Biopreparations for plant protection in Siberia: application and enhancement of activity

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This review is devoted to the most widespread biological preparations used for ecologically safe control of insect pests and plant diseases in Siberia. Special attention is given to the application of biopreparations on vegetable and soft fruit crops. The necessity of enhancement of control agent activity and relevant formulations is discussed. In most cases, data obtained by Siberian authors have been presented. Possible ways to enhance efficiency of biopreparations using the mixtures based on biocontrol agents of different origin or on the agents with nontoxic enhancers are shown. The role of host plant in the efficacy of biopreparations is emphasized, especially for future research.

Key words: biocontrol, biopreparation, enhancer, insect pest, plant disease.

Introduction

Siberia is an important region of Russia, where valued crops are grown in large areas. Siberia stretches from the Ural Mountains in the west nearly to the Pacific Ocean in the east. The temperature ranges from a low of approximately -50°C in the winter to the 30s in the short summer. This in itself presents a major challenge to growing any crop, especially fruits. Severe climatic conditions cause particular vulnerability of plants to stress; therefore, massive application of chemical pesticides for plant protection is undesirable. Biological pest control is indispensable alternative to chemical pesticides. Of biocontrol, however, application of biopreparations based on natural microorganisms or its metabolites is more preferable than release of beneficial insects due to sharp possible change of weather during a short period.

Historically, the first biological preparation in Russia was developed by Siberian researcher E. Talalaev (1959). He isolated *Bacillus thuringiensis* (*Bt*) subsp. *dendrolimus* (*sotto*) from larvae of a serious forest pest - the Siberian silkworm *Dendrolimus superans sibiricus*- during epizootics in Eastern

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Siberia. This *Bt* subspecies provided the basis of the first Russian bacterial preparation Dendrobacillin® for insect control. Initially, Dendrobacillin® was used for plant protection against pest insect in forestry. It was warranted because forest occupied the great area of Siberia and the maintenance of biodiversity of this biocoenosis by elimination of chemicals was very important. Later, the same formulation was used for lepidopteran insect control on agricultural crops as well. In the second half of 20th century, some research groups were organized in Eastern and Western Siberia (Irkutsk, Krasnoyarsk and Novosibirsk) for research and development biopreparations based on entomopathogenic microorganisms. Nowadays, Novosibirsk is a center of biocontrol research in Siberia, including research teams at State Agrarian University, some Institutes of Siberian Branch of Russian Academy of Sciences, and State Scientific Center of Virology and Biotechnology. The main directions of research are as follows: isolation and identification of potential biocontrol agents; mechanism of interaction of biocontrol agent with its target organism; application technology of ecologically safe preparations; enhancement of biocontrol efficacy.

It should be noted also that in 1960s the first Russian factory producing biological insecticides based on the most common biocontrol agent *Bt* was built near Novosibirsk. This fact accelerated the research on the application and improvement of the biological preparations (Shternshis, 1990). Together with *Bt*-formulations, preparations based on entomopathogenic viruses were considered as attractive ecologically safe alternatives to chemical insecticides. Several strains of baculoviruses were isolated by Siberian researchers from the serious forest pests, such as *D. superans sibiricus*, *Neodiprion sertifer* and *Aporia crataegi* (Gouli *et al.*, 1976). Again, it was a basis for development of agricultural plant protection technology including viral entomopathogenic preparations (Shternshis, 1988). For example, a serious polyphagous pest of vegetable and many other crops – the beet webworm *Pyrausta sticticalis,* was shown to be suppressed by specific granulosis virus (GV) and nucleopolyhedrovirus (NPV) (Ermakova and Shternshis, 1987). The GV appeared to be more virulent to larvae of beet webworm than NPV, therefore, viral preparation was developed using the GV (Shternshis *et al.*, 1997). Later, the efficacy of formulations based on entomopathogenic fungi or secondary metabolites of several useful microorganisms for plant protection in Siberia was shown as well (Kalvish, 1980; Ogarkov and Ogarkova, 2000; Shternshis, 2002).

Unfortunately, for a long period to the end of 20th century only one biological preparation against plant diseases was used in Siberia, namely Trichodermin® based on *Trichoderma viride (lignorum*) (Kolomnikova, 1988).

In recent decade, however, good results concerning the application of some biopreparations for disease control were obtained in Siberia (Tulpanova *et al*., 1997; Gromovykh *et al*., 1998). It should be noted that biological disease control may be occurred by two ways: 1) direct action on a causal agent; and 2) enhancement of plant resistance by some elicitors (Ozeretskovskaya, 1994).

This paper will focus on the application of ecologically safe preparations based on useful microorganisms and their secondary metabolites for biological plant protection in Siberian region. From the very beginning of biocontrol development, it was assumed that vegetable and soft fruit crops were the most preferable for biological plant protection due to their dietary and medicine importance. The vegetables and the fruits are important dietary components supplying a ready source of vitamin C as well as other nutritionally important factors for Siberians. Therefore, the discussion will concern the application of biopreparations on these crops most of all. The preparations produced by Russian firms only will be described. The aim of this review is to demonstrate the advantage and disadvantage of this ecologically safe technology in Siberia.

