Phomopsis control in sunflower using products of biogenic origin

I.I. Begunov[1], V.T. Piven[2], A.T. Podvarko[1], V.Y. Ismailov[1], T. Gulya[3]
[1]All-Russian Research Institute of Biological Plant Protection, 350039, Krasnodar, Russia, e-mail: vniibzr@internet.kuban.ru
[2]All-Russian Research Institute of Oil-Yielding Crops (VNIIMK), 350030. Krasnodar, Russia,e-mail: vniimk-centr@mail.ru
[3]Northern Crop Science Laboratory. Fargo, ND 58105 USA,
E-mail: ThomasGulya@ARS.USA.Cov

ABSTRACT
The regulation of growth and development in sunflower plants was studied. Resistance to Phomopsis and productiveness of sunflower plants were induced by treating seeds with a disease resistance inducer of elicitor nature based on chitosan together with plant growth regulators.

Key words: albite – chitosan – disease resistance inducer – growth regulator – Phomopsis – zircon.

INTRODUCTION
As the world literature indicates and also our data show, the fungus *Diaporte (Phomopsis) helianthi* (Munt.-Cvet et.al.), which affects both sunflower and wild plants, possesses a high infection potential that contributes to its expansion and injuriousness in Russia and other countries in which sunflower is cultivated. The available domestic and international sets of commercial and promising newly developed sunflower cultivars and hybrids are susceptible to this disease. Therefore, Phomopsis remains a problematic disease both for Russia and for other countries.

Within the current arsenal of pest control agents in crops, including those against Phomopsis in sunflower, chemical fungicides continue to be of priority. Research conducted in Yugoslavia, Romania, France, Russia and other countries demonstrated that the available range of chemical fungicides does not reduce the infection and, therefore, the effectiveness of protective measures remains low (Piven et al., 1997; Chaban, 1990).

In this connection, research on inducing the resistance based on strengthening the natural defense mechanisms of plants has very good prospects for developing environmentally safe and high-yielding crop production technologies. In fact, the concept of induced resistance appeared together with the purpose of using induced immunity in practice. The most important prerequisite to considering induced resistance as an actual phenomenon includes the fact that the defense reactions are triggered as a response to infection both in resistant and susceptible plants (Tyuterev, 1999).

Both synthetic and natural biologically active compounds may be used as disease resistance inducers. Among the latter, most attention is currently being paid to chitin and its derivative chitosan (Reunov, 2001; Reddy et al., 1999). For the first time, high activity of chitosan in protecting plants was demonstrated by L.A. Hadwiger in 1986. He determined that the seed treatment with chitosan protects the plants from fungal and bacterial diseases and increases their yields by an average of 20% (Hadwiger, 1989). Even in the recent past, the mechanism of the chitosan action on plants was practically unknown. Nowadays, convincing data have been obtained showing high effectiveness and revealing mechanisms of the chitosan action on biological objects. Some publications (Begunov et al., 2004). reported an effect of the induced biosynthesis of chitinases and chitisanases, that resulted from treating plant cells with chitosan; they showed very high resistance against plant-pathogenic fungi and bacteria. It was determined that not only the cells, but also the plants of rice, pea, carrot and other crops sprayed with chitosan synthesized pectins along with chitinolytic enzymes and their joint action led to the complex cell wall destruction of plant pathogens that prevented their invasion of plants. Long-term tests of chitosan under laboratory and field conditions conducted by Russian research institutes (All-Russian Research Institute of Biological Plant Protection, Krasnodar and All-Russian Research Institute of Plant Protection, St. Petersburg) showed positive results by using the models including root rot pathogens in cereals, vegetables, and rice and mildew in cucumber and other crops. The highest plant protection effect of using chitosan was produced due to seed treatments (Begunov et al., 2002).

Before our work started, no references to a complex application of disease resistance inducers and plant growth regulators against Phomopsis in sunflower were available in literature. Therefore, the work proposed by us that includes studying growth and development regulation, Phomopsis resistance induction and increase in sunflower yields by using the sunflower seed treatment with a disease resistance inducer of an elicitor nature based on chitosan, is especially important.

