Effect of Genetically Modified Bt Maize in an Artificial Diet on the Survival of Cydia pomonella (Lepidoptera: Tortricidae)

Authors: Stenekamp, Daleen, Pringle, Ken, and Addison, Matthew

Source: Florida Entomologist, 99(sp1) : 200-205

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.sp124

Effect of genetically modified Bt maize in an artificial diet on the survival of *Cydia pomonella* (Lepidoptera: Tortricidae)

Daleen Stenekamp, Ken Pringle and Matthew Addison*

Abstract

Genetically modified maize contains an insecticidal gene from the soil bacterium *Bacillus thuringiensis* (Bt), which is an important component in integrated pest management strategies against lepidopteran pests of maize. A project is being implemented in the Western Cape of South Africa against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a pome fruit pest, using an area-wide integrated pest management approach with a sterile insect technique component. The project requires rearing of large numbers of the target pest for which an artificial diet that contains maize meal as the main ingredient is used. Most of the maize produced in South Africa is Bt maize, which is known to be toxic to codling moth. The aim of this study was to assess the impact of Bt maize in the diet of codling moth on its production parameters. Codling moths were reared for a period of 44 d on artificial diets that contained 5 different concentrations of Bt maize meal and a control using non-Bt maize. The use of Bt maize in the larval diet resulted in larval mortality, delayed larval development and larvae leaving the diet prematurely. Delayed larval development seemed to be the response with most negative consequences. Since optimal rearing of codling moth is not feasible using meal from genetically modified maize with insecticidal properties, another nutritious meal lacking an insecticidal component must be substituted in the artificial diet.

Key Words: artificial diet; *Cydia pomonella*; larval development; larval mortality; *Bacillus thuringiensis*

Resumen

El maíz genéticamente modificado contiene un gen insecticida de una bacteria del suelo, *Bacillus thuringiensis* (Bt), que es un componente importante en las estrategias de manejo integrado de plagas contra las plagas lepidópteras del maíz. Se está implementando un proyecto en el Cabo Occidental de Sudáfrica contra el gusano de la manzana, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), una plaga de frutas de hueso, utilizando un enfoque de manejo integrado de plagas en toda la zona con un componente de la técnica de insecto estéril. El proyecto requiere la cría de grandes cantidades de la plaga objetivo para el que se utilizó una dieta artificial que contiene harina de maíz como ingrediente principal. La mayor parte del maíz producido en Sudáfrica es el maíz Bt, que se sabe que es tóxico para el gusano de la manzana. El objetivo de este estudio fue evaluar el impacto del maíz Bt en la dieta del gusano de la manzana en sus parámetros de producción. Los gusanos de la manzana fueron criados por un período de 44 días con dietas artificiales que contenía 5 concentraciones diferentes de harina de maíz Bt y un control utilizando el maíz sin Bt. El uso de maíz Bt en la dieta larval resultó en la mortalidad de las larvas, retraso en el desarrollo de las larvas y en larvas dejando la dieta antes de tiempo. El retraso en el desarrollo de las larvas parecía ser la respuesta con las consecuencias negativas. Dado que la crianza óptima del gusano de la manzana no es factible utilizando harina de maíz modificado genéticamente con propiedades insecticidas. Este debe ser sustituido en la dieta artificial con una otra comida nutritiva que no tenga de un componente insecticida.

Palabras Clave: dieta artificial; *Cydia pomonella;* desarrollo larval; mortalidad larval*; Bacillus thuringiensis*

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is a major pest of apples and pears throughout the temperate regions of the world (Barnes 1991; Boncheva et al. 2006; Soleno et al. 2008). Codling moth has been reared on a large scale at Stellenbosch, Western Cape, South Africa since 2002 for use in an area-wide integrated pest management (AW-IPM) program that includes a sterile insect technique (SIT) component (Addison 2005). Economical production and efficient rearing of large numbers of insects are a prerequisite in a program that uses sequential releases of sterile insects. In many rearing facilities selection is directed towards traits which result in high productivity such as high fertility and fecundity and a short life cycle (Sørensen et al. 2012), which sometimes has negative consequence in terms of low performance in the field (Simmons et al. 2010). An artificial larval diet, described by Guennelon et al. (1981), has been used in the facility for rearing codling moth and it includes maize meal as a major ingredient, making up 59% of the diet. Almost 30 years have passed since the first publication of the Guennelon diet but the availability, safety and quality of the original diet ingredients have changed.

