

ALL-UNION INSTITUTE OF PLANT BREEDING AND GENETICS, ODESSA,
 UKRAINIAN RESEARCH INSTITUTE OF PLANT PROTECTION, KIEV, USSR
 INSTITUTE OF MOLECULAR BIOLOGY AND GENETICS

U.S.S.R.

ACADEMY OF SCIENCES OF THE UKRAINIAN SSR, KIEV, USSR

1) Resistance of soybean cultivars and plant introductions to damage by soybean borer.

Due to considerable extending of soybean sowing areas and inculcation of it in new cultivation regions, the losses from diseases and pests are constantly rising. Rich biochemical composition of soybean is an excellent feeding medium for insects and mites, especially its reproductive organs. It is known that more than 500 species of pests parasitize leguminous crops, including more than 90 species of insects and mites.

Soybean borers (*Etiella zinckenella* Tr.) do great injury to soybean. This pest leads to the considerable losses of yield and quality and it is the main pest in this zone, together with the spider mite. The moth of the soybean borer has yellow-grey wings stretched with rusty yellow stripes. Lower wings are light grey with dark piping. Larvae are dirty green with nonclear dark red stripes. Pupae are dark brown and 9-12 mm long. This pest has 2-3 generations annually. Female moths put their eggs on young pods. Larvae of the first generations usually feed on grains of Siberian peashrub, pea and vetch, larvae of the second and third generations feed on soybean and false acacia grains. The biggest reproduction of the pest occurs on false acacia. Little round holes have been seen on injured pods and these pods are less plump and shrunken. Larvae live in the pods about a month, and sometimes migrate from one to another pod. After that they come down onto the surface of soil, transform into pupae and spend the winter in such form.

The possibility of creating pest-resistant forms of soybean has risen significantly in the last ten years in countries where soybean is cultivated, especially in the USA. First of all, there were found the sources of resistance to Mexican bean beetle (Elden et al., 1974; Van Duyn et al., 1971; Van Duyn et al., 1972). The number of adult beetles, eggs and larvae on the resistant forms was very small in the field as it was observed in South Carolina (Turnipseed and Sullivan, 1976). The higher mortality of adult larvae was pointed out when they were feeding on foliage of resistant forms in the laboratory. Their growth and productivity were reduced very much. The forms resistant to Mexican bean beetle (PI 171451, PI 227687 and PI 229358) were tolerant to *Cerotoma trifurcata*, *Heliothis zea*, *Epicauta vittata*, *Pseudoplusia includens* and *Spodoptera exigua* (Turnipseed and Sullivan, 1976). With these forms as a base, some USA states worked out a collective breeding program to create the pest-resistant soybean varieties. Now, there are some breeding lines from crosses of 'Bragg', 'Davis' and 'Forrest' with the donors of resistance mentioned above, which are characterized by resistance to two and more pests (Hatchett et al., 1978). These data show that it is possible to create prospective breeding material with group pest-resistance. The study of inheritance of resistance to Mexican bean beetle shows that it is quantitative (Sisson et al., 1976). Hybrid populations from crosses between resistant and susceptible forms had normal distribution at the third generation. The authors consider that there are two or three major genes affecting resistance level.

Considerable number of studies were made to analyze soybean resistance to corn earworm, *Heliothis zea* Boddie (Joshi and Wutoh, 1976; Joshi, 1977, 1978, 1979). These experiments demonstrated that the three forms resistant to Mexican bean beetle mentioned above were resistant to this pest, too. There were many resistant forms among 2797 plant introductions that were not injured by corn earworm during two and more years. The most resistant were varieties 'Ada', 'Portage', 'Peking', 'Arlington' and others.

Soybean has varietal tolerance to spider mite injury, too (Bailey and Furr, 1975; Carlson et al., 1979).

It is reported that soybeans have been damaged by soybean borer in the conditions of South Ukraine, but limited number of soybean forms were analyzed. Our experiments were made during 1980 and 1981 on fields of "Dachnaya," the base of All-Union Institute of Plant Breeding and Genetics. Soybean samples were sowed at 30-40 m from windbreak plantings of false acacia and Siberian peatree with the aim of creating hard infection background. After maturation, all plants were pulled up and analyzed in the laboratory. All pods of each variety were carefully examined to see if they had been damaged by larvae of soybean borer. The pods without injury were classified according to number of grains in them to simplify calculation of total grain sum. The damaged pods were opened and number of damaged grains was summarized. After that, all data were calculated to amount percentage of damaged pods and grains. In such way, 348 samples were analyzed in 1980, and more than 500 samples in 1981.