Biopreparations for plant protection of cabbage

Cabbage is the most common vegetable crop grown in Siberia. Lepidopteran pest insects, such as beet webworm, *Pyrausta sticticalis*, cabbage moth, *Mamestra brassicae*, diamondback moth, *Plutella xyllostella* and the large white butterfly, *Pieris brassicae* are all able to completely eliminate yield. Fortunately, all these pests are susceptible to formulations based on entomopathogenic bacteria *Bt* of pathotype A (Cannon, 1996). According to this susceptibility, the high efficacy of Russian preparations Dendrobacillin®, Entobacterin® (based on *Bt* subsp. *galleriae)*, Lepidocide® (based on *Bt* subsp. *kurstaki*) was shown for control of *P. sticticalis, P. brassicae* and *P. xyllostella* (Shternshis, 1987a; Shulgina and Shternshis, 2004). The larvae of *M. brassicae* are less susceptible to *Bt* than the larvae of other insect species. For biological suppression of this insect, increased doses of *Bt*-formulations mentioned above are needed. In addition, the usage of β-exotoxin-containing *Bt*-preparations –Bitoxibazillin® and Bicol® (*Bt* subsp. *thuringiensis*) and a specific viral NPV-formulation Virin-EKS is successful (Shternshis, 1988; Shternshis *et al*., 1995). It should be noted that complete replacement of chemical insecticides with microbial preparations would create favorable conditions for the beneficial insects that help control not only lepidopteran insects but also cabbage aphid *Brevicoryne brassicae*. For example, if 20 larvae of predatory hover fly (Syrphidae family) per one plant is observed, no treatment against aphid is needed (Shternshis *et al*., 1995). In addition, the successful application of Phytoverm® against all Lepidopteran on cabbage was shown in Eastern Siberia (Kuznetsova and Shternshis, 2000). Phytoverm® (Pharmbiomed, Moscow), a metabolite-based preparation of the soil microorganism, *Streptomyces avermitilis*, was developed and registered in Russia (Berezina *et al*., 1997). This preparation contains a complex of natural metabolites which were extracted from biomass of *S. avermitilis*. Phytoverm® was also highly effective against cabbage aphid in Krasnoyarsk region (Kuznetsova and Shternshis, 2000). At last, turnip maggot, *Delia floralis* is a harmful insect in some regions of Eastern Siberia. The first experiments have shown high efficacy of biopreparation based on entomopathogenic nematode, *Steinernema feltiae* SRP-18-91, for the maggot control in Yakutia (Sleptsov *et al*., 2003).

As to cabbage diseases, black leg caused by complex of agents including *Olpidium brassicae*, *Rhizoctonia solani* and *Pythium debaryanum* is important for seedlings, and two bacterial diseases caused by *Erwinia carotovora* and *Xanthomonas campestris* are harmful in vegetation period. In the former case, the treatment of seeds with Trichodermin® was recommended, and in the latter case, spraying of plants followed by seed treatment with Planriz® (*Pseudomonas fluorescens*) was successful for suppression of cabbage bacteriosis (Grigorjev, 2001; Kuznetsova, 2003).

After all these treatments of cabbage, larval mortality fluctuated from 65 to 95% depending on insect species and environmental factors, black leg was eliminated completely and bacteriosis was decreased to insignificant level. So far, there are no suitable biopreparations against the most early insects – flea beetles. As to flea beetles, high limitation of chemical spraying and, sometimes, its complete elimination, is achieved with preliminary seeding of some plants, attractive for the beetles, near cabbage field. A narrow strip of Indian mustard along one or two sides of cabbage field attracts the flea beetles before cabbage planting, and thus, prevents insect damage to cabbage (Osintseva, 1997). The total technology described above shows that it is possible to obtain ecologically safe cabbage heads for dietary consumption using biopreparations as starting mechanism for pest control by natural enemies.

Biocontrol of vegetable pests in greenhouses

Cold climates and short growing seasons in Siberia cause the need for vegetable growing in greenhouses. Under these conditions, some species of aphids, the greenhouse whitefly, *Trialeurodes vaporariorum* and two-spotted spider mite, *Tetranychus urticae* are especially damaging to cucumber and tomato. These sucking pests are not susceptible to commercial *Bt* preparations with the exception of the β-exotoxin-containing preparations (Bitoxibacillin® or Bicol®). Therefore, fungal preparations against the sucking pests that are of great importance in greenhouses could be useful. As a rule, fungi require a high relative humidity for the infectious process, which is available under greenhouse conditions. The most common fungi used for insect and mite control belong to the genera *Beauveria, Metarhizium, Paecilomyces, Verticillium, Aschersonia, and Conidiobolus*. Treatment with suspensions of *Verticillium (Lecanicillium) lecanii* (Verticillin®), and *Beauveria bassiana* (Boverin®) has resulted in efficient control of aphids and whitefly (Ogarkov and Ogarkova, 2000). In addition, the spider mite is also susceptible to these fungi, especially *V. lecani*, although for efficient mite control, the concentration of fungal suspension should be increased 5-10 fold (Andreeva and Shternshis, 1995). *Conidiobolus thromboides* (representative of the phylum Zygomycota), isolated from diseased pea aphids in the Novosibirsk region (Makhova and Rakshaina, 1980), was used for development of preparation designated as Pyriformin. The results of Pyriformin tests demonstrated the potential of *C. thromboides* for control of *T. vaporariorum*, *Myzus persicae*, *Aphis gossypie*, *Macrosiphum euphorbia*, *Aulacorthum solani*, *Brevicoryne brassicae* in glasshouses (Lobanova *et al*., 1989). The advantage of this fungus is a low relative humidity requirement (about 60% RH). In addition, Phytoverm® was also shown to be very effective against main pests of crops under greenhouse condition. The mortality of *A. gossypii* and *A. solani* after 3 days of application with 0.2% Phytoverm® suspension was very high under the conditions of Central Siberian Botanical Garden (Novosibirsk) (Andreeva and Shternshis, 2005). The high mortality of spider mites occurred simultaneously with aphids, was also observed, but unstable result was obtained for the whiteflies control.