The use of genetically modified (GM) maize that contains genetic material from *Bacillus thuringiensis* (Bt) targets stemboring Lepidoptera feeding on this crop. Bt maize is an important component in integrated management of maize pests in South Africa (Gore et al. 2002; Van Rensburg 2007; Kruger et al. 2012). *Bacillus thuringiensis* is a Gram positive bacterium which produces crystalline inclusions during sporulation (De Maagd et al. 1999; Boncheva et al. 2006; Konecka et al. 2007). These inclusions consist of delta-endotoxins or Cry proteins

Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
*Corresponding author; E-mail: daleen@hortgro.co.za

with insecticidal properties (De Maagd et al. 1999; Boncheva et al. 2006; Konecka et al. 2007). The Bt maize plant produces a protoxin that destroys cells in the insect's midgut. The toxin gains entry only if the insect ingests Bt-contaminated food causing direct mortality, indirect effects such as cessation of feeding (Fast & Regniere 1984; Harris et al. 2006) and increased dispersal of those larvae that recovered from the toxic effects (Halcomb et al. 2000; Gore et al. 2002; Harris et al. 2006).

In 2011, 72% of the total area planted with maize in South Africa was GM maize of which 45.2% contained single Bt gene events, 40.4% contained the combination of herbicide and Bt genes and 14.4% was herbicide tolerant (Anonymous 2012). South African milling companies do not distinguish between Bt and non-Bt varieties of maize and therefore, availability of commercial non-Bt maize meal is limited. *Bacillus thuringiensis* is known to be toxic to codling moth and Bt-crystal formulations are registered for the control of codling moth in the field (Andermatt et al. 1988; Falcon & Huber 1991; Boncheva et al. 2006). It is therefore hypothesized that the use of local maize meal as an ingredient of an artificial diet for mass-rearing codling moth represents a risk in terms of production volumes needed for SIT programs.

From 2005 to 2007, production in the SIT facility suffered from high larval mortality and fluctuations in numbers of moths produced (D. Stenekamp, personal observation). The presence of a virus infection was ruled out but various Bt concentrations were found in maize meal batches, which was thought to explain the fluctuations in the number of insects produced at the rearing facility. Therefore, the hypothesis that Bt toxins in the maize meal used in the codling moth diet was causing larval mortality was tested.

The purpose of this study was to evaluate the effect of Bt maize and commercially available organically produced maize meal on production parameters, i.e., larval mortality, development and larval dispersal, during the rearing of codling moth and to quantify toxicity of various Bt concentrations in maize meal diets.

Material and Methods

CODLING MOTH CULTURE

The codling moth colony originated from larvae that were collected in commercial apple orchards in Elgin, Western Cape during 2003. The moths reared from these larvae were used to initiate a laboratory colony in the SIT facility in Stellenbosch, Western Cape, South Africa where codling moth has been cultured on the diet described by Guennelon et al. (1981). Eggs were oviposited on wax paper and held at 25 ± 1 °C, 70 ± 10% RH and a photoperiod of 16:8 h L:D until the blackhead stage was reached. The egg sheets were sterilized in a 2% sodium hypochloride solution to reduce the risk of fungal contamination. These sterilized egg sheets were air-dried using a ventilated plastic box and kept in a closed plastic container until egg hatch. Wild codling moth larvae from apple orchards were introduced into the colony every second year to minimize inbreeding.

MAIZE MEAL IN THE DIET

Maize meal from Bt maize (Monsanto, Gauteng, South Africa; event Mon810; cultivars 'DKC 8012B' and 'DKC 7815B' used together), expressing Cry1Ab protein was used. This Bt maize meal was a mixture of 2 cultivars, and meal with the non-GM isoline was unavailable. Therefore, Bt free, organic maize meal (Bio-organic certified BDOCA 009GPhPrHa, Wensleydale Farms, Gauteng, South Africa) was used as the (non-Bt) control and in mixtures with the Bt maize meal to obtain a range of concentrations of Bt maize meal.

