THE RELATION OF DOSE RATE AND LIGHT INTENSITY TO THE EFFECT OF BAIT SPRAY FORMULATIONS WITH THE PHOTO-INSECTICIDE PHLOXINE B ON THE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (DIPTERA: TEPHRITIDAE)

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Summary

Adults of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) were tested at a range of light intensities with aqueous bait spray formulations of 4% yeast hydrolysate containing a range of concentrations of the photo-insecticide Phloxine B. Under a high light intensity (of approximately 500 $\mu Em^{-2}s^{-1}$), concentrations in the range 0.1–5.0% Phloxine B all resulted in a mean survival time of about 2 h and, in each case, over 85% of flies were killed within 5 h and virtually all flies within 24 h of ingesting the dye. Concentrations of 0.05% and 0.01% took 2 and 10 d respectively to kill all flies. The time taken to kill flies with a given concentration of Phloxine B was also dependent on light intensity, with the logarithm of time decreasing linearly with the logarithm of light intensity. In almost complete darkness (<0.1 $\mu Em^{-2}s^{-1}$), the dye remained potent in the insects for up to 10 d, killing the flies quickly when they were subsequently placed in strong light.

INTRODUCTION

Organophosphate insecticides such as Malathion® are normally used in bait sprays to control outbreaks of fruit flies (Roessler 1989). Malathion is a contact poison, and can compromise established biological control programs, as well as damage the natural arthropod predators and parasites of pest species; it has also gained an unfavourable reputation in the eye of the public for reasons of public health and property damage (Bergsten 1995). Viable alternatives are now being sought to replace Malathion in commercial use. Phloxine B is a hydrophilic red dye that is used as a histological stain and is known chemically as 2′,4′,5′,7′-tetrabromo-4′,5′,6′,7′-tetrachloro-fluorescein disodium salt. It has recently been shown to be toxic when ingested by insects (including several economically important tephritid fruit fly species) that are then exposed to light (Clement *et al.* 1980; Bergsten 1995, 1997; Mangan and Moreno 1995; Liquido *et al.* 1995ab; Pimprikar 1995; Dowell 1997; Wilson *et al.* 1997).

The dye's effect, when mixed with a range of food lures, was tested in the laboratory with the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), by Liquido *et al.* (1995a). Phloxine solutions of between 2% and 4% were shown to be lethal to flies several hours after ingestion, with more palatable baits (eg. 10% molasses) attracting more flies, and increasing overall mortality. Concentrations as low as 0.25% killed the majority of Mexican fruit flies, *Anastrepha ludens* (Loew), during preliminary field trials (Mangan and Moreno 1995). Similarly, Liquido *et al.* (1995b) showed that the water insoluble (non sodium salt) form of Phloxine B produced a lethal response in the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), when it was mixed with the male lure of the species (methyl eugenol). With a moderately high light intensity (200 $\mu Em^{-2}s^{-1}$), 100% mortality was noted within two hours of initial exposure to the chemical.

It is not clear how Phloxine B acts as a photo-insecticide. It is thought that the dye is able to transfer energy to oxygen molecules, converting them to volatile oxygen singlets, which then damage the tissues of the insect after ingestion. It is not known where this oxidation is focused, but death is likely to be a result of cumulative damage to an array of critical target sites (Bergsten 1995; Liquido *et al.* 1995a).

Because it is not a contact toxin, and lethal only after ingestion, the use of Phloxine B has the potential to reduce the damage that could be sustained by any non-target organisms (particularly invertebrates) that are not attracted to specific lures. It would also be harmless to species that are not exposed to light such as ground dwelling litter arthropods. Given the low dosage required to kill tephritids, Phloxine B is generally considered environmentally benign, and less hazardous than other pesticides now in use (Pimprikar 1995).

The trials reported here used various concentrations of Phloxine B in a 4% aqueous solution of yeast autolysate. A series of tests were designed to examine the dye's toxicity over a range of concentrations and to assess any differences in response under various light intensities.

MATERIALS AND METHODS

Subjects

All trials were conducted using the Queensland Fruit Fly, *Bactrocera tryoni*. The flies were obtained from a normal laboratory culture (Bateman 1967). Before the trial they were kept in cages under a 13:11

day:night cycle, at a temperature of 25°C (±1°C) and a relative humidity of 65% (±5%). During this period the flies had adequate supplies of water and sugar, but had been deprived of protein from the time they had emerged (at least 5 d before the trial began).

