Susceptibility to the Cry1F Toxin of Field Populations of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in Mediterranean Maize Cultivation Regions

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J. Econ. Entomol. 105(1): 214–221 (2012); DOI: http://dx.doi.org/10.1603/EC11213

ABSTRACT Maize hybrids expressing the Cry1F toxin provide efficient control of lepidopteran pests. The Mediterranean corn borer, *Sesamia nonagrioides* (Lefèvre), is one of the most damaging pests of maize in the Mediterranean basin. In this work we firstly determined the efficacy of maize hybrids expressing the Cry1F toxin (event TC1507) to control neonates of *S. nonagrioides.* Leaf tissue feeding bioassays revealed that TC1507 maize is highly effective against this pest, and the percentage mortality obtained was comparable to that obtained with a Cry1Ab-expressing maize hybrid (Compa CB, event 176), which is known to be highly efficacious against *S. nonagrioides.* Secondly, interpopulation variation in the susceptibility to the Cry1F insecticidal protein was established for nine field-collected populations of *S. nonagrioides* (three Spanish, two French, two Italian, one Greek, and one Turkish). Estimates of the susceptibility of larvae to the Cry1F toxin showed low variability in lethal concentrations and growth inhibition concentrations among field populations. Moreover, no significant differences were found when they were grouped by geographical areas [Western Mediterranean (Spain and France) versus Eastern Mediterranean (Italy, Greece and Turkey)] or by history of exposure to Bt plants (Spanish vs. other populations). Therefore, the minor differences found in field populations can be attributed to natural variation in sensitivity to Cry1F. The importance of establishing baselines of susceptibility for resistance detection is discussed. Future changes in susceptibility of *S. nonagrioides* populations to Cry1F could be documented based on this baseline data.

KEY WORDS transgenic maize, resistance, baseline susceptibility, corn borer, *Bacillus thuringiensis*

Cultivation of crop plants genetically modified started in 1996 in the United States and since then, global cultivated area has been constantly growing, reaching 148 million of hectares in 2010. Among these crops, insect resistant varieties engineered to express toxins derived from the bacterium *Bacillus thuringiensis* (Bt) that are toxic to insects pests occupied 58.6 million hectares (James 2010). Globally, maize genetically transformed to express the Cry1Ab toxin has been the dominant Bt maize planted for the control of corn borers since its deployment in 1996 in the United States and it has been the only Bt plant commercially cultivated in Europe since 1998. However, other Cry toxins have also proved to be effective against lepidopteran pests. One of them is Cry1F, with a mode of action similar to other Cry1 proteins (Bravo et al. 2007). Maize hybrids expressing the Cry1F toxin (event TC1507) have been commercially available in the United States since 2003 providing good efficacy against lepidopteran pests, such as *Ostrinia nubilalis* (Hübner), *Spodoptera frugiperda* (J.E. Smith), *Diatraea grandiosella* Dyar, *Striacosta albicosta* (Smith), *Agrotis ipsilon* (Hufnagel), and *Helicoverpa zea* (Boddie) (Buntin 2008, Siebert 2008, Higgins et al. 2009), and it is in pending for regulatory cultivation approval in the European Union (EU). Recently, this toxin has been expressed in a number of transformed maize varieties either as a single or stacked trait together with other insecticidal Cry toxins, to increase trait durability (Tabashnik et al. 2009, Sanahuja et al. 2011).

One of the most damaging pests of maize in Spain and the Mediterranean basin is the Mediterranean corn borer *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) (Tsitsipis 1989, Ortego et al. 1998). This species completes a variable number of generations per year depending on latitude, ranging from two in southern France to up to four in Morocco (Anglade 1972). The first generation is particularly devastating, because larvae tunnel throughout the maize stem from the first instar, causing great damage to maize seedlings and making their control particularly difficult. Bt maize expressing the Cry1Ab toxin is effectively protected from

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0022-0493/12/0214–0221$04.00/0

the damage caused by this pest (González-Núñez et al. 2000). Laboratory bioassays revealed that Cry1F is also toxic to *S. nonagrioides* (González-Cabrera et al. 2006), but the effectiveness of Cry1F-expressing maize to control *S. nonagrioides* is unknown.