DIET PREPARATION AND GENERAL METHODS

The diet was mixed in a stainless steel pot heated on a gas ring. Water (40 L) was boiled, agar-agar (800 g) was added and the mixture boiled again before cooling to 65 °C. The following ingredients were then added: ascorbic acid 266 g; benzoic acid 122.6 g; methylparaben 96 g; 4% formaldehyde 70 mL; wheat germ 1.89 kg; brewer's yeast 2.02 kg. To 1.5 L of the diet, 282 g of the following mixtures of organic maize meal and the Bt maize meal were added: (i) 0% Bt maize meal, 100% organic maize meal, (ii) 20% Bt maize meal, 80% organic maize meal, (iii) 40% Bt maize meal, 60% organic maize meal, (iv) 60% Bt maize meal, 40% organic maize meal, (v) 80% Bt maize meal, 20% organic maize meal, (vi) 100% Bt maize meal, 0% organic maize meal. The diet was dispensed into sterile 25 mL plastic cups. Once the diet had cooled and solidified, it was scarified before putting one first instar larva on the diet per container using a small (size 00) paint brush. These cups were covered with wax paper to maintain relative humidity and minimize contamination. The wax paper also reduced movement of the larvae, and hence, the probability of escape. For each concentration of Bt maize meal including the control, 50 cups with larvae were used.

QUANTIFICATION OF CRY1AB PROTEIN IN MAIZE MEAL

The concentrations of Cry1Ab protein in the maize meal was determined using a commercially available ELISA test kit (Agdia Inc., USA) and assayed according to the manufacturers' protocol. The positive control, which had a concentration of 40 µg/mL of Bt toxin, was suitably diluted to generate a standard curve. Maize meal samples were prepared by adding 1 mL sample buffer to 1 g of maize meal and allowing the Bt toxin to dissolve. Aliquots of 100 µL were used in the Bt ELISA test. The concentration of Bt toxin in the maize meal was calculated using the absorbance values obtained by reading the unknown values of the standard curve.

The Hoerl function (Daniel & Wood 1980):

$$y = a(x)^{b} \cdot e^{[c(x)]}$$

was used to describe the relationship between the percent Bt maize meal (x) and the amount of Bt in µg of Bt/g maize meal (y). The regression constants are a, b and c and e is the base of the natural logarithm.

INSECT BIOASSAY AND RESPONSES TO BT

Adult emergence started around day 30 and lasted for up to 2 weeks (day 44) at 27 °C, a long-day photoperiod 16:8 h L:D and 75% RH (Bloem et al. 1997). In the SIT rearing facility, larval trays were moved from the larval rearing room to the adult emergence room on day 28 when adult emergence was about to start and were kept until day 44 when most of the adults had emerged. Peak emergence occurred around day 35 (Bloem et al. 1997; Bloem et al. 2000).

Experiments were repeated twice on different dates using the 5 Bt maize meal concentrations, and the control. Because of the time interval between the 2 dates, different codling moth cohorts were used and seasonal differences in temperature and humidity were apparent: in experiment 1 larvae were kept in a rearing room at 27.0 ± 2 °C, 58 ± 10% RH and in experiment 2 at 28.8 ± 2 °C and 69 ± 10% RH. The photoperiod was 18:6 h L:D. Temperature and humidity were monitored continuously at 10 min intervals using a HOBO® H8 data logger. Observations were made until day 44 when adult emergence was completed (Bloem et al. 1997).

Previous observations showed that the presence of Bt maize meal in the larval diet increased larval mortality, and/or delayed larval development and/or larvae migrating out of the diet prematurely. Therefore, on day 44 the following responses were recorded for each of codling moth larvae: (i) larval mortality, (ii) larvae not reaching adulthood by

day 44 (delayed development), (iii) larvae leaving the diet, or (iv) total of the above 3 responses (total response). The concentrations causing 50% (LC_{50}) and 95% response (LC_{95}) and the 95% fiducial limits were determined by probit analysis using PoloPC (LeOra software 1987). To estimate the fiducial limits the heterogeneity factor was taken into account. This is given by

$$g = \frac{t^2}{b^2 S_{xx}},$$

where t is the Student's t-distribution value for the appropriate confidence level (95% in this case), b is the slope of the probit regression line and S_{xx} is the weighted corrected sum of squares of the independent variable (concentration of Bt in this case) (Finney 1971). For $g > 1$ the fiducial limits run from $-\infty$ to $+\infty$ (Finney 1971) and cannot be estimated. Probit lines from the 2 experiments were tested for being co-incidental. If they were not co-incidental, they were tested for parallelism (homogeneity of slopes). If they were co-incidental, the data were combined (Finney 1971).

