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Postharvest treatment research at USDA-ARS: Stored product fumigation

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ABSTRACT

The overall goal of this USDA-ARS research is to ensure the protection and quality of stored product foodstuffs. The results of this research directly enhance production, distribution, and safety of foodstuffs, promote and retain access of United States-grown crops to domestic and foreign markets, and protect the United States and trading partners from the agricultural, ecological and economic threat posed by quarantine and invasive pests. In general, USDA-ARS research related to the fumigation of stored products focuses on the development of techniques to rapidly disinfest raw products of field pests, control pests in processed products amenable to re-infestation and microbial infection, and reduce reliance on fumigation as a stand-alone measure for postharvest disinfestations and disinfections. Specific research objectives include: comparative evaluation of alternative fumigants to methyl bromide in postharvest applications, development of novel technologies to reduce and eliminate atmospheric emissions from chambers used in postharvest fumigation, and design production strategies that allow for a more strategic postharvest use of methyl bromide and alternative fumigants. Recent research findings will be presented and discussed, including: exposure requirements of phosphine on key stored product pests (as related to resistance management), the insecticidal efficacy of fumigant mixtures, and the juxtaposition of methyl bromide regulations and maximum residue level (MRL) regulations. For several key species of stored product insects endemic to California USA, detailed below are experimental details associated with the collection and preparation of eggs for fumigation studies.

Key words: Food safety, Food security, Phosphine resistance, Postharvest methyl bromide

The globalization of agriculture challenges political, regulatory, and industrial entities to balance consumer- and policy-driven demands for food security and food safety with human and environmental health concerns. For example, methyl bromide (MB), as a postharvest fumigant, is highly effective against insects and microorganism pests (Johnson et al., 2012; Bell et al., 1997). MB is also an atmospheric source of reactive bromine gases (Lary, 1996), which deplete stratospheric ozone (Solomon, 1990; Yung et al., 1980). Anthropogenic utilization of MB is regulated by international agreement under the Montreal Protocol, which specifically restricts the production and sales of MB. In instances where MB can no longer be purchased for the purpose of conducting postharvest fumigations, alternatives are required. Toxicological analyses must be conducted, environmental impacts of the treatment assessed, and the economics of accompanying cost(s) evaluated, for each agricultural sector where MB is replaced.

With respect to >2,000,000 tonnes of dried fruit and tree nuts produced annually in California, which includes >99% of the prunes, apricots, raisins, figs, dates, almonds, walnuts, and pistachios grown in the USA, the steady increase in the implementation of integrated pest management programs over the last decades has not been able to eliminate the reliance on postharvest fumigation. For handlers of these commodities, insect control can be generalized by two scenarios: the disinfestation of raw product from "production" pests within hours or days after harvest

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and the disinfestation of "stored product" from storage pests whenever the need arises. MB, which quickly penetrates commodity loads and has non-discriminating efficacy against targeted pests relative to other fumigants, was used successfully for this purpose over the last 2 decades to the extent that the infrastructural capabilities and logistics of the dried fruit and nut industry have evolved in concert with MB use. For either scenario, MB fumigations are typically conducted in a chamber at temperature >10°C and last 24 h or 4 h when conducted at normal atmospheric pressure or 100 mm Hg, respectively (Hartsell et al., 1991; Tebbets et al., 1986; Tebbets et al., 1978).

Sulfuryl fluoride, phosphine, propylene oxide, and ethyl formate are just a few fumigants that have been proposed as alternatives to MB for the control of insects in dried fruit and tree nuts from California. In general, eggs are relatively more tolerant toward these alternatives than other life stages. Here we report experimental details associated with the collection and preparation of eggs for fumigation studies involving the following species: *Carpophilus hemipterus* (L.) (Coleoptera, Nitidulidae), *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), and Carob moth (CbM), *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae).

MATERIALS AND METHODS

Insects and egg collection: Specimens were cultured in the insectary at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), San Joaquin Valley Agricultural Sciences Center, Parlier, CA (USDA, 2012). Cultures were housed in a 15.2 m^3 rearing unit maintained at 27 ± 1°C ($\bar{x} \pm s$) and 60 ± 5% r.h. ($\bar{x} \pm s$) with a photoperiod of 16:8 (L:D) h. Rearing procedures and diets, briefly mentioned below, were as reported in the Crop Protection and Quality Research Unit electronic rearing manual (USDA, 2007). Voucher specimens of *C. hemipterus*, *T. castaneum*, *L. serricorne*, *P. interpunctella*, *E. elutella*, *A. transitella*, and *C. pomonella* adults were preserved in 95% ethyl alcohol and deposited at the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 138, 139, 140, 141, 142, 143, and 144, respectively. Eggs were counted using a dissecting microscope (8 to 10 × magnification). Other methods for egg collection are detailed here.

