RADIATION INDUCED F_1 STERILITY IN *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE): POTENTIAL FOR POPULATION SUPPRESSION IN THE FIELD

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ABSTRACT

The potential of using F_1 sterility in a system to manage the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), was investigated in the laboratory and in field-cages. When 6-day old male pupae were treated with 200 Gy of gamma radiation, 71.5% developed into normal adults. However, radiation-induced reductions in fecundity and viability were expressed during the P_1, F_1, and F_2 generations. Sterility exceeded 60% in the P_1 and F_2 generations and 90% in the F_1 generation. The sex ratio was skewed in favor of males among F_1 and F_2 progeny. The percentages of metaphase spermatogonial cells with chromosomal aberrations were 86.9, 21.5 and 9.7 in the F_1, F_2, and F_3, respectively. No differences were observed in the sperm transfer between irradiated and unirradiated males. When treated males were released into field-cages at either a 5:1 or a 10:1 overflooding ratio with unirradiated moths, there was a significant reduction in the number of F_1 and F_2 adults emerging in the field-cages as compared to the control. A 50-60% reduction in the F_1 and 59-68% in the F_2 generation were observed. When irradiated females and males were released at a 5:5:1:1 overflooding ratio with untreated DBM, the decrease in F_1 adult emergence was not significantly different than for the control. However, adult emergence in the F_2 generation was reduced by almost 90%. This degree of suppression was significantly greater than that achieved in cages where only irradiated males had been released. The use of F_1 sterility in combination with releases of the parasitoid, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), in field-cages resulted in a 40% decrease in the DBM population in the F_1 and more than 90% in the F_2 generation. Nevertheless, additional research is needed to develop this system into an economically feasible strategy for managing early season populations of DBM.

Key Words: diamondback moth, *Cotesia plutellae*, inherited sterility, black stripe pupal mutant

RESUMEN

El uso potencial de la esterilidad F_1 como método de control para *Plutella xylostella* (L.) (DBM, "diamond back moth") se investigo en estudios de laboratorio y de campo. Cuando pupas macho de 6 días de edad se irradiaron con 200 Gy de radiación gamma, el 71.5% se desarrollaron como palomillas aparentemente normales. Sin embargo, en las generaciones P_1, F_1 y F_2 las reducciones en fecundidad y viabilidad fueron obvias. El nivel de esterilidad excedió el 60% en las generaciones P_1 y F_2 y el 90% en la F_1. En la progenie F_1 y F_2, la tasa sexual favoreció a los machos. El porcentaje de espermatogonias en metafase con aberraciones cromosomicas fue de 86.9, 21.5 y 9.7 en las generaciones F_1, F_2, y F_3, respectivamente. No se observaron diferencias entre machos irradiados y no irradiados en su habilidad en transferir esperma. Cuando se liberaron machos irradiados en jaulas de campo junto con palomillas fértiles en las tasas 5:1 o 10:1, el numero de adultos en las generaciones F_1 y F_2 se redujo significativamente. La reducción fue de 50-60% en la generación F_1 y 59-68% en la generación F_2. Cuando se liberaron insectos irradiados de ambos sexos en la tasa 5:5:1:1 con palomillas fértiles, la reducción en el nivel poblacional de la generación F_1 no fue diferente de lo observado en la jaula control. Sin embargo, se observo una reducción del 90% en la generación F_2. El uso combinado de la esterilidad F_1 con liberaciones de *Cotesia plutellae* (Kurdjumov) (Himenóptera: Braconidae) en jaulas de campo causaron que la población de DBM disminuyera mas del 40% en la F_1 y mas de 90% en la generación F_2. Sin embargo, sugerimos que es necesario continuar las investigaciones para lograr que este sistema sea económicamente factible para el control de las infestaciones tempranas de DBM.

The use of inherited or F_1 sterility as a component of population management programs for Lepidopterans has been studied by several researchers (Knipling 1970; North & Holt 1971; LaChance 1985; Carpenter et al. 1987). The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a serious pest of cruciferous crops throughout the world. Costs for its control are estimated at US$1 billion annually (Talekar 1992). Omar & Mansor (1993) report that radiation doses between 150 Gy and 200 Gy were suitable for inducing inherited sterility in DBM

males. Sutrisno et al. (1993) and Sutrisno & Hoedaya (1993) suggested that doses of 175 Gy or 200 Gy could be considered in a DBM suppression program. Preliminary results relating to the potential suppression of DBM populations in the field by the F_1 sterility technique are reported herein.