Results

QUANTIFICATION OF CRY1AB PROTEIN

The relationship, described by the Hoerl function, between percentage Bt maize meal and the amount of Bt in µg/g fit the data extremely well ($F_{3,6} = 397.37$; $P < 0.001$; $R^2 = 0.995$). The fitted equation, $y = 0.4012\ (x)^{0.7338} \cdot e^{[-0.0276(x)]}$, was used to estimate the amount (in µg) of Bt per g of maize meal for the 5 concentrations: 20% Bt maize meal = 0.8789 µg/g, 40% Bt maize meal = 1.7578 µg/g, 60% Bt maize meal = 2.6367 µg/g, 80% Bt maize meal = 3.5156 µg/g, 100% Bt maize meal = 4.3944 µg/g.

INSECT BIOASSAY AND RESPONSES TO BT

In the case of larval mortality and larvae leaving the diet (Fig. 1A & C, respectively), the lines for the 2 experiments were co-incidental ($\chi^2 = 1.131$, df = 2, $P = 0.568$ for mortality; $\chi^2 = 1.036$, df = 2, $P = 0.996$ for larvae leaving the diet). The lines for delayed larval development and total response (Fig. 1B & D, respectively) were not co-incidental ($\chi^2 = 46.754$, df = 2, $P < 0.001$ for delayed larval development; $\chi^2 = 14.957$, df = 2, $P < 0.001$ for total response) but were parallel ($\chi^2 = 2.037$, df = 1, $P = 0.154$ for delayed larval development and $\chi^2 = 0.080$, df = 1, $P = 0.778$ for total response). The data fit these probit models well except for the larvae leaving the diet that had a P-value for goodness of fit that was marginally non-significant at $P = 0.05$ (Table 1). The LC_{50} and LC_{95} values for delayed larval development and total response were lower in experiment 1 than in experiment 2 (Table 1). The fiducial limits for larval mortality and larvae leaving the diet could not be estimated (Table 1) because the values for g were greater than 1 (g for 95% fiducial limits = 2.341 and 2.384 for larval mortality and larvae leaving the diet, respectively).

All larvae in the control group developed to adults by day 44, while 58% and 24% (experiment 1 and 2 respectively) of the immature stages failed to develop to adults by day 44 in the 100% Bt maize group. Most of the codling moth individuals that failed to develop to adults by day 44 were still in the larval stage, except in the treatment containing 0.8789 µg/g Bt endotoxin where most were in the pupal stage (Table 2). The slopes of the probit regression lines for larval mortality and larvae leaving the diet were lower than those for delayed larval development and total response (Fig. 1).

Discussion

Our data clearly indicate that a Bt maize meal-based larval diet used in a codling moth production facility can adversely affect the number of moths produced. Of the 3 responses that can adversely affect colony production (larval mortality, delayed larval development and larvae leaving the diet), delayed larval development appeared to be the most important based on the slopes of the probit regression analysis. The slope for delayed larval development was higher (although not significant at $P = 0.05$) than the slopes for the other 2 responses (Fig. 1). In addition, the probit regression lines for delayed larval development more closely resembled the lines for total response than did those for larval mortality and larvae leaving the diet (Fig. 1). The lower slope of the regression line for larvae leaving the diet, suggests that an increase in the Bt concentration in the diet resulted in a limited increase in the response.

