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TOXICITY OF SULFURYL FLUORIDE (VIKANE®) TO FRUIT FLIES IN LABORATORY TESTS

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ABSTRACT

Exotic pests such as fruit flies pose the greatest threat to California's agricultural production. It is estimated that the economic impact of the Mediterranean fruit fly (medfly), *Ceratitis capitata,* would be as high as US$ 1.4 billion annually if it were to become established in the state. Additionally, direct costs of embargoed exports to Japan and other trading partners could total nearly US$ 500 million. The impending loss of methyl bromide (MB) poses a significant threat to quarantine security presently insured by MB treatments. Thus, alternative chemical fumigants are needed as commodity quarantine treatments for successful management of this pest and other exotics. One alternative fumigant is sulfuryl fluoride (SF). It is presently labeled for use as a structural fumigant against termites and wood boring beetles but does not yet have a food tolerance. Laboratory tests were conducted against life stages of the medfly, melon fly (MFF), *Bactrocera curcubitae,* and Oriental fruit fly (OFF), *Bactrocera dorsalis,* to determine if SF is sufficiently toxic to be used as a fumigant for control of these exotic pests. Results of 4-h atmospheric fumigations at 23.6ºC showed that 1st and 3rd instars of each species were relatively susceptible to SF with LC_{95} values ranging from 4.7 mg/L to 70.6 mg/L. One-hour old eggs of each species were relatively tolerant compared with 1st and 3rd instars with LC_{95} values ranging from 116.3 mg/L (medfly) to 224.2 mg/L(OFF). Susceptibility of eggs was directly related to egg age, the 1-day-old eggs being the most susceptible and the 24- and 48-h-old eggs more tolerant. Fumigations at fixed doses with variable exposure times were conducted against OFF eggs, which were the most tolerant among the stages tested. Exposures ranging from 2 to 12 h indicated that the relationship between concentration and time is nonlinear throughout the tested range, being weighted toward the time variable - favoring long exposure periods for efficient control. Thus, the large doses required for control of fruit fly eggs would preclude exposure times shorter than 48 h, at which time the eggs begin to hatch. These results indicate that SF has potential for controlling active life stages of fruit fly pests in short exposure periods but has little or no potential in quarantine treatments due to the relative tolerance of the egg stage.

INTRODUCTION

California produced 13.9 million tons of fruit and nut crops in 1998 that had a value of US$ 6.5 billion (Anon., 1998). Exotic pests such as Tephritid fruit flies pose the biggest threat to California agriculture. This threat is increasing because of increased international air transport, tourism, and human immigration from semitropical and tropical regions where many of these pests are indigenous. The economic impact of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), alone could be US$ 1.4 billion annually if it were to become established in the state with additional direct costs of embargoed exports to Japan and other trading partners of more than US$ 450 million (Orbach, 1995). With increased world trade and the need to protect U.S. exports, our dependence upon effective quarantine treatments to combat these invasions has never been greater.

Quarantine security for a variety of commodities and insect pests is presently ensured by methyl bromide (MB) treatments. The value of U.S. fruit, vegetable and nut imports in 1996 requiring MB fumigation was US$ 344.7 million, and the value of exported foods requiring MB fumigation was US$ 107.2 million (Anon., 1997). Indeed, 33 countries require MB as a treatment for importation of one or more U.S. commodities. However, due to recent regulatory action (UNEP 1992), MB production is being phased out for all uses except quarantine treatments. Because quarantine fumigations represent such a small part of overall MB usage, MB production may become economically unfeasible, even for quarantine treatment. Therefore, the development of MB alternatives is imperative to continued trade in agricultural commodities.

