Preliminary Studies on Inherited Sterility for Field Management of Diamondback Moth (*Plutella xylostella*) (Lepidoptera: Plutellidae) on Crucifers in Ghana

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Received: January 2009 Accepted: January 2010

Résumé

Ewusie, E. A., Nkumsah, A. K., Alimatu, S., Osae, M. Y. *Des études préliminaires sur la stérilité héritée pour la gestion du champ de la teigne à dos de diamant (Plutella xylostella) (Lepidoptera : Plutellidae) sur les crucifères au Ghana.* La teigne à dos de diamant, (TDD) (*Plutella xylostella* (L.) (Lepidoptera: Plutellidae) est le plus destructif ravageur insectes de crucifers au Ghana et au monde. Il est estimé à coûter environ un milliard de dollars pour contrôler chaque année dans le monde entier. Dépendance aux produits chimiques comme la mesure de contrôle exclusif pour la teigne à dos de diamant a entraîné le développement d'une multitude de problèmes, y compris la résistance, les niveax de résidus élevé sur le produit, la destruction des ennemis naturels et de la résurgence du nuisible entre autres. Stérilité héritée dans les insectes de lépidoptères a un potentiel pour la suppression des populations de TDD. Nous avons effectué cette étude pour évaluer l'utilisation de la technique pour gérer la teigne à dos de diament au Ghana. Quand 3-4 jours de pupes anciens ont été traités avec 130 Gy et 150 *Gy* du rayonnement gamma, 47 % et 46 % t des pupes mâles ont développé comme adultes normales respectivement tandis que 40 % et 17 % des femelles pupes ont développé comme adultes normales respectivement. Cependant, rayonnement induit des réductions dans la fécondité et la viabilité des œufs ont été exprimées dans les générations (F_1) parentales et les premières filiaux. Stérilité a dépassé 66 % dans le mâle parental traité et 92 % dans la femelle parentale traitée dans les deux traitements par rapport à 78 % et 95 % dans les traités F_1 mâle et femelle, respectivement. Le ratio du sexe était faussé en faveur des mâle de la descendance parental. Ces résultats indiquent la possibilité d'utiliser la stérilité héritée pour controller le TDD.

Mots-clés : La teigne à dos de diamant, *Plutella xylostella*, irradiation, stérilité hérité, F_1 stérilité.

Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most destructive insect pest of crucifers in Ghana and the world over. It is estimated to cost about USD 1 billion to control annually worldwide. Reliance on chemicals as the sole control measure for diamondback moth has resulted in the development of a myriad of

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problems including resistance, high residue levels on produce, destruction of natural enemies and pest resurgence among others. Inherited sterility in Lepidoptera insects has a potential for suppressing DBM populations. We conducted this study to evaluate the use of the technique to manage the diamond back moth in Ghana. When 3 – 4 day old pupae were treated with 130 Gy and 150 Gy of gamma radiation, 47% and 46% respectively of the male pupae developed as normal adults whiles 40% and 17% respectively of the female pupae developed as normal adults. However, radiation-induced reductions in fecundity and egg viability were expressed in the parental and first filial (F_1) generations. Sterility exceeded 66% in the treated parental male and 92% in the treated parental female in both treatments as compared with 78% and 95% in treated F_1 male and female, respectively. The sex ratio was skewed in favour of males in the parental progeny. These results indicate the possibility of using inherited sterility for DBM control.

Keywords: Diamondback moth *Plutella xylostella,* irradiation, inherited sterility, F_1 sterility.

Introduction

Cruciferous vegetables such as cabbage, *Brassica oleracea var capitata* L. and cauliflower *Brassica oleracea botrytis* L. are popular exotic vegetables grown both on small and large scales all-year-round in urban and rural areas (Osae, 2002). They have become an important source of income to many small-scale farmers in Ghana, and, a major dietary component for many Ghanaians. These and other vegetables are important sources of micronutrients, which can bolster the immune system and help the body fight diseases (Global Horticulture Initiative, 2007).