Materials and Methods

Laboratory Colonies

Diamondback moth. Field-collected DBM pupae from cabbage were used to found the colony. Emerging adults were paired in oviposition cages and provided with a 10% sucrose solution. Grooved aluminum foil egg sheets that had been dipped in autoclaved cabbage juice were used as oviposition substrates. Egg sheets were changed daily. Neonates were reared on leaves of *Tropaeolum majus* L. (Rhoedales: Tropaeolaceae), a wild host of DBM. The colony was maintained at 20 ± 2°C, 70-80% RH and a 14L:10D photoperiod. The black stripe pupal mutant (BSP) is a marker present in our DBM laboratory colony (see below).

Parasitoids. A colony of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), reared on DBM larvae is maintained at our laboratory in Dalat. Laboratory conditions are the same as for the DBM colony. The colony was founded with field-collected material.

Effects of Gamma Radiation on DBM

Six day-old colony pupae were irradiated with a dose of 200 Gy in a Co^{60} irradiator at the Dalat Nuclear Research Institute (dose rate 43.2 Gy/min). Insects were used in laboratory and field-cage tests. Percent emergence, percentage of moths without deformities and longevity of irradiated versus untreated DBM adults were recorded in the laboratory. An average of 200 pupae were used in each of three replicates for the first two parameters. A subset of 100 adults in each of three replicates were used in the longevity study.

Treated DBM males and females (from the P_1, F_1 and F_2 generations) were crossed with untreated counterparts and allowed to mate and lay eggs. Fecundity, fertility (percent sterile eggs), percent surviving to adulthood and sex ratio were recorded for all crosses. For fecundity and fertility data, an average of 20 moth pairs were used per replicate and three replicates were completed. For percent survival, an average of 100 larvae per replicate were used.

Chromosomal Aberrations

Testes of fourth instar DBM larvae from the F_1, F_2 and F_3 generations were dissected in Belar's saline solution and then squashed to release the germ cells. The slides were stained with aceto-orcein, sealed with clear nail polish and stored in a covered container in the refrigerator until examined for the presence of chromosomal aberrations.

Sperm Transfer

Irradiated and unirradiated DBM adult males were allowed to mate with unirradiated virgin females inside laboratory cages. Mating pairs were collected and mating was allowed to proceed until the insects separated. Males were reintroduced into the cages and exposed to virgin females after 24 h for 5 consecutive days. Females from each mating pair were dissected after oviposition to determine the presence of a spermatophore and to assess the ratio of eupyrene to apyrene sperm present in the spermatheca. The normal ratio of eupyrene to apyrene is considered to be 2:1 for *Trichoplusia ni* according to North & Holt (1971). Any ratio deviating from this is considered abnormal.

Effect of Mating Status and Type and Ratio of Sperm on DBM Oviposition

Unirradiated males were allowed to mate with unirradiated virgin females in laboratory cages. Mating pairs were removed from the cages. After separation, females were allowed to lay eggs in the laboratory. When oviposition was completed females were dissected to determine the presence of sperm and the ratio of apyrene:eupyrene sperm in the spermatheca. Virgin females were also held for oviposition as controls.

BSP Mutant

Individuals in our DBM laboratory colony possess the black stripe pupa or BSP mutant. We have isolated and maintained a vigorous and homozygous mutant culture using the method of Bartlett & Raulston (1982). Mutant individuals were inbred and a small colony was established. We attempted to determine the mode of inheritance of the BSP mutant using the method of Walder (1988). Reciprocal crosses were made between mutant (BSP) females and males, and wild type stock (BSP^+). Some F_1 adults from the initial crossed were used in reciprocal back-crosses with the mutant strain and the rest were inbred.

Evaluation of F_1 Sterility for Suppression of DBM in Field-Cages

Cabbage was planted inside four field-cages (2m × 2m × 4m); plants were irrigated regularly and received no other treatments during the experiments. Six-day old colony (BSP) pupae were irradiated as indicated above and pupae were sexed and allowed to emerge into separate containers. A wild strain (BSP^+ = pupae with no black stripe) was collected from the field as 4th instar

larvae and allowed to pupate and emerge in the laboratory. One-day old adults from both groups were used in the experiment.

Field-cages received the following ratios of irradiated BSP colony females (IF), irradiated BSP colony males (IM), feral BSP^+ females (UF) and feral BSP^+ males (UM), respectively: Treatment A = 0:0:1:1 (control), Treatment B = 0:5:1:1, Treatment C = 0:10:1:1 and Treatment D = 5:5:1:1. Cages were checked every two days and eggs deposited on the cabbage were tagged and counted. The population increase in each field-cage was determined by counting the number of eggs, pupae and adults for two generations. Three replications were completed.