This report quantifies the effects of Bt maize meal in an artificial diet on codling moth and it shows that larvae were negatively affected by even small amounts of Bt in maize meal, particularly in terms of larval development. This negative effect of Bt maize meal on codling moth productivity confirms earlier results on the toxicity of different δ-endotoxins and Cry1Ab to codling moth (Andermatt et al. 1988; Pasquier et al. 1997; Rang et al. 2000; Boncheva et al. 2006). Rang et al. (2000) determined the toxicity of Cry1Ab against codling moth with a LC_{50} of 2.92 x 10^{-1} µg/µL and Boncheva et al. (2006) at 0.078 µg/g of diet, which is less than in the present study, despite the mortality being determined after only 4 d. They used solubilized protoxins of Cry1 δ-endotoxins in a mixture with an artificial diet which could account for the difference in observed toxicity.

Delayed larval development was more pronounced in experiment 1 than in experiment 2 (LC_{50} and LC_{95} values in Table 1). This difference was most likely due to temperature related differences in potency of the toxin. Blomefield (2003) determined that the degree day units (DDU) for larval development from first to fifth instar at 25 °C (at a lower threshold temperature of 10 °C) were 321.6 °D for males and 329 °D for females. This resulted in a development time of 20–21 d. At the average temperature of 27.0 °C (experiment 1) the DDU were 357 ºD at day 21 and at 28.8 °C (experiment 2), the DDU were 378 ºD. Therefore, the larvae were expected to develop faster in experiment 2 than in experiment 1. By day 44, many of the larvae feeding on the Bt maize still had not reached the fifth instar stage (Table 2) in both experiments, although the DDU reached 748 ºD. This was more than double the expected time of 321.6 ºD for codling moth larvae (Blomefield 2003) to reach the fifth instar. Although there was a difference in temperature between the 2 experiments, the codling moth larvae in the control (0% Bt maize meal) still had a higher percentage emergence and faster development compared to the Bt maize meal-fed larvae in both experiments. The delayed larval development most likely was induced by ingestion of a sub lethal dose of Bt endotoxin which resulted in anorexia (Retnakaran et al. 1983; Harris et al. 2006). Dandekar et al. (1998) also reported a decreased rate of larval development in codling moth feeding on transgenic somatic walnuts with high levels of CryIA(c) gene expression, but low levels of CryIA(c) protein.

Larval dispersal can be associated with acute toxicity (Harris et al. 2006), and in our experiments, codling moth larvae were observed leaving the diet and it was assumed that they would die. Harris et al. (2006) found that light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) larvae stopped feeding after ingesting food contaminated with Bt and they showed an increased tendency to disperse after the larva recovered from the toxic effects. If the dose of the toxin was not lethal, the midgut was repaired in time to resume larval development (Harris et al. 2006).