The studies showed that damage by soybean borer very much reduced 100-grain weight, sometimes by 60% and more (Table 1). Some grains were fully eaten by pest, others a half or less. Most varieties had losses of 100-grain weight more than 50% as the result of damage (Table 1). When average soybean yield was 2000 kg/ha and level of damage was 10%, then 100 kg/ha of grain had been lost from damage of this pest. Plant introductions from various countries of the world had different levels of resistance to that pest (Table 2). Most had 3-7% injured grains. The main number of forms with less than 1% of injured grains was among Soviet varieties. As a rule, they are Far Eastern breeding. The forms from Japan, USA and China had weak tolerance. For instance, 91 Soviet varieties were studied; 8.8% of them had less than 1% of injury, and 3.3% had level of damage more than 10%. Varieties from USA and China had 3.2 and 12.7% and 3 and 12.1%, respectively. These data show that varieties of Far Eastern breeding have a high level of resistance to soybean borer. Resistant soybean forms are listed in Table 3, and very susceptible varieties are given in Table 4. Varieties K-5776, K-309, 'Amurskaya 401', 'Amurskaya 450', and 'Moneta' had high resistance. The most varieties with high susceptibility to this pest were from USA (Table 4). It must be pointed out that absolute meanings of damage percentage of varieties were different in various years, though their relative levels were in accordance with years. The results of these studies demonstrate that among plant introductions and cultivars of soybean are forms with high resistance to soybean borer. It is important to point out that such high yielding varieties as 'Zarnitsa', 'Belosnejka', 'Severnaya 2', and 'Hodgson' have resistance to this pest and they have complex economical and useful characters. These forms can be used not only as donors of resistance to this pest, but as donors of many other valuable characters, too.

Table 1. The level of soybean grain losses from soybean borer injury

Variety	Weight of 100 grains		Loss percentage
	Undamaged	Damaged	
Ontario	16.5	7.1	57.0
Beechwood	13.9	7.9	43.2
Bukuria	9.9	4.9	50.0
Krasnodarskaya 585	11.1	4.9	55.9
Hybrid 438	10.2	3.9	61.8
K-1187	13.3	9.5	28.6
073-2	13.6	8.5	37.5
Hybrid 646	14.8	5.6	62.2
Peremoga	12.0	6.1	49.2
Lumina	12.4	5.8	53.2
Zora	11.8	5.3	55.1
PI 189995	9.7	3.8	60.8
Nordic 138	11.7	5.9	49.6

Table 2. Distribution of introduction varieties of soybean due to grains damage by soybean borer depending on origin (percent of total amount of tested numbers)

Country	Number of tested varieties	Percent of damage										
		0-1	1.1-2	2.1-3	3.1-4	4.1-5	5.1-6	6.1-7	7.1-8	8.1-9	9.1-10	More than 10
USSR	91	8.8	4.4	6.6	18.7	5.5	16.5	19.8	7.7	5.5	3.3	3.3
USA	63	3.2	3.2	9.5	11.1	11.1	15.9	7.9	7.9	11.1	6.3	12.7
China	33	3.0	6.1	9.1	15.2	18.2	9.1	9.1	6.1	9.1	3.0	12.1
West and East Europe	30	3.3	10.0	20.0	13.3	3.3	10.0	13.3	3.3	13.3	10.0	0
Canada	10	0	0	20.0	0	20.0	20.0	0	10.0	20.0	0	10.0
Japan	3	0	0	0	0	33.3	0	0	0	0	0	66.7
Others	26	0	0	11.5	15.4	11.5	23.1	7.7	19.2	0	7.7	3.8

Table 3. Soybean forms resistant to soybean borer

Variety	Origin	Damage percent			
		of pods		of grains	
		1980	1981	1980	1981
Amurskaya 411	Far East of the USSR	3.3	4.6	2.4	5.3
Manchu	North China	4.0	0	3.1	0
Severnaya 2	Far East of the USSR	0.5	0	0.3	0
K-5776	North China	0.3	0	0.3	0
K-309	North China	0.2	0.6	0.3	0.5
Pavlikeni 2	Bulgaria	2.7	2.1	2.9	1.7
Varshavskaya	Poland	3.2	2.8	2.5	2.6
Amurskaya 401	Far East of the USSR	0	1.3	0	0.9
K-6334	Brazilia	1.3	1.2	1.0	1.2
Record Severniy	Far East of the USSR	1.6	0.4	0.9	0.5
Herb 620	DDR	0.9	2.4	0.8	2.5
Amurskaya 450	Far East of the USSR	0	1.3	0	1.1
K-4355	DDR	1.2	2.1	0.9	1.4
Hodgson	USA	0.6	3.8	0.3	3.2
Zarnitsa	UkSSR	0.2	1.3	0.1	1.2
Belosnezhka	UkSSR	1.2	2.1	0.9	2.1
Peterson 2090	USA	2.5	0	1.8	0
Moneta	Latvian SSR	0.2	0.9	0.2	0.8
Amurskaya 147	Far East of the USSR	0.9	3.1	0.5	3.0
Amurskaya yellow	Far East of the USSR	1.3	2.5	0.8	1.3
PI 189863	USA	1.2	0.8	1.2	0.7