A major concern of the widespread cultivation of insect-resistant Bt maize hybrids is the potential selection for Bt resistance in target pests, as field populations are exposed to a constantly high selection pressure. The long-term success of growing Bt plants is thought to depend on the implementation of effective resistance management programs (Gould 1998). In this context, the establishment of baseline susceptibility of target pest populations to the Bt insecticidal proteins provides a benchmark against which future changes in susceptibility can be measured when monitoring for the development of resistance (Siegfried et al. 2007, Sivasupramaniam et al. 2007). Baseline data for a particular insecticidal protein should be recorded before widespread release of plants that produce that protein, from appropriate agro-ecological areas across the geographical range of the target species. Afterwards, 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 timely implement appropriate resistance management strategies. Baseline susceptibility studies in Europe have been performed with Cry1Ab for *O. nubilalis* and *S. nonagrioides* (Marçon et al. 1999, González-Núñez et al. 2000, Farinós et al. 2004, 2011, Saeglitz et al. 2006) and with Cry1F for *O. nubilalis* (Gaspers et al. 2011), but data on baseline susceptibility of *S. nonagrioides* to Cry1F are not available.

The aim of this study was firstly to determine the efficacy of Cry1F-expressing Bt maize to control neonates of *S. nonagrioides.* Secondly, we assessed the interpopulation variation in the susceptibility of *S. nonagrioides* neonates to the Cry1F toxin, by comparing the susceptibility of field populations of *S. nonagrioides* collected in nine locations from five Mediterranean countries (three of Spain, two of France and Italy, and one of Greece and Turkey), where maize cultivation is intense and *S. nonagrioides* frequently causes yield loss. To obtain comparable results, a standardized methodology based on diet overlay bioassays with purified toxin was used during the 2-yr study (2004–2005). Regression lines obtained from lethal concentration (LC) values will serve as baselines of susceptibility for *S. nonagrioides* in postmarket resistance monitoring plans if Cry1F-expressing maize hybrids are ever introduced in these areas.

Materials and Methods

Plants. All maize plants were grown in growth chambers (Conviron S10H, Controlled Environments, Winnipeg, Canada) at 26 ± 1°C, 80 ± 10% RH and 16:8 h (L:D) photoperiod until eight-leaf stage. An illumination of 675 μmol/m^2/s at plant level (using fluorescent and incandescent lamps) was used. Three maize genotypes were tested: transgenic maize expressing the Cry1F toxin (event TC1507); its near isogenic line 2722 (Mycogen); and a commercially grown Bt maize cultivar expressing the Cry1Ab toxin (Compa CB, event 176, Syngenta), which has provided effective control of *S. nonagrioides* (Farinós et al. 2004), as a positive control. TC1507 was developed jointly by Dow AgroSciences and Pioneer Hi-Bred International.

Insects. *Insect Culture.* A laboratory population of *S. nonagrioides* established in Spain (Centro de Investigaciones Biológicas, Madrid) was maintained as described in González-Núñez et al. (2000). Larvae were fed on a meridic diet, modified from Poitout and Bùes (1970) by the addition of 1.6 g Wesson's salt mixture and 1 g methyl p-hydroxybenzoate per liter of diet. Groups of 50 larvae were placed in plastic boxes of 21 × 16 × 4 cm and vermiculite was added when they reached last instar to facilitate pupation. A minimum of 100 pairs (batches of six pairs) of *S. nonagrioides* adults were confined in ventilated plastic cylinders (12 cm diameter × 30 cm high) containing 5–7 maize seedlings for oviposition. Egg masses were removed from the maize leaf sheaths and placed in plastic containers (9.5 cm diameter × 3.5 cm high), provided with moistened filter paper until hatch. Neonates (larvae <24 h after hatching) were used in the bioassays. All the life stages were kept in growth chambers (Sanyo MLR-350 H, Sanyo, Osaka, Japan) at 25 ± 0.3°C, 70 ± 5% RH and a photoperiod of 16:8 h (L:D).