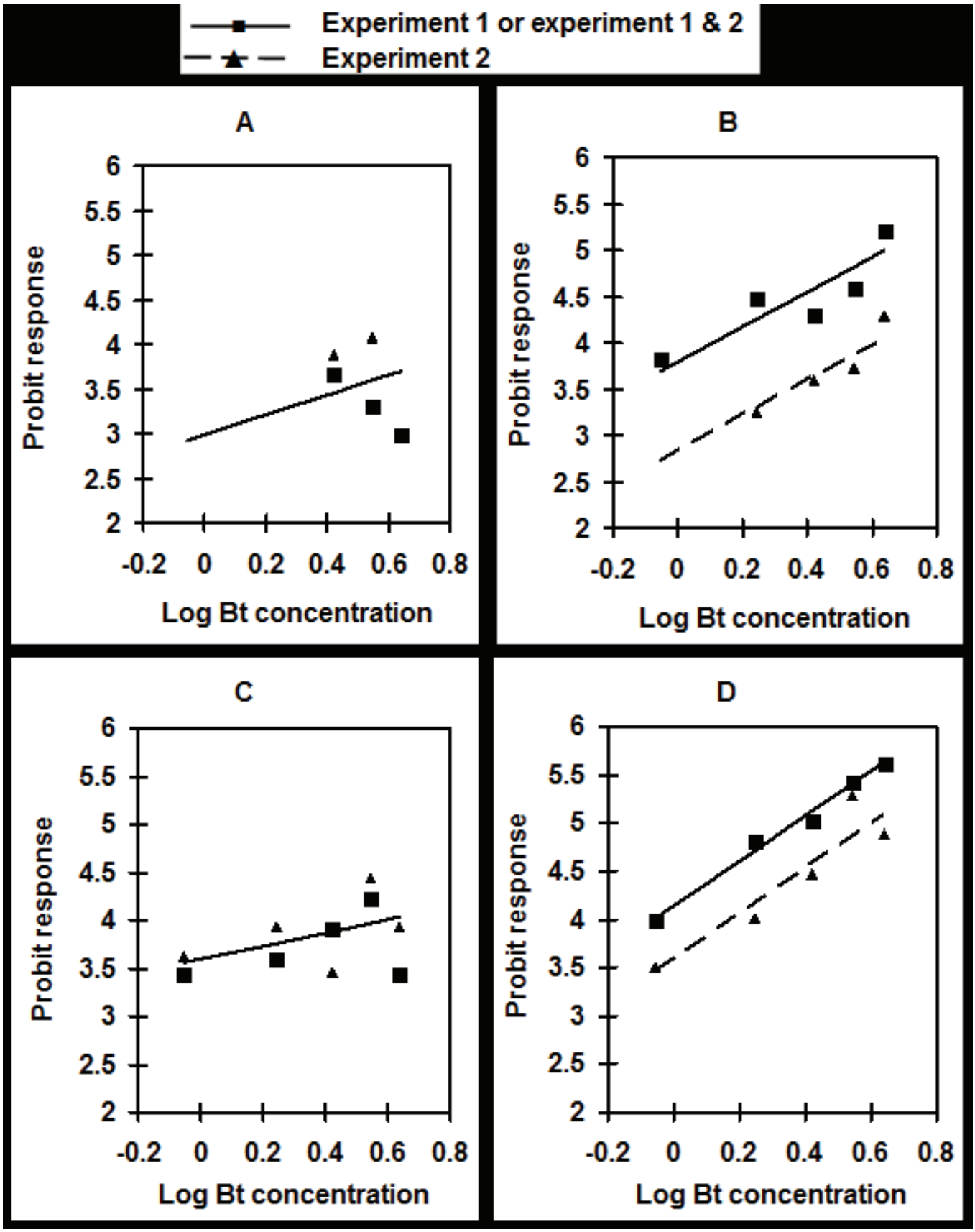

Fig. 1. Probit response on log Bt concentration of *Cydia pomonella* larvae reared on a diet containing maize where the responses were: (A) larval mortality Y = 2.980 + 1.27(X); (B) delayed larval development, Y = 3.789 + 1.886(X) for experiment 1 and Y = 2.840 + 1.886(X) for experiment 2; (C) larvae leaving the diet, Y = 3.595 + 0.681(X); (D) total response or response A + B + C, Y = 4.133 + 2.344(X) for experiment 1 and Y = 3.592 + 2.344(X) for experiment 2. Y is the probit response and X is the log Bt concentration.

Table 1. Chi-square (χ^2) goodness of fit values with the degrees of freedom (df) and probability levels (*P*) for the co-incidental and parallel probit regression lines of 4 responses on log Bt concentration, with the LC_{50} and LC_{95} values and their 95% fiducial limits (FL).

Response	Experiment	χ^2 (df)	*P* value	LC_{50} (95% FL)	LC_{95} (95% FL)
Mortality	1 & 2	12.600 (8)	0.126	36.745 (no estimates)	581.917 (no estimates)
Delayed development	1	9.540 (7)	0.216	4.383 (3.391 to 6.858)	32.643 (15.598 to 167.780
	2			13.805 (8.333 to 39.098)	102.815 (37.034 to 989.963)
Larvae leaving diet	1 & 2	15.450 (8)	0.051	115.771 (no estimates)	30166.00 (no estimates)
Total	1	6.465 (7)	0.487	2.349 (1.991 to 2.733)	11.803 (8.449 to 20.153)
	2			3.989 (3.352 to 4.943)	20.079 (13.303 to 38.964)

Table 2. The number of *Cydia pomonella* larvae and pupae recorded on day 44 after exposure to different amounts of *Bacillus thuringiensis* (Bt) endotoxin in the 2 experiments combined giving a total of 100 *C. pomonella* in each treatment.