One MB alternative is sulfuryl fluoride, presently marketed under the trade name Vikane7 (Schneider, 1993). This fumigant has been registered for structural fumigations against termites, wood boring beetles and pantry pests for nearly 40 years (Stewart, 1956). It has been used to fumigate a variety of buildings and non-edible commodities but as yet has not been used in food premises because of the lack of food tolerances. Recent studies showed that sulfuryl fluoride has potential to control a wide variety of postharvest pests (Reichmuth *et al.,* 1997; Reichmuth *et al.,* 1999; Bell and Savvidou, 1999; Bell *et al.,* 1999; Schneider and Hartsell, 1999) including quarantine pests (Zettler *et al.,* 1999; Zettler and Arthur, 2000). Because of its performance against these pests, we tested the toxicity of sulfuryl fluoride against the medfly, the melon fly, *Bactrocera cucurbitae*, and the oriental fruit fly, *B. dorsalis*, to determine if this fumigant is a suitable replacement for MB in quarantine treatments to control these exotic fruit flies

MATERIALS AND METHODS

Insects

Eggs of *C. capitata*, *B. dorsalis*, and *B. cucurbitae* were reared at the USDA-ARS Pacific Basin Agricultural Research Center, Hilo, Hawaii. They were collected from mated females during a 2-h egging period and placed on moist filter paper in petri dishes (Vargas *et al.,* 1985). The eggs were held at 24°C to obtain the late embryonic stage of egg development, which occurred at 48, 24, or 24 h for *C.□apitata, B. dorsalis* or *B. cucurbitae*, respectively. First instars were obtained from newly hatched eggs. Feeding third instars were obtained by placing eggs on larval rearing diet (Tanaka *et al.,* 1970) held at 24°C. *Ceratitis capitata*, *B. dorsalis*, and *B. cucurbitae* developed into third instars at 8, 9 or 7 days, respectively (Roger

Vargas, unpublished data). Larvae were separated from the diet by floatation in a saturated sugar-water solution (Jang, 1986).

A comparison between 1- and 48-h old *C. capitata* eggs was conducted to determine the most tolerant age to the fumigant. Eggs on moist filter paper in petri dishes were exposed to the fumigant. Following aeration, fumigated treatments and non-fumigated controls were placed separately into perforated ziplock® bags, held at 24°C, and checked daily for egg hatch. Larvae in rearing diet in 12- by 24-mm vials fitted with screen covers were exposed to the fumigant. Following aeration, treated and control larvae were removed from the vials and placed on fresh larval diet in plastic cups (60 mL) and held in screened containers with sand (about 1 cm deep) at the bottom for larval pupation. The sand was sifted after 2 to 3 weeks to collect pupae. Insects that pupated were counted as survivors.

FUMIGATION

Fumigation chambers were made from wide mouth Mason® jars (0.95 L). Each jar was sealed with a rubber gasketed lid secured tightly with a metal screw ring. The lid was fitted with an injection port consisting of a short length of copper tubing fitted on the outside with rubber tubing that could be closed by pinch clamp. Sulfuryl fluoride gas was taken by syringe directly from a commercial compressed gas cylinder (Dow AgroSciences) and, depending on the intended dose, injected directly into either the fumigation jar or a 3.8 L dose jar, similarly fitted with an injection port, for dilution and subsequent dosing of the treatment jars. Prior to dosing the treatment jar, a volume of air equivalent to 1.5-X the fumigant volume was removed with a syringe. After dosing, the partial pressure in the treatment jars was allowed to equalize to normal atmospheric pressure and the injection port was closed. Gas concentration readings for each treatment jar were made at the beginning and end of each exposure to verify actual sulfuryl fluoride concentrations achieved (Zettler *et al.,* 1999).

Toxicity treatments

All treatments were conducted at the Pacific Basin Agricultural Research Center. The fruit fly life stages were exposed to a selected range of concentrations of sulfuryl fluoride during a 4 h exposure to obtain dose-response data. A second series of treatments consisted of exposing *B. dorsalis* eggs to selected sulfuryl fluoride concentrations at exposure times ranging from 2 to 12 h. For each test, a sample of 100 eggs or larvae was used in each treatment and in the untreated control. Each test was replicated at least 3 times (range = 3–11). When possible, all life stages were tested together in the same treatment jar. However, in many cases, only one or two life stages could be tested at the same time. At the completion of each test, the fruit fly life stages were removed from the treatment jars and allowed to aerate under a fume hood and then held for pupation counts. Dose response data were analyzed in order to construct dosage mortality regression lines (SPSS Inc., 1997) at each concentration and exposure period.