Dried fruit beetle (DFB), *C. hemipterus* (L.), was originally collected in 1978 from Italian Swiss Colony Winery in Fresno County, California USA and cultured in 946 mL glass jars on ripened banana placed atop soil substrate. An oviposition unit (OU) was developed based on the finding that DFB females will oviposit into crevices. One OU was comprised two glass slides (75 l × 25 w × 1 h mm). A thin smear of lima bean-based agar diet (codling moth diet; USDA 2007) was centered along the length of each slide. Four wax paper strips (75 × 20 mm) were each folded in half lengthwise (75 × 10 mm). Along each length of the slide, two of the folded strips were aligned with the creases spanning the center of the slide and paralleling the diet. Two identically prepared slides were placed together sandwiching the diet and wax paper between them. All the edges were aligned and the slides held together by a rubber band (#16) stretched lengthwise down the center. Four OUs were stood upright inside a 236 mL glass jar. DFB adults (n = 75 to 100) were transferred to the jar. The glass jar was sealed with a filter paper (Whatman® #1, 70-mm diameter), followed by a wire screen (U.S. #40 mesh, 70-mm diameter), and both were secured a top the jar with a threaded metal ring. The jars were transferred to the rearing unit for an oviposition period of 12 to 18 h. Afterwards, the OUs with eggs were retrieved and the adults were brushed off and back into the jar using a soft, horsehair brush. The respective number of eggs per OU were counted and recorded within a duration of ~ 2 h. Prior to fumigation treatments, or concomitant use as untreated controls, the OUs with eggs < 20 h old were transferred to an empty 236 mL glass jars with only a #40 mesh stainless-steel screen lid (70-mm diameter) secured as above.

Red flour beetle (RFB), *T. castaneum* (Herbst), was obtained from a laboratory colony maintained at USDA, Manhattan, KS in 1967. RFB adults (200 to 300) were transferred to a 946 mL glass jar filled with 20 to 25 g of sifted white flour (U.S. #70, 0.21 mm^2 openings). The jar was sealed with filter paper (Whatman® #1, 90mm diameter) followed by a wire screen (U.S. #40 mesh, 90mm diameter) and both were secured a top the jar with a threaded metal ring. The jar was transferred to the rearing unit for a 72 h ovipositional period, after which, eggs were separated from the adults and flour using a stack of sieves (Seedburo Equipment Company, Des Plaines, IL). The contents of the jar were poured into the top sieve (U.S. #25, 0.71 mm^2 openings) and shaken vigorously for a few minutes. The eggs were retrieved, along with a small amount of flour remnant, from the underlying sieve (U.S. #70, 0.21 mm^2 openings) by decanting

them into a glass Petri dish (100 diameter × 15 mm high). Using a small, horsehair brush, 100 to 200 eggs were transferred onto black velour paper that lined the inside of a 35 mm diameter Petri dish (Falcon, Oxnard, CA). Wheat bran diet (5 g), prepared as described in the rearing manual (USDA 2007), was placed in each 10 cm diameter plastic Petri dish cages. The diet was spread concentrically to the outer edge of each dish bottom and a single 35 mm Petri dish, containing the < 72-h old eggs, was placed in the center of the void. After fumigation treatment, or concomitant use as non-treated control specimens, lids were placed atop the 10 cm diameter plastic Petri dish cages.

Cigarette beetle (CB), *L. serricorne* (F.) was originally obtained from whereabouts unknown circa 1967 and cultured in 946 mL glass jars filled with ~225 g of rice bran diet. CB adults (200 to 300) were transferred to a 946 mL glass jar filled with 20 to 25 g of sifted white flour (U.S. #70, 0.21 mm^2 openings). The jar was sealed with filter paper (Whatman® #1, 90mm diameter) followed by a wire screen (U.S. #40 mesh, 90-mm diameter) and both were secured a top the jar with a threaded metal ring. The jar was transferred to the rearing unit for a 72 h ovipositional period, after which, eggs were separated from the adults and flour using a stack of sieves (Seedburo Equipment Company, Des Plaines, IL). The contents of the jar were poured into the top sieve (U.S. #25, 0.71 mm^2 openings) and shaken vigorously for a few minutes. The eggs were retrieved, along with a small amount of flour remnant, from the underlying sieve (U.S. #70, 0.21 mm^2 openings) by decanting them into a glass Petri dish (100 diameter × 15 mm high). Using a small, horsehair brush, 100 to 200 eggs (< 72 h old) were transferred onto black velour paper that lined the inside of a 35 mm diameter Petri dish. CB eggs were caged and prepared for testing as described above for RFB eggs.