One of the constraints in the production of cruciferous vegetables is the infestation by a group of lepidoteran pests such as; the diamondback moth (DBM), *Plutella xylostella* (Linnaeus), the cabbageworm, *Pieris rapae* (Linnaeus), the cabbage looper, *Trichoplusia ni* (Hubner), the corn earworm, *Helicoverpa armigera* (Hubner), the cabbage webworm, *Hellula undalis* (Fabricius), the common cutworm, *Spodoptera litura* (Fabricius), the cabbage head caterpillar (CHC) *Crocidolomia binotalis* (Zeller). Other important insects that damage the cruciferousvegetables include beetles such as striped flea beetle, *Phyllotreta striolata* (Fabricius). These insects greatly reduce both yield and quality of the produce (Morallo-Rejesus and Sayaboc, 1992).

The diamondback moth (DBM) is the most destructive insect pest of crucifers throughout the world. Cost of its control is estimated at US$ 1 billion annually (Talekar, 1992). In Ghana it is an important limiting factor in the production of cruciferous vegetables.

The larvae feed on the foliage from seedling stage to harvest and could cause 100% yield loss if not controlled (Talekar and Shelton, 1993). Because these crops are of high economic value, farmers who grow them use intensive cultivation practices to produce high quality vegetables. In Ghana and many parts of the world, farmers frequently apply mixtures of pesticides on their cabbage crop about twice a week (Obeng-Ofori, 1998). Reliance on chemicals as a control measure for the diamondback moth has resulted in the development of resistance to many insecticides (Talekar and Shelton, 1993). Additionally, high insecticide use has led to excessive residue on produce. Because monitoring for pesticide residue is not practiced in most developing countries including Ghana, insecticide-contaminated crucifers often pass easily through marketing channels (Talekar and Shelton, 1993).

Due to these problems, crucifer production all over the world is now limited by DBM (Talekar and Shelton, 1993). The damage caused by DBM emphasized the need for other alternative control measures and also their use within an integrated pest management approach. Mixtures of *Bacillus thuringiensis* (*Bt*) spray formulations derived from subspecies kurstaki (with Cry1Aa, Cry1Ab, Cry1Ac, Cry2A and Cry2B toxins) and *B. thuringiensis* subspecies aizawai (Cry1Aa, Cry1Ab, Cry1C and Cry1D and Cry2B) have been used as part of an IPM approach for controlling DBM since the late 1980s (Heckel *et. al.,* 2004), but due to genetic resistance in populations to some *Bt* strains (Tabashnik *et al.,* 1987), attention has again been turned to developing alternative control measures. Insecticide resistance, elimination of natural enemies by increased insecticide use, the mounting concern over pesticide pollution and the desire to effectively manage *Plutella xylostella* both within fields and on an area wide basis have encouraged scientists to identify and develop new methods of controlling the pest (Abro *et al.,* 1988; Schmutterer, 1992; Syed, 1992; Kadir, 1992; Omar and Mansor, 1993 and Talekar and Shelton, 1993).

Even though the sterile insect technique (SIT) has been used for Lepidoptera, insects in this order are radio-resistant, presumably due to their holokinetic chromosomal configuration (Bauer, 1967). Therefore, lepidopterans require high doses of radiation for sterilization, leading to somatic damage and reduced competitiveness in the irradiated insect. A favoured alternative to using fully sterile moths in SIT is to use F_1 sterility (Seth and Sharma, 2001).

Sterile F_1 progeny is produced when sub-sterilizing doses of radiation are applied to the parental (P) males. The resulting progeny are more sterile than the irradiated parent, and the irradiated moths are more competitive as a result of receiving a lower dose of radiation. Inherited sterility in the progeny of treated males has been shown to have potential in suppressing populations of lepidopteran pests (North and Holt, 1969; Knipling, 1970; North, 1975; LaChance, 1985).