mg/g Bt toxin	Larvae (all < 5th instar)	Pupae
0	0	0
0.8789	1	5
1.7578	11	6
2.6367	9	7
3.5156	13	9
4.3944	28	13

These results demonstrate that Bt maize meal has a deleterious effect on codling moth development even at low concentrations. It has a direct toxic effect causing larval mortality as well as an indirect effect resulting in delayed development. Both effects are of major concern in mass-rearing facilities where lepidopteran insects are reared. In our experiments, ideally the non-Bt isoline of maize should have been used as the control diet. However, the Bt maize was a mixture of 2 Bt cultivars and meal of the non-Bt isoline was unavailable. The nutritional properties of the organically grown maize meal may have differed from those of the Bt- maize meal. However, our data indicate that the risk of using South African milled maize meal in an artificial diet for rearing codling moth is high. The alternative is to find a suitable replacement for commercial maize meal, which can include organic maize meal, whole wheat flour (Brinton et al. 1969) or soybean flour (Howell 1972; Hansen & Anderson 2006). However these ingredients are more expensive. Extensive testing, evaluation and GM status of these products are needed before being accepted as a suitable replacement. Optimization of insectary-rearing of codling moths may be enhanced through modification of diet ingredients, and thus the latter have important implications for pest management.

Acknowledgments

We would like to thank Christina Gonzalves for technical assistance, Monsanto for providing the Bt maize meal and Professor Dirk Bellstedt, Department of Biochemistry, Stellenbosch University for performing the ELISA tests. Thanks go to the University of Stellenbosch and Hortgro Science for providing funds to support the research. This work was part of the FAO/IAEA Coordinated Research Project on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control.

References Cited

Anonymous 2012. Biotech Facts and Trends. http://www.isaaa.org/resources/publications/biotech_country_facts_and_trends/download/Facts%20and%20Trends%20-%20South%20Africa.pdf [accessed on 24 January 2012].

Addison MF. 2005. Suppression of codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) populations in South African apple and pear orchards using sterile insect release. Acta Horticulturae 671: 555-557.

Andermatt M, Mani E, Wildboltz T, Lüthy P. 1988. Susceptibility of *Cydia pomonella* to *Bacillus thuringiensis* under laboratory and field conditions. Entomologia Experimentalis et Applicata 49: 291-295.

Barnes MM. 1991. Codling moth occurrence, host race formation and damage, pp. 313-328 *In* Helle W [ed.], Torticid Pests: Their Biology, Natural Enemies and Control. World crop pests, Vol. 5, Elsevier, Amsterdam, The Netherlands.

Bloem S, Bloem KA, Calkins CO. 2000. Incorporation of diapause into codling moth mass rearing: Production advantages and insect quality issues, pp. 329-335 *In* Tan KH [ed.] Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May-5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.

Bloem S, Bloem KA, Fielding LS. 1997. Mass-rearing and storing codling moth larvae in diapause: a novel approach to increase production for sterile insect release. Journal of the Entomological Society of British Columbia 94: 75-81.

Blomefield TL. 2003. Bionomics, behaviour and control of the codling moth in pome fruit orchards in South Africa. PhD (Agric) dissertation, Stellenbosch University, Stellenbosch, South Africa.

Boncheva R, Dukiandjiev S, Minkov I, De Maagd RA, Naimov S. 2006. Activity of *Bacillus thuringiensis* δ-endotoxins against codling moth (*Cydia pomonella* L.) larvae. Journal of Invertebrate Pathology 92: 96-99.

Brinton FE, Proverbs MD, Carty BE. 1969. Artificial diet for mass production of the codling moth, *Carpocapsa pomonella* (Lepidoptera: Olethreutidae). Canadian Entomologist 101: 577-584.

Dandekar AM, McGranahan GH, Vail PV, Uratsu SL, Leslie CA, Tebbets JS. 1998. High levels of expression of full-length cryIA(c) gene from *Bacillus thuringiensis* in transgenic somatic walnut embryos. Plant Science 131: 181-193.

Daniel E, Wood FS. 1980. Fitting Equations to Data. John Wiley and Sons, New York, NY.

