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EFFECT OF 20 KRAD OF GAMMA IRRADIATION ON REPRODUCTION OF A TRANSGENIC STRAIN (EGFP) AND A NONTRANSGENIC STRAIN OF PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE) AND THEIR F1 PROGENY

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Abstract

Responses to irradiation treatment with 20 krad of gamma irradiation with the transformed strain of PBW containing the *EGFP* genetic marker show no potential reproductive advantage over its wild type ancestors, the APHIS stain of PBW. Conversely, the data indicated that the reproductive potential of EGFP stain parents treated with a 20 krad dose of irradiation would be lower that that of the nontransgenic APHIS strain of PBW due to lower fecundity rates and mating frequencies. Therefore, this suggests a lower risk of transmitting unexpected traits to wild field populations than initial projections based on APHIS strain reproductive potential.

Introduction

The successful use of sterile insects (radiation induced) for insect pest control, as a barrier defense, exclusion or eradication tool is well documented in the literature and includes Sterile Insect Technique (SIT) programs to control screw worm fly (*Cochliomyia hominivorax* Coquerel), Mediterranean Fruit Fly (*Ceratitis capitata* Wiedemann) , Melon Fly (*Dacus cucurbitae* Coquillett)), Mexican Fruit Fly (*Anastrepha ludens* Loew),Codling moth (*Cydia pomomella* L.) and Pink Bollworm (PBW) (*Pectinophora gossypiella* Saunders). The PBW-SIT program in the San Joaquin Valley of California is one of most successful long-term sterile release programs in the U.S. However, inherent in the PBW program as in all SIT programs is loss of insect quality as a result of the radiation dose required to cause sterility. This debilitation directly affects the competitiveness of the sterile release insects, thus requiring high ratios of sterile to native insects to successfully accomplish the intended purpose of a program. Improvement in the quality of mass reared insects should lead to more efficient use of this technology through expanding the size of the release area while using the same number of insects due to their improved competitiveness.

The recent developments in molecular biology of genetic transformations of insects other that *Drosophila* and the discovery of alternative gene vectors for gene transfer other than *Drosophila* p-elements offer the possibility of significantly altering the traditional strategies used in SIT programs. Klassen (et al. 1970) and more recently, Fryxell and Miller (1995) report on the merits of implementing this type of strategy to control pest populations.

Peloquin (et al. 2000) reported on a genetically engineered strain of pink bollworm with an enhanced green fluorescent protein (*EGFP)* marker gene derived from a jellyfish. Miller (et al. 2001) reported on the stability of the gene and fitness of the PBW EGFP strain under laboratory rearing conditions. However, the uncertainties of what impact a genetic transformation may have on the biology of the insect, particularly an insect with pest status is of great concern to the general public, government officials and the scientific community. Therefore, before genetically transformed insects can be considered for use in SIT programs, extensive studies must be conducted to learn more about their behavior and performance under both laboratory and field conditions.

We report here on laboratory tests comparing the effect a 20-krad dose of irradiation on a transgenic strain (EGFP*)* of PBW and a nontransgenic strain of PBW.

Methods and Materials

Experiments were conducted within the confines of the United States Department of Agriculture (USDA), Plant Protection and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST), Phoenix Quarantine Laboratory. A partially interogressed transgenic EGFP strain of pink bollworm was originally obtained from John Peloquin at UC Riverside and selectively interogressed to homozygosity over 15 generations in our facility. At the initiation of this study the strain had been maintained in our laboratory for 26 generations. The comparative nontransgenic control strain was obtained from the Pink Bollworm Rearing Facility (PBWRF) in Phoenix, AZ, and is the parental ancestors of the EGFP stain.