The use of inherited sterility also referred to as delayed sterility as a component of population management programmes for lepidopterans has been studied by several researchers (Knipling, 1970; North and Holt, 1971; LaChance, 1985; Carpenter *et al.,* 1987; Annoh *et al.*, 2000). Inherited sterility has been studied in the diamondback moth elsewhere. Omar and Mansor (1993) reported that radiation doses between 150Gy and 200Gy were suitable for inducing inherited sterility in DBM males. Sutrisno *et al.* (1993) and Sutrisno and Hoedaya (1993), suggested that doses of 175Gy or 200Gy could be considered in a diamondback moth suppression programme. Nguyen and Nguyen (2001) suggested that radiation dose of 200Gy has the potential for population suppression of DBM in the field. None of those studies were carried out in sub-Saharan Africa and the effects of doses below 150Gy were not stated. There is, therefore, the need to evaluate the potentials of using inherited sterility for the local populations of diamondback moth and also determine if doses smaller than 200Gy could be used to induce inherited sterility.

The purpose of this study was to determine the best dose for inducing inherited sterility in populations of diamondback moth in Ghana, with the aim of using the technique for its sustainable management in the field.

Materials and methods

Colony establishment

The study was conducted using a colony established from field collections around Kwabenya in the Abokobi-Madina District of the Greater Accra Region in Ghana. Once a week, samples of cabbage plants on farmers' fields were inspected and diamondback moth larvae and pupae were collected. The larvae were reared on detached cabbage leaves, while emerging adults were paired in plastic cylindrical oviposition vials (2.5 x 2.7cm) with perforated screw cups and fed on a 10% (v/v) honey-water solution on cotton swabs and provided with strips of cabbage leaves as oviposition substrates. The colony was maintained at 25±1°C, 70±5% relative humidity and a 12h light: 12h dark regimen.

Effects of Gamma radiation on DBM pupae

Three to four days old unsexed pupae were irradiated with doses of 130 Gy and 150 Gy in a calibrated Cobalt-60 gamma irradiator (SLL-515, Hungary) at the Radiation Technology Centre of Ghana Atomic Energy Commission at a dose rate of 2.36 KGy/h in air and the absorbed dose was confirmed by Fricke's dosimetry. A control, not treated, (0Gy) was also set up. There were three replicates of 30 pupae for each dose. Both irradiated and control pupae were then observed for adult emergence, deformities in the adults that emerge and longevity of irradiated and unirradiated DBMs. Malformations in wing structure, legs, abdomen and body colour were considered deformity, using Hilkinson 3" (75mm) Nickel Rim hand magnifier with a magnification of x2.

Mating of adults

For each group of irradiated pupae, the resulting adults were paired with unirradiated adults of the opposite sex, in the following pairings [{Control - Normal male (NM) and Normal female (NF)}, {Irradiated male (IM) and Normal female (NF)}, {Irradiated female (IF) and Normal male (NM)}, {(F_1 male (F_1M) and Normal female (NF)}, and {F_1 female (F_1F) and Normal male (NM)}] and allowed to mate and lay eggs. Fecundity (number of eggs laid per female) which was counted under x10 magnification with Olympus microscope, egg sterility (number of eggs that failed to hatch into larvae), survival of adults (adults surviving to at least day 6) and sex ratio were recorded for all crosses. For fecundity and sterility data, we used 3 replicates of 10 moth pair each.

Statistical analysis

All count data were transformed using natural log ($\log_n$) and all percentage data transformed using Arcsine transformation ($y = \sin^{-1}(\sqrt{x/n})$). Data were analysed by comparing means using analysis of variance (ANOVA) and where there were significant differences; means were separated by Duncan's Multiple Range Test (DMRT). All data were analysed at the 0.05 confidence level using SPSS v16 soft ware.

