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Susceptibility of *Ostrinia nubilalis* to *Bacillus thuringiensis* var. *kurstaki*

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Abstract

Baseline susceptibility of European corn borer larvae, *Ostrinia nubilalis* (Hübner), to a commercial formulation of *Bacillus thuringiensis* var. *kurstaki* was determined for different strains in 7 geographic locations in the corn area of North Italy (Po Valley). For the bioassay 7 different doses, raised on a meredic diet, were used and the data was analysed with the Probit analysis. Differences in the susceptibility among the northern Italian populations of *O. nubilalis* were recorded, which can be attributed to natural variability. The ECB collected near Como and Torino was the most susceptible, respectively with LC50 of 0.23 and 0.57 mg/ml. The most tolerant ones were the larvae coming from the area of Vicenza (LC50 = 3.40 mg/ml). Baseline susceptibility was also determined for one population originally from Iowa but reared for more than 20 years in the laboratory and which had never experimented Bt. The LC50 calculated for this last one was compared to the Italian situation.

Key words: European corn borer, Bt, insecticide resistance, *Ostrinia nubilalis, Bacillus thuringiensis.*

Introduction

The European corn borer [ECB, *Ostrinia nubilalis* (Hübner)] ranks amongst the most important pests of *Zea mays* L. in Italy and in several other European countries, however, being extremely polyphagous it can attack many other plants, both agricultural and spontaneous (Maini *et al.,* 1998). Amongst the micro-organisms applied for the biological control of Lepidoptera, *Bacillus thuringiensis* (Bt) is actually one of the most used and its employment is progressively intensified in Italy (Burgio and Maini, 1991; Deseö and Rovesti, 1992; Molinari *et al.,* 1995). Commercial products based on Bt are used against ECB. In fact Burgio *et al.* (1993) claim that *B. thuringiensis* has a similar efficacy, sometimes even superior, to chemical insecticides in the control of *O. nubilalis* in corn fields. Analogous results were recorded in the case of ECB on green beans (Curto, 1996). However similar to other insecticides and despite its complex mechanism of action, *B. thuringiensis* does not escape from the possibility of adaptation and resistance of target insects. Cases of natural resistance are actually limited only to *Plutella xylostella* (L.). Moreover, an increase tolerance to Bt treatments was recorded in other Lepidoptera as *Plodia interpunctella* (Hübner), *Heliothis virescens* (F.), *Helicoverpa zea* (Boddie) (Shelton *et al*., 1993; Tabashnik, 1994; Perez *et al.,* 1995; Huang *et al.,* 1999). These results have led to the worry that also Bt transgenic crops, expressing a single δ-endotoxin, could have a strong selective pressure increasing the probability of development of resistance to Bt crop and to Bt commercial products too (Gould, 1998). Normally the bioassay used to test the Bt activity is based on reference standard microbial strain and *Ephestia kuehniella* (Zeller) or on *Trichoplusia ni* (Hübner) as test insects (Navon, 1993). However, in view of the different biological answer as well as intra and inter specific, it is necessary to test the susceptibility directly on target species and on different strains of it. In fact both the type of the intra specific variation within, as well as among, population of target pest have an important implication for its management (Kennedy, 1993). Knowing the baseline susceptibility of all different strains of the pest is really important to forecast the resistance development. This research intends to detect any difference in the susceptibility of different population of ECB from North Italy to *B. thuringensis* var. *kurstaki*.

Figure 1. Sampling localities with selected corn fields near: 1)Torino, 2)Novara, 3)Como, 4)Rovigo, 5)Vicenza, 6)Treviso, 7)Pordenone.