RESULTS

Third instars

Sulfuryl fluoride was toxic to third instars of the three fruit fly species at relatively low doses (Fig. 1, A–C). The data in each scatter plot fit a sigmoidal logistic function with R^2 = 0.64, 0.82, and 0.69 for *B. cucurbitae*, *B. dorsalis*, and *C. capitata*, respectively. The lowest dose causing the highest mortality was 39.8 mg/L (94.1 % mortality) for *C. capitata* (1C), 90.5 mg/L (100% mortality) for *B. dorsalis* (1B), and 87.7 mg/L (99.9% mortality) for *B. cucurbitae* (1A). Relative susceptibilities were *B. dorsalis* > *C. capitata* > *B. cucurbitae* with LC_{99} Ct's of 363.2, 549.2, and 717.6 mg.h/L, respectively (Table 1).

First instars

Toxicity of sulfuryl fluoride to first instars is shown in Fig. 1, D–F. The data in each scatter plot fit a sigmoidal logistic function with R^2 = 0.91, 0.96, and 0.79 for *B. cucurbitae*, *B. dorsalis*, and *C. capitata*, respectively. The lowest dose causing the highest mortality was 57.4 mg/L (100% mortality) for *B. cucurbitae* (1D), 273 mg/L (100% mortality) for *B. dorsalis* (1E), and 387.8 mg/L (100% mortality) for *C. capitata* (1F). The most susceptible species was *B. cucurbitae* with a Ct of 36 mg.h/L (Table 1). Regression line slopes for *B. dorsalis* and *C. capitata* were extremely flat (b = 0.56 and 0.50, respectively). The LC_{99}'s for these two species were extremely large, resulting in Ct's of 3,136 and 6,316 mg·h/L, respectively. The Chi Square values for first instars were large and, consequently, confidence intervals for the LC values could not be calculated.

Eggs

Fruit fly eggs were consistently the most tolerant life stage tested. The data in each scatter plot fit a sigmoidal logistic function with R^2 = 0.79, 0.98, 0.78, 0.93, and 0.89 for 24-h-old *B. cucurbitae*, 1-h-old *B. dorsalis*, 24-h-old *B. dorsalis*, 1-h-old *C. capitata*, and 48-h-old *C. capitata*, respectively) (Fig. 2). The lowest dose causing the highest mortality was 104 mg/L (72.8% mortality) for 1-h-old *B. dorsalis* (2B), 218.4 mg/L (100% mortality) 1-h-old for *C. capitata* (2D), 384 mg/L (100% mortality) for 24-h-old *B cucurbitae* (2A), 487.7 mg/L (99.8% mortality) 48-h-old for *C. capitata* (2E), and 593.7 mg/L (99.9% mortality) for 24-h-old *B. dorsalis* (2C). The most susceptible egg stage was the 1-h-old *C. capitata* egg with an LC_{99} Ct of 628 mg·h/L; the most tolerant was the 24-h-old *B. dorsalis* egg with an LC_{99} Ct of 2,316 mg·h/L (Table 1).

Lethal time tests

Because *B. dorsalis* eggs were the most tolerant life stage tested, we conducted lethal time (LT) tests on them (Fig. 3). The exposure times, in 2-h intervals ranging from 2 to 12 h, showed that the relationship between concentration and time was nonlinear throughout the tested range. The data points fit an exponential decay curve with R^2 = 0.875. The LC_{50} concentration was reduced from 243.8 mg/L at 2 h to 59.1 mg/L at 12 h. Fig. 4 shows that exposure times between 2 and 6 h produced LC_{50} Ct's that were consistently lower than the relatively constant LC_{50} Ct for 6 to 12 h exposures.

