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Gamma irradiation as a quarantine treatment for apples infested by codling moth (Lep., Tortricidae)

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Abstract: Effects of gamma radiation on the fifth instar codling moth, *Cydia pomonella* (L.), larvae were examined. Mature larvae were exposed to a series of gamma radiation doses ranging from 50 to 250 Gy and survival to pupae and adults was examined. The results showed that pupation and adult emergence decreased with increasing radiation dose. The results also showed that diapausing larvae were more sensitive to irradiation treatment than non-diapausing larvae, and females were more sensitive than males. A dose of 150 Gy reduced adult emergence to less than 2% in non-diapausing larvae, while a dose of 200 Gy completely prevented it. Furthermore, none of the emerging moths exposed to a dose of 150 Gy were females; at 100 Gy dose, the percentage of females was less than 14%. Irradiating larvae in apple fruit in a small-scale laboratory experiment produced similar results. Tests in which >100 000 larvae (in the fifth instar) were irradiated in an artificial rearing medium with a dose of 200 Gy resulted in no adult emergence. Similar results were also obtained when >32 000 larvae in the same stage were exposed in apple fruit to the same dose. The results indicate that the use of ionizing radiation as a quarantine treatment for codling moth infested fruits is feasible and requires a relatively low dose.

1 Introduction

The codling moth, *Cydia pomonella* (L.), is a key pest of pome and stone fruits throughout most deciduous fruit growing areas of the world (Chapman, 1973). Its larvae infest apples, pears, walnuts, and many other deciduous fruit crops and cause hundreds of millions of dollars in losses to the fruit industry every year (Barnes, 1991). This species is also of quarantine importance in Japan, Korea, Taiwan and parts of China (Shel'deshova, 1967). Strict quarantine measures are applied to prevent its entry and/or establishment in these countries. The insect overwinters as a diapausing larva in cocoons that can be transmitted in fruit containers to importing countries. It can also be transmitted as eggs or larvae on or in fruits (Moffitt, 1989). As a result, quarantine regulations are imposed on fruit exported to codling moth free countries.

The continued use of methyl bromide (MB), a chemical fumigant highly effective against this pest, has been debated for several years because it has been identified as an important atmospheric ozone-depleting substance and the industry is looking seriously for an alternative (Ross, 1999). Ionizing radiation has been recognized as an alternative to MB for treating fresh agricultural products in order to overcome quarantine barriers in trade. Several authors have presented reviews of this subject in the last decade (Burditt, 1994; Nation and Burditt, 1994; Hallman, 1998, 2000, 2001; Johnson and Marcotte, 1999).

Studies on the codling moth have indicated that gamma radiation could be an effective quarantine treatment against eggs and mature larvae (Burditt and Moffitt, 1985; Burditt et al., 1985; Burditt, 1986; Toba and Moffitt, 1996). Burditt and Moffitt (1985) estimated the projected dose necessary to prevent 99.9968% emergence of adult codling moth for the fifth instar larvae. They calculated that a dose of 225 Gy would be required to meet the probit 9 (LD99.9968) security level for diapausing larvae in fibreboard strips and 206 Gy for non-diapausing larvae in fruits. On the contrary, Burditt (1986) found that diapausing larvae were more susceptible to irradiation than non-diapausing larvae and a dose of 230 Gy was estimated to prevent adult emergence of non-diapausing larvae. Furthermore, Burditt and Hungate (1989) exposed 79 540 non-diapausing larvae to a dose of 153 Gy in apples with no adult emergence. However, most of these larvae were still in the third and fourth instars, which are more sensitive to irradiation treatment than the fifth instar. The potential use of gamma irradiation as a quarantine treatment against codling moth larvae was also investigated by Toba and Moffitt (1996). They calculated that a dose of 147 Gy would be required to prevent 99% of the fifth instar larvae from developing into adults. Calculation of this dose, however, was based on irradiating just a small number of larvae (80 larvae) in an artificial diet.