The general diseases of vegetables in Siberian greenhouses caused by *Fusarium oxysporum* f.sp. *lycopersici*, *Phytophthora infestans*, *Pythium debaryanum, Erysiphe cichoracearum* and *Sclerotinia scleotiorum* are controlled by preparations based on *T. viride, T. harzianum, Bacillus subtilis, P. fluorescens, P. aureofaciens* at more or less level. For example, the Fusarium wilt of tomato and root rots were successfully controlled by Trichodermin® in greenhouses of Novosibirsk, Krasnoyarsk and Irkutsk regions (Kolomnikova *et al*., 1990). Of very harmful organisms in Siberian greenhouses is the root-knot nematode *Meloidogyne incognita*. Many efforts were made by Siberian researchers to investigate the possibilities of application of preparation based on predatory fungus *Arthrobotrys oligospora* against this pest (Tepljakova, 1977; Gushchin and Tepljakova, 1985; Shternshis *et al*., 1999). In some cases, successful results were obtained, but the efficacy of this preparation depended on environmental factors very seriously. Therefore, in spite of considerable achievements of biocontrol under greenhouse conditions, it is no doubt that there are much more possibilities for spreading the use of biopreparations in Siberian greenhouses.

Biopreparations against pests on soft fruit crops

Soft fruits, such as black currant, red raspberry, strawberry, are the common crops in Siberia. Shortly after the successful application of biopreparations on vegetable crops, the attempts were made to use those preparations for pest control on soft fruit crops in Siberia (Prokofjev, 1987; Shternshis, 1988).

Black currant

As to black currants pests, all Lepidopteran insects including gooseberry pyralid moth, *Zophodia convolutella*, currant moth, *Abraxas grossulariata*, currant leaf roller, *Pandemis ribeana*, are susceptible to *Bt*-preparations. *Tetranychus urticae*, often damaged the black currant, would be controlled with *β*-exotoxin- based preparations as described above. Some successful attempts were made to control currant bud mite, *Cecidophyes ribis* Bitoxibazillin® (5 kg/ga) was applied during release of the mite from the buds coincided with black currant flowering, in this period, chemical pesticides are forbidden (Berger, unpublished data). Recent research showed that *Z. convolutella* was effectively suppressed with liquid preparation Lepidocide®, and both *Z. convolutella* and *T. urticae* are successfully controlled with Phytoverm® in Novosibirsk region (Vaskin and Shternshis, 2004). These treatments did not influence beneficial insects which helped to kill other pests.

Although there are some possibilities to use biopreparations against blackcurrant diseases, such as powdery mildew and septoriosis, a few reports on this subject are known. For example, the treatment of plants with *P. fluorescens*-formulations considerably reduced the severity of blackcurrant septoriosis (Ermakova and Shternshis, 1994).

Red raspberry

Red raspberry is also damaged by *T. urticae* and can be controlled by Bitoxibazillin®. However, the most harmful insect pest in Siberia is the raspberry cane midge *Resseliella theobaldi*. In 1990s preparation based on *Bt* subsp. *israelensis* pathogenic to Dipteran insects was successfully applied against rice midge (*Cricotopus silvestris*) (Kandybin *et al*., 1995). Because the cane midge is a member of the order Diptera, research was stimulated on field testing of commercial preparation Bacticide® (Sibbiopharm, Novosibirsk) based on *Bt* subsp. *israelensis* (Shternshis *et al*., 2002a). It should be noted that midge damage to the raspberry plants is usually associated with mycoses caused by *Didymella applanata*, *Botrytis cinerea* and some other plant pathogens. This syndrome is known as raspberry midge blight (Pitcher and Webb, 1952). Under field conditions, it is not possible to separate damage due to the cane midge from that due to fungi. Thus, the efficacy of Bacticide® was assessed by estimating the severity of midge blight. The data presented in Table 1 show that the reduction of midge blight severity by *Bt*-preparation was comparable to the reduction by the chemical insecticide Actellic®, the product traditionally applied on raspberry. In addition, Phytoverm® is considered to have potential for control of raspberry midge blight (Shternshis *et al*., 2002a). Experimental results showed significant reduction in midge blight severity by Phytoverm®.

Table 1. Effect of Bacticide® on raspberry midge blight severity.