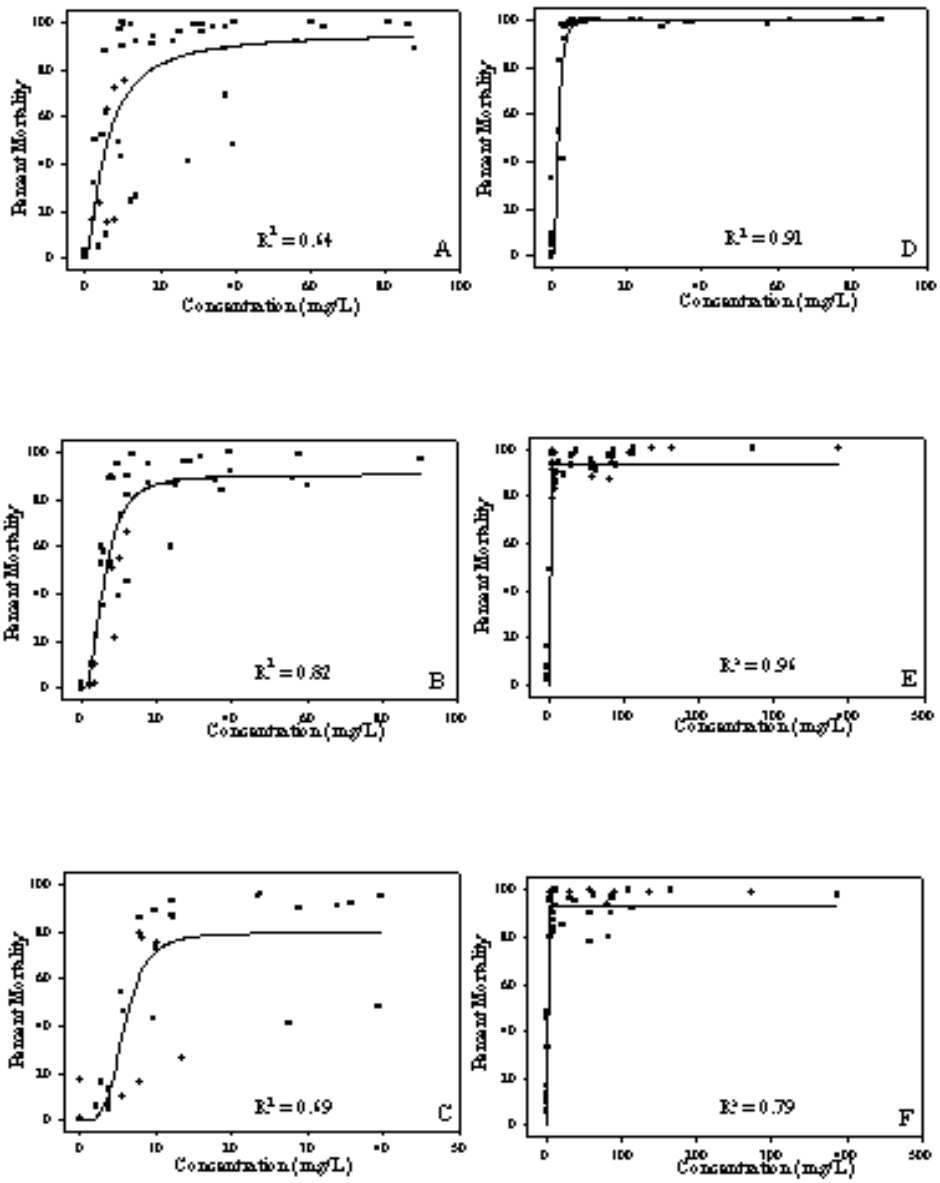

Fig. 1. Toxicity of sulfuryl fluoride to fruit flies after 4 h fumigations at 23.6°C: third instars of (A) melon fly, *Bactrocera cucurbitae*, (B) oriental fruit fly, *B. dorsalis*, and (C) Mediterranean fruit fly, *Ceratitis capitata*; and to first instars of (D) melon fly, *B. cucurbitae*, (E) oriental fruit fly, *B. dorsalis*, and (F) Mediterranean fruit fly, *C. capitata*.