Dose rates and light intensities

There were 3 experiments. The first, conducted under a high light intensity of approximately 500 $\mu E\ m^{-2}s^{-1}$, tested the toxicity of 6 concentrations of Phloxine B (0.01, 0.05, 0.1, 0.5, 1.0 and 5.0%) in 4% yeast autolysate, against a control (4% yeast autolysate only). The second experiment examined the effect of various light intensities on flies that had been fed with 0.1% Phloxine B. The light intensities used were 350, 25, 10 and 0.75 $\mu Em^{-2}s^{-1}$. The third experiment compared the toxicity of a 0.5% solution with the control in conditions of extremely low light (<0.1 $\mu Em^{-2}s^{-1}$). The flies were monitored for 10 days in these conditions and then introduced to full light (500 $\mu Em^{-2}s^{-1}$).

The Phloxine B used in each formulation was of the water soluble form of 95% dye content (ICN Biomedicals). The metal arc lights used throughout these trials have a spectrum of emission similar to that of sunlight. Light intensities were measured with a LiCor meter and sensor. The two highest light intensities used are typical of those found in bright, direct sunlight. We chose 25 $\mu Em^{-2}s^{-1}$ as typical of the light intensity within a dense canopy of a tree that was also in the shade. Light intensities in the range 10–0.75 $\mu Em^{-2}s^{-1}$ are typically experienced in the field from sunset to approximately 1 h later (i.e. 'dusk' illumination).

Experimental procedure

For each treatment in each experiment, approximately 100 flies of each sex were placed into separate cages (250×150×125 mm) covered in black nylon mesh. Each 'spray' formulation was tested with one cage of males and one cage of females. The flies were supplied with water and sugar *ad libitum*.

To administer a test formulation, a folded paper facial tissue was soaked in the solution until it was saturated. The excess was then removed to prevent dripping and the tissue was placed in the centre of the cover, on top of each cage; the flies had free access to the tissue for at least two hours before it was removed. Most flies fed on the solution in the tissue within minutes of administration.

Generally, the flies were exposed to temperature and relative humidity conditions similar to those they had experienced before the trial.

Recording of data

The flies were observed at regular intervals, and the number of dead or 'knocked down' individuals counted. During the trials, flies were observed every 30 min for the first 2 h, hourly for the next 3 h, and then once a day until the completion of the trial. Flies were scored as knocked down (unable to fly and unable to walk in a normal manner) or dead (no movement of limbs after prodding). All flies scored in the first category at one inspection were invariably dead at the next inspection or the one thereafter.

During the experiment that compared the effect of different concentrations of Phloxine B in very bright light, the flies were exposed to a light regime of L:D 13:11 h. On the first day, they were exposed to light for 6.5 h after the treatment had been administered. For the experiment involving different light intensities, exposure to light was continuous for the first 9 hours (during which all flies died except in the treatment at 0.75 $\mu Em^{-2}s^{-1}$). In the case of the flies kept in almost complete darkness (<0.1 $\mu Em^{-2}\ s^{-1}$) inspections were carried out in a light intensity of 0.75 $\mu Em^{-2}s^{-1}$, hence they were unavoidably exposed to this higher intensity for about 2 min at each inspection.

Analyses

The mean time of death at each concentration was calculated, treating the death of each fly as a single observation at the midpoint of the interval between each recording period.

One way analyses of variance were conducted to examine the differences between this measure at every concentration, and each light intensity (P=0.05). Prior to these analyses, homogeneity of variance was examined using Bartlett's test, and transformed accordingly. The mean time of death of controls was estimated from the proportion found dead at the end of each trial.

RESULTS

Effects of dose rate of Phloxine B

Phloxine concentrations of 0.1, 0.5, 1.0 and 5.0% caused the majority of subjects (at least 85%), regardless of sex, to die within five hours of initial exposure to the dye when exposed to illumination of 500 $\mu Em^{-2}s^{-1}$. After 24 h, virtually all subjects were dead. Of those that were still living (8 individuals out of approximately 700), only one had consumed the dye (as indicated by its reddened abdomen). All those that had not eaten the bait were in the 1.0% and 5.0% treatments. Survival time was longer in the case of the flies given the two lowest concentrations, total mortality being 2 d and 10 d for the 0.05 and 0.01% treatments respectively.

