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Effect of MON 810 *Bt* transgenic maize diet on stored-product moths (Lepidoptera: Pyralidae)

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Abstract

The transgenic hybrid MON 810-YieldGard® was developed to protect maize against herbivorous Lepidoptera larvae in the field. Although the hybrid kernels contained 20 times less of the Cry1Ab toxin than leaves, they had been shown to be toxic to some stored product pests, indicating a protective effect of Cry1Ab during maize storage. However, the characterization of the resistance level and benefits of the Cry1Ab kernels during their storage are still incomplete. In this study, we compared the suppressive effect of the diets obtained from the MON 810-YieldGard® hybrid to four species of stored product moths: *Ephestia kuehniella*, *Ephestia elutella*, *Cadra cautella* and *Plodia interpunctella*. The diets originated from kernels that were obtained at two different experimental fields containing the same concentration of Cry1Ab ($0.35 \pm 0.056\,\mu g\, g^{-1}$), and caused 100% mortality in *E. ellutella*, *C. cautella* and *P. interpunctella*, and 65% mortality in *E. kuehniella*. The comparison of LD_{50} (time when 50% individuals died) and larval relative growth rate (RGR) among the tested species revealed that *P. interpunctella* was the most sensitive species followed by *E. elutella*, *C. cautella* and *E. kuehniella*. The lowest toxic concentration of Cry1Ab in the diet of *E. kuehniella* larvae was determined by mixing the diets from hybrid kernels containing Cry1Ab with diets from control kernels without Cry1Ab. The mortality of *E. kuehniella* larvae decreased with decreasing Cry1Ab concentration, and the LD_{50} (concentration when 50% individuals died) was $0.20\,\mu g$ Cry1A g^{-1} of diet. Similarly, the larval RGR decreased with decreasing logarithmically transformed concentrations of Cry 1Ab in the diet. These results show that MON 810-YieldGard® hybrid kernels are protected during their storage against feeding by stored product moths.

Keywords: Maize MON 810; *Ephestia*; *Plodia*; *Cadra*; Storage; Cry1Ab; *Bacillus thuringiensis*

1. Introduction

Stored-product moths are very common pests of stored food and food-processing facilities. Moth larvae cause damage to stored food and products by feeding or by contamination with spinning silk. The bodies of adult insects are an unwanted addition to the food-processing line (Sedlacek et al., 1995; Mohandass et al., 2007). Moreover, the stored-product moth species *Ephestia kuehniella* is of medical importance due to its allergen production (Makinen-Kiljunen et al., 2001; Armentia et al., 2004). *E. kuehniella* feeds on flour and is, together with *Plodia interpunctella* and *Cadra cautella*, also a pest of other cereal products including corn grain (Sedlacek et al., 1995). All of these moths are also found in cosmopolitan areas. *Ephestia elutella* is a pest of stored tobacco (Ryan, 1995), but also infests cereals, especially in warmer regions (Sedlacek et al., 1995).

In storage facilities, one can find grain either in stored grain mass, or in grain residues and debris. The grain residues are in the store corners or machinery as a result of improper cleaning and sweeping. This "unwanted form of grain" is usually formed by cracked grain and grain dust (Hubert et al., 2006). It is well documented that grain residues harbor many insect species (Reed et al., 2003). In addition, the insect population in grain residues in empty stores might increase substantially between consequent harvests (Kucerova et al., 2003). Thus, grain residues are

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an ideal insect shelter and reproduction site and, thus, there is an effort to control and eliminate pests in these residues before infestation of harvested grain.

Stored-product moths are mostly controlled chemically, especially due to the desirable cost/efficiency ratio of chemical control (Zettler and Arthur, 2000). However, the increasing resistance to some chemical insecticides, as well as the presence of insecticidal residues after chemical treatment, advocate for alternatives to chemical pesticides, including biological control and transgenic plants (Arthur, 1996; Kramer et al., 2000; Flinn et al., 2006). Stored-product moths are susceptible to *Bacillus thuringiensis* (*Bt*) (Johnson and McGaughey, 1984; Calogero et al., 1989): commercial formulations of *Bt* endotoxins (e.g. Dipel, a mixture of Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa) are usually suppressive to these moths, although tolerance or resistance has been reported for some strains (Salama et al., 1991; Johnson and McGaughey, 1996; Johnson et al., 1998; Oppert et al., 1997). Mixtures of Cry1Aa, Cry1Ac and Cry2Aa effectively suppressed the larvae of *E. kuehniella* (Tounsi et al., 2005).

