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# Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas

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A three year (2003–2005) field study compared the susceptibility to the Cry1Ab toxin, expressed in Bt maize, of Mediterranean corn borer (MCB) *Sesamia nonagrioides* populations collected from areas with different adoption rates of Bt maize in Spain with Bt-free areas in Greece. Spain is the only European country where the cultivar Compa CB derived from the event Bt176 was commercially grown, from 1998 to 2005. The large decrease of the titer of the toxin in this cultivar at later growth stages represented the worst-case scenario for resistance development of MCB, since larvae of the second and third generations were exposed to sublethal concentrations of Cry1Ab toxin. Our data revealed that the variation in susceptibility to Cry1Ab for the MCB Spanish field populations analyzed in the three years was very low, with $LC_{50}$ values fluctuating between 12 and 30 ng Cry1Ab/cm$^2$, regardless of the region of origin, the type of maize (Bt or non-Bt) and the year. Furthermore, no significant differences were found when comparisons were made with a laboratory population ($LC_{50}$ values: 18–26 ng Cry1Ab/cm$^2$) or with field populations from Greece (Bt-free areas), which displayed $LC_{50}$ values ranging between 22 and 27 ng Cry1Ab/cm$^2$. Standardizing bioassay protocols proved to be essential for obtaining comparable results. These findings suggest that resistant MCB populations did not evolve in those Spanish maize areas where Compa CB was largely cultivated for eight years, contradicting the expected rapid development of resistance under these unfavourable conditions. Additionally, our results can be used as baseline indices in post-market resistance monitoring programs if Bt maize is introduced in Greece. Further studies should continue, since the insights gained from a resistance monitoring program may help to enhance the durability of Bt maize.

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## 1. Introduction

The Mediterranean corn borer (MCB) *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) causes large yield losses in the Mediterranean area, particularly in Spain and Greece (Ortego et al., 1998; Zanakis et al., 2009). Genetically modified maize expressing Cry1Ab δ-endotoxin from the soil bacterium *Bacillus thuringiensis* is effectively protected from the damage caused by this corn borer (González-Núñez et al., 2000). Cultivation of transgenic crops is broadly extended in some American countries, lead by the USA with 64 million hectares in 2009. However, the adoption of transgenic crops has been limited in European countries, with less than 0.1 million hectares cultivated in the same year (GMO Compass, http://www.gmo-compass.org). Hybrids based on the event Bt176 (Syngenta) were planted for the first time in Spain in 1998. Compa CB was the only commercial Bt176 derived variety grown between 1998 and 2002, being gradually substituted between 2003 and 2005 by hybrids based on the event MON810 (Monsanto). Planting of Bt176 hybrids was disallowed in 2005, and since 2006 only MON810 hybrids can be commercially grown (Ortego et al., 2009). At present, more than 120 MON810 derived varieties are registered for their cultivation in Spain. In 2009, Spain maintained the largest growing area of Bt maize in the EU (76,057 ha, representing 22% of the total maize area). Bt maize hybrids are cultivated to a lesser extent in the Czech Republic, Portugal, Romania, Poland and Slovakia (GMO Compass, http://www.gmo-compass.org), and they

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have never been commercially planted in other European countries such as Greece.

Deployment of insect-resistant transgenic maize has raised concerns about their potential to select Bt resistance in field populations of corn borers, as they apply intense selection pressure, which could result in widespread Bt resistance. As such, the development of resistance in target pests to Bt plants is considered the main risk for long-term success of this technology. So far, field-evolved resistance to Bt maize has been documented in two species, the African stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (Van Rensburg, 2007; Kruger et al., 2009) and the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Matten et al., 2008; Storer et al., 2010). In addition, laboratory selection assays have shown that laboratory populations of *Ostrinia nubilalis* (Lepidoptera: Crambidae) can develop low to moderate levels of resistance under intense selection pressure from Bt toxins (Bolin et al., 1999; Chaufaux et al., 2001; Farinós et al., 2004; Alves et al., 2006; Crespo et al., 2009).

One of the key factors for successful resistance management is the timely implementation of monitoring programmes to detect early changes of susceptibility in field populations, jointly with the implementation of resistance management strategies to prevent or delay the emergence of resistant populations. Different strategies have been proposed to delay insect resistance to Bt crops, but the "high dose/refuge strategy" (HDR) is the most widely used, and it is mandatory in most countries where Bt crops are grown. This strategy combines the use of Bt crops that express high concentrations of Cry toxins and the planting of refuges of non-Bt crops near Bt crops, with the aim to reduce heritability of resistance (Tabashnik et al., 2004; Bravo and Soberón, 2008; Carrière et al., 2010). However, hybrids derived from the event Bt176 do not appear to satisfy the requirements recommended for resistance management purposes, since the titer of the Bt toxin decreased as the crop reached maturation (Fearing et al., 1997) allowing exposure to sublethal doses of the toxin and representing a favourable scenario for the development of insect resistance (Gould, 1998; Onstad and Gould, 1998).