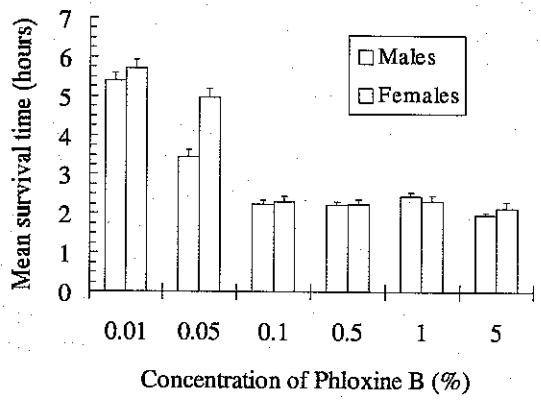

Figure 1. Survival time of flies under bright illumination (500 $\mu Em^{-2}s^{-1}$) after ingesting solutions of 0.01, 0.05, 0.1, 0.5, 1 and 5% Phloxine B mixed with 4% yeast hydrolysate. The control contained 4% yeast hydrolysate only. Means plus 95% confidence limits (n ≈ 100).

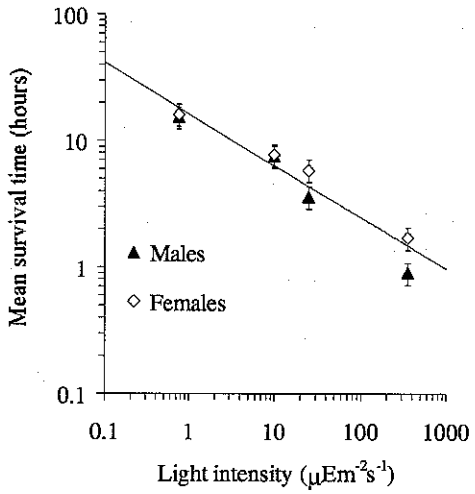

Figure 2. Time for knock down (after ingestion of bait spray mixture with 0.1% Phloxine B) related to light intensity. Means plus 95% confidence limits (n ≈ 100).

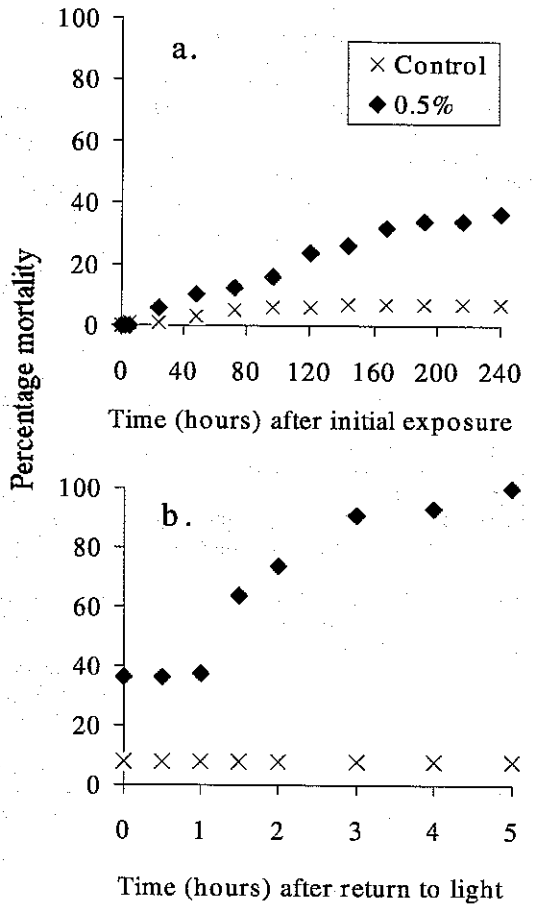

Figure 3. Percentage mortality of a cohort of 100 female flies: (a) over ten days at very low light intensity (< 0.1 $\mu Em^{-2}s^{-1}$) after ingesting bait spray mixture with 0.5% Phloxine B, and (b) after transfer of the cohort to strong illumination (500 $\mu Em^{-2}s^{-1}$).

