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Gamma irradiation for postharvest disinfestation of *Ctenopseustis obliquana* (Walker) (Lep., Tortricidae)

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Abstract: Gamma irradiation was examined for its potential as a quarantine treatment for eggs and larvae of the leafroller *Ctenopseustis obliquana*. One-day-old eggs were more radiosensitive than 5-day-old eggs, requiring 55.9 Gy and 269 Gy for 99% mortality, respectively. Effective disinfestation of *C. obliquana* could be achieved with a dose as small as 70.1 Gy, which would inhibit any oviposition by adults developing from irradiated 1-day-old and 5-day-old eggs, and 1st, 3rd and 5th instar larvae. Further quarantine security for these life stages could be achieved with a dose of 150 Gy which would inhibit adult emergence, or 215 Gy which would inhibit larvae from entering pupation. As these doses are unlikely to damage host fruit, gamma irradiation provides a potential alternative to methyl bromide fumigation.

1 Introduction

The leafroller *Ctenopseustis obliquana* (Walker) is native to New Zealand and can be found on a variety of crops including stone-fruits, kiwi fruit, pip fruit and persimmons. It is of quarantine importance in countries such as Australia, Japan and USA, and crops with infestations are currently treated by fumigation with methyl bromide. As well as damaging fruit quality (Ito and Hamilton, 1980; Batchelor et al., 1985; Beever and Yearsley, 1987, Harman et al., 1990), methyl bromide has been identified as an ozone depleting chemical and is anticipated to be phased out by the year 2001 in the United States (Anonymous, 1993). A possible alternative, non-chemical disinfestation treatment is gamma irradiation.

Irradiation has been proposed as a disinfestation method for a number of arthropod pests on a range of horticultural produce (Jona ad Arzone, 1979; Burditt and Hungate, 1988; Burditt, 1994). The use of gamma irradiation as a disinfestation method is generally limited to sublethal effects resulting from treatment, as doses high enough to kill arthropod pests are often damaging to crops (Burditt, 1994). Sublethal effects of irradiation include an inability to pupate, emerge from pupation, or oviposit viable eggs as an adult. An irradiation treatment that inhibits the emergence of a range of fruit fly genera is being tested as a quarantine treatment for papaya grown in Hawaii and exported to the continental US (Anonymous, 1989).

Our aim in this study was to determine the gamma irradiation dose which prevents egg hatch, larval pupation or emergence, and adult oviposition of *C. obliquana*. Two egg ages and three larval instars were examined.

2 Materials and methods

2.1 Egg and larval collection

C. obliquana were obtained from a laboratory colony (Clare and Singh, 1988) maintained at 20°C, 60% RH and photoperiod of 16:8 (L:D) h at the Horticulture and Food Research Institute of New Zealand Ltd (Auckland). Eggs were laid on plastic sheets within gauze oviposition cages (modified from Singh et al., 1985) over a 15 h period. The egg sheets were collected and irradiated within 5 h (referred to as 1-day-old eggs), or held at 20°C for a further 4-days (5-day-old eggs) before irradiation. 1st instar <24 h old were individually placed on artificial diet (Singh, 1983) in 5 ml test tubes with cotton wool caps and either irradiated, or reared to 3rd or 5th instar (12 days and 21 days, respectively from egg hatch).

2.2 Irradiation treatment

A cobalt-60 gamma radiation source (Gammacell) was used to irradiate eggs and larvae with a dose rate of 51.3 ± 0.8 Gray (Gy)/min as determined using the Fricke method (Spinks and Woods, 1964). Between 99 and 265 1- and 5-day-old eggs were placed in plastic tubes and irradiated at 0, 20, 40, 60, 80, 120, 140 and 160 Gy. Three replicates of each dose were done for each egg or larval stage.

2.3 Post-irradiation treatment

After treatment, eggs were placed into plastic containers, and stored at the aforementioned rearing conditions. Eggs were counted immediately after irradiation, and after they hatched to determine the egg fertility (total eggs laid less the infertile eggs) and fertile-egg mortality (those eggs which did not hatch but which contained preformed larvae). All larvae that hatched from eggs were individually placed into test tubes with artificial diet. These larvae, and those which were irradiated at 1st, 3rd and 5th instar were maintained at rearing conditions until pupation. Pupae were collected and stored until adult

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emergence. Five newly emerged adult females were placed individually in small plastic pots, each with two males, for mating and oviposition. Eggs were laid on plastic strips which lined the pots. Plastic strips were removed and replaced every 3–4 days until the female died. Egg fertility and fertile-egg mortality were assessed as above.