Field Collection. A total of nine field populations of *S. nonagrioides* were collected from geographically distinct regions in 2004 [Albacete and Ebro (Spain), Toulouse (France)] and 2005 [Badajoz (Spain), Landes (France), Sardinia and Metaponto (Italy), Nigrita-Serres (Greece) and Adana (Turkey)] (Fig. 1). Collections were performed by randomly collecting a sample of 300–500 last instars at 2–5 non-Bt maize fields of each region (Table 1). Only one larva per plant was taken to minimize the possibility that sibs were collected. In the laboratory the larvae were dipped in a solution of 1% chlorine bleach to minimize pathogen contamination. Most of the larvae collected were in diapause. They were maintained in batches of 50 larvae in plastic boxes (21 × 16 × 4 cm) containing vermiculite and meridic diet at diapause conditions of 12 ± 1°C, 70 ± 5% RH and 12:12 h (L:D) photoperiod. When required, diapause was disrupted by placing larvae at 28 ± 1°C, 70 ± 5% RH and continuous light. Adult mating, oviposition, egg collection, and larva incubation were performed as explained above at the same environmental conditions. Neonates of the F1 generation were used for all bioassays.

Bt Toxin. The Bt toxin used in overlay diet bioassays was provided by Mycogen (Dow AgroSciences, Indianapolis, IN). The insecticidal toxin consisted of proteolytically truncated Cry1F (35% active ingredient), which was heterologously produced in culture of *Pseudomonas fluorescens* using the Cry1F gene isolated from *Bacillus thuringiensis* variety *aizawai.* The toxin was provided as a lyophilized powder and subsequently suspended in a 0.1% solution of Triton X-100 buffer in distilled water, and three different stock

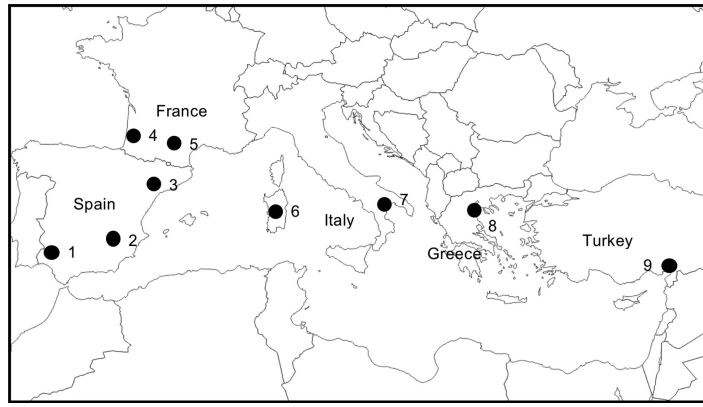

Fig. 1. Sampling sites where field populations of *Sesamia nonagrioides* were collected: Badajoz (1), Albacete (2), Ebro (3), Landes (4), Toulouse (5), Sardinia (6), Metaponto (7), Nigrita-Serres (8), and Adana (9).

suspensions (one per replicate) were prepared with it. Then serial dilutions in 0.1% Triton X-100 were prepared from the stock suspension to produce between 0 (control) and 100% mortality.

Feeding Bioassays. *Leaf Disks.* Leaf disks of ≈1 cm^2 were cut with a cork borer from eight-leaf stage plants of the three different maize genotypes tested: Compa CB (Cry1Ab), TC1507 (Cry1F), and its near isogenic line 2722. A minimum of 100 *S. nonagrioides* neonates of the laboratory population per treatment were placed singly with a fine brush in cylindrical plastic cages (2.5 cm diameter × 1.5 cm high) with moistened filter paper and leaf disks, which were replaced every 2 d. The assay was performed in a growth chamber (Sanyo MLR-350H, Sanyo, Osaka, Japan) and the conditions were photoperiod 16:8 h (L:D) and temperature 26 ± 1°C (day) and 20 ± 1°C (night). Daily inspections were made to check survivorship and development.

Plant Seedlings. Maize plants [Compa CB (Cry1Ab), TC1507 (Cry1F), and its near isogenic line 2722] were infested with neonates from the laboratory population at the eight-leaf stage. Two *S. nonagrioides* neonates per plant were confined with a polythene bag (7 cm diameter × 22 cm length), and sealed at the base of the plant with a tape band. A total of 50 plants per genotype were used; plants were held in a growth chamber (Conviron S10H) at similar environmental conditions as above and arranged in a completely randomized block design. Larvae were allowed to feed on each plant genotype for 5 d. Then, the polythene bags were removed and the plants were dissected to identify, by visual inspection, both the number of surviving larvae per plant and their developmental stage. A minimum of 100 neonates were tested in each of the three treatments.