Results and Discussion

Effects of Gamma irradiation on DBM

Effects of gamma irradiation at 130Gy and 150Gy on emergence are in Table 1. No significant differences were observed in the emergence of adults in all treatments. When 3-4 day old pupae were irradiated at 130Gy, 47 and 40% of the male and female pupae respectively developed as normal adults, while the respective corresponding emergence for similar pupae irradiated at 150Gy were 46 and 17%.

A significant dose-dependent effect was observed on adults surviving to day 6 (Table 2). Both males and females treated at both 130Gy and 150Gy had significantly smaller per cent survival compared to the control. Significantly more treated males survived to day 6 than females at both doses. The sex ratio was skewed in favour of males in the original irradiated population.

No female adults showed any deformities for all treatments (Table 3). This differs from the report of Nguyen and Nguyen (2001). The difference

Table 1. Emergence of adults from pupae irradiated at two different doses using a Cobolt-60 source.

Dose (Gy)	*% Emergence*	*No. of females*	*No. of males*	*% Development (♀:♂)*	*Sex ratio ((♀:♂))*
0 (Control)	80±5.0[a]	6±2	18±2	21:59	1:3
130	87±2.2[a]	12±1	14±0	40:47	1:1
150	63±1.5	5±3	14±2	17:46	1:3

Means followed by the same letter in a column are not different ($p < 0.05$) (DMRT).

Table 2. Per cent survival of adults emerging from pupae irradiated at two different doses using a Cobolt-60 source.

Dose (Gy)	*% survival*	
	Males	*Females*
0 (Control)	85.6 ± 4.0[a1]	84.4 ± 2.2[a1]
130	75.6 ± 2.9[ab1]	60.0 ± 3.8[b2]
150	65.6 ± 6.3[b1]	47.8 ± 2.9[c2]

Means followed by the same letter in a column are not different ($p < 0.05$) (DMRT).

Means followed by the same letter in a row are not different ($p < 0.05$). Paired sample *t*- Test.

might be due to lower doses used and age of pupae at irradiation. Nguyen and Nguyen (2001) irradiated 6 days old pupae at 200Gy and obtained up to 30% deformity. Male adults exhibited deformities for both 130Gy and 150Gy (Table 3). Statistically smaller levels of deformity were observed in males that emerged from 3-4 day old pupae irradiated at 130Gy compared to those of 150Gy (Table 3).

Table 3. Per cent deformity of adults emerging from pupae irradiated at two different doses using a Cobolt-60 source.

Dose (Gy)	*% Deformity*	
	Males	*Females*
0 (Control)	0.00 ± 0.00[b]	0.00 ± 0.00[a]
130	1.11 ± 1.11[b]	0.00 ± 0.00[a]
150	3.33 ± 0.00[a]	0.00 ± 0.00[a]

Means followed by the same letter in a column are not different ($p < 0.05$) (DMRT).

Inherited sterility in Plutella xylostella

The effects of substerilizing doses of gamma irradiation on the fecundity and sterility of parental crosses of DBM are presented in Table 4. In the parental generation, the mean numbers of eggs laid by the control (0Gy) were significantly more than when irradiated females were crossed with normal males (P>0.05). Males irradiated at 130Gy and crossed with normal females produced significantly greater numbers of eggs compared with those irradiated at 150Gy (P>0.05). The mean sterilities for irradiated parental males and females were about 78.65±2.90 and 96.21±1.63% respectively for 130Gy and 66.95±22.77 and 92.33±3.96% respectively at 150Gy compared to about 48% and in the control (Table 4).

Table 5 shows the results of the effects of radiation doses on fecundity and sterility of F_1 DBM crosses. Fecundity of F_1 was not affected by the doses of radiation, and this agrees with what is reported by Sutrisno and Hoedaya (1993). F_1 sterility was significantly greater in all the irradiated males and females mated to opposite sexes than the control. Nguyen and Nguyen (2001) suggested that if partially sterile females are released, they could contribute a significant fraction to the progeny produced in the target population. North and Holt (1971) also suggested that the maximum economic efficiency in the use of inherited sterility for suppression of lepidopteran populations requires the release of both sexes of partially sterile moths. Our preliminary results support these assertions.