The *Bt* endotoxins have been incorporated into transgenic plants to increase their resistance against herbivorous insects (Shelton et al., 2002). Monsanto has developed the transgenic YieldGard® maize event MON 810, which produces the *Bt* endotoxin Cry1Ab. The maize is protected against the feeding of herbivorous insects, such as *Ostrinia nubilalis*, *Diatraea grandiosella* and *Sesamia cretica* (as reviewed by Shelton et al., 2002; Glaser and Matten, 2003). The highest concentration of the Cry1Ab toxin is found in the leaves, while its concentration in the kernels is up to 20 times lower. However, the MON 810 kernels are toxic to *P. interpunctella* and *Sitotroga cerealella* despite the lower concentration of the toxin (Giles et al., 2000; Sedlacek et al., 2001; Hanley et al., 2004). The study of Sedlacek et al. (2001) indicates that the MON 810 kernels could be beneficial in the reduction of stored-product losses due to infestation with pest moths. They recommended more research to fully characterize the tolerance or resistance of the stored-product insect pests against *Bt* toxins. Due to the toxicity of MON 810 kernels, one can expect that the residues formed by these kernels will not be the sources of moth infestation in granary stores.

Before its registration, the MON 810-YieldGard® hybrid maize was experimentally tested in the Czech Republic. The aim of our study was to characterize the resistance of transgenic kernels MON 810 to *E. kuehniella*, *E. elutella*, *C. cautella* and *P. interpunctella.* The toxic effect of the kernels to moths is described in terms of their mortality, larval developmental period and larval weight increase. In addition, we determine the lowest toxic concentration of Cry1Ab to *E. kuehniella.*

2. Materials and methods

2.1. Insects and rearing conditions

The following species of stored-product pest moths (Lepidoptera: Pyralidae) were tested in the experiments: Mill moth *E. kuehniella* Zeller, 1879, Cacao moth *E. elutella* Hubner, 1796, Almond moth *C. cautella* Walker, 1863, previously known as *E. cautella*, and Indian meal moth *P. interpunctella* Hubner, 1810. The laboratory culture of *E. kuehniella* originated from Boršov near České Budějovice (South Bohemia) and was collected in September 2001. The *P. interpunctella* strain originated from an infestation of storage facilities inside the Research Institute of Crop Production, Prague (Central Bohemia), and was collected in August 2000. The strains of *C. cautella* and *E. elutella* originated from laboratory strains reared at the Institute for Stored Product Protection, Federal Biological Research Centre for Biology, Agriculture and Forestry, Berlin, Germany.

The species were reared on the following standard diets: P. *interpunctella* and *E. elutella*—wheat groats, wheat germ, glycerol and Pangamin (Rapeto a.s., Prague, Czech Republic) at the ratio of 10:10:1:0.5 (wt/wt/wt/wt); *E. kuehniella*—wheat germ, oat flakes, glycerol and Pangamin at the ratio of 10:2:1:0.5 (wt/wt/wt/wt); C. *cautella* wheat groats, wheat bran, glycerol and Pangamin at the ratio 10:10:1:0.5 (wt/wt/wt/wt/). The moths were reared in glass jars (1 l volume) covered by a textile mesh. Eggs of the moth were collected by confining approximately 50 adults in 500 ml plastic vials (Aliachem a.s., Pardubice, Czech Republic) with their bottoms covered with 0.6 mm mesh, which were placed in larger collecting vials, into which the eggs fell through the mesh. Eggs were transferred at 24-h intervals to the rearing jars. The larvae were reared at $27\pm1\,^{\circ}C$ and 60% relative humidity in continuous darkness.

2.2. Maize hybrids and isolines

Hybrid MON 810-YieldGard® and the non-transgenic isoline (control) of maize (*Zea mays* L.) were each cultivated in two experimental fields in the Czech Republic: (i) at the Research Institute of Crop Production, Prague, Central Bohemia, sown on 28 April 2003 and harvested on 30 September 2003; and (ii) in Ivanovice na Hané-South Moravia, sown on the 29 April 2003 and harvested on the 25 September 2003. After the harvest, the maize kernels (F_2 generation) were dried for 8 h at 50 °C and stored in a refrigerator (5 °C) in plastic bags until the start of the experiments, as described by Sedlacek et al. (2001). The kernels were cracked for 30 s by using a blender, because the tested moth larvae fed mainly on cracked kernels (Sedlacek et al., 1995, 2001).

In a preliminary observation (unpublished), we did not find any significant changes in the mortality and length of the development time of the tested moth species between their standard rearing diets and the powdered maize isoline kernels. To find the lowest functional concentration of Cry1Ab with insecticidal activity to *E. kuehniella*, the diets from hybrid kernels were diluted by isoline kernel diets (both originating from the Research Institute of Crop Production field) to the following concentrations of