately 129 eggs per female at 20°C and approximately 156 at 25°C. However, Wilkinson and Daugherty (1970. Ann. Entomol. Soc. Am. 63: 656-660) observed an average of only 75 eggs per female with a range of 12 to 156 eggs and no variation in fecundity with increased rearing temperature. Kennedy (1974) attributed these differences to genetic variation or nutritional deficiency in the Wilkinson and Daugherty (1970) technique.

We have described an inexpensive—estimated cost to raise 100 to 200 larvae in a shell vial was <13¢ for consumables including glassware, agar, cotton balls, and amortized equipment costs and <38¢ for labor costs—culture method for establishing and maintaining colonies of *B. impatiens.* Shell vials were reusable and vial storage racks and tissue culture racks were easily constructed from scrap lumber. Startup and maintenance costs were thus minimized. Agar culture vials were quickly prepared and easily stored in the rearing room for several weeks (or for longer periods under refrigeration). Developing larvae were more easily observed in glass shell vials containing agar where the insects remain on the substrate, rather than beneath it, as with manure or soil-containing substrates (Kennedy, 1973). Most sciarid rearing methods have used a fungus as the primary larval food source making observation of the larvae difficult due to mycelial growth. Using alfalfa meal did not require a substrate which supports fungal growth so it was unnecessary to supplement the nonnutritive bacto-agar substrate with another medium, such as the corn meal agar used by Steffan (1966), to augment fungal growth. The sterilized alfalfa meal prevented contamination by mites and other microorganisms which frequently occurs with the use of manure and other decaying substrates.

Our method further produced 100 to 200 larvae per culture vial in a small rearing room without using environmental chambers. Most larvae were at the same stage of

development. The method was easily adapted to meet experimental requirements. Also, eggs, early first instars, and larvae of known sex were produced for testing by isolating females on agar slants without alfalfa meal. While we observed a slight seasonal variation in development times, probably attributable to average room temperature changes, we found a mean development time, egg to adult, for *B. impatiens* of 15 to 16 d.

We have maintained two self-sustaining colonies of *B. impatiens* for 34 and 50 generations—1.75 and 2.5 years, respectively—producing 35,000 to 50,000 larvae per wk for laboratory bioassays with no observed decline in culture viability. We also have cultured several generations of shore fly, *Scatella stagnalis* Fallen (Diptera: Ephydridae) using this method. Gillespie (1986. J. Entomol. Soc. Brit. Columbia 83: 45-48) maintained cultures more than 3 yr with occasional supplemental introduction of wild flies collected from greenhouses, but Kennedy (1973) found such additions were unnecessary in his method, rearing 20 laboratory generations of *B. impatiens* with no significant decline in culture viability.

The authors thank Elmer Gray and the Medical and Veterinary Laboratory staff of the Department of Entomology, Clemson University for assistance at various stages of this project. Partial funding was provided by Abbott Laboratories, North Chicago, IL. This is South Carolina Agriculture and Forestry Research System Clemson University Technical Contribution Nr. 4443.