Treatments	Concentrations of preparation, %	Midge blight severity (means of four replicates), %
Bacticide®	0.20	10.8*
Actellic®	0.20	9.8*
Control	0	20
LSD		3.8

*Significant at P< 0.05; LSD – the least significant difference

Based on model experiments which showed the efficacy of different species of entomopathogenic fungi against blackcurrant cane midge (Borisov and Goncharova, 1994), we conducted similar experiments to control the raspberry cane midge of Siberian population (Shternshis *et al*., 2005). In these experiments, the efficacy of the treatments with three fungal isolates belonging to genus *Beauveria* was estimated as percent of adults emerging from the soil. The results showed that only 1-3.5 % of adults emerged from the soil treated by all isolates studied, while 82% of adults emerged from the soil treated with water (control)[*].

Because the midge blight is able to be suppressed with biopreparations influenced the one member of this complex (insect), it seemed to be reasonable of application biopreparation influenced another member, such as *D. applanata*. Therefore, it was shown that treatment of raspberry canes with chitinase (Chi) (enzyme preparation of microbial origin) also was effective for suppression of raspberry midge blight (Shternshis *et al*., 2002a). Further laboratory and field experiments confirmed the ability of Chi to control *D. applanata* and independent raspberry spur blight caused by this fungi (Shternshis *et al*., 2004a). Data on the influence of chitinase on spur blight lesion development under the treatment with Chi are presented in Table 2. Chi was also shown to be effective for *Botrytis cinerea* control, as one more member of the raspberry midge blight (Shpatova *et al*., 2003). Furthermore, Phytoverm® significantly reduced spur blight (Shternshis *et al*., 2002a). This result suggests an influence of Phytoverm® on *D. applanata* that could be associated with dual effects of microbial metabolites (avermectins) against insects and fungi. The dual effect of microbial metabolites could be explained with its actions like elicitors as mentioned in introduction. Our data also pointed out to the possibility of Ketomium-mycofungicide® usage for control of raspberry spur blight (Shternshis *et al*., 2004b).

Table 2. Influence of chitinase on cane lesion due to spur blight development.

Treatments	**Area of lesion, cm^2, days after treatment (mean±SE)**	
	7	30
Chitinase	0,2±0,1	0,2±0,1
*Control	0,2±0,1	10,5±3,8
Untreated canes	0	1,6±1,6

*Control includes inoculation by the fungus lacking sprayed Chitinase.

Strawberry and sea-buckthorn

Some pests of strawberry important for Siberia were shown to be controlled by biopreparations. It is also *T. urticae* damaged other fruit soft crops described above. The more serious pest mite is the strawberry mite *Tarsonemus pallidus*. Of the same importance is the strawberry blossom weevil *Anthonomus rubi*. So far, the possibility of Bitoxibacillin® and Phytoverm® usage against all these pests have been shown in Novosibirsk region (own unpublished data). Also the successful attempts were made for control of the fungi *B. cinerea* and *Ramularia tulasnei* causing the most harmful diseases of strawberry in Siberia. For example, *T. viride* and *T. harzianum*-based biopreparations significantly decreased the disease severity with spraying of fungal suspension (Bokova, 1999). In addition, efficacy of Planriz® against *B. cinerea* has been shown (Grigorjev, 2001).

Sea-buckthorn is a valuable small fruit crop of dietary and pharmaceutical importance in Siberia. The sea-buckthorn fly *Rhagoletes batava* is the most harmful insect of this crop, especially in West Siberia. Control potential of *B. bassiana* against the soil dwelling stages of the sea-buckthorn fly was demonstrated. The introduction of *B. bassiana* into the soil beneath sea buckthorn bushes during the period of larval pupation was very effective in controlling this pest (Kalvysh, 1980). In Eastern Siberia, the sea-buckthorn moth, *Gelechia hippophaella* is known to damage this crop very significantly. As a lepidopteran insect, this pest was controlled by *Bt*-preparations, especially Bitoxibazillin® (Kalvish *et al.,* 1986). Therefore, only a few examples of application of biopreparations on this crop are known so far, although great necessity for biocontrol on the sea buckthorn occurs.

Overcoming obstacles to wide spreading of biopreparations

The merits of biological preparations for plant protection are well known. They concern high safety of biological agents for non-target organisms and environment and low probability of pest resistance development. Nevertheless, in Siberia (as all over the world), the use of biopreparations for protection of agricultural crops, especially vegetable and soft fruit ones, is not as widespread as desirable. Analysis of this situation reveals some explanations: relatively high dependence of bioagents on environmental factors; usually short shelf-life of biopreparations; narrow spectrum of host pest (the merit from ecological point of view and the demerit from the farmer position interested in economical profit); more variable efficacy and field stability than chemicals; biopreparations can be more expansive comparing with chemical pesticides.

Certainly, these obstacles have to be overcome. Research has been carried out using entomopathogens and relative biopreparations used in Siberia. Among the most harmful of abiotic factors which can cause undesirable changes in entomopathogens (and antagonistic microorganism as well) is solar radiation in the field (Shternshis, 2001). To avoid the destruction, additives are usually applied to protect the biocontrol agents (Couch, 2000). In our research, we consider solar damage to the entomopathogens during field application as a free radical process, therefore, antioxidants have been suggested as protectants (Shternshis and Gouli, 1985). For example, the antioxidant 2,4-dioxybenzophenon appeared to be the most appropriate for *Bt* subsp. *kurstaki* to increase resistance to UV-radiation (Shternshis and Solodova, 1989). These substances were also suggested for protection of *Bt* and NPV from some oxygen forms during storage (Shternshis, 1995b). Significantly prolonged shelf life was obtained for liquid formulation of viral

(Virin-EXS) and bacterial (Dendrobacillin®, LEST®) insecticides by introduction of antioxidants in low concentrations.