Diet Bioassay and Purified Toxin. All field-collected populations of *S. nonagrioides* were tested in the first generation after the winter diapause period. A laboratory culture of this corn borer was used as control. The diet overlay bioassays were carried out in accordance with the method described by Farinós et al. (2004). Eight different concentrations of Bt toxin that ranged between 0 (control) and 384 ng Cry1F/cm^2, established from preliminary trials, were tested, and 50 μl of the suspension were applied on the surface of 1 ml of solidified diet dispensed in each well of the plastic trays (Bio-Ba-128, Color-Dec Italy, Capezzano

Table 1. Source description and no. of field-collected larvae of *S. nonagrioides* to establish the baseline susceptibility to Cry1F

Population (country)	Number of larvae collected	Locations of collection	Number of fields	Collection date
Ebro (Spain)	315	Huerto	3	Oct. 2004
Albacete (Spain)	305	Albacete	2	Oct. 2004
		Casasimarro	1	Oct. 2004
Badajoz (Spain)	342	Guadalperales	1	Sept. 2005
		Zurbarán	2	Sept. 2005
Toulouse (France)	400	Toulouse	1	Nov. 2004
		Cazères/Rieumes/Mauzac	3	Dec. 2004
		Muret	1	Dec. 2004
Landes (France)	424	Pomarez	1	Nov. 2005
		Peyrehorade	1	Nov. 2005
Sardinia (Italy)	378	Olmedo	2	Oct. 2005
		Marrubiu	1	Oct. 2005
Metaponto (Italy)	423	Pisticci	2	Oct. 2005
		Bernalda	1	Oct. 2005
Nigrita-Serres (Greece)	438	Nigrita/Serres	2	Sept. 2005
Adana (Turkey)	223	Adana	3	Oct. 2005

In all cases larvae were collected from non-Bt maize fields at the last larval stages.

Pianore, Italy). The control consisted of diet surface treated with 0.1% Triton X-100 solution. The trays were let dry in a laminar flow hood. One neonate (<24 h old) was placed in each well and confined with a cover (Bio-Cv-16, Color-Dec Italy, Capezzano Pianore, Italy). Trays were maintained in growth chambers at 25 ± 1°C, 70 ± 5% RH, and constant dark. Three replicates were performed for each field population and for the laboratory control colony. Within each replicate, 32 single neonates were tested. Bioassays were scored after 7 d. The determination of death was a larva not developing to the second instar during the seven-day assay. Growth inhibition (GI) was also estimated in populations collected in 2005 by weighting individually all live larvae in a Mettler-Toledo AX205 analytical balance (Mettler-Toledo International Inc., Madrid, Spain).

Data Analysis. The survival of larvae in feeding bioassays with leaf disks was evaluated by a Kaplan–Meier survival analysis, and the distributions were compared by the Mantel log-rank test. In the plant seedlings feeding bioassays, a χ^2 test was used to determine the significance of the differences in mortality.

The results obtained for mortality at different concentrations of Cry1F in the diet bioassays were adjusted by probit weighted regression lines. Only replicates with mortality in the control below 20% were included in the analyses. The lethal concentrations for 50% (LC_{50}) and 90% (LC_{90}) of each population were estimated together with their 95% fiducial limits using the POLO-PC program (LeOra Software 1987).

Field populations were grouped according to two criteria: 1) by geographical areas, Western Mediterranean (WM) (Spanish and French populations) versus Eastern Mediterranean (EM) (Italian, Greek and Turkish populations), because we found in a previous population genetic study that *S. nonagrioides* populations from these countries were clustered in these two groups (de la Poza et al. 2008); and 2) by history of exposure to Bt plants (Spanish versus the other populations). Mortality values of each area at different concentrations were pooled and analyzed together by probit analysis as described above, to obtain LC_{50} and LC_{90} values and their 95% fiducial limits. The significance of population susceptibility was assessed by the 95% fiducial limits of lethal concentration ratios (LCR) at the LC_{50}, being significantly different ($P < 0.05$) if the LCR 95% fiducial interval did not include one (Robertson et al. 2007).