EGFP PBW pupae were divided into 2 male and female groups of 250 pupae in each group. Each group was then evenly distributed in the bottom of a liter size plastic container and placed in an environmental chamber maintained at 25.1°C and 80

RH until all pupae eclosed. The control APHIS strain PBW pupae were handled in a like manner. After eclosion, one group of EGFP moths and one group of APHIS moths were randomly selected for irradiation treatment. The remaining two groups (1 APHIS strain and 1 EGFP strain) were held as unirradiated groups. The EGFP strain insects were maintained under quarantine conditions, and were moved from the quarantine facility to the irradiator held in the PBWRF under a variance in our permit requiring that the insects be triple contained. Plastic containers containing the insects were placed them in a paper box sealed with tape. This was then placed in a Biomailer for transport to the PBWRF irradiation room. In the irradiation room two plastic containers containing moths were removed from the boxes and irradiated with 20 Krad from $cobalt_{60}$ gamma irradiation. After irradiation plastic containers were returned to the containment boxes and returned to the quarantine facility. Once in the quarantine facility the four plastic containers were removed from the boxes and placed in the refrigerator held at 10°C for ½ hour or until insects were fully immobilized to facilitate handling. Moths were transferred to 5.7-liter containers with cardboard sides, a plastic bottom and screen lid (Stewart 1984). Each cage was clearly labeled in the following manner 1) EGFP 20KR, 2) EGFP 0KR, 3) APHIS 20KR 4) APHIS 0KR. The cages were then placed in an environmental chamber maintained at 25.1°C and 80 RH. Within the chamber the cages were isolated from each other to prevent the chance of egg/larvae contamination between cages.

Oviposition substrate was placed on the screened lid of the caged adults and eggs were harvested daily from each cage for 10 days. The donut shaped oviposition substrate consisted of (in order of placement on the cage): an 18 X 16 mesh fiberglass screen, an embossed paper towel (American Tissue Chicago, IL), and a silicon weight to insure complete contact with the screen top of the cage. Placed in the center of the donut shaped oviposition substrate was a 5 x 6.4 cm sanitary napkin saturated with a 6% sucrose solution containing 0.1% methyl paraben to prevent microbial growth. Sucrose served as the adult stage food source and the pads were changed daily. Eggs harvested on the 4^{th}, 5^{th} and 6^{th} day were incubated for 2 d in a 17.8 cubic foot growth chamber (Precision Instruments Growth Chamber model 818, Winchester, VA) held at 26.7°C, 45 % RH, 24-h dark cycle. After the 2-day incubation, egg pads were placed, egg side facing up, in the bottom of a 13.4 x 13.4-cm thermoformed plastic tray made of 25-mil PVC plastic. PBW diet (≈ 240 g) was placed over the eggs. Immediately after diet placement, the top of the tray was covered and sealed with a multiple layer paper/plastic material (part #35563 Oliver Products, Grand Rapids, MI). The paper lid was sealed to the tray using a hot iron, which activated a heat sensitive adhesive imbedded in a matrix on the paper material. After sealing, the lids on each tray were perforated using a metal roller fitted with 24-0.6cm long x 0.09-cm diameter pins. Trays with the inoculated diet were placed in Precision growth chambers, one contained the irradiated treated insects the other the unirradiated insects maintained at 29°C, 50 ±10% RH, 24-h dark cycle for 10 days. After 10 days the trays were removed from the chambers and each tray placed in a vertical position in 3.02-liter Rubbermaid containers. The bottom of the Rubbermaid container contained a piece of honeycomb 8.9 X 15.3 cm in size placed over a piece of cardboard of the same dimension. The honeycomb/cardboard substrate served as a pupation site for the larvae as they "cutout" from the tray. In the field, PBW feeds to larval maturity, then chews its way out of a cotton boll and drops to the ground where it pupates in soil cracks or plant litter at the base of the plants. Insects reared on artificial diet placed in a rearing container exhibit the same behavior. The Rubbermaid containers were held in a second pair of Precision Growth Chambers maintained at 29°C, 50 ±10% RH, 24-h light cycle at 4 –6 lux.

Pupae were harvested by separating the honeycomb sheets from the cardboard backing. This allowed the pupae to fall from the honeycomb into a collection tray. Diet in the containers was examined to insure that all F_1 were collected. Collected pupae were counted, sexed, then placed individually in plastic tubes (10 X 75 mm) with cotton stoppers. All F_1 adults that eclosed were sexed again, then paired in one of the following combinations: 1) F_1 X untreated 2) F_1 X F_1. 3) Untreated X untreated. Single or multiple pairs were placed in 473-ml oviposition cages depending on the number available.