Combined Releases of Irradiated DBM and *Cotesia plutellae* in Field-Cages

Cotesia plutellae (CP) parasitoids from our colony (see above), one day-old 200 Gy treated colony (BSP) DBM adults (I) and unirradiated wild (BSP^+) moths (U) were released into field-cages in the following ratios: Treatment 1 = 0 CP: 0 I: 1 U (control), Treatment 2 = 5 CP: 0 I: 1 U, Treatment 3 = 0 CP: 5 I: 1 U, and Treatment 4 = 2.5 CP: 2.5 I: 1 U. In Trt. 4, the release of one day-old irradiated moths occurred at day one and was followed ten days later with the release of one-day old parasitoid adults. Field-cages were checked every two days and the cabbage leaves on which eggs had been deposited were tagged and eggs were counted. The size of the DBM population inside the field-cages was determined by counting DBM eggs, pupae and adults. The size of the parasitoid population was determined by counting the number of parasitoid cocoons present in each cage.

Statistical Analysis

Data were subjected to either the pooled t-test or to analysis of variance. Differences between means were tested for significance using Tukey's Honest Significant Difference (HSD) (Minitab 9.2; Minitab Statistical Software). Deviations from expected ratios were tested by chi-square analysis.

Results and Discussion

Effects of Gamma Radiation on DBM

The results of our study on the effect of 200 Gy on percent emergence, percentage of adults without deformities and longevity of DBM are presented in Table 1. In general, DBM females appear to be more sensitive to gamma radiation than males. When 6-day old pupae were treated with 200 Gy, 71.5% of the male pupae developed as normal adults and 65.3% of these survived to the 6th day, while the corresponding percentages for treated females were 70.6 and 46.5. Adult longevity of both males and females was reduced by about one-third as compared to controls.

Table 2 shows the results of measuring fecundity, sterility, percent survival and sex ratio of DBM irradiated with 200 Gy and their F_1 and F_2 progeny. Significant differences were found in all measured parameters when compared to controls. These significant effects persisted in the F_1 and F_2 progeny. In the P_1 generation, the mean number of eggs laid by the controls was 154. This number decreased to an average of 84 eggs when the female was crossed to an irradiated male. Further, the number of eggs produced by untreated females mated to F_1 males was reduced to about one-fifth the number laid by the controls. However, when F_2 males were mated to untreated females, the number of eggs produced per female was similar to the number produced when irradiated males were mated to normal females. The sterility of treated males was 62.1%, while that of F_1 males was 94.8% and that of F_1 females was 91.1%. In the F_2 generation sterility ranged from

Table 1. Emergence, percentage of adults without deformities and longevity of *Plutella xylostella* adults which had been irradiated with 200 Gy as 6-day-old pupae.

Sex	Mean ± SD			
	Percent emergence[1]	Percent adults without deformities[1]	Percent survival to day 6[2]	Longevity (days)[3]
		Control		
Male	94.1 ± 3.1[a]	89.3 ± 2.7[a]	84.2 ± 5.3[a]	12.3 ± 2.4[a]
Female	95.2 ± 1.8[a]	91.1 ± 5.8[a]	85.1 ± 4.6[a]	11.9 ± 1.4[a]
		200 Gy		
Male	85.4 ± 6.3[a]	71.5 ± 7.2[a]	65.3 ± 7.4[b]	8.9 ± 0.8[a]
Female	83.2 ± 4.1[a]	70.6 ± 7.8[a]	46.5 ± 8.5[a]	7.9 ± 1.0[a]

Means followed by the same letter within a column for each treatment are not significantly different ($P > 0.05$; pooled t-test).
[1]Average of 200 pupae, 3 replications.
[2]Moths, which did not survive up to the sixth day usually, were not able to mate.
[3]Average of 100 moths, 3 replications.

TABLE 2. FECUNDITY, STERILITY, PERCENT SURVIVAL AND SEX RATIO OF *PLUTELLA XYLOSTELLA* IRRADIATED WITH 200 GY AS 6-DAY-OLD PUPAE AND THEIR F_1 AND F_2 PROGENY.