Navel orangeworm (NOW), *A. transitella* (Walker), was originally obtained in 1966 from the University of California, Berkeley and cultured in 3.8 L glass jars filled with ~400 g of wheat bran diet (Tebbets et al., 1978). NOW eggs were obtained by transferring adult moths (150 to 200) into a 2.8 L stainless-steel can (16.5 cm diameter). The can was covered with a white paper towel, which served as the substrate for oviposition. The paper towel was held in place with two rubber bands (size 18). The can was laid on its side on a shelf in the rearing unit with the paper towel lid facing towards the light sources. After 72 h, the paper towel was removed. The < 72h old eggs were harvested by counting and cutting sections of paper towel to contain 200 to 300 eggs each. Petri-dish cages were prepared and diet was concentrically introduced as described above for RFB, however, NOW eggs attached to a paper towel section were placed on top of an inverted plastic weigh dish (41 × 41 × 8 mm) placed at center of each cage bottom.

Codling moth (CM), *C. pomonella* (L.), was originally obtained in 1984 from an apple orchard in North Fork, Fresno County, CA. Approximately, 120 adult *C. pomonella* were aspirated from 30 mL rearing cups containing lima bean agar diet and were transferred into a 2.1 L stainless-steel can, which had the bottom removed. The inner surface of the can was lined with velour poster board (Hygloss Products Inc., Wallington, NJ). Both ends of the can were closed with wax paper, which was secured with plastic snap cap lids. The can was transferred to the rearing unit for a 72 h ovipositional period. The adults were tapped to one side of the can and the < 72 h old eggs were harvested from the opposite end of the can by removing the wax paper. The wax paper was trimmed into sections containing 200 to 300 eggs each and each section was placed in Petri-dish cages on top of an inverted plastic weigh dish (41 × 41 × 8 mm) as described above for NOW.

Indian meal moth (IMM), *P. interpunctella* (Hübner), was originally collected from a walnut packing house in Modesto, Stanislaus County, California in 1967 and cultured in 3.8 L glass jars filled with ~400 g of wheat bran diet (USDA, 2007). To collect eggs, adults (ca. 200 to 300) were aspirated and transferred to a 1.9 L glass jar. Inside the jar, each of two paper towel strips (7.6 cm × 22.5 cm) were taped to the bottom in a manner that allowed them, upon inversion (vide infra), to hang in parallel without touching the screen lid. Each glass jar was covered with a wire screen (size, U. S. #40 mesh) that was secured using a threaded metal ring. The jar was transferred to the rearing unit, inverted, and aligned atop a paper clip spacer, which sat inside the bottom of a Petri dish (90 d × 20 h mm) lined with a single filter paper (Whatman® #1, 90mm diameter). Following a 72 h ovipositional period, the side of the jar was tapped several times, which allowed the < 72 h old eggs to drop through the mesh screen for collection on the filter paper. The jar and the paper clip were removed from the Petri-dish. Thereafter, eggs were separated from other contents atop the filter paper using a stack of sieves (Seedburo Equipment Company, Des Plaines, IL). The contents were first poured into the top sieve (U.S. #25, 0.71 mm^2 openings) and shaken vigorously for a few minutes.

The eggs were retrieved from the underlying sieve (U.S. #60, 0.25 mm^2 openings), sorted into groupings containing 200 to 300 specimens, and then caged as described above for RFB eggs.

Tobacco moth (TM), *E. elutella* (Hübner), was originally collected in 1969 from the USDA Tobacco Investigations Laboratory, Richmond, VA and cultured in 3.8 L glass jars filled with ~400 g of wheat bran diet (USDA, 2007). The < 72 h old TM eggs were collected as described above for IMM; however, the groupings containing 200 to 300 specimens were transferred onto double-sided sticky tape attached to the bottom of a 10 cm diameter plastic Petri-dish cage. After fumigation treatment, or concomitant use as non-treated control specimens, lids were placed atop the 10 cm diameter plastic Petri dish cages.

Carob moth (CbM), *E. ceratoniae* (Zeller), was originally obtained in 2009 from Dr. Tom Perring at the University of California, Riverside and cultured in 3.8 L glass jars filled with ~400 g of wheat bran diet (Tebbets et al., 1978). CbM eggs were collected and prepared for fumigation as described for NOW.

RESULTS AND DISCUSSION

Results of fumigation studies involving sulfuryl fluoride, propylene oxide, sulfuryl fluoride –propylene oxide mixtures, and ethyl formate will be reported in separate manuscripts.

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