GI was calculated by transforming individual larval weights to percentage of growth by the equation $GI_x = 100\text{-}(W_x{*}100)/W_o$, taking into account the mean larval weight (W) at every concentration (x) in relation to a control (o). Dead larvae were recorded as 100% inhibition of larval growth. The percentages of GI at the different concentrations tested were adjusted by probit weighted regression lines. The GI_{80} expressed the concentration at which weights of treated larvae had decreased 80% relative to the weights of control larvae.

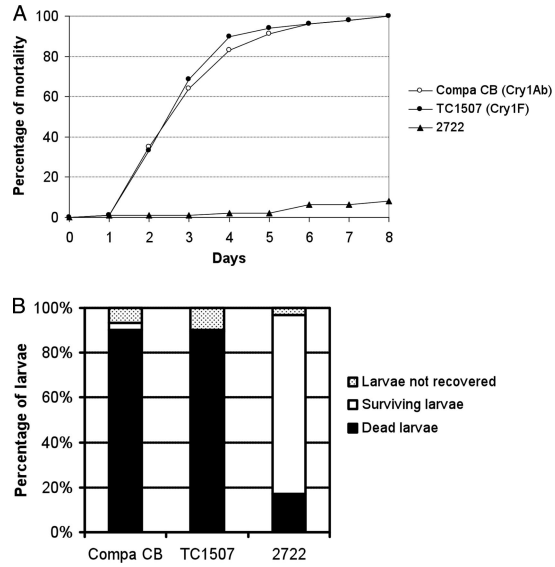

Fig. 2. Mortality of *Sesamia nonagrioides* neonates fed on the three different maize plants: Compa CB (expressing Cry1Ab), TC1507 (expressing Cry1F) and its near isogenic line 2722. (A) Larvae fed on leaf disks. (B) Larvae fed on maize seedlings after 5 days of exposure. A minimum of 100 larvae per treatment was used in both assays.

Results

S. nonagrioides neonates readily accepted detached leaf disks, but significant differences in survivorship among the three treatments were observed (Log-Rank test: $\chi^2 = 171.2$, $P < 0.00$). Larval mortality was observed from the second day after feeding on the events Compa CB (expressing Cry1Ab) and TC1507 (expressing Cry1F) (35 and 33%, respectively; Fig. 2A). Mortality was over 90% after 5 d on Compa CB (91%) and TC1507 (94%), relative to those reared on the control 2722 (2%) and reached 100% after 8 d of feeding on leaves of both transgenic events. In contrast, only 8% of larvae died in the near isogenic line 2722 at day 8 and all surviving larvae had molted to second or third instar.

In the plant seedling feeding bioassay, significant differences in mortality were also found for neonates fed on the three different corn plants tested ($\chi^2 = 201.3$, $P < 0.00$). Five days after infestation 83% of larvae were found alive in the isogenic line 2,722, and all of them had molted to second or third instar. In contrast, only 3 and 1% of larvae survived in Compa CB and TC1507 maize, respectively (Fig. 2B). These surviving first instars, recovered from transgenic plants, were maintained on leaf disks of the corresponding genotype, but they all died before day 7. In all the treatments a small percentage of larvae (ranging from 3 to 10%) were not recovered at the end of the experiment, likely because of death inside the plant in an early stage of the experiment or to escaping.

The susceptibility to Cry1F toxin of Mediterranean field populations of *S. nonagrioides* based on lethal concentrations in diet bioassays is listed in Table 2.

Table 2. Susceptibility of field populations of *S. nonagrioides* from Mediterranean region to Cry1F toxin based on lethal concentrations

Population (country[a])	*n*	Slope ± SE	LC_{50}[b] (95% FL)	LC_{90}[b] (95% FL)	χ^2	df
Ebro (SP)	320	2.40 ± 0.39	11.5 (2.4–19.0)	39.3 (24.3–126.2)	24.2	8
Albacete (SP)	448	1.76 ± 0.20	29.6 (18.8–44.0)	157.7 (91.8–468.9)	31.2	12
Badajoz (SP)	569	2.12 ± 0.20	10.0 (4.5–15.6)	40.3 (25.2–103.7)	94.9	16
Toulouse (FR)	384	2.00 ± 0.22	18.8 (11.2–27.0)	81.9 (54.6–159.5)	20.3	10
Landes (FR)	382	1.39 ± 0.17	12.8 (2.6–25.5)	106.7 (48.5–1376.1)	55.4	10
Sardinia (IT)	574	1.72 ± 0.17	12.4 (9.1–15.9)	68.9 (53.2–96.4)	14.0	16
Metaponto (IT)	384	2.24 ± 0.26	10.9 (3.4–18.5)	40.7 (23.0–302.5)	65.7	10
Nigrita-Serres (GR)	383	2.17 ± 0.28	11.2 (3.0–18.9)	43.6 (24.6–359)	54.4	10
Adana (TU)	515	2.07 ± 0.17	27.4 (21.2–34.4)	114.2 (85.1–172.3)	25.1	16