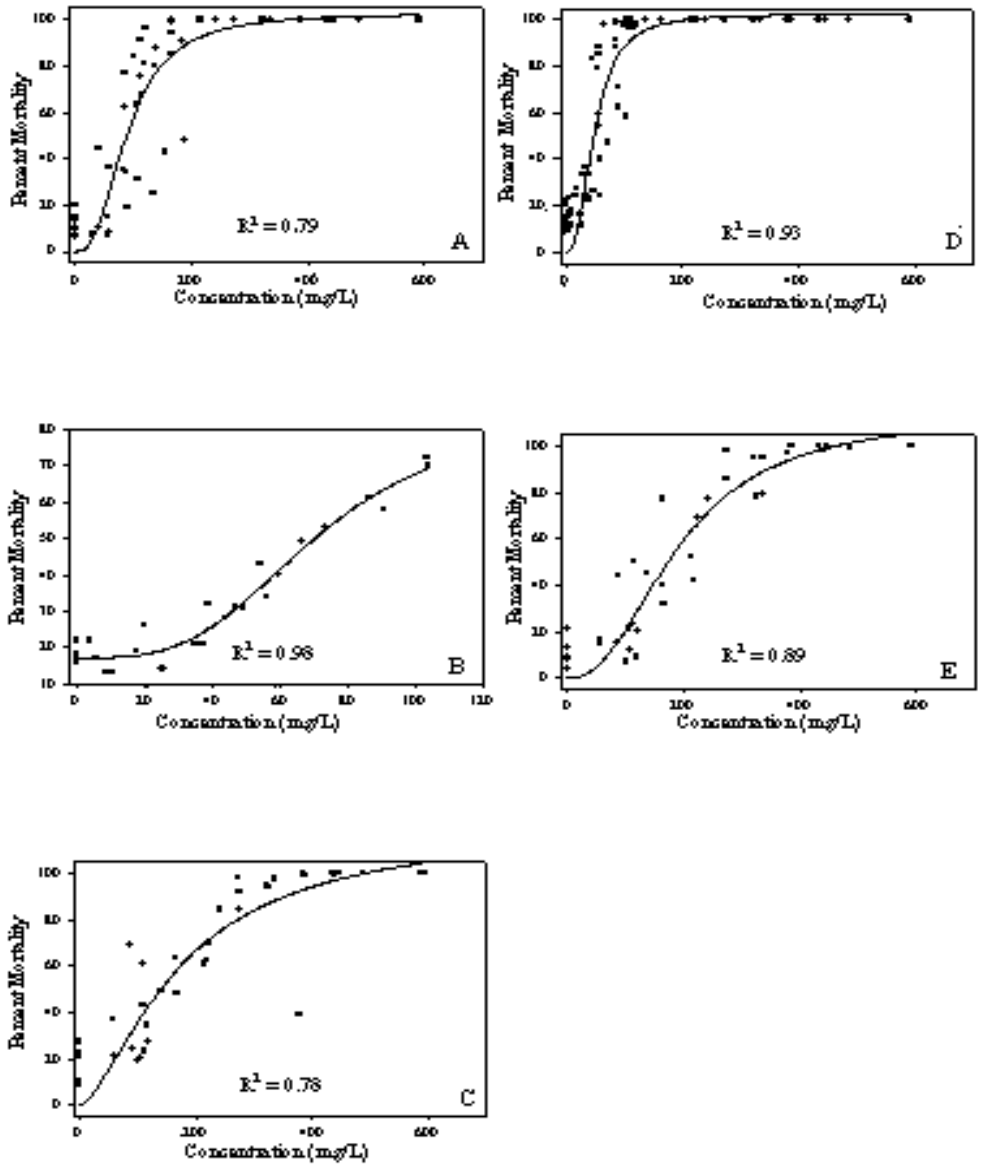

Fig. 2. Sulfuryl fluoride toxicity to eggs of fruit flies after 4 h fumigations at 23.6°C: (A) melon fly, *Bactrocera cucurbitae*, 24-h-old; (B) oriental fruit fly, *B. dorsalis*, 1-h-old; (C) *B.□dorsalis*, 24-h-old; (D) Mediterranean fruit fly, *Ceratitis capitata*, 1-hr-old and (E) 48-h-old.