A widely used method to detect the development of field resistance is to check on shifts in the susceptibility of the target pests to Cry1Ab through time in relation to the original susceptibility of the population (McGaughey et al., 1998; González-Núñez et al., 2000; Farinós et al., 2004). It is well established that the first step of the monitoring for the detection of resistance development is the establishment of the baseline of susceptibility of target pest's populations to the Bt proteins expressed in the crop. Thereafter, resistance monitoring ought to be performed on a regular basis in those areas where Bt maize is cultivated more extensively, so that if a resistant population evolves, it could be detected quickly to implement appropriate management strategies. Another complementary strategy for assessing resistance is by showing that a population with a history of relatively high exposure to an insecticide is less susceptible than conspecific populations that have had less exposure (Tabashnik, 1994).

A post-market resistance monitoring program for Bt maize hybrids derived from the event Bt176 (cv. Compa CB) was started in Spain in 1998, when Bt maize was first deployed. This allowed establishment of the baselines of susceptibility to Cry1Ab toxin of *S. nonagrioides* and *O. nubilalis* populations from the most representative maize growing regions (González-Núñez et al., 2000). Moreover, an annual resistance monitoring plan was carried out from 1999 to 2002, which found no consistent shifts in susceptibility to the toxin in Spanish populations of both species of corn borers after 5 years of cultivation (Farinós et al., 2004). During the period 2003–2005, Compa CB was gradually substituted by commercial varieties derived from the event MON810 and the adoption rate of Bt maize increased from 7 to 13% of the total maize growing area, although regional rates of adoption were quite variable. In those areas, such as the Northeast corner of Spain (Ebro basin: Aragon and Cataluña), where there was a significant yield advantage of Bt maize over conventional maize (Gómez-Barbero et al., 2008), Bt hybrids were rapidly adopted, reaching 36.5% of the total maize area in 2005, whereas they were planted to a lesser extent in other Spanish regions. The cultivation of Compa CB for eight years could be considered as a favourable scenario for Spanish MCB populations to develop field resistance to Cry1Ab toxin, since *S. nonagrioides* larvae were exposed to sublethal concentrations of Bt toxins, which may have affected both the borer's performance and the development of Bt resistance (Eizaguirre et al., 2005). By contrast, Greek MCB populations represent a valuable control for comparison, since they have never been exposed to Bt toxin and there is a limited genetic exchange between Spanish and Greek *S. nonagrioides* populations (De la Poza et al., 2008).

The aim of this study was to compare the susceptibility to the Cry1Ab toxin, expressed in Bt maize, of field populations of *S. nonagrioides* collected from areas with different adoption rates of Bt maize and Bt-free areas. Specifically, we have: i) investigated the differences in susceptibility of populations collected in three regions in Spain with different adoption rates of Bt maize, and three regions in Greece where Bt maize has never been cultivated; and ii) compared the susceptibility of conspecific populations collected in Bt and non-Bt maize fields from the same areas in Spain. To obtain comparable results, a standardized methodology was used during the three year field study reported here. In addition, the $LC_{50}$ and $LC_{90}$ values obtained from Greek populations will serve as the baseline of susceptibility for post-market monitoring plans if Bt maize is ever introduced in Greece.

## 2. Materials and methods

### 2.1. Insect collection

MCB larvae from Spain were collected in commercial Bt maize fields (cv. Compa CB, Event 176) or non-Bt maize fields of three growing regions, Ebro, Centre and Southwest from 2003 to 2005 (Fig. 1). MCB larvae from Greece were collected in 2004 from conventional maize fields of three Bt-free regions where this crop is regularly cultivated: Thessaloniki, Serres and Larissa (Fig. 1). For each population, between 300 and 500 last instar larvae were collected by dissecting maize stalks before harvesting. Only one larva per plant was taken to minimize the possibility that sibs were collected.

Field-collected larvae were dipped in a solution of 1% chlorine bleach to prevent pathogen contamination, and placed in plastic

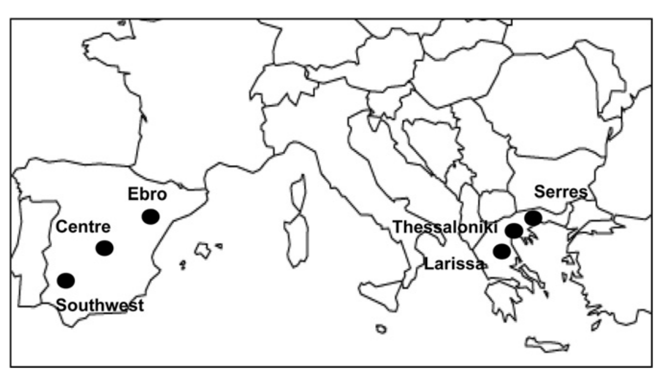


**Fig. 1.** Geographical locations in Greece and Spain where *Sesamia nonagrioides* field populations were sampled.

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