2.4 Statistical analysis

Dose-mortality data were analysed using the complementary loglog (cloglog) model (PREISLER and ROBERTSON, 1989). This transformation gave approximate linearity, and the line was used to determine the estimated dose for 50% and 99% mortality (LD_{50} and LD_{99}, respectively). A model was fitted using a robust version of the generalized linear model analysis available in S-PLUS (STATISTICAL SCIENCES INC., 1991). Variance was assumed proportional to a binomial distribution.

The irradiation dose estimated to inhibit egg lay was calculated using linear or quadratic interpolation of the percentage decrease of the number of eggs laid by treated adults at each dose relative to untreated control adults. Percentage data was subjected to the arcsine transformation (1-day-eggs) or to the logit transformation (1st, 3rd and 5th instars) before interpolation. For 5-day-eggs the dose was log transformed. Means and confidence intervals were calculated from interpolates from separate replicates.

3 Results and discussion

3.1 Egg hatch

The LD_{50} and LD_{99} estimates to prevent egg hatch for 5-day-old eggs of *C. obliquana* were 115 Gy (n = 3 243; 95% CI, 46.4–289) and 269 Gy (n = 3 243; 95% CI, 159–455) respectively. 269 Gy is well within the gamma irradiation tolerance of many crops (KADER, 1986). The 1-day-old eggs were much less tolerant to gamma irradiation, and the LD_{50} and LD_{99} were estimated at 18.8 and 55.9 Gy (n = 1 689) respectively. No confidence intervals were calculated for 1-day-old eggs, as the high mortality of eggs at even very low doses of gamma irradiation prevented confident estimates of error.

The dose that inhibits hatch of 1-day-old and 5-day-old eggs of lightbrown apple moth (*Epiphyas postvittana*) has been reported to be similar at 70 and 258 Gy, respectively (BATCHELOR et al., 1984). However, 1400 Gy is necessary to kill 5-day-old eggs of codling moth (*Cydia pomenella*) (TOBA and BURDITT, 1992), and eggs of twospotted spider mite (*Tetranychus urticae* Koch) require doses higher than 1200 Gy for complete kill (GOODWIN and WELLHAM, 1990). These pests are found on many of the same crops as *C. obliquana*, and the high doses necessary to kill their eggs exceed the maximum legal dose of gamma irradiation in countries such as the United States (ANONYMOUS, 1984). For this reason detailed examination of the dose required to kill *C. obliquana* larvae was not undertaken, as our preliminary studies indicated that the dose required to kill 1st, 3rd and 5th instar was approximately 2400, 3300 and 2700 Gy, respectively (data not shown).

3.2 Pupation

A dose of 215 Gy was required to inhibit 99% of 5th instar larvae developing to pupae, and other instars showed a similar response with overlapping confidence intervals (table 1). first and 3rd instars required slightly lower doses of 192 and 193 Gy. The most tolerant egg stages tested appeared to be the 5-day-old eggs with an LD_{99} of 159 Gy, compared to 81.4 Gy for 1-day-old eggs (table 1).

BATCHELOR et al., (1984) reported that pupation of irradiated egg stages, 1st and 3rd instars of lightbrown apple moth was inhibited with a slightly higher dose of 254 Gy, but•5th instars, which were identified as the most tolerant life stage, required 537 Gy.

3.3 Emergence

Adult emergence was inhibited by a dose of 111 Gy for 5-day-old eggs, or 41 Gy for 1-day-old eggs. 1st instar larvae required the highest dose (150 Gy) to inhibit emergence. Other larval instars showed a similar response but required slightly lower doses for inhibition of emergence (table 2).

Much research has been undertaken on the use of irradiation as a quarantine treatment to inhibit emergence (BURDITT, 1994), and it is probably the most likely quarantine treatment for gamma irradiation. Emergence of all life stages of the most tolerant fruit fly species examined to date, *Bactrocera dorsalis*, was inhibited by 150 Gy (with the exception of 1 adult emerging from a test population of 197 041). However, the emergence of some fruit fly species can be inhibited by a dose as low as 18 Gy (BURDITT, 1994). The 5th instar was the life stage of lightbrown apple moth which required the highest dose (199 Gy) of gamma irradiation for inhibition of emergence (BATCHELOR et al., 1984; DENTENER et al., 1990). Adult emergence of codling moth can be inhibited by irradiating larvae or eggs with a dose of 187 Gy (PROVERBS and NEWTON, 1962; BURDITT and HUNGATE, 1989; TOBA and BURDITT, 1992), although a much larger dose of 600 Gy is necessary to stop development of twospotted spider mite to the adult stage (GOODWIN and WELLHAM, 1990). 100 Gy has been shown to kill the adult form of the thrips *Frankliniella pallida* (WIT and VAN DE VRIE, 1985).