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Security levels for insect quarantine treatments are based on probit 9 (LD99.9968) mortality standards proposed by Baker (1939). At this level of accuracy, a very high number of insects (tens of thousands) should be treated in the commodity at the recommended dose without any survivor before a treatment can be accepted by quarantine officials (Couey and Chew, 1986; Hallman, 2000).

Reviewing the previous studies indicates that more research is needed before any dose can be adopted for quarantine security of fruit potentially infested by the codling moth. The objective of this study was to investigate the potential use of irradiation as a quarantine treatment against codling moth larvae and to establish a database for calculating the quarantine dose that will provide a probit 9 (LD99.9968) security level.

2 Materials and methods

2.1 Insects

Insects used in this research were obtained from a colony of *C. pomonella* that had been reared for over 30 generations on an artificial medium similar to that reported by Brinton et al. (1969). The colony originated from moths collected at several locations near the city of Damascus in the summer of 1994. Codling moth males from the local natural populations in the same area were periodically introduced into the colony every summer. Larvae were reared in plastic trays ($18 \times 15 \times 6$ cm), each containing about 800 g of the larval rearing medium. Pupation occurs at the top of the diet in this rearing system. Non-diapausing larvae were reared at 26 ± 2°C, 40–60% RH and 16 : 8 (L : D) cycle. Under these conditions, the larval stage requires about 1 month in diet and rearing on apples takes place about the same time. Diapausing larvae, on the other hand, were obtained by rearing them at 24 ± 2°C and 8 : 16 (L : D) cycle. Corrugated cardboard strips (10-cm long and 1.5-cm wide) were used to collect mature larvae leaving the rearing medium for pupation. After irradiation, all rearing was done at room temperature (26 ± 3°C).

2.2 Irradiation

Two Co-60 gamma irradiation sources were used in this study and two kinds of tests (small-scale laboratory tests and large-scale confirmatory ones) were conducted. The mean dose rate of the first source was approximately 13.5 Gy/min while the dose rate of the second one was approximately 71.0 Gy/min. The absorbed dose was measured using an alcoholic chlorobenzene dosimeter. Experiments on the effects of gamma radiation on larval survival in the small-scale laboratory tests were done using the first source. Tests on the effects of gamma radiation on larvae in the large-scale confirmatory tests, however, were done using the second source.

2.3 Effects of gamma radiation on codling moth larvae

2.3.1 Small-scale laboratory tests

Irradiating larvae in cardboard strips: Batches of mature larvae, 50 each, were irradiated in plastic vials (5×7 cm) and each treatment was replicated four times. A series of gamma radiation doses between 50 and 250 Gy at 50-Gy increments was used and a group of un-irradiated larvae was held as a control. Directly after irradiation, larvae were returned to the laboratory in order to continue their development to the adult stage. The cardboard strips were held for 10 weeks to ensure that the emergence was complete and the number of formed pupae, emerged adults and their sex was recorded.

Irradiating larvae in host fruits: Apple fruit 'Golden Delicious', about 200 g each, were washed in water, air dried and placed in cardboard boxes. The fruits were infested with codling moth larvae by covering them for about 16 h with sheets of waxy paper carrying codling moth eggs in the black head stage. Infested fruits were incubated at 26 ± 2°C, examined periodically, removing decaying ones, and irradiated at the end of 4 weeks (late fifth instar). Before irradiation, fruits were divided into five groups, 35 apples each, and each group was exposed to gamma radiation. A series of gamma radiation doses between 100 and 250 Gy at 50-Gy increments was applied and each dose was replicated four times. Irradiated fruits were placed in paper bags containing cardboard strips to collect mature larvae leaving the fruits for pupation. For 2 weeks, the cardboard strips were removed every 3–5 days and replaced with new ones. Collected larvae were held for 6 weeks and the number of both formed pupae and emerged adults was recorded.

