Journal of Asia-Pacific Entomology 15 (2012) 13–16

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Laboratory evaluation of the chemosterilant lufenuron against the fruit flies *Ceratitis capitata*, *Bactrocera dorsalis*, *B. cucurbitae*, and *B. latifrons*

Chiou Ling Chang [a], Il Kyu Cho [b], Qing X. Li [b,*]

[a] U.S. Pacific Basin Agricultural Research Center, 64 Nowelo Street, Hilo, HI 96720, USA
[b] Department of Molecular Biosciences and Bioengineering, University of Hawaii, 1955 East-West Road, Honolulu, HI 96822, USA

ARTICLE INFO

Article history:
Received 8 March 2011
Revised 26 July 2011
Accepted 29 July 2011

Keywords:
Lufenuron
Fruit fly
Ceratitis capitata
Bactrocera dorsalis
Bactrocera cucurbitae
Bactrocera latifrons

ABSTRACT

Four species of tephritid fruit flies, *Ceratitis capitata*, *Bactrocera dorsalis*, *B. cucurbitae*, and *B. latifrons* were evaluated for toxic, developmental, and physiological responses to the chemosterilant lufenuron. No significant mortality of laboratory strains of the first three species was observed after their exposure up to 50 μg/mL of lufenuron in agar adult diet, whereas *B. latifrons* adults fed with 50 μg/mL of lufenuron in the diet caused significant mortality compared to the control. Fertility of *C. capitata* adults fed on 50 μg/mL lufenuron-fortified diet between 7 and 12 days of age was approximately 46% of the no lufenuron control. Fertility of *B. dorsalis* and *B. latifrons* adults fed on 50 μg/mL lufenuron-incorporated diet was about 45% and 62% of the control, respectively. Lufenuron did not significantly affect fertility of *B. cucurbitae* adults. Lufenuron did not affect fecundity of *C. capitata* and *B. dorsalis*. Fecundity of *B. cucurbitae and B. latifrons* was not evaluated due to difficulty to count the eggs laid deep in the agar diet. Larvae fed on a liquid larval diet with ≤0.1 μg/mL of lufenuron were also evaluated. Pupal recovery, adult emergence, adult fliers, mating, egg hatch, and egg production of *C. capitata* were significantly decreased, while for *B. dorsalis*, pupal recovery, larval duration and adult emergence were affected. No effect of lufenuron on *B. cucurbitae* larvae was observed. *B. latifrons* was not performed because shortage of eggs at the time of this research. Lufenuron is a potential agent for management and control of *C. capitata* and *B. dorsalis*.

Introduction

Tephritid fruit flies are among the most important insect pests in subtropical and tropical climates around the globe and cause a substantial loss of agricultural products. The sterile insect technique (SIT) is one of the most effective methods for tephritid fruit fly control, in which sterile male fruit flies are released into the field to compete with wild males for wild females leading to reduced fruit fly populations. Currently, radiation is the most broadly used method to sterilize the males. Radiation is associated with issues of cost-ineffectiveness, facility requirement and maintenance, personnel safety, and product irradiation safety (Torres-Rivera and Hallman, 2007). It would be beneficial if there were some other means to safely induce sterility via larval or adult diets during the rearing process.

Pesticides have also been effective for fruit fly control. Most effective insecticides for fruit fly control today include malathion, fipronil, spinosad, and Suredye. They are used in the field and, thus, are labor intensive (Alcantara-Licudine et al., 2002). The insecticides used in the field either as baits or sprays will end up in the environment, whereas those used in rearing diets can be collected and disposed of safely.

Lufenuron (a chitin-synthesis inhibitor) has remarkable effects on the development and reproduction of *Drosophila melanogaster* (Wilson and Cryan, 1997). Wilson and Cryan (1997) found that the eggs from *D. melanogaster* adults exposed to 10 ppm of lufenuron failed to hatch and examined the embryos. Mosson et al. (1995) reported that lufenuron disrupted the molting process, prevented egg hatch, and eradicated the German cockroach, *Blattella germanica* after 12 months of spraying in a commercial freight containers that had been specially modified to serve as a simulated domestic environment. However, similar parameters were not completely effective against the oriental cockroach, *Blatta orientalis;* incorrect dosage is suspected as the key factor (Mosson et al., 1995). Lufenuron was also tested for toxicity to the cotton bollworm, *Helicoverpa armigera* (Butter et al., 2003). They reported that different larval instars significantly differed for LC_{90}, but not significantly for LC_{10} and LC_{50}. They also reported that lufenuron-treated larvae had swollen heads and were significantly smaller than the control. Larval weight, pupal length, pupal weight, and pupal duration were all significantly increased by lufenuron; and pupal deformities also occurred. Avila et al. (1999) studied the effect of lufenuron on fecundity and egg

* Corresponding author at: Department of Molecular Biosciences and Bioengineering, University of Hawaii, 1955 East-West Road, Honolulu, HI 96822, USA. Tel.: +1 808 956 2011; fax: +1 808 956 3542.
E-mail address: qingl@hawaii.edu (Q.X. Li).

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doi:10.1016/j.aspen.2011.07.012

viability of cucurbit beetle, *Diabrotica speciosa* (Coleoptera), under laboratory conditions and found that fecundity was significantly reduced for 4 days and egg viability was significantly reduced for 8 days if both males and females were treated with lufenuron. Moya et al. (2010) recently evaluated the chemosterilising effect of lufenuron against four economically important Latin American fruit flies species: *Anastrepha ludens*, *A. obliqua*, *A. serpentina* and *A. striata* (Diptera: Tephritidae). They reported that lufenuron is a potentially viable agent for controlling *A. striata* (guava fruit fly) because the egg hatch was reduced 40% when crossing treated males with untreated females, whereas treated *A. ludens* (Mexican fruit fly) females that mated with untreated males produced sterile offspring in a similar fashion to those when both sexes were treated.

