EFFECTIVENESS OF Bt NEWLEAF® POTATO TO CONTROL LEPTINOTARSA DECEMLINEATA (Say) (COLEOPTERA: CHRYSOMELIDAE) IN BULGARIA

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ABSTRACT
Laboratory and field studies were conducted in 2000 and 2002 in Bulgaria to evaluate the efficacy of transgenic Bt potato (Newleaf®) against the Colorado potato beetle (CPB) Leptinotarsa decemlineata. Younger larvae of CPB were more susceptible to Bt potato leaves than older and died 100% during one day. The least defoliation of potato plants by CPB and the lowest number of overwintering adults of this pest were observed in Bt plots. Results from two year trials demonstrated that Bt potato plants were highly effective and provided better CPB control than insecticides Vaztak® and Regent®.

Introduction
Colorado potato beetle L. decemlineata is the major arthropod pests of potatoes in Bulgaria. In addition to CPB there are several other groups of pests including aphids (Stenorrhyncha: Aphididae) and wireworms (Coleoptera: Elateridae). Unlike the major pest, these pests require fewer specific control measures because they cause less consistent damage and are often controlled by chemical sprays against CPB. This pest requires specific annual control measures to prevent economic damage. Traditional interventions include treatment with a range of broad spectrum insecticides. Reliance on conventional insecticides has resulted in multiple resistance in CPB and variety of untoward effects on nontarget organisms and the environment (1, 7). Some insecticides for example aldicarb (Temik®) and methomyl (Lannate®) have high toxicity not only for nontarget organisms but also for vertebrates and other organisms (2, 8).

Advances in plant molecular biology and biochemistry in past two decades have allowed the development of modern genetic engineering technology that offers the potential to improve agronomic traits of crop cultivars. Several species of crops have been modified with genetic engineering methods to express genes from various subspecies of Bacillus thuringiensis Berliner (Bt) that encode Crystalline (Cry) proteins (δ - endotoxins). These Cry proteins confer effective protection to the crop plants from damage by certain phytophagous insect pest including CPB. In this article, we report the results of a laboratory and field studies conducted in Bulgaria to evaluate the efficacy of a transgenic Bt potato cultivar on CPB.

Materials and Methods
Laboratory experiments. Adults of CPB were collected in June from the conventional (non-Bt) potato fields. After transfer to the laboratory they were reared in glass jars 750 ml. In every glass jar there were 5 pairs which were fed with Bt (Newleaf®)
or non-Bt potato (Santana®) leaves taken from the field. Fifteen pairs (30 adults) were fed with Bt and other fifteen pairs with non-Bt potato leaves. The test was continued until all adults died.

The eggs of CPB were collected in June from non-Bt potato fields too. The larvae hatched from these eggs were used in the experiments. Four experiments were done with larvae:

1. The larvae were fed on Bt (Newleaf®) leaves from 1st instar
2. Newly hatched larvae were reared on non-Bt potato (Santana®) leaves and from 2nd instar they were fed on Bt potato leaves.
3. Larvae 1st and 2nd instars were reared on non-Bt potato leaves and from 3rd instar they were fed on Bt leaves.
4. Larvae 1st, 2nd and 3rd instars were reared on non-Bt potato leaves and from 4th instar they were fed on Bt potato leaves.

Laboratory experiments were done at a temperature of 22 ± 2 °C and relative humidity 45 – 72 %. Data were analyzed by the LSD test (Least significant different test, \( \alpha = 0.05 \)).

Field observations. In 2000 the investigated fields were situated at 900 m a.s.l. 20 kms from Samokov (western Bulgaria). Transgenic potatoes containing Bt-toxin (Superior Newleaf®) were planted in monocultute field of area 1.6 ha. The potatoes were planted in end of April. Approximately 80 – 100 m from this field separated by a bare land there was a control field with conventional cultivar (Santana® 4 ha) which was sprayed twice in the season (8th and 26th July) by pyrethroid alfa-cipermethrin (Vaztac® - 10 EC, 100 ml/ha). Non-Bt potatoes were planted in middle of May. Both fields were almost free of weeds. The potatoes were harvested in the beginning of September.

In 2002 the investigated fields were situated of 900 m a.s.l. near Samokov. Experiments were carried out in 1.5 ha field separated into three part of 0.5 ha. In the middle part were planted Bt potatoes. Of both sides of Bt plot were planted non-Bt potatoes (classic cultivar Sante® of one side and Santana® of other side). These conventional potatoes were sprayed once in the season (17th July) by fipronil (Regent® - 400 WG, 0.02 kg/ha). Ten meters from this field separated by a dirt road and field boundary there was a control field (0.5 ha non-Bt potatoes Santana® with no intervention). All potatoes were planted in end of May and harvested in the beginning of September.

Defoliation estimates were made visually in 2002 using the index sistem of Sinden et al. (12). The 25 plants were selected randomly in the central part of each plot. These plants were marked and each plant was carefully examined for damages on June 29, July 14 and July 28. Damage was scored on a 0 to 5 scale: 0, no feeding; 1, a few leaflets partially eaten; 2, some leaflets completely eaten; 3, at least one stem defoliated; 4, all stems nearly defoliated; 5, complete defoliation.

Sampling for overwintering beetles was conducted on October 19, 2002. Eight 0.05 m³ soil samples (50 cm x 50 cm x 20 cm deep) were taken in central part of each plot. The soil samples were sieved through a 0.6 cm mesh screen and adults of CPB were counted.