2.3.2 Large scale confirmatory tests

In order to demonstrate probit 9 mortality standard at the 95% confidence level, subsequent confirmatory tests were carried out. To synchronize larval development in these tests, egg collections were made over a 12–16 h period and egg sheets carrying eggs in the black head stage were left on the rearing media for about the same time. The following two experiments were conducted:

Irradiating larvae in the larval rearing medium: Plastic trays ($18 \times 15 \times 6$ cm) were seeded with codling moth eggs at a rate of about 600 eggs/tray and incubated at 26 ± 2°C for 4 weeks. At this stage of development (late fifth instar), batches of larvae were exposed to a dose of 200 Gy on the larval rearing medium on eight different occasions and five trays from each batch were held as controls. Immediately after irradiation, trays were returned to the laboratory and placed in cardboard boxes sealed from the top with clear plastic sheets. For 6 weeks, the boxes were checked every 2–3 days for any emerging moths resting on the plastic cover and the trays were examined carefully at the end of the experiment for the presence of any exuvae. The number of treated larvae/replicate was estimated from the number of emerging adults in the control. This was done by placing control trays in a cardboard box identical to those that held irradiated trays. At the end of the experiment (after 6 weeks), the number of exuvae left in each tray was counted and the average number of moths emerging from each tray calculated. To be on the safer side, we assumed that the number of larvae/tray equals the number of emerging adults (in reality it is more).

Irradiating larvae in apple fruit: Apple fruit, about 100 g each, were washed in water, air dried and arranged in four layers within cardboard boxes ($45 \times 35 \times 30$ cm). The fruits were infested with codling moth larvae by placing strips of waxy paper carrying codling moth eggs in the black head stage among them for about 20 h. Infested fruits were stored at room temperature (26 ± 3°C), examined periodically and decaying ones removed. After 4 weeks of storage, the fruits were exposed to a dose of 200 Gy and 1000 fruits were held as a control. Irradiation was done in a semi-commercial irradiation facility. Fruits were placed in boxes on a rotationary table in the irradiation chamber and the radio-

active source was raised into the chamber. For dose uniformity, the table was rotated 180° after the passage of half of the time needed to achieve the required dose. Irradiated fruits were returned to the rearing room and cardboard strips were distributed among them for collecting mature larvae leaving the fruits for pupation. For 2 weeks, the cardboard strips were collected every 4–6 days and replaced by new ones. Collected cardboard strips were held for 6 weeks and the number of emerging adults was recorded. The total number of treated larvae was estimated from the number of collected larvae in the control.

2.4 Data analysis

Data from these experiments were subjected to analysis of variance. Means were separated by Fisher's multiple range tests.

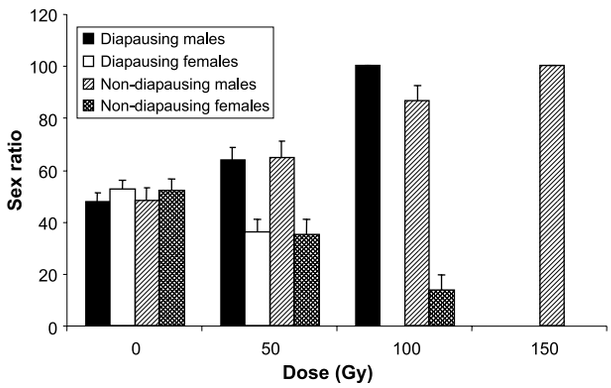

Fig. 1. *Effects of gamma radiation on sex ratio in mature non-diapausing codling moth*