Results and Discussion

The results of our study indicate that irradiation with 20 Kr of gamma irradiation had similar effects on the fertility of the EGFP strain PBW (transgenic) and the APHIS strain PBW(control). Fertility of F1 eggs produced by irradiated parents were significantly reduced by 99.6% in the EGFP strain and 99.8% in the APHIS strain when compared to their respective untreated controls (Table 1). Irradiation also significantly reduced female fecundity in both strains with a reduction of 69.9 % in the EGFP strain and 67.9 % in the APHIS strain when compared to their respective untreated controls (Table 2). As indicated in figure 1 female egg production by irradiated females of both strains the first two days of egg collection are similar to their untreated female counterparts. However, egg production on days 3-10 shows a rapid decline by irradiated females of both strains when compared to their untreated counterparts. Furthermore, fecundity levels of the irradiated female group and untreated female group of the EGFP strain PBW were 25.9% and 20.9% lower, respectively than fecundity levels of irradiated and untreated female groups of the APHIS strain (Table 2). This loss in female fecundity of the untreated group of EGFP strain PBW when compared to the APHIS strain untreated group is consistent with results reported by Miller (et al 2001).

Irradiation significantly reduced the frequency of mating as determined by the number of spermatophore found in the female *bursa copulatrix* with reductions of 24.2% and 29.3% in the EGFP and APHIS strains, respectively, when compared to their

untreated controls. More importantly, mating frequency of the untreated EGFP strain was 19.5% lower than that of the untreated APHIS strain (Table 2).

The 500-EGFP female moths irradiated with 20-krad of gamma irradiation and mated with a similar number of irradiated EGFP males produced 2 F_1 male moths while the same number of irradiated females produced 8 male and 5 female F_1 moths (Table 3). Of the 13 F_1 moths produced by the APHIS stain, 8 or 69.25% of them emerged with malformed wings or legs. One of the 2 F_1 moths emerged from the EGFP strain was deformed, whereas none of the 115 F_1 moths from the untreated APHIS strain parents contained deformities (Table 3).

When F_1 progeny of irradiated parents from the EGFP strain or the APHIS strain were mated in a self cross mating or out crossed with untreated moths no F_2 progeny were produced.

Conclusions

The transgenic EGFP strain of PBW showed no potential reproductive advantage over their wild type ancestors, the APHIS strain after irradiation treatment with 20-krad of gamma irradiation. The data show that reproductive potential of EGFP stain parents treated with the 20-krad dose would be lower than that of the nontransgenic APHIS strain due to lower fecundity rates and mating frequencies in the transgenic strain. This shows a lower risk of transmitting unexpected traits to field populations of PBW than initial projections based on the APHIS strain reproductive potential, the strain that is routinely released in the San Joaquin SIT program. The production of small numbers of F_1 progeny containing a high degree of adult deformities and sterility from parents irradiated with 20 krad in a laboratory environment is consistent with an earlier study by Miller (et al 1983). However, the authors of the 1983 study also reported that no F_1 progeny were produced in a cotton field cage study were 2.25 million PBW irradiated moths were released over the course of a cotton-growing season.

References

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Table 1. Comparison of fertility of two strains of PBW after irradiation with 20-Krad of gamma irradiation.

Type mating	# eggs examined	# eggs hatched	% hatch
I- EGFP X I- EGFP	1427	4	0.3 a
U- EGFP X U- EGFP	2282	1903	83.4 b
I- APHIS X I- APHIS	2144	4	0.2 a
U- APHIS X U- APHIS	2872	2512	87.5 b

U, Untreated; I, irradiated at 20 Krad. of gamma irradiation.
Means in columns followed by the same letter are not significantly different, ANOVA with means separated by a Tukey Test at the 0.05 level.

Table 2. Comparison of mating and fecundity of different strains of PBW, both non irradiated females mated with non irradiated males and irradiated females mated to irradiated males treated with an irradiation dose of 20-Krad of gamma irradiation.

Type mating	# females tested	Avg. eggs / female	Avg. # spermatophore /mated female	% females mated
I- EGFP X I- EGFP	500	47.5 a	2.5 c	91.9
U- EGFP X U- EGFP	500	157.8 b	3.3 b	96.9
I- APHIS X I- APHIS	500	64.1 a	2.9 b	91.0
U- APHIS X U- APHIS	500	199.6 b	4.1 a	97.9

U, Untreated; I, irradiated at 20 Krad of gamma irradiation.
Means in columns followed by the same letter are not significantly different, ANOVA with means separated by a Tukey Test at the 0.05 level.