MATERIALS AND METHODS

In 2006 research was conducted using two sunflower cultivars: a susceptible cultivar Rodnik - growing season duration: 80 days, potential yield: 3.2 t/ha, oil content: 55%; and a tolerant cultivar Master - growing season duration: 94 days, potential yield: 4.0 t/ha, oil content: 54%. The experiment layout, as well as phenological, phytopathological and biometric studies were designed according to conventional methods (Begunov et al., 2004). The plot area was 100 m^2, three replicates and randomized block design were used. The experiment plots were planted on May 15. The soil of the plots included deep low-humic leached chernozem. The humus level in the arable soil layer was 3.4-4.1 %, pH_{salt} was 6.5, pH_{water} was7.5. Winter wheat was a predecessor.

The experiment included the use of a biogenic resistance inducer based on chitosan – chitosanium glutaminium succinate - in the mixture with phytohormonal plant growth regulators based on hydroxycinnamic (zircon), polybetabutyric (albite), heteroauxinic (IAA) acids and trace elements of chelate form (hydromics). Hydromics, zircon, chitosanium glutaminium succinate are recommended in the Russian Federation to be used for sunflower seed treatments, while albite and IAA are new experimental plant growth regulators for sunflower plants.

The tested disease resistance inducer and plant growth regulators were applied as different combinations for the seed treatments of both sunflower cultivars a day before planting. The product Maxim (active ingredient: fludioxonyl) was used as a standard. A treatment without the application of the above products served as control. Experiment design, applied compositions and application rates are shown in Table 1.

The progress of Phomopsis in sunflower plants was evaluated using the VNIIMK scale.

Table 1. Experiment design

#	Experimental treatment	Rate of application, kg,l/t of seeds
1	Chitosanium-glutaminium succinate + zircon	0.2 + 0.2
2	Chitosanium-glutaminium succinate + phloroxan + IAA + zircon	0.2+0.0005 + 0.006+0.2
3	Chitosanium-glutaminium succinate + albite	0.2 + 0.1
4	Chitosanium-glutaminium succinate + phloroxan + IAA + albite	0.2 + 0.0005 + 0.006 + 0.1
5	Chitosanium-glutaminium succinate + hydromics	0.2 + 0.15
6	Chitosanium-glutaminium succinate + phloroxan + IAA + hydromics	0.2 + 0.0005 + 0.006 + 0.15
7	Maxim (standard)	5.0
8	Control	Untreated

Biological effectiveness was calculated with the formula:

$$B = \frac{Pc-Pt}{Pc} \times 100\ \%$$

where B – biological effectiveness, %;
Pc – Phomopsis progress in control;
Pt – Phomopsis progress in an experimental treatment.

The sunflower was harvested with a Sampo combine.

The agrometeorological conditions during the sunflower growing season were rather favorable both for sunflower growth and Phomopsis progress. The hydrothermic coefficient for the growing season was 1. The mathematical data processing was done using the variance analysis method.

RESULTS

Research on determining biological parameters of the chitosanium-glutaminium succinate application against Phomopsis showed that the highest disease resistance induction of 56-60 % was caused in sunflower plants as a result of treating their seed, *i.e.* when the defense mechanisms were launched at the early plant ontogeny stages. The optimum rate of application was 0.2 kg/t of seeds (Begunov et al., 2004). In addition, it was shown that chitosan has good hydrophylic property, complex formation ability, film-forming capacity, absence of toxicity and broad-spectrum biological activity.

All these characteristics of chitosan provided a stimulus to search for the possibilities to enhance its biological activity, add new useful properties to this polymer and create its novel compositions with non-phytotoxic biologically active compounds.

The sunflower seed treatments using chitosanium-glutaminium succinate with zircon, IAA, albite and hydromics stimulated germination and activated initial growth and development of plants. For the Rodnik cultivar, the root growth stimulation reached 170% (Treatment 6) and the stem growth stimulation reached 140% (Treatment 1). For the cultivar Master, the maximum root and stem stimulation values were recorded in the Treatments 2 and 1 and they reached 150 and 119%, respectively. On the whole, the growth-stimulating process at the first ontogeny stages was more active in the early-ripening cultivar Rodnik than in the late-ripening cultivar Master (Table 2).