Recent studies have shown that lufenuron is very potent to the Mediterranean fruit fly (medfly), *Ceratitis capitata* in multiple-year field trials at a rate of 10–50 g/ha in Spain (Navarro-Llopis et al., 2004, 2007). Lufenuron as a chemosterilant showed good control results to reduce medfly populations. When *C. capitata* females ingest bait containing 1000 μg/g lufenuron, hatching of the subsequently laid eggs was inhibited. Females that mated with lufenuron-treated males (5 mg/g AI in diet) laid non-viable eggs (Katsoyannos et al., 1999). However, to our knowledge, laboratory test toxicity studies of lufenuron to tephritid fruit flies are very limited. The objective of the present study was to evaluate the effectiveness of lufenuron on sterility of four fruit fly species in Hawaii through artificial liquid larval diet and adult diet in the laboratory. In this study, we attempted to develop a fruit fly sterility procedure using the chemosterilant lufenuron. Both males and females were reared on the lufenuron-incorporated diet.

Materials and methods

Insects

Newly laid eggs and newly emerged laboratory strain adults of *C. capitata* (Wiedemann), *B. dorsalis* (Hendel), *B. cucurbitae* (Coquillett), and *B. latifrons* (Hendel) from the USDA/ARS Pacific Basin Agricultural Research Center facility in Hilo, Hawaii were used in this study.

Chemicals and diet preparations

Lufenuron standard (99.5%) was diluted to 100 and 500 μg/mL in HPLC grade acetone for later use. For adult diet, 1 mL of 100 μg/mL or 500 μg/mL lufenuron solution was incorporated into 10 g agar diet (sugar:hydrolyzed yeast, 1:3) to make a final concentration of 10 and 50 μg/g diet. For larval diet, 20, 50, 100, 150 and 200 μL of 100 μg/mL lufenuron solution was incorporated, respectively, to each 200 mL of fruit fly liquid larval diet as described by Chang et al. (2006, 2007) to achieve final concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1 μg/mL. Acetone (500 μL) was used as a control.

Effect of lufenuron through incorporation into adult agar diet

All four fly species were tested in the same way as described below.

Mortality test

Newly emerged adults were sexed within 24 h of emergence. Twenty five adults of each sex were transferred to a 500-mL wax cup designed for holding adults. The above mentioned lufenuron-incorporated agar diets were provided along with adequate water as a standard operational procedure. Agar diets were replenished daily. Mortality was observed and recorded daily; and dead flies were removed daily. Three independent repeat experiments and four replicates per treatment in each repeat experiment were conducted with three different batches of flies (12 replicates per treatment).

Fecundity and fertility

Four hundred pairs of newly emerged adults were sexed and transferred into a metal adult holding cage for mating and egging. At sexual maturity ages (*B. cucurbitae* 11-d-old, *B. dorsalis* 11-d-old and *C. capitata* 6-d-old) an egging apparatus was inserted into cages for egg collection and egg hatch. Eggs were continuously collected for 7 to 10 days. The total volume of eggs collected was divided by collection days and number of females to obtain the eggs per female per day (eggs/female/d). Four sets (replicates) of 100 eggs per treatment were then seeded on a blotting paper to measure fertility.

Effect of lufenuron through incorporation into liquid larval diet

One millilter of 6-h-old eggs was seeded onto 0.01, 0.025, 0.05, 0.075 and 0.1 μg/mL lufenuron-incorporated liquid larval diet. Pupal production, larval duration, pupal weight, adult emergence, adult fliers, egg production, egg hatch, and percent mating were used as evaluation parameters as described by Chang et al. (2006, 2007).

All data present here were repeated four times from each batch of insect. Data were presented as mean ± standard errors, and subjected to analysis of variance. Means separations for significant factors were done using a protected least significant difference test (SAS 2008).

Results and discussion

Effect of lufenuron through incorporation into adult agar diet on fruit fly performance

In this study, effects of lufenuron on adult fly mortality, fecundity and fertility of the four fruit fly species were compared.

Mortality

There were no significant mortalities before reaching sexually mature ages in *C. capitata, B. dorsalis,* and *B. cucurbitae*. These results have led to speculation that: (1) the dosage tested was not high enough to cause any mortality, so further tests may be needed. Mosson et al. (1995) found that lufenuron affected adult mortality of the German cockroach, *Blattella germanica*, but not the oriental cockroach, *Blatta orientalis,* even after 18 months exposure to lufenuron bait or spray treatments. (2) Lufenuron simply may not have high acute toxicity to the tested species through oral ingestion. However, in our last test with *B. latifrons*, 30 and 50 μg/mL of lufenuron in the adult diet, caused significant mortality compared to the control (data not shown). A mortality of 8.5% for females and 14% for males was found for adults fed on diets with no lufenuron while 40.5% and 43.5% mortality were found for the 30 μg/mL level, and 41.5% and 44.5% mortalities were found for the 50 μg/mL level for females and males, respectively.

Fecundity and fertility

Among the four species tested, lufenuron decreased fertility (egg hatch) of *C. capitata*, *B. dorsalis* and *B. latifrons* adults in comparison with the respective no lufenuron control (Table 1). The results showed that *C. capitata, B. dorsalis,* and *B. latifrons* adults fed on the 50 μg/mL lufenuron adult diet were significantly different from the other concentrations in terms of mean egg hatch (Table 1). Lufenuron had no significant effects on *B. cucurbitae* egg hatch (Table 1). However, control (0 μg/mL) adults yielded similar results on egg hatch through 8–10 consequent days of egg collections for *C. capitata* (Fig. 1) and *B. cucurbitae* (Fig. 2).