Results and Discussion

Laboratory experiments. Transgenic Newleaf potatoes were highly effective against CPB larvae. Newly hatched larvae were the most susceptible to Bt potatoes and died 100 % during one day (Tab. 1 and Tab. 2). Larvae 2nd and 3rd instar lived 3 – 8 days and died 100 % too. Only 65 – 82 % of larvae 4th instar died after 4 – 9 days. A part of larvae 4th instar pupated and successfully imaginated. However the weight of resulting adults was vary low - 12.9 ± 3.3 mg (n=14, min. - 7.8, max. - 18.8 mg). These adults lived only 6 – 10 days and
TABLE 1

The effect of Bt and conventional potato leaves on Leptinotarsa decemlineata in 2000

<table>
<thead>
<tr>
<th>Potatoes</th>
<th>larvae 1st instar</th>
<th>larvae 2nd instar</th>
<th>larvae 3rd instar</th>
<th>larvae 4th instar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (rep.)¹</td>
<td>Long.² (min-max)</td>
<td>Mort.³ (%)</td>
<td>n (rep.)¹</td>
</tr>
<tr>
<td>Bt</td>
<td>30 (3)</td>
<td>1.0 ± 0.0 (1 - 2)</td>
<td>30 (100)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>Conventional</td>
<td>30 (2)</td>
<td>3.6 ± 0.6 (3 - 5)</td>
<td>3 (10)</td>
<td>30 (3)</td>
</tr>
</tbody>
</table>

1 – replications; 2 – longevity in days; 3 - mortality

During this period they did not copulate and laid no eggs. The weight of adults reared on non-Bt leaves was 76.6 ± 19.3 mg (n=30, min – 38.8, max. 108.1 mg).

Field observations. Defoliation surveys also indicated that nearly 100 % of conventional potato plants not protected by any insecticides were defoliated to end of July, whereas no Bt potato plants were defoliated during this period and during all the season (Tab. 3). Some leaves of conventional potato plants treated with Regent were completely eaten too. Defoliation rating (Tab. 3) followed the number of eggs and larvae of CPB (Fig. 1, Fig. 2). Maximum eggs and larvae were observed on June 29 and after that during one mount non-Bt potatoes with no intervention were defoliated by CPB larvae and newly emerged adults.

Overwintering beetles were at highest densities in control plot (Table 4). The next highest densities of CPB beetles were found in Regent-treated conventional plots. Only 0.25 beetles/0.05 m² were found in Bt plot.

Results from the two years of laboratory and field trials indicated that transgenic Newleaf potatoes were highly effective in reducing the abundance of CPB populations.

Older larvae were less susceptible to Bt than do first instar. Larvae and adults of CPB eat only 2 – 5 min on Bt leaves. After that their longevity depends of their energetic reserves. Younger larvae have lower energetic reserves and live shorter. These results correspond with effect of microbial Bt on CPB larvae and adults (4, 6, 14, 15).

Some researchers have recommended that applications of microbial Bt be timed to coincide with CPB egg hatch or when early instars predominate. Humidity, temperature and solar radiation affect activity of microbial agents. Reed et al. (11) reached a good effect against CPB with four microbial Bt sprays. Ferro et al. (5) reported rapid decline in larvicidal activity of microbial Bt under field conditions. To minimize solar deactivation, in this study the authors applied...
Fig. 1. Abundance of different stages of Colorado potato beetle during the season 2000. (TBt – transgenic Bt potato, CV – non-Bt treated with Vaztak).
Fig. 2. Abundance of different stages of Colorado potato beetle during the season 2002. (TBt – transgenic Bt potato, CV – non-Bt treated with Regent, C – non-Bt with no control measures).
TABLE 3
Defoliation rating in plots during 2002. (Means within the same column followed by the same letter are not significantly different (LSD test, P < 0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean defoliation rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June 28</td>
</tr>
<tr>
<td>Conventional (no intervention)</td>
<td>1.1 ± 0.3a</td>
</tr>
<tr>
<td>Conventional (Regent)</td>
<td>1.4 ± 0.2a</td>
</tr>
<tr>
<td>Bt-transgenic (Newleaf®)</td>
<td>0 b</td>
</tr>
</tbody>
</table>

TABLE 4
Average number of overwintering Colorado potato beetles per 1 m² in different plots during 2002. (Means followed by the different letters are significantly different (LSD test; P < 0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional (no intervention)</td>
<td>3.5 ± 0.5a</td>
</tr>
<tr>
<td>Conventional (Regent)</td>
<td>1.4 ± 0.5b</td>
</tr>
<tr>
<td>Bt-transgenic (Newleaf®)</td>
<td>0.25 ± 0.1c</td>
</tr>
</tbody>
</table>

microbials in early evening. Bt potatoes have an advantage over microbial Bt because the activity of Bt protein toxins is not altered in transgenic crops during the season.

Results from the field trials indicated that Bt potatoes provided better control than insecticides Vaztak and Regent. The number of overwintering beetles in Regent treated plots was relatively high. This is probably due to the fact that when control plot was defoliated the insecticide-treated plots offered the only local sources of suitable vegetation. Some beetles probably migrate also to Bt plot and due to unsuitable food and weather conditions they stay to overwinter there.

Newleaf potatoes were highly effective against CPB larvae and adults, and more effective than weekly sprays of Bt-based microbial insecticides, bi-weekly applications of permethrin, or early and mead-season applications of systemic insecticides phorate and disulfoton (11). Something more, Newleaf potatoes appear to have an advantage over broad-spectrum foliar applied insecticides in promoting the role of natural enemies. In contrast to the insecticides, Bt potatoes have not significant effect on the abundance of beneficial predators and secondary potato pests (9, 11, 13). These findings are not surprising because the Cry 3A protein is highly selective in its activity, affecting only Coleoptera in the family Chrysomelidae (3, 10).

Acknowledgements
This project was supported by the grant No B-1105 of the Bulgarian National Research Fund; Bulgarian Ministry of Agriculture and Forestry, and by Monsanto Europe, Sofia.

REFERENCES