Table 2. Evaluation of the growth-stimulating action on sunflower plants produced by the tested formulations under field conditions

Treatment #	Cultivar Rodnik Germination on the 14th day				Cultivar Master Germination on the 14th day			
	Root (cm)	Percentage of control	Stem (cm)	Percentage of control	Root (cm)	Percentage of control	Stem (cm)	Percentage of control
1	9.3	137	22.0	140	9.4	130	17.5	119
2	8.7	128	19.7	125	10.7	150	16.4	111
3	9.6	140	19.8	125	9.0	120	17.0	116
4	8.5	125	19.3	120	10.5	140	15.4	105
5	9.4	138	19.3	120	10.5	140	16.3	110
6	11.6	170	18.2	115	10.5	140	16.3	110
7	8.5	125	17.7	110	10.5	140	16.0	110
8	6.8		15.8		7.3		14.7	
Control								
HCP_{05}	0.55		1.34		0.50		0.68	

Our further phenological observations showed that the sunflower plants of both cultivars formed the second pair of true leaves in the treatments where the tested compositions had been applied two or three days earlier than in the standard (Maxim) and control treatments. Also, the accelerated budding and flowering stages were recorded for the plants whose seeds had been treated with the tested compositions.

Effects of the compositions of the tested disease resistance inducer and plant growth regulators were tested under natural conditions at the field. It should be noted that the weather conditions were especially favorable for the progress of Phomopsis at all the developmental stages of the plants, from germination to flowering. For that period, 21 rainy days were recorded with 250 mm of total rainfall. The disease incidence and severity were evaluated at the budding, flowering and physiological ripeness stages.

The first symptoms of the Phomopsis leaf form were detected in the control plot at the budding stage on June 20 for the cultivar Rodnik and on June 29 for the cultivar Master. By July 29, 6% of the sunflower plants of Rodnik and 4% of Master had been affected by Phomopsis. The stem form of Phomopsis appeared in the plants of Rodnik on July 10 and in the plants of Master on July 24.

Table 3 shows the results of the evaluation of Phomopsis stem symptoms at the physiological ripeness stage. The cultivar Rodnik showed 42.3% of diseased plants, with 23.6% of disease severity; for the cultivar Master these values were 26.7 % and 12.2 %, respectively.

The data analysis showed that the combined application of chitosanium-glutaminium succinate togeteher with IAA, phloroxan, zircon, and albite produced the most effective protective action on plants. Induced biological effectiveness in these experimental treatments (2 and 4) was 71-73 % for Rodnik and 81-88 % for Master. Therefore, it was shown that plant growth regulators such as albite and zircon in combination with chitosanium-glutaminium succinate strengthened the defense mechanism of sunflower plants against Phomopsis.

Among the tested plant growth regulators, the seed treatments with the combination of disease resistance inducer and zircon (Treatments 1 and 2) and albite (Treatments 3 and 4) contributed to obtaining higher yields from both cultivars.

Table 3. Biological and economic effectiveness of the tested formulations

Treatment	Rodnik				Master			
	Incidence (%)	Severity (%)	Biological effectiveness (%)	Yield (t/ha)	Incidence (%)	Severity (%)	Biological effectiveness (%)	Yield (t/ha)
1	16.2	12.1	49	2.19	12.9	5.9	52	2.21
2	8.7	6.3	73	2.15	8.5	2.3	81	2.11
3	12.0	8.0	73	2.20	5.2	4.9	52	2.10
4	10.3	6.9	71	2.04	5.2	1.5	88	2.11
5	9.3	6.1	74	1.95	7.9	3.5	71	2.02
6	12.1	8.4	64	2.10	14.7	5.3	57	2.06
7	14.9	16.2	31	1.90	26.0	11.2	8	1.85
8 (Control)	42.3	23.6	-	1.89	26.7	12.2	-	1.76
HCP_{05}				0.083				0.085

DISCUSSION

The combined application of the tested biogenic disease resistance inducer and phytohormonal plant growth regulators albite and zircon enhances the Phomopsis resistance of sunflower plants and increases their yields.

Thus, this area of research on improving disease resistance in plants without changing their genome is a connecting link between fundamental immunology and practical crop protection. In this connection, using the products based on chitosan is very promising, and the induced resistance caused by them may be considered as one of the biological methods for disease control.

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