Table 3. F_1 and F_2 progeny of PBW parents irradiated with 20 Krad of gamma irradiation.

		No. moths test		Total eggs implanted	No progeny produced @ indicated life stage			
Progeny	Mating Type	M	F	on diet	Pupa	Adult	% eclosion	% adults deformed
F_1	I-EGFP X I- EGFP	250	250	15,013	7	2	28.6	50.0
	APHIS X I- APHIS	250	250	19,178	36	13	36.1	53.8
	U-EGFP X U-EGFP	250	250	29,111	15,425	14,855*	96.3	2.9
	U- APHIS X U-APHIS	250	250	40,200	28,991	27,919*	96.3	0.9
F_2	I-EGFP male X U-EGFP female	2	4	1	0	0		
	I-APHIS male X I-APHIS female	1	2	1	0	0		
	I-APHIS male X U-APHIS female	1	2	15	0	0		
	U-APHIS male X U-APHIS female	1	1	152	115	115	100.0	0

U, Untreated; I, irradiated at 20 Kr with cobalt $_{60}$ or F_1 progeny of parents irradiated at 20 Kr.
* Estimated number of adults. Due to the large number of F1 progeny in the 0 Kr treatments, 100 pupae per replicate (600 total per treatment) were set up to determine eclosion and deformity rates.

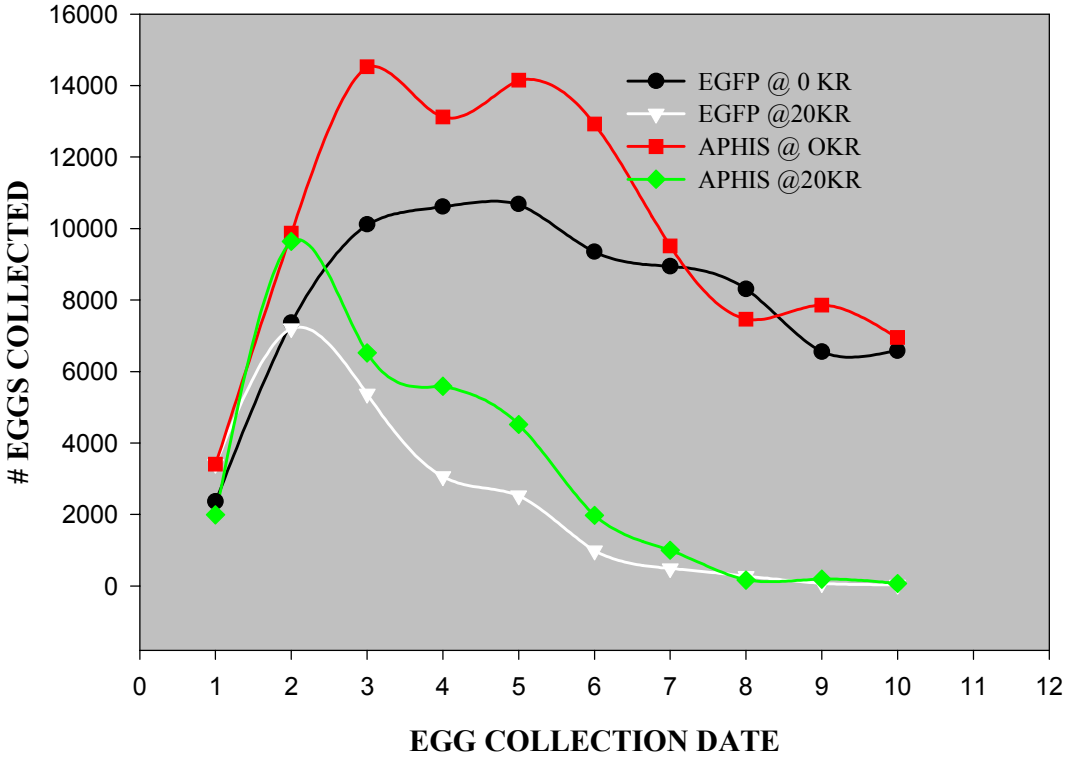

Figure 1. Impact gamma irradiation (20Krad) has on female fecundity in APHIS and EGFP strain PBW. Egg totals from 500 females per treatment.