Material and methods

Collection and insect culture

The research was carried out between 1999 and 2001 on different strains of ECB collected from different geographical areas of North Italy (figure 1). One strain collected more than 20 years ago in Iowa (USA) and since that moment reared in the laboratory of Entomological Institute of Bologna University (by Prof. Maini) was also included. Three hundred larvae were randomly collected in the field at fifth instar, then brought to the laboratory for the overwintering period. Diapause was broken in climate chamber at 25 ± 1 °C and 18:6 h (L:D) and 75 ± 5 % RH. Pupae, after sexual determination were put in cylindrical cages; on their top a white paper was put for ovipositing the egg masses. Adults were fed with water and honey. Egg masses were transferred in a plastic box containing a meredic diet for feeding the neonates not used for the bioassay. To continue the insect culture, a corrugated cardboard was inserted in the box as a site of pupation. *O. nubilalis* was reared following the protocol described previously (Manachini, 2002; Lozzia and Manachini, 2003).

Bioassay

The bioassay was done by treating the neonate larvae with different doses of a commercial product based on *B. thuringiensis* var. *kurstaki.* Seven different doses, determined by preliminary observations to detect an approximate LC50 as suggested for the probit analysis, were used: dose 1 = 0.1 mg/ml; dose 2 = 1.0 mg/ml; dose 3 = 2.5 mg/ml; dose 4 = 5.0 mg/ml; dose 5 = 10.0 mg/ml; dose 6 = 25.0 mg/ml; dose 7 = 50.0 mg/ml; Control = 0 mg/ml. The appropriate dilutions were prepared with 0.05 % Triton X 100.

The meredic diet (120 ml) was put in a container and before solidification a plastic mesh was inserted forming 50 small squares of 1.35 cm^2 and high 15 mm. For each square 26 µl of solution of the different doses were directly put, using a micro-pipette (Eppendorf), on the surface of the diet. Once the solution was dry 1 neonate larva, from the first laboratory generation, was put into a single cell. Four replicates, with 50 larvae for each dose and the control were done. In total 14000 larvae were used for the bioassay. Because the neonates do not immediately have an endophytic behaviour, but initially eat pollen (Lozzia and Manachini, 2003), the bioassay was performed laying the Bt on a meredic diet rather than integrating the bio insecticide into the food.

The test containing the larvae was covered to avoid the escape of ECB and put in climate chamber at rearing condition (25°C, 75 ± 5 % RH) but in the dark. Mortality was checked five days later, using a small brush to detect any live stimuli.

Data analysis

The probit analysis (SPSS version 11.0) was used to calculate the LC50 and to describe the susceptibility curves with a confidence level of 95%. As the data of LC50 and percentage of mortality in relation to the doses were skewed, Kruskal-Wallis one way analysis (SPSS version 9.0) was calculated to detect differences in the susceptibility of the strains to *B. thuringiensis.*

The test t-student was applied to find out the differences in the susceptibility of the different populations analysed in couple when the variance was homogenous; otherwise the χ^2 test was applied.

Results and discussion

Baseline larval susceptibility to commercial Bt of 8 ECB strains was evaluated. Table 1 shows the results of the probit analysis and indicates which function better describes the biological answer to the increment of toxin concentration. It is possible to note that the relationship dose-mortality was well described by a linear function only in the case of the strains from Torino and Rovigo. While for all other strains the biological answer to toxin increment was drawn by exponential curves.

Table 1. Results of probit analysis on susceptibility to *B. thuringiensis* of European corn borer collect on different corn regions and results of Goodness-of-fit test.

Strain	Regression curves	χ^2	df	P
Torino	$y = 0.52x + 1.70$	11.54	1, 6	0.420
Novara	$y = x^{0.90} + 2.30$	19.05	1, 6	0.002
Como	$y = 0.46x^{1.68}$	41.97	1, 6	0.000
Rovigo	$y = 0.63x + 1.68$	10.60	1, 6	0.060
Vicenza	$y = 0.80x^{1.97}$	19.22	1, 6	0.002
Treviso	$y = 0.80x^{2.10}$	13.10	1, 6	0.022
Pordenone	$y = 0.80x^{2.10}$	12.48	1, 6	0.025
Iowa	$y = 0.47x^{1.28}$	34.50	1, 6	0.001