Table 4. Soybean varieties highly susceptible to damage by soybean borer

Variety	Origin	Damage percent			
		of pods		of grains	
		1980	1981	1980	1981
Heimkraft	DDR	10.4	22.5	8.8	13.1
Early Hachubu	Japan	24.4	19.2	18.4	17.5
PI 153294	USA	32.5	14.3	25.9	7.8
PI 189995	USA	20.2	11.6	15.1	7.2
PI 153245	USA	35.2	16.8	24.0	12.5
Earlyana	USA	13.8	13.7	9.1	8.4
VNIISK 8012	Krasnodarskij kraj	16.9	10.6	11.8	7.6
K-1286	North China	20.2	10.0	16.8	7.6
K-1657	North China	11.0	19.7	11.3	10.6
Nairn	Canada	18.3	20.7	18.1	18.5
K-434432	unknown	37.8	23.3	20.8	17.8

References

- Bailey, J. C. and R. E. Furr. 1975. Reaction of 12 soybean varieties to the two-spotted mite. *Environ. Entomol.* 4,5:733-734.
- Carlson, E. C., B. H. Beard, R. Tarailo and R. L. Witt. 1979. Testing soybeans for resistance to spider mites. *Calif. Agr.* 33, 9.
- Elden, T. C., J. A. Schillinger and A. L. Steinhauer. 1974. Field and laboratory selection for resistance in soybeans to the Mexican bean beetle. *Environ. Entomol.* 3:785-788.
- Hatchett, J. H., G. L. Beland and T. G. Kilen. 1979. Identification of multiple insect resistant soybean lines. *Crop Sci.* 19(4):557-559.
- Joshi, J. M. and J. G. Wutoh. 1976. Evaluation of commercial soybean cultivars for leaf feeding resistance to *Heliothis zea*. *Soybean Genet. Newsl.* 3:43-46.
- Joshi, J. M. 1977. Field screening of soybean germplasm (maturity groups 00 to IV) against *H. zea* damage. *Soybean Genet. Newsl.* 4:46-50.
- Joshi, J. M. 1978. Evaluation of soybean germplasm for resistance to corn earworm. *Soybean Genet. Newsl.* 5:49-59.

- Joshi, J. M. 1979. Evaluation of soybean germplasm for resistance to corn earworm - III. Soybean Genet. News1. 6:75-77.
- Sisson, V. A., F. A. Miller, W. V. Cambell and J. W. Van Duyn. 1976. Evidence of inheritance of resistance to the Mexican bean beetle in soybeans. Crop Sci. 16(6):835-837.
- Turnipseed, S. and J. Sullivan. 1976. Plant resistant in soybean insect management. In B. D. Hill (ed.) World soybean research, Proc. of the World Soybean Res. Conf. The Interstate Printers and Publishers, Danville, IL.
- Van Duyn, J. W., S. G. Turnipseed and J. O. Maxwell. 1971. Resistance in soybeans to the Mexican bean beetle. I. Source of resistance. Crop Sci. 11(4):572-573.
- Van Duyn, J. W., S. G. Turnipseed and J. D. Maxwell. 1972. Resistance in soybeans to the Mexican bean beetle. II. Reactions of the beetle to resistant plant. Crop Sci. 12(5):561-562.

V. I. Sichkar
O. A. Grikun
V. N. Lobko
V. F. Marj'ushkin

2) Activity of trypsin and chymotrypsin inhibitors of soybean forms with different resistance to soybean borer.

In a previous investigation, the wide amplitude of plant resistance of different soybean varieties to soybean borer has been discovered. Our further experiments are directed at the discovery of the main mechanisms of this resistance. It is known that the phenomenon of plant resistance to insects may be classified into three components: nonpreference, antibiosis, and tolerance (Painter, 1951). We use the term nonpreference when the plant is an unfavorable object for feeding and oviposition. When there is antibiosis, the pests have an abnormal biology of development that leads to inhibition of their growth and survival rate and depression of reproductive functions. Tolerance is the capability of plants to withstand damage without particular detriment to the yield.

Results of the study of well-known resistant soybean donors to leaf-feeding pests showed that the resistance was suggesting nonpreference and antibiosis (Elden et al., 1974; Van Duyn, 1972; Beland and Hatchett, 1976; Schillinger, 1976).

Numerous experiments proved that the feeding of different groups of animals on unheated soybean grains leads to the inhibition of their growth and to the appearance of toxicity symptoms. The main reason of this phenomenon is the presence of such antinutritional factors as trypsin and chymotrypsin inhibitors, lectins, phenols and other substances.

