N O T E

A Method for Ovipositor Cleaning of Field-Collected *Lygus lineolaris* (Palisot de Beauvois)[1]

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Wild tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), from many fields in Mississippi are contaminated with microorganisms, especially *Aspergillus niger* (pers. observ., USDA, ARS Robert T. Gast Rearing Laboratory, Mississippi State, MS isolate). Those collected from the field to establish or invigorate a laboratory colony often cause contamination. Because *A. niger* spores are air-borne and thrive at the growth conditions used in rearing many insects, it is difficult to control contamination by this microorganism in laboratory-reared insect colonies (Funke 1983, Bull. Entomol. Soc. Am. 29: 41-44; Gifawesen et. al. 1975, J. Econ. Entomol. 68: 441-444; Clark et. al. 1961, J. Econ. Entomol. 54: 4-9; Singh and Bucher 1971, Entomol. Exp. Appl. 14: 297-309; Ouye 1962, J. Econ. Entomol. 55: 854-857). Gel packets used for oviposition (Patana 1982, J. Econ. Entomol. 75: 668-669) by field-collected *L. lineolaris* are contaminated with *A. niger* at a rate of 75% in the Gast Rearing Laboratory (unpubl. data). Although F1 generation eggs hatch, the microorganism becomes established in the insect colony. A method for cleaning the ovipositors of field-collected tarnished plant bugs is described in this paper.

Lygus lineolaris used in this study were collected from weeds in Clay Co., MS. Voucher specimens were placed in the Mississippi State University Entomology Museum. Experimental set-up for the insects was the same as previously described by Alverson and Cohen (2002, J. Econ. Entomol. 95: 256-260) with the following modifications. Insects collected from the field were divided into two cages with approximately 500 insects each for the first replicate and 200 insects each for the following two replicates. Differences in number of insects used in replicates was due to availability. Three replications were performed. Blocks consisted of replication in time. Cages used were Rubbermaid® 7.8-L Servin'Saver™ (Wooster, OH) rectangular plastic storage boxes. Cages were topped with 1.0-mm mesh fiberglass screen held tight by the box's snap-on top that had a 23 × 34 cm opening cut into it. Growth conditions were a light-dark cycle of 16:8 h, temperature of 27°C (±2.0°C), and relative humidity of 60% (±3%). Cages were placed on wire racks in a rearing room

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that allowed air circulation and light to reach each cage. Gel oviposition in Parafilm® (Pechiney Plastic Packaging, Menasha, WI) packets developed by Patana (1982, J. Econ. Entomol. 75: 668-669) were used in this study. Feeding packets and gel packets were made from 20.8 × 10 cm strips of Parafiim, folded and sealed with a heat sealer (Deni Freshlock Turbo II, Keystone Manufacturing, Co., Buffalo, NY) along two sides to create a 10.4 × 10 cm packet with an open top for filling. The diet used in these experiments was the artificial diet developed by Cohen (2000, J. Entomol. Sci. 35: 301-310). A 4% gel made from Gelcarin® (FMC-Food Ingredients Division, Rockland, ME) and tap water and then autoclaved, was used for oviposition (Cohen 2000, J. Entomol. Sci. 35: 301-310). Gel packets with no antimicrobial agents added were provided to the field-collected insects when the cages were first set up to confirm the presence of *A. niger* contamination. All of the cages containing the field-collected insects were contaminated as evidenced by *A. niger* growth within the oviposition gel packets after eggs were laid in them and the packets incubated for 5 to 8 d at 27°C. Oviposition packets were changed every 24 h. Control insects continued to be given gel packets with no antimicrobials added for 13 d. Test insects were given gel packets with 0.2% propionic acid added for 1 day, and then were given regular gel packets with no antimicrobials added for the following 12-d experiment period.

We observed a dramatic decrease in the *A. niger* contamination rate in the oviposition gel packets from the test insects. No *A. niger* contamination was found in the oviposition gel packets from the test insects either on the first day with the propionic acid-treated oviposition gel packets, or in the untreated gel packets from the subsequent 12-d experiment period (Table 1). Thus, using propionic acid for only 1 day in the oviposition gel packets resulted in the oviposition gel packets for the subsequent 12-d period remaining free of *A. niger* contamination. The oviposition gel packets from the control insects with no antimicrobials added had a 76.9 ± 4.4% (mean ± SE over 3 test replications) contamination rate (Table 1). This was not significantly different from the contamination rate that had been previously observed in the colony (75%, unpubl. data), but was significantly different from the zero contamination rate seen in the test insects (ANOVA, Fisher's protected least significant difference (LSD) mean comparison, SAS version 8.01, 2000).

Eggs from the test insects and the control insects laid in the untreated oviposition gel packets hatched at a rate of 55% (data not shown). Eggs from the test insects laid in the propionic acid-treated gel packets hatched at a lower rate (35 to 40%, data not shown). The average hatch rate for the Gast Rearing laboratory *L. lineolaris* colony is 55% (unpubl. data).

Table 1. *Aspergillus niger* contamination rate in oviposition gel packets

Replicate	Control*	Test*
1	10+, 3–	0+, 13–
2	9+, 4–	0+, 13–
3	11+, 2–	0+, 13–
Experimental Means**	76.9% ± 4.4 SE a	0% b

* Number of gel packets positive (+) or negative (–) for *A. niger* contamination.

** Means ± SE followed by the same letter were not significantly different (LSD test, $P < 0.01$, SAS Institute, Inc., software version 8.01, 2000).

For each of the three replications of the experiment, 20 nymphs hatched from gel packets contaminated with *A. niger* (60 total) and 20 nymphs hatched from uncontaminated gel packets (60 total) were sacrificed and plated individually on Sabouraud's Dextrose Agar (SDA) (Difco, BD Diagnostics Systems, Sparks, MD) and incubated at 27°C for 5 to 8 d. In all cases, the nymphs hatched from eggs in gel packets contaminated with *A. niger* supported *A. niger* growth (100% contamination rate), and the nymphs hatched from eggs in gel packets not contaminated with *A. niger* did not grow the mold after incubation on SDA at 27°C for 5 to 8-d (zero contamination rate). Using only oviposition gel packets uncontaminated with *A. niger* to seed a new cage of *L. lineolaris* is, thus, a crucial means to eliminate this fungal contaminant from the insect colony.

Insectary insects are often outcrossed to wild insects, and if wild insects are not quarantined for several generations, they may introduce many microorganisms that may be difficult to eliminate from the insectary such as *Serratia marcescens, Pseudomonas* spp., and *Aspergillus* spp. (Sikorowski and Lawrence 1994, Am. Entomol. 40: 240-253). *Aspergillus niger* has been shown to decrease fecundity, increase nymphal mortality, and decrease biomass in *L. hesperus* (Alverson 2002, J. Entomol. Sci. 37: 338-344). Other scientists have also shown that *A. niger* contamination in insectaries is associated with high mortality of young insects, a prolonged development time, and smaller than normal larvae and adults (Howell 1971, J. Econ. Entomol. 64: 631-636; Singh and Bucher 1971, Entomol. Exp. Appl. 14: 297-309; Sikorowski et. al. 1980, J. Econ. Entomol. 73: 106-110). Controlling *A. niger* contamination in insect colonies is desirable to reduce impact on insect health and to maintain productivity of quality insects in the insectary. The development of techniques such as the described method of ovipositor cleaning for *L. lineolaris* help in achieving that goal.

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