Based on LC50 the ECB collected near Como and Torino was the most susceptible, respectively with LC50 of 0.23 mg/ml and 0.57 mg/ml, while the more tolerant ones were the larvae coming from the area of Vicenza (LC50 = 3.40 mg/ml) (figure 2). On average the LC50 of Italian population could be considered as 1.96 mg/ml. Whereas, considering the range between the maximum and the minimum it is possible to note that all values have at least a portion overlap. Regarding the strain from Iowa the confident limits are wider (min = 0.01 mg/ml e max =19.28 mg/ml) but the LC50 being 1.85 mg/ml it is comparable to the one calculated for the Italian *O. nubilalis*. Considering them on a whole significant differences in susceptibility among the ECB strains were recorded (χ^2 = 20.99; df = 7, 56; P = 0.004). In fact, also comparing the susceptibility of the ECB strains in couples, we can notice that the susceptibility of *O. nubilalis* from Como to Bt was found to be significantly higher (about 14 fold) than the ones come from Vicenza. A higher level of susceptibility compared to Vicenza ECB was noticed also for the populations from Torino (5 fold), while the ratio of LC50 with the one of the other strains was always less than 2 (table 2).

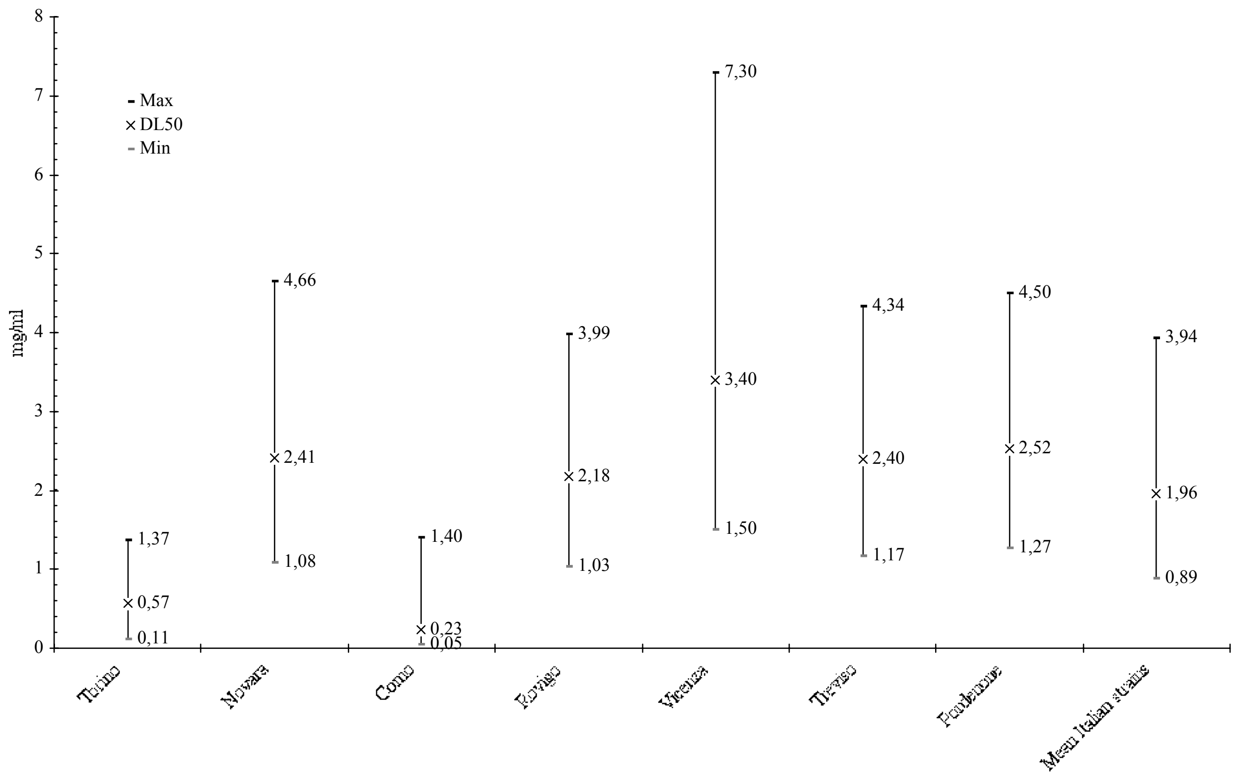

Figure 2. LC50, minimum and maximum of confident range, at the significant 5% level, for all Italian ECB strain and average of North Italian populations.