	Mean ± SD			
Mating type	Fecundity (total # of eggs)[1]	Sterility (% of eggs that failed to hatch)[1]	Survival (%)[2]	Ratio of males/females
		P_1 generation		
UM × UF	156 ± 10[a]	12.7 ± 7.8[a]	78.5 ± 8.9[b]	1.01 ± 0.01[a]
IM × UF	84 ± 3[b]	62.1 ± 6.8[b]	57.8 ± 7.6[a]	1.98 ± 0.07[b]
		F_1 generation		
UM × UF	145 ± 8[b]	10.3 ± 0.6[a]	85.1 ± 2.2[c]	0.97 ± 0.05[a]
[A]F_1M × UF	33 ± 4[a]	94.8 ± 1.3[b]	11.1 ± 3.7[a]	1.78 ± 0.20[b]
[B]F_1F × UM	28 ± 2[a]	91.1 ± 2.0[b]	32.1 ± 10.5[b]	1.63 ± 0.17[b]
		F_2 generation		
UM × UF	140 ± 7[b]	11.4 ± 3.4[a]	75.7 ± 78[a]	1.03 ± 0.14[a]
F_2AM × UF	84 ± 3[a]	63.5 ± 7.1[b]	64.7 ± 9.7[a]	1.18 ± 0.11[a]
F_2AF × UM	80 ± 7[a]	61.8 ± 8.2[b]	65.3 ± 13.3[a]	1.42 ± 0.13[b]
F_2BM × UF	87 ± 6[a]	64.8 ± 11.8[b]	62.9 ± 12.3[a]	1.25 ± 0.12[ab]
F_2BF × UM	86 ± 3[a]	62.4 ± 5.6[b]	70.1 ± 10.6[a]	1.34 ± 0.10[b]

I = irradiated, U = unirradiated.
Means followed by the same letter within a column for each treatment are not significantly different ($P = 0.05$; Tukey's Honest Significant Difference).
[1]Average of 20 pairs of moths, 3 replications.
[2]Percent survival of 100 neonate larvae to adult, 3 replications.
[A, B]Male and female progeny from each F_1 cross were followed separately.

61.8% to 64.8%. Survival to adulthood in progeny of untreated moths varied between 75-85%, while it was 57.8% in the F_1 generation, from 11.1% to 32.1% in the F_2 and from 62.9% to 70.1% in the F_3. The sex ratio was biased in favor of males among F_1 and F_2 progeny.

These results differ from those reported by Sutrisno et al. (1993) and Sutrisno & Hoedaya (1993). We report higher levels of sterility than those reported by these authors. In addition, the fecundity of untreated females mated to irradiated, F_1 and F_2 males was lower than for untreated controls in our experiments, while Sutrisno et al. (1993) and Sutrisno & Hoedaya (1993) report that fecundity in these crosses was equal to that observed in the untreated controls. The reasons for these differences remain unclear, but might be explained by differences in DBM strain, rearing methods and dose calibration.

Chromosomal Aberrations

Cytological examination of chromosomal aberrations in the primary spermatocytes in 4th-instar larvae (Table 3) showed abnormal chromosomes evident in 86.9%, 21.5% and 9.7% of larvae from the F_1, F_2 and F_3 generations respectively, while none were evident in the untreated controls. DBM have 31 pairs of chromosomes, individually recognizable during metaphase-I. Reciprocal translocations in the form of rings or chains, involving four or six chromosomes were observed in the F_1 generation. These particular aberrations are the main cause of inherited sterility.

TABLE 3. PERCENTAGE OF METAPHASE NUCLEI WITH VISIBLE CHROMOSOMAL ABERRATIONS IN PROGENY OF *PLUTELLA XYLOSTELLA* MALES WHICH HAD BEEN IRRADIATED WITH 200 GY AS 6-DAY-OLD PUPAE AND THEIR F_1, F_2 AND F_3 MALE PROGENY.

Generation	No. of nuclei examined[1]	No. with chromosomal aberrations	Frequency of aberrations (%)
F_1	92	80	86.9
F_2	79	17	21.5
F_3	72	7	9.7
Control	77	0	0.0

[1]Testes from mature larvae were dissected in Belar's solution and then squashed to release the germ cells. The slides were stained with aceto-orcein, sealed and stored in the refrigerator until examined.

Carpenter (1991) and Zhang et al. (1993) report that incidence of visible chromosomal aberrations in F_1 and F_2 larvae of *Helicoverpa zea* and *Ostrinia furnacalis* is dose dependent, and that aberrations occur most frequently in the F_1 and progressively less frequently in subsequent generations. North (1975) reported that male progeny of *H. zea* treated with 200 Gy showed at least one translocation. Our observations on DBM agree with results reported by these authors for other Lepidoptera.