To overcome the narrow spectrum of activity of some biopreparations, especially viral ones, mixture with other biological agents is useful in some cases. For instance, in Thailand, combinations of viral preparation Spod-X with *Bt* achieved commercially acceptable levels of vegetable protection compared with virus alone (Kolodny-Hirsch *et al*., 1997). Although the aim of these authors was to improve the efficacy of NPV, other possible Lepidopteran insects on the crop could be controlled by that mixture. In Siberia, it was shown that to control all Lepidopteran insects on cabbage, the mixture of NPV-based Virin-EXS and *Bt*-preparation was useful to overcome very narrow spectrum of Virin-EXS activity (Spichenko *et al*., 1980; Shternshis, 1987a).

The main problem is an enhancement of the pest-killing capability of bioagent for effective suppression of target organism. Our approaches to this problem are based on an influence on the mechanism of interaction of the agent with its host. Some biochemical responses, such as intensification of lipid peroxidation of cell membranes has been demonstrated in insects under the influence of both *Bt* and baculoviruses (Shternshis, 1987b). The products of lipid peroxidation (free radical process) have been shown to accumulate in midgut and fat body cells of some lepidopteran species after treatment with *Bt* or NPV. In order to strengthen the efficacy of this process, some initiators of free radical formation were added to bacterial and virus preparations. For example, the addition of low concentrations of a known initiator ($FeSO_4$), to *M. brassicae* NPV resulted in significant enhancement of insect mortality (Shternshis, 1995b).

A common approach for enhancement of insecticidal activity of both bacterial and viral biocontrol agents is to increase the permeability of host insect cell membranes. This can be achieved by the addition of dimethylsulfoxide (DMSO). DMSO is a well known non-toxic compound that is capable of penetrating cell membranes, and thereby facilitating the penetration and movement of other biologically active agents to their targets in living organisms (Knubovez *et al*., 1987). DMSO has been demonstrated to serve as an enhancer of insecticidal activity of NPV and *Bt* (Shternshis, 1995b; Shternshis and Gouli, 2000). One more aspect of this approach is the exogenous application of enzymes, which are capable of damaging insect peritrophic membranes (PM). Addition of exogenous chitinase led to destruction of chitin of the PM and thereby increase the permeation of the *Bt* preparation. Our attempts were made to test this approach on such biocontrol agent, as *Bt*, baculoviruses and entomopathogenic fungi.

We used the same enzyme preparation (Chi) as described above for raspberry disease control but at a very low concentration. The addition of Chi to *Bt* subsp. *sotto* doubled the mortality of *P. sticticalis*. Also, the insecticidal activity of two GV (of *P. sticticalis* and *Cydia pomonella)* and NPV of *M. brassicae* was significantly increased by the addition of the enzyme (Duzhak *et al*., 1995). These results showed the possibility of reducing the doses of baculoviruses by the addition of a very low amount of exogenous Chi. This observation has served as the basis for development of improved viral formulations. Such improved formulations were successful in field tests against *C. pomonella* and *M. brassicae* in Novosibirsk region. However, when *B. bassiana* and *V. lecanii* with enzyme were tested, contradictory data were obtained that pointed to complexity in interactions between exogenous chitinase and an endogenous fungal one. Experiments showed inhibition of the fungal growth in the presence of Chi. Thus, the specific effect of chitinase depended on the nature of both the insect, and the biocontrol agent as well. It should be noted that data concerning the influence of Chi on the efficacy of entomopathogens and their metabolites have been published by different authors. Recently, Thamthiankul *et al*. (2004) reported the results showed that chitinase was able to increase activity of *Bt* subsp. *aizawa* against *Spodoptera exigua*. Using scanning electron microscope, the authors observed clear perforations in PM of larvae incubated with chitinase. They confirmed that the addition of chitinase, which enhances penetration of the PM by entomopathogens, could also enhance their insecticidal effects by destroying the structure of the PM. An enhancer effect of the enzyme on the activity of cloned Cry protein was reported by Regev *et al*. (1996). Therefore, PM destruction with Chi may explain the universal action of this enzyme in providing an enhancer effect on the activity of separate NPV and *Bt*. This effect was expressed in the increased insect mortality without any toxic effect of the chitinase alone. At the same time, the prolonged period of entomopathogen activity was significantly reduced. The above-mentioned data regarding synergism between *Bt* and NPV together with enhancers served as an basis for development of a triple mixture for control of all lepidopteran insects on cabbage (Shternshis *et al*., 2002b).