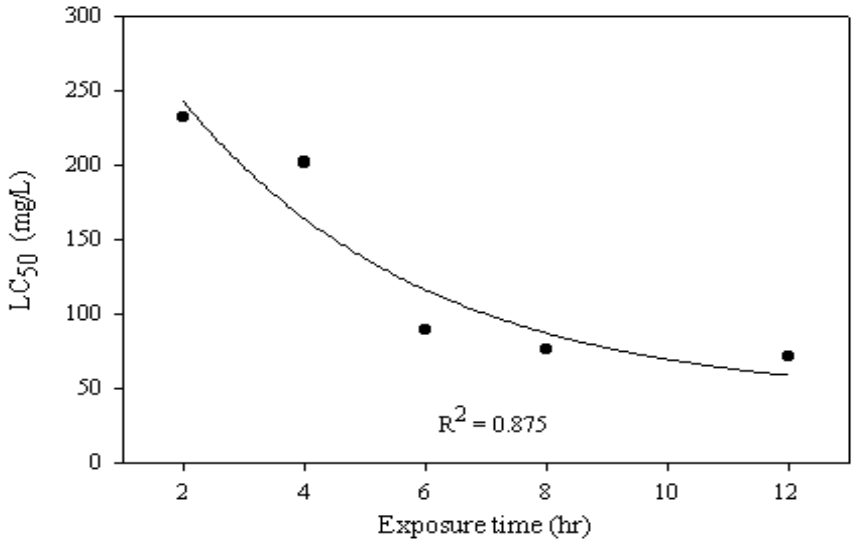

Fig. 3. Effects of concentration and exposure time on the LC_{50} toxicity of sulfuryl fluoride to 24-h-old eggs of oriental fruit fly, *Bactrocera dorsalis*.

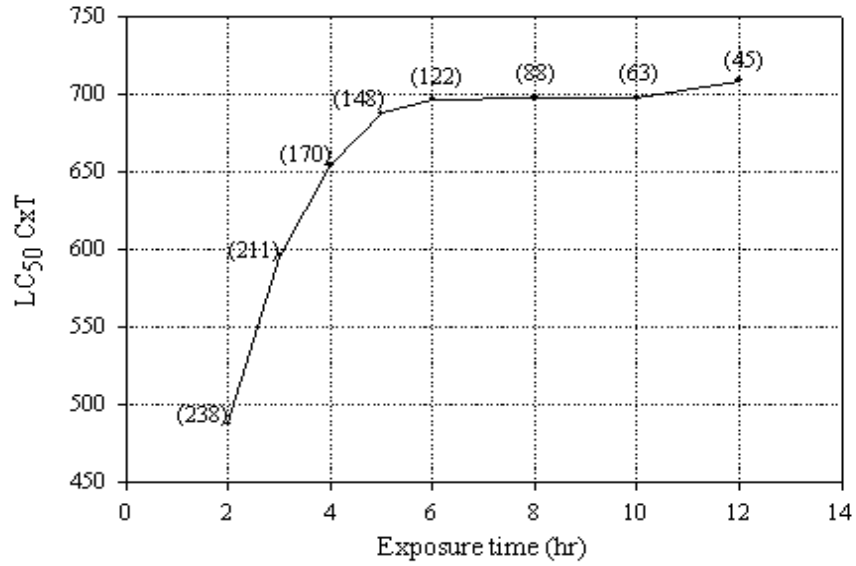

Fig. 4. Effects of exposure time on LC_{50} Ct products of sulfuryl fluoride toxicity to 24-h-old eggs of oriental fruit fly, *Bactrocera dorsalis.* Concentration (C) of CxT in parentheses for each exposure time.