Sperm Transfer

Table 4 shows the percentage of matings on each of five consecutive days by irradiated (200 Gy) and unirradiated DBM males. This percentage diminished for both groups with each successive mating. Lepidopteran males produce apyrene and eupyrene sperm that is transferred to the female during mating. Eupyrene sperm are nucleate and therefore capable of fertilization, while apyrene sperm are smaller and anucleate. Both types are present in the spermatophore and migrate to the spermathecae in the female after copulation is complete (North & Holt 1971). The percentage of treated and untreated DBM males that transferred sperm in each of five consecutive matings is given in Table 4. No reduction in sperm transfer was evident among the first, second and third matings for either male group. The percentage of males that transferred a normal ratio of eupyrene to apyrene sperm remained fairly high during the first three consecutive matings for both groups.

In *Trichoplusia ni* Holt & North (1970) report that the ability of fully sterile males to transfer sperm was considerably lower than in unirradiated males. They found that when sperm were deposited into the bursa copulatrix, the eupyrene sperm lacked motility, while the apyrene sperm were able to migrate to the spermatheca. In contrast, treating DBM pupae with a substerilizing dose of radiation (200 Gy) resulted in males able to transfer normal ratios of eupyrene:apyrene sperm. Our results for DBM are similar to those reported by El-Naggar et al. (1984) on *Agrotis ypsilon*, Carpenter et al. (1987) on *Helicoverpa zea*, and Sallam & Ibrahim (1993) on *Spodoptera littoralis*.

Effect of Mating Status and Type and Ratio of Sperm on Oviposition

North & Holt (1971) reported that when *T. ni* females mated with an irradiated male they often failed to deposit a normal number of eggs. They suggested that this might be caused by inadequate sperm or accessory gland fluid transfer to the female during mating. Table 5 shows the effect of mating status and type and ratio of sperm on oviposition in DBM. Females that received a normal sperm complement laid significantly (twice as many) more eggs than those receiving an abnormal sperm ratio. It appears that type and quantity of sperm transferred significantly influences fecundity in DBM females.

Holt & North (1970) reported that early in copulation the spermatophore in *Trichoplusia ni* becomes filled with a clear fluid even in females

TABLE 4. ABILITY OF *PLUTELLA XYLOSTELLA* MALES IRRADIATED WITH 200 GY OF TO TRANSFER SPERM.

No. of consecutive matings	No. males tested[1]	Percent mated	% of mated males which transferred sperm	Percent of males which transferred normal ratio eupyrene:apyrene sperm[2]
		Unirradiated male		
1	92	91.3	92.8	89.7
2	84	75.0	92.2	83.3
3	63	69.8	90.9	80.0
4	44	34.1	53.3	32.5
5	15	33.3	0.0	
		Irradiated male		
1	127	88.9	92.1	88.9
2	113	74.3	92.7	83.4
3	84	65.4	91.1	79.5
4	55	32.7	51.2	31.2
5	18	0.0		

[1]Irradiated and unirradiated males were allowed to mate with unirradiated virgin females on each of five consecutive days. Mating pairs were removed from the cages. Mated females were dissected after oviposition to determine the presence of a spermatophore and the ratio of apyrene to eupyrene sperm in the spermatheca.

[2]The normal ratio of eupyrene to apyrene is considered to be 2 eupyrene:1 apyrene. Any ratio below 1:1 is considered abnormal (North & Holt 1971).

TABLE 5. EFFECT OF MATING STATUS AND TYPE AND RATIO OF SPERM ON *PLUTELLA XYLOSTELLA* OVIPOSITION IN THE LABORATORY.

Sperm transferred	No. females	No. eggs/female (Mean ± SD)
Normal eupyrene:apyrene[1]	77	234 ± 26[d]
Abnormal eupyrene:apyrene[1]	55	112 ± 16[c]
No sperm[1]	32	73 ± 14[b]
None (virgin females)	42	47 ± 11[a]

Means followed by a different letter within a column are significantly different (P = 0.05; Tukey's Honest Significant Difference).
[1]Unirradiated males were allowed to mate with unirradiated virgin females in laboratory cages. The mating pairs were removed from the cages. When the mated females had finished oviposition, they were dissected to determine the presence of sperm and the ratio of apyrene and eupyrene sperm in the spermatheca. Virgin females were held for oviposition as controls.

that receive no sperm, and that this may elicit the laying of a significant number of eggs. This observation appears to hold true for DBM. In our experiments mated females that received no sperm laid significantly more eggs than virgin females, which suggests that fecundity in DBM is also influenced by the secretions from the accessory gland transferred during mating.