As already noted, the most harmful of these pests are the larvae of the cabbage moth, diamondback moth, and large white butterfly for cabbage in Siberia. Moreover, all of these species are frequently found in fields simultaneously. In Siberia, the populations of cabbage moth are less susceptible to the different *Bt* subspecies than the populations of large white butterfly or diamondback moth. At the same time, the *M. brassicae* NPV (MbNPV) appeared to be an effective biological agent against the most harmful cabbage pest, the cabbage moth, but not for other Lepidoptera. Despite the merits of the NPV, such as high specificity and transgenerational transmission, the infection by this entomopathogen requires a latent period of up to 10 days. This disadvantage could be overcome partly by the addition of *Bt*, or enhancers to the NPV. Therefore, the development of a mixture containing *Bt*, MbNPV and certain enhancers for biocontrol of all lepidopteran pests of cabbage could increase control efficacy of these pests.

The addition of Chi to the mixture of *Bt* and NPV was proposed in order to develop the optimum composition of these three ingredients for improved microbial control of all Lepidopteran pests on cabbage. The formulation the original mixture of *Bt* subsp. *galleriae*, MbNPV, and Chi to control lepidopteran pests on infested cabbage in the field was suggested. Field trials were conducted on cabbage plots infested with the three major species of lepidopteran pests simultaneously. The results demonstrated the efficacy of the mixture against such a complex of lepidopteran pests. The mortality of the larvae varied from 74.1 to 80.4 percent depending on the insect species. Furthermore, the addition of the Chi to the *Bt* and NPV composition provided the acceleration of insect mortality at a considerably reduced concentration of entomopathogens. The composition consisted of a 5-fold lower amount of *Bt* and 10-fold lower amount of polyhedra per ml compared with the values recommended for the standard commercial formulations.

Improvement of insect control may be realized in two ways: either as tank mixtures of biopreparation, enhancers and protectors immediately before application, or by developing a formulation containing all these components. In 1950s, tank mixtures of commercial biological preparations with sublethal dosages of chemical insecticides were suggested (Telenga, 1956). Some demerits of this concept have been discussed (Shternshis, 2004). Because of these demerits, tank mixtures of biopreparations may be composed by replacement of chemical pesticides with non-toxic enhancers, such as DMSO or Chi. As a rule, plant growers prefer formulations which include all necessary ingredients instead of tank mixtures. Partly, our research was devoted to development of formulations, which are designed in accordance with principles of enhancement of biopreparation. Some formulations were developed for this purpose (Shternshis, 1995a). A liquid *Bt*-formulation containing DMSO and an antioxidant was characterized by high insecticidal activity together with an increased shelf life (Shternshis *et al*., 1981.) A solid formulation based on *Bt* subsp. *kurstaki* was designated as LEST® (Shternshis and Zurabova, 1987). This formulation also contained an enhancer and an antioxidant that facilitated a reduced dose of *Bt,* and better persistence during storage and application. The improvement of the formulation based on *P.*

fluorescens was made as well (Ermakova and Shternshis, 1994). In this case, replacement of liquid formulation suggested for Planriz® to solid ceolite – containing RIZ® led to prolongation of shelf-life from 2 weeks to 1 year. It is also well known that quality of formulation is closely connected with the techniques for application of biopreparation. For instance, thorough treatment of the leaf surface is indispensable condition for better consumption of entomopathogen during feeding by lepidopteran insects. In order to enhance such a treatment, joint efforts of researchers from several Institutes of Siberian Branch of Russian Academy of Sciences resulted in aerosol technology for biopreparations application (Kirov *et al*., 1988). This technology provided high efficacy of bacterial and viral insecticides and of its mixture against Lepidopteran insects on cabbage. The advances consist of thorough distribution of agents on the leaf surface, sharp shortening of treatment timing and some decrease of biocontrol agent doses.

Finally, sometimes, the plant itself or its allelochemicals can influence negatively on the efficacy of biopreparations. Gukasjan (1958) and Poltev and Peshcherskaya (1967) were among the first to focus on this problem in Siberia. They showed that efficacy of *Bt* against insect pests varied with different species of coniferous and deciduous trees. Plant volatiles and other allelochemicals may inactivate agents for control of the insect or plant disease. With regard to entomopathogenic fungi, volatiles of bird cherry and long-rooted onion were demonstrated to sharply reduce the viability of *B. bassiana* in Eastern Siberia (Gromovykh, 1982). It was also shown that the mortality of beet webworm and spider mite was depended on the host plant (Shternshis *et al*., 2004c). The maximum larvae mortality under the treatment with *Bt* subsp. *kurstaki* -based formulation was observed on alfalfa. In order to achieve such efficacy on carrot, sharp increase of dosage against *P. sticticalis* was necessary. The mortality of the spider mite under the influence of *L. lecani* was much more on cucumber than on strawberry. Certainly, the impact of plant allelochemicals is needed to take into account in order to achieve good results concerning the application of biopreparations.

Conclusion

Crops grown in Siberia, require ecologically safe pest control, including application of biopreparations. Particularly, it concerns vegetable and berry crops to avoid chemical residues in fresh fruits as dietary food most of all. The use of ecologically safe agents for pest control promotes the biodiversity of other natural enemies useful for insect and plant disease control. Although biopreparations are started for Siberian crop protection several decades ago, in some cases, application is still in its infancy. Some observed disadvantages in the usage of biopreparations could be reduced by enhancing their potency with one or more additives. However, to provide environmental safety, the mixture of microbial agents with chemical pesticides should be avoided, even at sublethal dosages of the latter, because of accumulation of chemicals in the environment and increase the probability of development of insect resistance to these compounds. Mixtures combining biocontrol agents with low concentrations of ecologically friendly components are more preferable to enhance biocontrol activity. More research is required concerning the development of suitable mixtures including one biocontrol agent for insect control and another one for disease control to provide integrated pest management.