Figure 1 compares the mean survival times associated with the various treatments. There were no significant differences between the means associated with concentrations of between 0.1 and 5.0% Phloxine B, but there was a significant difference between this group of means and those pertaining to the lowest two concentrations which also differed significantly from each other ($P<0.01$).

Light intensity and toxicity

Figure 2 shows that when a given concentration (0.1%) of Phloxine B is administered, the time taken to kill the flies is dependent on light intensity, with the logarithm of time decreasing linearly with the logarithm of light intensity. Over the range tested here (350–0.75 $\mu Em^{-2}s^{-1}$), each decrease in light intensity resulted in a significant decrease in knock-down rate ($P<0.01$).

When flies fed with 0.5% Phloxine B were kept at a very low light intensity (<0.1 $\mu Em^{-2}s^{-1}$) less than 40% died within 10 d. When they were then placed under full light, the rate of mortality increased so that within 5 h, no individuals remained alive. Figure 3 shows the results for a cohort of female flies; the results for males were almost identical.

The mean time of death of the controls in all trials was estimated to be about 1600 hours (66 days), based on extrapolation of the daily survival rate over the period of observation. This corresponds well with other estimates of the normal life-span of *B. tryoni* (Meats 1998).

DISCUSSION

In the presence of strong light, Phloxine B was toxic to *B. tryoni* at concentrations of between 0.1 and 5.0%, killing all but a few subjects within 24 h. This was also true of more dilute solutions, although the time required to produce this response was longer, being 2 d and 10 d for concentrations of 0.05% and 0.01% respectively. No substantial differences were seen between the sexes. These results are in agreement with other studies of Phloxine B on other species (Liquido *et al.* 1995a, 1995b; Mangan and Moreno 1995). However the dramatic relationship of survival time with light intensity has not been demonstrated previously.

At higher concentrations (1.0 and 5.0%), the Phloxine B solution appeared to become unpalatable to the flies, possibly due to the high salt load associated with the dye. Although only a small proportion did not consume these solutions, lower concentrations (0.1 or 0.5%) appeared to work as effectively. In a commercial sense this may be of added benefit, reducing the costs of using Phloxine B as an insecticidal spray, and reducing the risk of phytotoxicity.

The light intensities used during these experiments were chosen to represent a wide array of natural light conditions, ranging from those experienced in direct sunlight (500 and 350 $\mu Em^{-2}s^{-1}$), in deep shade on a sunny day (25 $\mu Em^{-2}s^{-1}$), dusk and almost complete darkness (10–0.75 and <0.1 $\mu Em^{-2}s^{-1}$ respectively). We can conclude that under almost any light intensity, Phloxine B is toxic and that the logarithm of survival time is linearly related to the logarithm of light intensity. The very low death rate in almost complete darkness over 10 days may have been even lower had not the flies been exposed to light at dusk levels during inspections. We cannot discount, however, either the possibility of there being a slight toxic effect that is not due to an interaction with light or the possibility that the linear relation shown in Figure 2 could break down at very low light intensities for some other reason such as a threshold phenomenon.

Bait sprays (used as spot-sprays for tephritid fruit flies) incorporating yeast hydrolysate or yeast autolysate as 'attractants', are generally held to work because 'protein starved' flies are attracted to the application and feed on it (as in this study). These flies would be immature. This would be an advantage since it is better to kill them before they breed and lay eggs. However, they mature rapidly after access to protein (Fletcher 1987). Thus, when considering Phloxine B as a substitute for Malathion® in these formulations, one would recommend a strength that would kill within 24 hours rather than several days.

Phloxine B has potential for use as a commercial insecticide against tephritid fruit flies such as *B. tryoni*, achieving mortality at relatively low concentrations. If its insecticidal power depends upon exposure to light, it would be expected to be harmless to invertebrates deep in soil and perhaps leaf litter. Further, in a spot-spray it would have a very low dosage rate per hectare and is considered relatively benign, with no known hazards to public health (Pimprikar 1995). More work is required to explore its efficacy in the field, and to establish whether is has any impact on non-target organisms (Thomas and Meats 1999).

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