BSP Mutant

Reciprocal crosses made between BSP and/or BSP^+ virgin females and males confirm that the gene responsible for the mutation black stripe pupa (BSP) is dominant and most likely located on an autosome.

Evaluation of F_1 Sterility for Suppression of DBM in Field-Cages

Evaluating the results of inherited sterility as a method for population suppression is particularly complex. Released partially sterile individuals mate with released untreated individuals to produce partially sterile progeny. The progeny continue to do the same until the experiment is stopped and effects are ascertained. Several marking techniques (such as radioisotopes, externally applied fluorescent powders and internal oil soluble dyes) have been used to identify released Lepidopteran insects. Unfortunately, these markers do not allow the descendants of a released insect to be tracked through subsequent generations. Bartlett (1967) suggested that mutations could be used as biological markers. Furthermore, he suggested that dominant and co-dominant mutants are most useful, as they can identify not only the released individuals but also their progeny and descendants in subsequent generations. The presence of the BSP mutant in our DBM colony made it possible for us to examine the mating interactions of the irradiated DBM through several generations.

The results of our field-cage experiments are shown in Table 6. When treated males (Trts. B and C) were released at either a 5:1 or a 10:1 overflooding ratio with unirradiated moths, there was a significant reduction in the number of F_1 and F_2 adults emerging in the field-cages as compared to the control (Trt. A). A 50-60% reduction in the F1 and 59-68% in the F2 generation were observed for field-cages receiving Trt. B and C, respectively. When irradiated females and males were released at a 5:5:1:1 overflooding ratio (Trt. D), the decrease in F_1 adult emergence was not significantly different than for the control (<3%). However, adult emergence in the F_2 generation was reduced by almost 90%. This degree of suppression was significantly greater than that achieved in cages where only irradiated males had been released. Nonetheless, 1.4 times more neonates were produced by Treatment D in the F_1 generation as compared to the control, and these neonates caused greater host plant damage.

Several investigators have formulated hypotheses concerning the role that released irradiated females might play in the suppression of populations subjected to the sterile insect technique (Whitten & Taylor 1970, Allam & Galun 1976). If partially sterile females are released they could contribute a significant fraction of the progeny produced in the target population. This increase in the number of F_1 progeny may benefit a release program when the released females carry lethal chromosomal changes that will be transferred to the target population. However, the major suppression of the target population is deferred to (but greatly enhanced in) the second generation. North & Holt (1971) suggested that the maximum economic efficiency in the use of inherited sterility for the suppression of lepidopteran populations requires the release of both sexes of partially sterile moths. Our results support the above analyses and findings.

In our field-cage studies BSP individuals were identified among the progeny in all cages except the control. The ratio of BSP to BSP^+ in the F_1 generation was 2.21, 3.41 and 8.94 in Treatments B, C, and D, respectively. The ratio of BSP to BSP^+ in the F_2 generation was 0.11, 0.09 and 0.07 in Trts. B, C, and D, respectively (Table 6). All individuals expressing the black stripe pupal mutant were

TABLE 6. INFLUENCE OF RELEASING IRRADIATED (200 GY) AND UNIRRADIATED BSP *PLUTELLA XYLOSTELLA* ADULTS AT DIFFERENT RATIOS ON THE DEMOGRAPHIC PARAMETERS OF THE F_1 AND F_2 GENERATIONS IN FIELD-CAGES.

Treatment (No. of IF:IM:UF:UM per cage)	Mean ± SD				Ratio
	No. eggs	No. neonates	No. pupae	No. adults	
			F_1 generation		
A-control (0:0:50:50)	3243 ± 767[a]	3037 ± 64[c]	1015 ± 167[b]	921 ± 5[b]	0
B (0:250:50:50)	2653 ± 667[a]	2238 ± 338[b]	542 ± 124[a]	459 ± 87[a] (50.16%)	2.21
C (0:500:50:50)	2397 ± 645[a]	1490 ± 166[a]	415 ± 90[a]	362 ± 51[a] (60.69%)	3.41
D (250:250:50:50)	8781 ± 1138[b]	4325 ± 206[d]	1120 ± 194[b]	895 ± 118[b] (2.82%)	8.94
			F_2 generation		
A	8348 ± 459[b]	7766 ± 198[d]	2345 ± 160[c]	2154 ± 145[c]	0
B	4246 ± 361[a]	3166 ± 323[c]	923 ± 74[b]	879 ± 82[b] (59.19%)	0.11
C	3619 ± 212[a]	1523 ± 105[b]	729 ± 70[ab]	684 ± 68[b] (68.25%)	0.09
D	4370 ± 466[a]	688 ± 270[a]	648 ± 47[a]	266 ± 43[a] (87.65%)	0.07

Means followed by the same letter within the same column are not significantly different (P = 0.05; Tukey's Honest Significant Difference).