DISCUSSION

The majority of previous reports on sulfuryl fluoride toxicity has been restricted to termites, wood boring beetles, and selected postharvest insect pests (Stewart, 1956; Thoms and Scheffrahn, 1994; Bell and Savvidou, 1999; Reichmuth *et al.,* 1997; Reichmuth *et al.,* 1999; Schneider and Hartsell, 1999; Zettler *et al.,* 1999). This is the first report on the effectiveness of sulfuryl fluoride against exotic fruit flies. Based on 4 h fumigations, sulfuryl fluoride was toxic to third instars at doses slightly higher than those of larval instars of other insect species (Thoms and Scheffrahn, 1994; Reichmuth *et al.,* 1997). Additionally, calculated LC_{99} Ct's were higher than those required for MB quarantine treatments for deciduous fruits and nuts to control these three fruit flies (Anon., 1992), or for sulfuryl fluoride fumigation of walnuts to control codling moth (Zettler *et al.,* 1999; Leesch and Zettler, 2000).

Unexplained variability in assays precluded meaningful conclusions about sulfuryl fluoride toxicity to first instars. However, the *t* statistics for the regressions were significant ($P = 0.05$) and represented a linear relationship with concentration (Robertson and Preisler, 1992). In spite of the extreme variation among Ct's for first instars, this life stage does not appear to be more tolerant to sulfuryl fluoride than the active life stages of other insect pests (Stewart, 1956; Thoms and Scheffrahn, 1994; Reichmuth *et al.,* 1997; Zettler *et al.,* 1999).

Previous research has shown that eggs are the most tolerant life stage to sulfuryl fluoride (Kenaga, 1957; Su and Scheffrahn, 1990; Thoms and Scheffrahn, 1994; Drinkall *et al.,* 1996; Bell *et al.,* 1999; Zettler *et al.,* 1999). Our data show that eggs were the most tolerant life stage of the fruit fly species we tested. LC_{99} Ct values ranged from a low of 628 mg•h/L to a high of 2,316 mg•h/L (Table 1). Susceptibility of eggs was directly related to egg age. For example, 24-h-old *B.□dorsalis* eggs were 1.7-fold more tolerant than were 1-h-old eggs and 48-h-old *C.□capitata* eggs were 3.1-fold more tolerant than were 1-h-old eggs. These data support other studies that showed the insect egg varies in susceptibility to sulfuryl fluoride depending upon age (Thoms and Scheffrahn, 1994; Bell and Savvidou, 1999; Bell *et al.,* 1999; Reichmuth *et al.,* 1999; Zettler *et al.,* 1999).

Su *et al.* (1989) determined that exposure time is slightly more important than concentration in controlling Formosan termite, *Coptotermes formosanus.* In our LT study with 24-h-old eggs of *B. dorsalis*, we found that the LC_{99} Ct at 2 h (2,373 mg•h/L) was reduced by 40% when compared with that at 12 h (1,442 mg•h/L). Between the 6 and 12 h exposure times, the concentration-time relationship followed the model $C^n t = k$, where $n = 1$. However, at exposures shorter than 6 h, this model consistently overestimated the concentration and resulted in a toxicity index (Winks, 1984) of $n<1$. Therefore, in short exposures less than 6 h, exposure time appears to be the more important component of the Ct relationship and beyond 6 h exposure time, both components are in parity.

One of the characteristics of an ideal quarantine fumigant is that it is quick acting (Chakrabarti, 1996). Indeed, the MB quarantine treatments for fruit flies are typically 2 to 4 h in duration (Anon. 1992). Our fumigations were conducted at 4 h to simulate those standard treatments. However, sulfuryl fluoride may be more efficient at longer exposure times, particularly if potential infestations in the treated commodity include eggs. The tolerance of eggs to sulfuryl fluoride may be caused by the inability of the fumigant to rapidly penetrate the egg shell. For example, Outram (1967a, b) showed that it took more than 24 h for the fumigant to penetrate *Schistocerca gregaria* eggs. Therefore, fumigations of less than 24 h may not be

able to control fruit fly eggs at concentrations low enough to prevent damage to the commodity. In practice, the large doses required to control fruit fly eggs may be mitigated by exposure periods of up to 48 h, at which time the eggs begin to hatch and then develop into the more sensitive larval stage. Additionally, two fumigations aimed at the active life stages rather than a single one aimed at the eggs (Anon. 1999) may provide a more efficient control. However, a double fumigation would be too time consuming to be an efficacious quarantine treatment to control fruit flies in fresh commodities.

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