3 Results

Data on the effects of gamma radiation on mature codling moth larvae irradiated in cardboard strips are presented in table 1. The data show that increasing radiation dose caused consistent decrease in pupation and adult emergence. Percentage of pupating larvae, exposed to a dose of 250 Gy, decreased from about 90% in the control to 9.5 and 20.5% for diapausing and non-diapausing larvae, respectively. When adult emergence was used as a criterion for measuring survival, however, the effect of gamma radiation was extremely severe. A dose of 50 Gy reduced adult emergence significantly in non-diapausing larvae ($P < 0.001$; d.f. = 5, 3; $F = 942.9$) and 200 Gy completely prevented it. Table 1 also shows that diapausing larvae were more susceptible to irradiation treatment than the non-diapausing larvae. While no adults emerged from diapausing larvae exposed to 150 Gy dose, 1.5% of the non-diapausing larvae exposed to the same dose were able to continue their development into the adult stage. Sexual differences in radiosensitivity were also noticeable (fig. 1). Examination of adults from irradiated non-diapausing larvae showed that larvae destined to become female moths were significantly more susceptible to irradiation injury than males ($P < 0.001$; d.f. = 5, 3; $F = 85.8$). For instance, 64.5% of adults developed from larvae exposed to 50-Gy dose were males compared to 48% for adults developed from untreated larvae while none of the moths from larvae exposed to 150 Gy were females. Examination of pupae showed that, at this dose level, female larvae were unable to develop into the adult stage. Female diapausing larvae were even more sensitive to irradiation treatment than the non-diapausing larvae (fig. 1). A dose of 50 Gy significantly reduced the number of females ($P < 0.001$; d.f. = 5, 3; $F = 346.2$) and 100 Gy completely eliminated them.

Tests on larvae inside natural host fruit in the small-scale laboratory experiment (table 2) showed similar results. From an estimated number of 420 larvae exposed to a dose of 200 Gy, only 41.6% pupated compared to 96.8% of the larvae in the control and none of the pupating larvae reached the adult stage.

Results of the large-scale confirmatory tests on larvae in the larval rearing medium showed that a dose of 200 Gy completely prevented adult emergence in 460 trays containing an estimated number of >100 940 fifth instar codling moth larvae while the average number of emerging adults in control trays was over 219 moths/tray. Examination of the diet showed that most of the insects died before reaching the pupal stage. Some, however, were able to continue their development to late pupae and a few pupae contained fully developed adults. However, these adults were not able to break the pupal case. As the results of irradiating larvae in natural host fruit may differ from irradiating them in the larval rearing

Table 1. *Effects of gamma radiation on mature diapausing and non-diapausing codling moth larvae*

	%			
	Diapausing		Non-diapausing	
Dose (Gy)	Pupation	Emergence	Pupation	Emergence
0	$87.0^{a} \pm 5.3$	$79.5^{a} \pm 3.4$	$94.5^{a} \pm 6.2$	$87.5^{a} \pm 2.4$
50	$77.5^{b} \pm 4.3$	$49.5^{b} \pm 4.7$	$85.0^{b} \pm 4.2$	$65.0^{b} \pm 4.2$
100	$64.5^{c} \pm 3.4$	$09.5^{c} \pm 1.9$	$82.5^{b} \pm 6.2$	$19.3^{c} \pm 2.8$
150	$49.5^{d} \pm 4.4$	$00.0^{d} \pm 0.0$	$69.0^{c} \pm 8.1$	$01.5^{d} \pm 1.9$
200	$11.0^{e} \pm 4.8$	$00.0^{d} \pm 0.0$	$30.5^{d} \pm 7.2$	$00.0^{d} \pm 0.0$
250	$09.5^{e} \pm 3.0$	$00.0^{d} \pm 0.0$	$20.5^{e} \pm 4.4$	$00.0^{d} \pm 0.0$

Means followed by the same letter within a column are not significantly different ($P > 0.05$; Fisher's LSD test).

Table 2. *Effects of gamma radiation on the fifth instar non-diapausing codling moth larvae irradiated in apples*

	%	
Dose (Gy)	Pupation	Emergence
0	$96.8^{a} \pm 4.8$	$90.0^{a} \pm 7.1$
100	$79.9^{b} \pm 10.4$	$18.7^{b} \pm 7.8$
150	$67.8^{c} \pm 4.7$	$04.3^{c} \pm 4.5$
200	$41.6^{d} \pm 9.9$	$00.0^{d} \pm 0.0$
250	$37.4^{e} \pm 3.4$	$00.0^{d} \pm 0.0$

Means followed by the same letter within a column are not significantly different ($P > 0.05$; Fisher's LSD test).

medium, where water content and oxygen level is different, a large-scale confirmatory test with infested fruits was conducted. The results of this test, however, were also similar to irradiating larvae in the artificial rearing medium. None of an estimated number of > 32 193 larvae exposed to a dose of 200 Gy in apples reached the adult stage, while 96.3% of the estimated number of larvae in the controls developed into adults.