[1]Values in parentheses indicate the percent decrease in the moth population as calculated by the formula: ((U-R)/ U) where U is the number of emerged moths in the control population, and R is the number of moths which emerged in the treated population.

[2]BSP^+ = wild moth and BSP = mutant moth.

Three replications were completed.

descendants of released DBM, and it was these individuals that transmitted the sterility factors into subsequent generations. In fact, the ratios of BSP to BSP^+ in the various field-cages are the ratios of substerile to fertile DBM in the F_1 and F_2 generations. The presence of BSP-marked individuals is the most compelling evidence of the mating interaction between released substerile and wild moths. The usefulness of mutant markers as a tool to monitor the outcome of sexual interactions between irradiated (BSP) and unirradiated (BSP^+) DBM has been demonstrated in these experiments.

Even though our results would suggest that male only releases should be considered for DBM, no efficient technique is currently available to separate large numbers of male and female pupae. As a consequence, simultaneous release of treated males and females is unavoidable at this time. In our experiments, population suppression in the F_2 generation for bisexual releases was significantly higher than that observed in the F_2 for male only releases, even at a ratio of 10:1. However a higher amount of crop damage by F_1 larvae was observed when both irradiated substerile male and female DBM were released into the field-cages. One potential solution to avoid excessive crop damage would be to rear the DBM F_1 generation in the laboratory and release F_1 adults instead of their irradiated parents. This would reduce crop damage because of the lower fertility in the F_1 as compared to the P_1 generation. However, the lower fertility in the F_1 generation would call into question the economic feasibility of this option.

Combined Releases of Irradiated DBM and *Cotesia plutellae* in Field-Cages

Knipling (1979) suggested that combining parasitoid releases with sterile insect release might yield both additive and synergistic effects. Although the modes of action of both tactics are different, the effectiveness of the sterile insect technique increases the ratio of adult parasitoids to adult hosts, while the action of parasitoids increases the ratio of sterile to fertile insects. Even greater suppression could be expected if parasitoids are combined with the releases of partially sterile insects. Carpenter (1993) demonstrated that the economic benefits of combining inherited sterility and parasitoids would be greatest when the ratio of irradiated to unirradiated moths is ≤10:1 and the ratio of parasitoid to hosts is ≤5:1.

Our objective was to investigate the potential of using combined releases of partially sterile DBM with releases of *Cotesia plutellae*, a specific larval parasitoid, to suppress wild DBM populations. The results are summarized in Table 7. In cages receiving partially sterile DBM (Trts. 3 and 4), the number of F_1 eggs was significantly (1.5

TABLE 7. INFLUENCE OF RELEASING *COTESIA PLUTELLAE* (CP), IRRADIATED (I) (200 GY) AND UNIRRADIATED (U) *PLUTELLA XYLOSTELLA* ADULTS AT DIFFERENT RATIOS ON THE DEMOGRAPHIC PARAMETERS OF THE F_1 AND F_2 GENERATIONS IN FIELD-CAGES.

Treatment[1] CP:I: U	Mean ± SD			
	No. eggs	No. neonates	No. pupae	No. adults[3]
		F_1 Generation		
Treatment 1 (0:0:10)	298 ± 67[a]	262 ± 42[a]	127 ± 33[b]	108 ± 25[a]
Treatment 2 (50:0:10)	279 ± 40[a]	252 ± 34[a]	87 ± 10[ab]	78 ± 17[a] (27.78%)
Treatment 3 (0:50:10)	417 ± 21[b]	255 ± 41[a]	115 ± 25[ab]	104 ± 16[a] (0%)
Treatment 4[2] (25:25:10)	432 ± 20[b]	262 ± 41[a]	66 ± 15[a]	63 ± 15[a] (41.67%)
		F_2 Generation		
Treatment 1	2796 ± 415[b]	2534 ± 204[c]	1572 ± 297[d]	1431 ± 2214[d]
Treatment 2	2267 ± 308[b]	2018 ± 195[b]	1108 ± 109[c]	1002 ± 86[c] (29.98%)
Treatment 3	1432 ± 289[a]	288 ± 56[a]	261 ± 48[b]	257 ± 51[b] (82.04%)
Treatment 4[2]	843 ± 152[a]	195 ± 8[a]	52 ± 4[a]	40 ± 5[a] (96.58%)