Table 4. Fecundity of parental *P. xylostella* irradiated pupae at two different doses of Cobolt-60 source, and sterility of F_1 eggs.

Dose (Gy)	*Cross*	*No. of eggs*	*F_1 sterility (%)*
0 (Control)	NM & NF	934 ± 113^{a}	48.61 ± 8.70^{b}
130	IM & NF	956 ± 149^{a}	78.65 ± 2.90^{ab}
130	IF & NM	636 ± 101^{ab}	96.21 ± 1.63^{a}
150	IM & NF	326 ± 111^{b}	66.95 ± 22.77^{ab}
150	IF & NM	673 ± 116^{ab}	92.33 ± 3.96^{a}

Means followed by the same letter in a column are not different ($p < 0.05$) (DMRT).

Table 5. Fecundity of F_1 and egg sterility of F_2 *P. xylostella* pupae at two different doses of Cobolt-60 source.

Dose (Gy)	*Cross*	*Fecundity*	*F_2 sterility (%)*
0 (Control)	NM & NF	934 ± 113^a	48.61 ± 8.70^b
130	F_1M & NF	800 ± 207^b	77.95 ± 8.72^a
130	F_1F & NM	609 ± 29^a	92.21 ± 5.55^a
150	F_1M & NF	712 ± 55^a	95.28 ± 3.03^a
150	F_1F & NM	645 ± 69^a	90.84 ± 4.59^a

Means followed by the same letter in a column are not different ($p < 0.05$) (DMRT).

The phenomenon of F_1 sterility in *P. xylostella* has been examined over a range of substerilizing doses by several researchers (Sutrisno and Hoedaya, 1993; Nguyen and Nguyen, 2001). In general, Lepidoptera require doses between 350Gy and 500Gy to be fully sterilized (Carpenter *et al*., 2005). In DBM, a dose of 200Gy has been reported to induce inherited sterility in 6-day old *P. xylostella* pupae (Nguyen and Nguyen, 2001). The level of sterility and the number of individuals in the F_1 and F_2 generations are controlled by the dose of radiation administered to the parental generation, and whether only irradiated males or both irradiated males and females are released (Carpenter, 1993). We selected these two doses because lower doses of radiation used for F_1 sterility increases the quality and competitiveness of released insects (North, 1975). In addition, because F_1 sterile progeny are produced in the field, the release of partially sterile insects offers greater suppressive potential than the release of fully sterile insects (La Chance, 1985). The reduced reproductive performance of the treated moths resulted from the combined effects of reduced fecundity and reduced egg viability. A greater reduction in reproductive performance was observed at 150Gy dose than the 130Gy dose. This demonstrates that 150Gy could be the minimum dose for inducing inherited sterility in DBM. Ghana is privileged of having one of the biggest irradiation facilities in West Africa. This facility which is at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) could potentially be used to induce inherited sterility in DBM for release as part of an integrated control programme, thereby reducing pesticide usage. This would provide an effective solution to the conservation of agro-ecosystem diversity, safe environment and sustainable crop protection system.

Overall, our preliminary data suggest that inherited sterility may be used in combination with other management strategies for the suppression/control of DBM populations in crucifers. Nonetheless, further work needs to be undertaken in this regard to obtain the optimum irradiation dose for DBM and to develop this strategy into an economically viable control option in Ghana.

Acknowledgements

We thank Messrs M. Ofori-Appiah and J. E. Ansah of the Radiation Technology Centre, GAEC, for the operation of the gamma facility. We are also grateful to the vegetable farmers who allowed us to use their farms for this research.

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