[a] Countries are: SP, Spain; FR, France; GR, Greece; IT, Italy; TU, Turkey.
[b] Lethal concentrations to 50% (LC_{50}) and 90% (LC_{90}) of the pop and their 95% fiducial limits (FL 95%) are expressed in ng Cry1F/cm^2 diet.

The LC_{50} values ranged from 10.0 ng Cry1F/cm^2 (Badajoz, Spain) to 29.6 ng Cry1F/cm^2 (Albacete, Spain) and the LC_{90}s from 39.3 ng Cry1F/cm^2 (Ebro, Spain) to 157.7 ng Cry1F/cm^2 (Albacete, Spain). The lowest LC values were comparable to that of the laboratory population, with LC_{50} of 9.6 ng Cry1F/cm^2 and LC_{90} of 23.8 ng Cry1F/cm^2 (slope: 3.26 ± 0.47; $\chi^2 = 23.7$; df = 13). The difference between the most tolerant and the most susceptible field populations to Cry1F was three-fold at LC_{50} level and four-fold at LC_{90} level.

When the populations were grouped by geographical area (Western Mediterranean, WM and Eastern Mediterranean, EM), the LC_{50} values of WM (15.2 ng Cry1F/cm^2) and EM (14.2 ng Cry1F/cm^2) were very similar, not revealing significant differences when they were compared by lethal concentration ratios (Table 3). Likewise, no significant differences were obtained when the populations were grouped by history of previous exposure to Bt toxins: Spain (LC_{50} = 14.8 ng Cry1F/cm^2) versus rest of the populations (LC_{50} = 14.6 ng Cry1F/cm^2).

The Cry1F toxin inhibited larval growth at concentrations lower than those that caused mortality. The percentage of growth inhibition produced by Cry1F ranged between 56% (Adana, Turkey) and 68% (Sardinia, Italy) at the lowest concentration tested in these populations (6 ng/cm^2) (data not shown). Values of GI_{80} calculated for the six field populations collected in 2005 ranged between 6.9 (Metaponto, Italy) and 17.4 ng/cm^2 (Adana, Turkey) (Fig. 3).

Discussion

The genetically modified maize TC1507 expressing the insecticidal protein Cry1F has proved to be very effective to control larval stages of a wide spectrum of lepidopteran pests that affect maize production (Buntin 2008, Higgins et al. 2009). However, no data were previously available on the efficacy of Cry1F-expressing maize to control *S. nonagrioides*, a major pest that negatively affects the quality and the yield of maize crops in the Mediterranean region. The feeding bioassays performed revealed that TC1507 maize is highly effective against *S. nonagrioides* neonates, and its level of protection is comparable to that obtained with Compa CB (event 176, Cry1Ab toxin), a hybrid commercially cultivated from 1998 to 2005 that provided a successful control of *S. nonagrioides* in Spanish maize growing areas (González-Núñez et al. 2000, Farinós et al. 2004). It is noteworthy that *S. nonagrioides* larvae tunnel throughout the maize stem from the first instar, feeding on leaves for a short time. In our studies, similar results were obtained for both, TC1507 leaf disks and seedlings feeding bioassays. Hence, leaf disks bioassays appear to be a good option when assessing the efficacy of a new Bt-resistant plant to control this corn borer. Additionally, our results indicate that, although the susceptibility of *S. nonagrioides* to Cry1Ab toxin was ≈10-fold higher than that to Cry1F toxin (González-Cabrera et al. 2006), this species presented the same level of mortality in maize hybrids expressing either Cry1Ab or Cry1F toxins.