De Maagd RA, Bakker P, Staykov N, Dukiandjiev S, Stiekema W, Bosch D. 1999. Identification of *Bacillus thuringiensis* delta-endotoxin Cry1C domain III amino acid residues involved in insect specificity. Applied and Environmental Microbiology 65: 4369-4374.

Falcon LA, Huber J. 1991. Biological control of the codling moth, pp. 355-369 *In* Helle W [ed.], Tortricid Pests: Their Biology, Natural Enemies and Control. World Crop Pests, Vol. 5. Elsevier, Amsterdam, The Netherlands.

Fast PG, Regniere J. 1984. Effect of exposure time to *Bacillus thuringiensis* on mortality and recovery of the spruce budworm (Lepidoptera: Tortricidae). Canadian Entomologist 116: 123-130.

Finney DJ. 1971. Probit Analysis. Cambridge University Press, London, UK.

Gore J, Leonard BR, Church GE, Cook DR. 2002. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. Journal of Economic Entomology 95: 763-769.

Guennelon G, Audemard H, Fremond JC, Idrissi Ammari AM. 1981. Progrès réalisés dans l'élevage du carpocapse (*Laspeyresia pomonella* L.) sur milieu artificiel. Agronomy 1: 59-64.

Halcomb JL, Benedict JH, Cook B, Ring DR, Correa JC. 2000. Feeding behavior of bollworm and tobacco budworm (Lepidoptera: Noctuidae) larvae in mixed stands of nontransgenic and transgenic cotton expressing an insecticidal protein. Journal of Economic Entomology 93: 1300-1307.

Hansen JD, Anderson PA. 2006. Mass rearing codling moths: improvements and modifications. Journal of the Entomological Society of British Columbia 103: 33-36.

Harris MO, Marwick N, Sandanayake M. 2006. Is resistance to *Bacillus thuringiensis* endotoxin Cry1Ac associated with a change in the behavior of light

brown apple moth larvae (Lepidoptera: Tortricidae)? Journal of Economic Entomology 99: 508-518.
Howell JL. 1972. Rearing codling moth on a soya, wheat germ, starch medium. Journal of Economic Entomology 65: 636-637.
Konecka E, Kaznowski A, Ziemnicka J, Ziemnicki K. 2007. Molecular and phenotypic characterization of *Bacillus thuringiensis* isolated during epizootics in *Cydia pomonella* L. Journal of Invertebrate Pathology 94: 56-63.
Kruger M, Van Rensburg JBJ, Van den Berg J. 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Entomology 136: 38-50.
LeORA Software 1987. Polo-PC. A Users' Guide to Probit or Logit Analysis. Berkeley, CA, USA.
Pasquier D, Charmillot PJ, Scalco A, Renard D. 1997. Biological control of codling moth *Cydia pomonella* with *Bacillus thuringiensis* (BT): From laboratory to orchard. Revue Suisse de Viticulture Arboriculture Horticulture 29: 233-238.
Rang C, Lacey LA, Frutos R. 2000. The crystal proteins of *Bacillus thuringiensis* subsp. *thompsi* display a synergistic activity against the codling moth, *Cydia pomonella*. Current Microbiology 40: 200-204.
Retnakaran A, Lauzon H, Fast P. 1983. *Bacillus thuringiensis* induced anorexia in the spruce budworm, *Choristoneura fumiferana*. Entomologia Experimentalis et Applicata 34: 233-239.
Simmons G, Carpenter J, Suckling M, Addison M, Dyck A, Vreysen MJB. 2010. Improved quality management to enhance the efficacy of the sterile insect technique for lepidopteran pests. Journal of Applied Entomology 134: 261-273.
Soleno J, Anguiano L, De D'Angelo AP, Cichon L, Fernandez D, Montagna C. 2008. Toxicological and biochemical response to azinphos-methyl in *Cydia pomonella* L. (Lepidoptera: Tortricidae) among orchards from the Argentinian Patagonia. Pest Management Science 64: 964-970.
Sørensen JG, Addison MF, Terblanche JS. 2012. Mass-rearing of insects for pest management: challenges, synergies and advances from evolutionary physiology. Crop Protection 38: 87-94.
Van Rensburg JBJ. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. South African Journal of Plant and Soil 24: 147-151.