Overall, more males emerged from treated larvae and eggs than female. Combining data from all larval instars dosed at 0 Gy, 56% of those that emerged were male (n_t = 450 test insects, n_e = 401 emerged). Of larvae irradiated at 80 Gy, 89% of those that emerged were male (n_t = 450, n_e = 104), and of those irradiated at 120 Gy, 92% of emerged adults were male (n_t = 450, n_e = 13). The five adults which emerged from all larvae irradiated at 140 Gy were male. This result has been observed for a number of other lepidopteran genera (BATCHELOR et al., 1984; BURDITT and HUNGATE, 1989). Consequently the LD_{99} estimates calculated to inhibit pupation and emergence are likely to be biased towards the males.

3.4 Oviposition

The LD_{99} for inhibition of oviposition was lowest (35.6 Gy) for 5-day-old eggs, and highest (70.1 Gy) for 5th instar which was the most tolerant life stage for this treatment (table 3). The dose required for 1-day-eggs to

Table 1. *LD_{50} and LD_{99} for inhibition of pupation, with 95% confidence intervals*

Life stage	n	LD_{50} (95% CI) (Gy)	LD_{99} (95% CI) (Gy)
1-day-old eggs	1106	31.1 (–)	81.4 (–)
5-day-old eggs	3027	73.1 (30.2–177)	159 (125–202)
1st instar	940	110 (72.0–170)	193 (116–320)
3rd instar	997	101 (67.0–154)	192 (166–222)
5th instar	904	109 (104–114)	215 (156–269)

Table 2. *LD_{50} and LD_{99} for inhibition of adult emergence, with 95% confidence intervals*

Life stage	n	LD_{50} (95% CI) (Gy)	LD_{99} (95% CI) (Gy)
1-day-old eggs	1405	25.3 (–)	41.2 (–)
5-day-old eggs	2605	50.9 (21.4–121)	111 (67.4–183)
1st instar	888	62.3 (38.5–101)	150 (84.3–266)
3rd instar	850	74.5 (67.3–82.5)	126 (113–138)
5th instar	770	71.0 (67.4–78.0)	117 (106–128)

Table 3. *LD_{50} and LD_{99} for inhibition of oviposition, with 95% confidence intervals*

Life stage	n	LD_{50} (95% CI) (Gy)	LD_{99} (95% CI) (Gy)
5-day-old eggs	10980	16.7 (15.6–17.9)	35.6 (33.2–38.2)
1st instar	9898	17.7 (4.66–67.4)	55.4 (25.2–122)
3rd instar	13935	18.1 (12.5–26.0)	64.8 (51.2–82.0)
5th instar	10300	28.8 (16.4–50.8)	70.1 (54.0–91.1)

inhibit egg lay was not calculated due to insufficient data. Analysis of the dose for the production of only fertile eggs was not performed, as there was a high proportion of eggs still fertile at the high doses, even where there was significant reduction in the number of eggs oviposited.

Twospotted spider mite are more tolerant than *C. obliquana*, with 2106 Gy being required to inhibit oviposition. However, 351 Gy will result in any diapausing or non-diapausing adult mites developing from treated juvenile stages being sterile (GOODWIN and WELLHAM, 1990; LESTER and PETRY, 1995). In conclusion, with codling moth and lightbrown apple moth 351 Gy will inhibit oviposition, adult emergence and pupation. A dose of ≈350 Gy appears to have potential as a post-harvest quarantine treatment for several pests found on New Zealand horticultural produce. For *C. obliquana* this dose appears to inhibit pupation, emergence and oviposition, although large scale trials will need to be conducted on fruit to confirm these findings. Research on the effects of such a dose on pipfruit and stonefruit has indicated that fruit quality is not adversely affected (KADER, 1986), and in some cases shelf life can be extended (AKAMINE and MOY, 1983). Thus, as an alternative to methyl bromide fumigation, gamma irradiation could be a viable and effective quarantine treatment.

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