Table 3. Susceptibility to Cry1F of field populations of *S. nonagrioides* grouped according to two different criteria

Criterium adopted	Countries	*n* (controls)	Slope ± SE	LC_{50}[a] (95% FL)	LCR (LC_{50})[b] (95% FL)	LC_{90}[a] (95% FL)	LCR (LC_{90})[b] (95% FL)	χ^2	df
(A) Geographical area	Turkey, Greece, Italy	1856 (373)	1.8 ± 0.1	14.2 (10.5–18.0)	1	74.7 (54.9–116.0)	1	254	58
	Spain, France	2103 (352)	1.9 ± 0.1	15.2 (11.5–19.1)	1.07 (0.91–1.26)	73.9 (56.1–107.0)	0.99 (0.79–1.23)	347	64
(B) History of exposure to Bt maize	Spain	1337 (224)	2.0 ± 0.1	14.8 (9.9–20.1)	1	64.6 (45.3–110.3)	1	276	40
	Rest of countries[c]	2622 (501)	1.7 ± 0.1	14.6 (11.5–17.9)	0.98 (0.84–1.17)	80.7 (62.4–113.7)	1.25 (1.00–1.55)	352	82

(A) The geographical area (Eastern vs. Western Mediterranean regions); and (B) the history of exposure of field populations to Bt maize (Spain vs. the other countries).
[a] Lethal concentrations to 50% (LC_{50}) and 90% (LC_{90}) of the pop and their 95% fiducial limits (FL 95%) are expressed in ng Cry1F/cm^2 diet.
[b] Lethal concn ratio (LCR) at LC_{50} and LC_{90}. LCRs significantly different ($P < 0.05$) if the 95% confidence interval does not include 1.
[c] France, Italy, Greece, and Turkey.

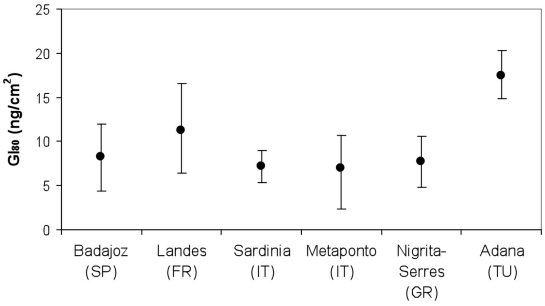

Fig. 3. Growth inhibition of six field populations of *Sesamia nonagrioides* collected in 2005 upon neonate exposure to Cry1F. GI_{80} is the concentration causing 80% growth inhibition in terms of larval weight relative to the control. Countries are Spain (SP), France (FR), Greece (GR), Italy (IT) and Turkey (TU). Bars indicate 95% fiducial limits.

These findings suggest that Cry1F-expressing maize hybrids might be a good biotechnological option for the control of the noctuid *S. nonagrioides.* Yet, it should be integrated in pest management strategies, because insects have demonstrated the capacity to develop resistance to Bt toxins, as it has been recently reported for the Cry1F insecticidal protein for field populations of the noctuid fall armyworm, *S. frugiperda* (Storer et al. 2010). As a resistance management practice it is important to establish the target pest's baseline susceptibility to the toxin expressed in the plant, which could be used for monitoring changes that might occur with the deployment of Bt maize expressing Cry1F toxin. Susceptibility is typically measured in laboratory bioassays testing the progeny of field-sampled insects for responses to the insecticidal protein. Laboratory bioassays using diet overlay bioassays, the one used in this study, require lower amount of toxin than diet-incorporated toxin, enabling long-term monitoring programs to be conducted at reasonable costs, because protein purification is very expensive and quantities are limited (Blanco et al. 2008). Susceptibility estimates to the Cry1F toxin for the nine populations *S. nonagrioides* showed very low variability, being three-fold the difference between the LC_{50} of the most tolerant and the most susceptible field population. These results are in accordance with other studies previously reported. Gaspers et al. (2011) informed of natural interpopulation variations in susceptibility of *O. nubilalis* to Cry1F across American and European populations and among the populations within each continent, and differences between the most tolerant and the most susceptible field populations were three-fold and five-fold in European and U.S. populations, respectively. Similarly, variation in susceptibility to Cry1F in field populations of *H. virescens* from the southern and central United States was limited to a three-fold change in LC_{50} values (Blanco et al. 2008).