Investigations by Jansen and Juster (1976) with *Callosobruchus maculatus* beetle showed that the adding of bean lectins to cowpea meal without this substance leads to a high death rate of larvae. If, in control without lectins, about 4-6 beetles per cowpea grain survived, the addition of 0.1, 1 and 5% of lectins reduced the number of beetles to 3.2, 0.4 and 0,

respectively. With this reason, authors consider that leguminous lectins play an important role in the protection of the grain from the pests.

Since larvae of the soybean borer feed on raw soybean grains, it is very interesting to know the dependence of resistance upon the content of trypsin and chymotrypsin inhibitors.

Activity of the above mentioned inhibitors has been determined by casein method, based the inhibition of casein hydrolysis by trypsin and chymotrypsin (Levitsky, 1979).

Table 1 shows that the content of trypsin inhibitors in mature soybean grains changed significantly (12.6-53.6 mg/g) depending on varieties. The variability of chymotrypsin inhibitor was significantly lower (14.3-25.3 mg/g).

Results demonstrated an absence of association between resistance and the activity of inhibitors in soybean grains. Although correlation coefficient with trypsin inhibitors was positive, it had a very low meaning and was not significant (0.24), but it was -0.05 with chymotrypsin inhibitor. For example, varieties resistant to soybean borer (K-4355, 'Peterson 2090', 'Pavlikeni 2') had reduced activity of trypsin inhibitors, while K-4867, K-1390, K-309, 'Zarnitsa' and 'Hodgson' combined high resistance to given pest and increased activity of trypsin inhibitors. The same picture happened to the second type of inhibitor.

As soybean borer is a special pest of leguminous crops, apparently in this case there was the prolonged conjugate evolution of pest and host plants. During this evolution, soybean borer has developed adaptations that play an important role in the feeding on this grain. The normal assimilation of raw soybean grains by larvae shows that trypsin and chymotrypsin inhibitors, present in large quantities in soybean grains, assimilate like other nutrient proteins and don't influence the activity of main digestive enzymes.

It is possible that this pest has several peptide-hydrolases at the first stages of protein proteolysis that are dissimilar from trypsin and chymotrypsin. It isn't out of the question that proteases of soybean borer have some other inhibitors, although they are still unknown.

References

- Beland, G. L. and J. H. Hatchett. 1976. Expression of antibiosis to the boll-worm in two soybean genotypes. *J. Econ. Entomol.* 69:557-560.
- Elden, T. C., J. A. Schillinger and A. L. Steinhauer. 1974. Field and laboratory selection for resistance in soybeans to the Mexican bean beetle. *Environ. Entomol.* 3,5:785-788.
- Jansen, D. H. and H. B. Juster. 1976. Insecticidal action of the phytohemagglutinin in black beans on a bruchid beetle. *Science* 192, 4241: 795-796.
- Levitsky, A. P. 1979. Methods of determination of trypsin inhibitors. In: Biochemical research methods of breeding materials. Odessa, All-Union Institute of Plant Breeding and Genetics, USSR.
- Painter, R. H. 1951. Insect resistance in crop plants. University Press of Kansas, Lawrence, and London.

Table 1. Content of trypsin and chymotrypsin inhibitors in the seed of different soybean varieties resistant to soybean borer

Variety	% average infected grains	Activity of an inhibitor	
		Trypsin	Chymotrypsin
Amurskaya yellow	1.0	23.6	23.5
Amurskaya 147	1.7	24.2	24.2
Severnaya 2	0.2	30.3	21.7
K-254	7.8	47.5	19.2
K-4355	1.1	20.5	24.6
K-678	1.2	23.6	15.4
K-4867	3.0	53.6	19.7
Hybrid A-6-71	8.9	39.7	18.9
Hybrid 681	10.6	24.2	17.5
Hybrid 467-127	10.0	44.2	18.6
Hybrid 117-9	8.3	40.4	17.2
K-1390	2.7	46.7	16.5
K-309	0.4	52.7	19.7
Hybrid 466-214	2.8	36.7	18.6
Peterson 2090	0.9	12.6	20.0
Pavlikeni-2	2.3	16.7	14.7
Moneta	0.5	25.9	14.3
Virginia	2.4	30.9	14.7
VNIISK 8012	9.7	14.4	18.6
Zarnitsa	0.6	44.6	20.4
Hodgson	1.7	46.2	19.7
PI 153245	18.2	46.7	22.9
PI 189995	11.1	37.2	16.1
K-6468	1.5	29.5	18.4

Schillinger, J. 1976. Host plant resistance to insects in soybeans. In: L. D. Hill (ed.) Proc. World Soybean Res. Conf. The Interstate Printers and Publishers, Danville, IL.

Van Duyn, J. W., S. A. Turnipseed and J. O. Maxwell. 1972. Resistance in soybeans to the Mexican bean beetle. II. Reactions of the beetle to resistant plants. Crop Sci. 12(5):561-562.

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