Means followed by the same letter within a column are not significantly different ($P = 0.05$; Tukey's Honest Significant Difference).
[1]CP = *Cotesia plutellae*; I = irradiated BSP moth; U = unirradiated BSP^+ moth.
[2]Irradiated DBM were released on day 1 and were followed 10 days later with the release of *Cotesia plutellae*.
[3]Values in parentheses indicate the percent decrease in the moth population as calculated by formula: (U-R)/ U) where U is the number of moths which emerged in the control population, and R is the number of moths which emerged in treated population.
Three replications were completed.

times) higher. However, because dominant lethal mutations in the irradiated moths killed many embryos during development, no significant difference was found in the number of neonates among treatments. Thus, the level of host damage caused by larvae in all four populations was almost identical. The number of adult moths was lowest in Treatment 4, although differences among treatments were not significant. However, in the F_2 generation the number of eggs in Trts. 3 and 4 was significantly lower than the number of eggs in Trts. 1 and 2. Treatment 4 had the lowest number of eggs at 843. The number of neonates for each of the treatments was significantly lower than the number of neonates in the control. Furthermore, the number of neonates in Trt. 3 (288) and Trt. 4 (195) was significantly lower than the number of neonates in Trt. 2 (2018). As a result, the level of damage on host plants was the lowest in cages treated with both irradiated males and females and parasitoids.

Each of the treatments significantly suppressed population growth as compared to the control. However, population suppression was significantly greater in cages receiving a combination of irradiated (200 Gy) DBM males and females followed by a single release of *C. plutellae*. When only one tactic was used, population suppression was significantly higher in cages receiving irradiated DBM than in those receiving parasitoids.

The numbers of parasitoids, BSP, and BSP^+ marked moths recovered from each of the treatments are summarized in Table 8. In Treatment 2 (parasitoids only), the population of *C. plutellae* decreased by about 20% in the F_1 generation and increased by about 75% in the F_2 generation. However, in Trt. 4 (irradiated DBM + parasitoids) the size of the parasitoid population increased by about 50% in F_1 generation and declined by about 15% in the F_2. These observations might suggest that *C. plutellae* may have had a higher survival rate on irradiated F_1 (BSP) larvae than on the wild type (BSP^+) larvae. However the greater number of *C. plutellae* in the F_2 generation in Trt. 2 is largely due to lower number of DBM larvae in Trt. 4 (195 neonates).

Our data suggest that the use of both tactics, inherited sterility and releases of the parasitoid, *C. plutellae*, may be feasible for managing early-season populations of DBM. Partially sterile DBM adults could be released to produce large numbers of F_1 sterile larvae on early-season annual hosts. These F_1 larvae could in turn serve as hosts for *C. plutellae* and other parasitoids present in the field. In this way the next generation of parasitoids would be increased, and any surviving partially sterile larvae would become

TABLE 8. NUMBER OF PARASITOIDS, BSP AND BSP+ MARKED *PLUTELLA XYLOSTELLA* OBSERVED IN FOUR POPULATIONS SUBJECTED TO ONE RELEASE OF IRRADIATED *BSP* MOTHS AND OF THE PARASITOID, *COTESIA PLUTELLAE*.

Population (CP:I:U)[1]	Mean ± SD[1]		
	Parasitoids	BSP	BSP+
		F_1 generation	
Treatment 1 (0:0:10)			108 ± 25
Treatment 2 (50:0:10)	41 ± 8		78 ± 17
Treatment 3 (0:50:10)		93 ± 12	11 ± 5
Treatment 4 (25:25:10)	38 ± 3	56 ± 13	7 ± 2
		F_2 generation	
Treatment 1			1431 ± 214
Treatment 2	70 ± 6		1002 ± 86
Treatment 3		11 ± 5	246 ± 45
Treatment 4	32 ± 3	21 ± 4	28 ± 7

[1]*BSP*+ = wild moth; *BSP* = mutant moth; I = irradiated *BSP* moth; U = unirradiated *BSP*+ moth; CP = *Cotesia plutellae*. Three replications were conducted.

partially sterile adults (Carpenter et al. 1996). Further studies are needed to measure the in-field effects of these combined tactics on the early-season population dynamics in DBM.

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