Two of the factors that may contribute to differences in the susceptibility to Bt toxins are genetic differentiation, because of geographic separation, and previous exposure to toxins that may confer cross-resistance. The genetic analysis of *S. nonagrioides* populations in Europe using RAPDs markers indicates that populations in Spain and southwest France were in closer contact to each other than with populations from Italy, Greece, and Turkey (de la Poza et al. 2008). However, no significant differences in the susceptibility to Cry1F were found when comparing the WM versus the EM populations. These results suggest that evolutionary divergences with respect to this character have not occurred, despite the limited genetic exchange between populations (de la Poza et al. 2008). None of the field populations of *S. nonagrioides* analyzed in this study have been previously exposed to the Cry1F toxin. However, the Spanish populations collected in 2004 and 2005 have been exposed to transgenic Bt maize expressing the Cry1Ab toxin since 1998, whereas no Bt maize was commercially cultivated in Italy, Greece, and Turkey during this period and only 200 hectares in France (James 2005). The susceptibility to Cry1F of Spanish populations was not significantly different from the rest of the countries considered in this study. Actually, the lethal concentrations of the population from Ebro (Spain) was in the lower range of the values obtained, though Bt maize in this area represented ≈70% of the transgenic maize cultivated in Spain in 2004–2005. This finding is consistent with the lack of resistant development to Cry1Ab in Spanish populations (Farinós et al. 2004, 2011). Moreover, binding site analysis performed with *S. nonagrioides* larvae revealed that Cry1F and Cry1Ab proteins have different high-affinity binding sites (González-Cabrera et al. 2006), making the development of cross-resistance unlikely.

Although most resistance monitoring has focused on survival of neonates, growth inhibition determined by larval body weight can also be a useful indicator of susceptibility. The ingestion of Cry1F by neonates strongly inhibited larval growth at all the doses tested in all the populations. The range of variation in Cry1F susceptibility indicated by GI_{80} in the six populations analyzed (2.5-fold) was very similar to that indicated by LC_{50} in the same populations (2.7-fold). Analogous results have been reported in susceptibility to Cry1Ab toxins of the corn pests *O. nubilalis* and *H. zea*, whose range of variation indicated by growth inhibition was similar to that indicated by mortality (Marçon et al. 1999, Siegfried et al. 2000). Nonetheless, other studies with *H. armigera* found that values of GI and LC are not always correlated, and variations in susceptibility determined by mortality can be much higher than those measured by larval weight (Wu et al. 1999, Brevault et al. 2009). Though less amount of insecticidal protein is required to measure GI than LC values, the work load (time and labor) to measure GI is considerably higher than the evaluation of LC values. Therefore, we would recommend the use of mortality as endpoint of dose–response bioassays to test effects of Bt toxins on corn borers.

In summary, we have obtained the first baseline susceptibility from field-collected populations of *S. nonagrioides* of representative maize agro-ecological areas in Spain, France, Italy, Greece, and Turkey to the

Cry1F toxin. The use of diet-overlaid bioassay, mortality, or growth inhibition as endpoints, and the same batch of Cry1F toxin provided reliable data on the interpopulation variation in the susceptibility of *S. nonagrioides* populations. Our findings suggest that the observed susceptibility differences reflect natural variation in Cry1F susceptibility among *S. nonagrioides* populations rather than variation caused by prior exposure to selection pressures or because of geographical isolation. Baseline susceptibility data generated for a number of geographic populations of *S. nonagrioides* can serve as a benchmark for monitoring resistance, if Cry1F-hybrids are approved for cultivation in the EU.

Acknowledgments

We are grateful to A. Alves (Pioneer Hi-Bred International) for many helpful comments on the manuscript, and to S. Arpaia (Italy) and B. Naibo (France) for their help to collect *S. nonagrioides* larvae. We also thank Pioneer Hi-Bred International and Dow AgroSciences for financial funding, for provision of Cry1F toxin and the transgenic maize TC1507 and its near isogenic line 2722, as well as for technical assistance for field sampling of Turkish and Greek populations of *S. nonagrioides*. Thanks also to Syngenta for providing Compa CB seeds.

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Received 1 July 2011; accepted 28 October 2011.