4 Discussion

The idea of using gamma radiation as a quarantine treatment for agricultural products is more than 70 years old (Koidsumi, 1930) and considerable amount of research was done in this area in the last 50 years. The practical applications, however, were delayed for years because of scientific and technical reasons (Hallman, 2001). Developments in the last decade, particularly those related to the phasing out of MB (Anonymous, 1998), revived interest in this technique and this method has been recognized as an acceptable alternative to chemical fumigation (Burditt, 1994).

In this report, the effects of gamma radiation on pupation, adult emergence and sex ratio in codling moth mature larvae from the Syrian strain were examined. In addition, the required dose for quarantine treatment of this pest at the probit 9 (LD99.9968) security level was investigated.

Our results show that increasing radiation dose caused consistent decrease in pupation and adult emergence, and a dose of 200 Gy prevented adult emergence entirely. The results also show that diapausing larvae were more susceptible to irradiation treatment than non-diapausing larvae, which is consistent with results of Burditt (1986) and Toba and Moffitt (1996). However, they contradict the data reported by Burditt and Moffitt (1985), which showed that non-diapausing larvae were more sensitive to irradiation treatment than diapausing larvae. The results also show that females were more radiosensitive than males, which appears to be generally the case in insects (Hallman, 2000). Furthermore, the results show that when the lack of pupation was used as a criterion for measuring effectiveness of irradiation treatment, a relatively high dose of gamma radiation (> 250 Gy) was required. This dose could be injurious to treated fruits (Drake et al., 1998), particularly when applied on a commercial scale, where the dose uniformity ratio could be as high as 3 : 1. However, when adult emergence, which is an acceptable objective (Hallman, 2001), was used as a criterion for effectiveness, the results were more promising. A dose of 200 Gy applied to the fifth instar larvae completely prevented adult emergence and reduced the pupation to about 30%; at a dose of 150 Gy, females were completely eliminated (all emerged moths were males). Consequently, the lack of adult emergence should be used as a criterion for effectiveness.

Security levels for insect quarantine treatments are based on the probit 9 (LD99.9968) mortality standards proposed by Baker (1939). This security level was first suggested for fruit flies; however, it was later adopted for other insects of quarantine importance (Burditt, 1982). At this level, not more than 32 insects are accepted to emerge as adults for each one million treated individuals. The total number of individuals to be treated without any survivors is determined by the level of security desired by the importing country. Couey and Chew (1986) showed that demonstration of probit 9 security at the 95% confidence level (CL) requires cumulative tests on 93 613 individuals in fruits without survivors. However, no survivors from a treated population of 30 000 individuals (LD99.99 at the 95% CL) is considered adequate by many countries (Hallman, 2000). Exposure of > 100 000 fifth instar larvae in their larval rearing medium to a dose of 200 Gy and > 32 000 larvae in apples did not result in a single adult. Therefore, it seems that a dose of 200 Gy applied to the fifth instar codling moth larvae will be adequate for quarantine security of apples against this pest.

In summary, this study indicates that the use of ionizing radiation as a quarantine treatment for codling moth infested fruit is feasible and requires a relatively low dose. In such a process, prevention of moth emergence should be used as a criterion for effectiveness and a dose of not more than 200 Gy should be adequate. In fact, as females are completely eliminated at 150 Gy, a lower effective dose may be feasible. It should be noted, however, that hypoxia can increase insect tolerance to irradiation. Therefore, this factor should be accounted for when gamma irradiation is used to treat apples stored under controlled atmosphere (low oxygen) conditions.

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