The results of the statistical analysis made in couples have enhanced significant differences in the susceptibility of ECB from Novara compared to the ones from Como (P = 0.03) and from Vicenza (P = 0.04). The last ones had different susceptibility also with the ECB from Torino (P = 0.01), Como (P = 0.01) and Pordenone (P = 0.03). Highly significant differences were recorded comparing the susceptibility of *O. nubilalis* from Vicenza and Torino (P = 0.004), and Como (P = 0.006). Other statistical differences in the mortality of the two populations are illustrated in table 2. Thus in general the susceptibility recorded for the ECB of Como and Torino is significantly lower compared to an hypothetical North Italian population of *O. nubilalis*.

The answer to the increment of Bt doses is similar also for the Lepidoptera that were reared for more than 20 years in the laboratory and that have never had a contact with Bt for all these years.

Table 2. Ratio of LC50 for all populations, for Iowa strain and for the estimated mean of Italian populations.

	Torino	Novara	Como	Rovigo	Vicenza	Treviso	Pordenone	Mean IT	Iowa
Torino	1.00	4.23	0.40	3.82	5.96*	4.21	4.42*	3.44*	3.25
Novara	0.24	1.00	0.10*	0.90	1.41*	1.00	1.05	0.81	0.77
Como	2.48	10.48*	1.00	9.48	14.78*	10.43*	10.96*	8.52*	8.04*
Rovigo	0.26	1.11	0.11	1.00	1.56	1.10	1.16	0.90	0.85
Vicenza	0.17*	0.71*	0.07*	0.64	1.00	0.71*	0.74*	0.58	0.54
Treviso	0.24	1.00	0.10*	0.91	1.42*	1.00	1.05	0.82	0.77
Pordenone	0.23*	0.96	0.09*	0.87	1.35*	0.95	1.00	0.78	0.73
Mean IT	0.29*	1.23	0.12*	1.11	1.74	1.23	1.29	1.00	0.94
Iowa	0.31	1.30	0.12*	1.18	1.84	1.30	1.36	1.06	1.00

Conclusions

Differences in the susceptibility to Bt in the North Italian populations were recorded. Regional differences in susceptibility to *B. thuringiensis* of other insects have been recorded also by other workers, but in most cases the differences were small (Wu *et al.,* 1999). Our results are comparable with the ones of Huang *et al.* (1997). The level of susceptibility recorded in this research may reflect the susceptibility of the field corn borer populations. Although significant differences in the susceptibility were recorded between some strains, they should not have an impact on the field efficacy of Bt as a commercial insecticide as is not regularly used in Italy against ECB in corn fields, due to economic reasons. In addition, considering the rather high amounts of Bt used in fields on other alternative hosts of ECB or on corn in other countries, the adaptive answer of insects has to be considered feeble both for the number of species and for the intensity of the phenomena. This seems to indicate that the use of bacteria as leaf insecticide determines a selective pressure but not so strong. However laboratory bioassays have shown that the continuous exposition to Bt products, more or less purified, can determine a quick increment of resistance higher than one observed in nature (Navon, 1993; McGaughey and Johnson, 1994; Muller-Cohn *et al*., 1994; Moar *et al.,* 1995). However, from the results, it is evident that, even though the differences in the susceptibility is not so high, a particular attention has to be paid to the ECB population from Vicenza, especially in the case of the use of Bt corn or intensive use of Bt traditional insecticides. Forecasting the resistance development is important for two reasons: preserving the biotechnology and preserving the use of *B. thuringiensis* in biological and IPM agricultural crops. For this reason it is important to verify the susceptibility of *O. nubilalis* to Bt toxins in the commercial products before they are used in genetically modified organisms, especially in the ones that should express more than one toxin. This research provides important baseline information on the susceptibility of *O. nubilalis* to *B. thuringiensis,* which is critical to the development of an effective monitoring program and implementation of Bt resistance management.

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