In some cases, the microbial metabolite preparations have some advantages over living organism-based preparations. First, metabolites are less susceptible to environmental factors, such as temperature, humidity, UV-radiation, *etc.* Furthermore, shelf life is typically longer for metabolites than for propagules. Metabolite preparations also appear to have wider spectrum and quicker action. At the same time, metabolite-based preparations are environmentally safe and are not subject to accumulation in fruits as compared with synthetic pesticides. In addition, dual properties of these products concerning both insect and disease control observed in some cases, are rather valuable for plant protection. Another aspect of enhancement of insecticidal activity is protection of control agents from the negative effect of some environmental factors, especially UV-radiation. The selection of a suitable protectant provided an enhancement of biocontrol activity. Enhancement for biopreparation activity may be realized not only as a mixture which is produced just before spraying, but as an improved formulation with enhancers. At last, the influence of plant or its allelochemicals should be taken into account while application of biopreparations on different crops. Recent research in this direction pointed out the importance of tritrophic interaction for determination of the most relevant agent and its exact dosage.

References

Andreeva, I.V. and Shternshis, M.V. (1995). Microbial preparations for spider mite control in greenhouses. Plant Protection (Moscow), 11: 41.

Andreeva, I.V. and Shternshis, M.V. (2005). The efficacy of Phytoverm against sucking pests in greenhouses. Siberian Bulletin of Agricultural Sciences (in press).

Beresina, N.V., Chizhov, V.N., Drinyaev, V.A., and Jurkiv, V.A. (1997).Perspectives of avermectin-containing formulations for insect pests control. Gavrish (Moscow), 2: 10.

Bokova, J.V. (1999). Biopreparations for pest control on strawberry. In: Actual problems of agroecology in integrated pest management, Penza (ed. A.I.Ivanov), 29.

Borisov, B.A., and Goncharova, N.G. (1994). The efficiency of entomopathogenic fungi against the black currant pests. In: The Ecologically Safe and Pesticide-Free Technologies of Plant Growing Production Obtaining, Proceedings of All-Russian Conference, Pushchino, 103.

Cannon, R.J.C. (1996). *Bacillus thuringiensis* use in agriculture: a molecular perspective. Biological Review, 71: 561.

Couch, T.L. (2000). Industrial fermentation of entomopathogenic bacteria. In: Entomopathogenic Bacteria: From Laboratory to Field Application, (ed. J.-F. Charles, A.R.M. Delecluse, and C.A. Nielsen-LeRoux), Kluwer Academic Publisher, The Netherlands, 297.

Duzhak, A.B., Salganik, R.I., Shternshis, M.V., Ermakova, N.I., Tsvetkova, V.P., Andreeva I.V., and Kushnikova T.A. (1995). The influence of chitinase enzyme on the activity of biopreparations. Siberian Journal of Ecology, 5: 457.

Ermakova, N.I., and Shternshis, M.V. (1987). The infectivity of baculoviruses isolated from *Pyrusta sticticalis.* Agricultural Biology, 4: 34.

Ermakova, N.I. and Shternshis, M.V. (1994). New formulation RIZ for plant disease control. Plant Protection (Moscow), 12: 18.

Gouli, V.V., Shternshis, M.V., and Koltunov, E.V. (1976). Perspectives of viral control of insect pests. Siberian Bulletin of Agricultural Science, 5: 36.

Grigorjev, V.N. (2001). Stages of the development. Plant Protection and Quarantine (Moscow), 12: 8.

Gromovykh, T.I. (1982). Entomopathogenic fungi in Forest Protection, Nauka, Novosibirsk.

Gromovykh, T.I., Gukasjan, V.M., Golovanova, T.I., and Shmarlovskaya, S.V. (1998). *Trichoderma harzianum* Rifai as a factor for enhancement of tomato resistance to root rot. Mycology and Phytopathology, 3 2: 72.

Gukasyan, A.B. (1958). Bacterial control of insect pests. Bulletin of Siberian Branch of the Academy of Sciences of the USSR, 7: 21.

Gushchin, F. L. and Tepljakova, T.V. (1985). Interaction between predatory fungi and soil microbial antagonists. In: Integrated pest and disease control in Siberia, (ed.V.V.Gouli), 158

Kalvysh, T.K. (1980). The influence of soil fungistasis on the muscardine fungi. Bulletin of Siberian Branch of the Academy of Sciences of the USSR, 3: 8.

Kalvish, T.K., Sanjimitupova, R.,D., Sharapov, V.M. and Khodyrev, V.P.(1986). Fungi for the sea buck thorn control. In: Microbial Pest Management, Novosibirsk, 110.

Kandybin, N.V., Ermolova, V.P. and Smirnov, O.V. (1995). Bactoculicide spectrum of activity. Plant Protection, 5: 32.

Kirov, E.I., Makarov, V.M., Kutsenogii, K.P., Khodyrev V.P., Larionov, S.V. Shternshis M.V., Osintseva, L.A. Zaguljaev, G.N., Oskolkov, D.P. and Fidosov, N.I. (1988.) Aerosole application biopreparations against cabbage pests (ed. M.V.Shternshis). Novosibirsk

Knubovez, T.L., Zinchenko, V.D., and Sabeldina, L.A. (1987). The effect of dimethylsulfoxide on cell membrane measured with NMR- method. Biological Membranes (Moscow), 4: 84.

Kolodny-Hirsch, D.M., Sitchawat, T., Jansiri, T., Chenrchaivachirakul, A. and Ketunuti, U. (1997). Field evaluation of a commercial formulation of the *Spodoptera exigua* nuclear polyhedrosis virus for control of beet armyworm on vegetable crops in Thailand. Biocontrol Science and Technology, 7: 475.

Kolomnikova, V.I. (1988). Disease biocontrol on vegetable crops in greenhouses. In: The first Bulgarian-Russian Symposium on microbial pesticides, Plovdiv, Bulgaria, 24-26 October 1988, 71.

Kolomnikova, V.I., Kostyleva, E.V., Kosulnikova, L.P. and Novikova, A.G. (1990). Application technology of *Trichoderma*-based preparations against tomato wilt. In: Agroecological problems of crop protection in Siberia and Kasakhstan, 93.

Kuznetsova, I.A., and Shternshis, M.V.(2000). Biological suppression of phytophagous insects on cabbage in Krasnoyarsk region. Siberian Bulletin of Agricultural Sciences, 3: 50.

Kuznetsova, I.A. (2003). Biopreparations for cabbage protection against pests under the condition of Krasnoyarsk, PhD Thesis, Novosibirsk State Agrarian University.

Lobanova, N.V., Rakshaina, M.T., and Shternshis M.V. (1989). Production and Application of Pyriformin, Siberian Branch of Academy of Agricultural Sciences, Novosibirsk.

Makhova, N.M., and Rakshaina, M.T.(1980). Fungi of Entomophthorales for the sucking insects control. Bulletin of Siberian Branch of the Academy of Sciences of the USSR, 3: 107.

Ogarkov, B.N., and Ogarkova, G.R. (2000) Entomopathogenic Fungi of Eastern Siberia, State University, Irkutsk.

Osintseva, L.A. (1997). Increasing plant diversity for cabbage protection against flea beetles. Agricultural Biology, 1: 95.

Ozeretskovskaya, O.L. (1994). Induced resistance of plants against phytopathogens effected by elicitors. Applied Biochemistry and Microbiology, 30: 325.

Pitcher, R., S., and Webb, P.S. (1952). Observations on the raspberry cane midge *Thomasiniana theobaldi* . 1. Biology. Journal of Horticultural Sciences. 2: 95.

Poltev, V.I. and Peshcherskaya G.L. (1967). The effect of phytoncides on entomopathogens. In: Microorganisms and Green Plant, Nauka, Novosibirsk, 67.

Prokofjev, M.A. (1987). Insect control of fruit crops in Siberia, Agropromisdat, Moscow.

Regev, A., Keller, M., Strizhov, N., Sneh, B., Prudovsky, E., Chet, I., Ginzberg, I., Koncz-Kalman, Z., Koncz, C.,Schell, I., and Zilberstein, A. (1996). Synergistic activity of a *Bacillus thuringiensis* endotoxin and a bacterial endochitinase against *Spodoptera littoralis* larvae. Applied and Environmental Microbiology, 62: 3581.

Shpatova, T.V., Shternshis, M.V. and Beljaev, A.A. (2003). Evaluation of the effects of biological preparations on phytopathogenic fungi *Didymella applanata* and *Botrytis cinerea.* Applied Biochemistry and Microbiology, 1: 36.

Shternshis, M.V., Gouli, V.V.,Ivanov, G.M., Pakhtuev, A.I., Rakshaina, M.T., Koltunov, E.V., Spichenko, L.N.(1981). The prolongation of activity of bacterial preparations under storage conditions. In: Microorganisms in Plant Protection. Novosibirsk, 84.

Shternshis, M.V. and Gouli, V.V.(1985). Antioxidants as ingredients of entomopathogenic formulations. Bulletin EPS IOBC, 10: 43.

Shternshis, M.V. (1987a). Microbial insect control under the intensive crop cultivation technology. Bulletin of Agricultural Science (Moscow), 9, 54.

Shternshis, M.V. (1987b). The principles of intensification of microbiological insect control. Mededelingen Faculteit Landbouwwetenschappen Rijks Universiteit Gent, 52: 395.

Shternshis, M.V. and Zurabova, E.R. (1987). New formulation of Lepidocde. Plant Protection (Moscow), 9, 39.

Shternshis, M.V.(1988). Microbial insect control in Siberia and Far East, Agropromisdat, Moscow.

Shternshis, M.V., and Solodova, K.V.(1989). Search of effective antioxidant for protection of bacterial entomopathogen. In: All-Union Conference on Bioantioxidant, Institute of Chemical Physics, Moscow, 100.

Shternshis, M.V. (1990). Factors of optimisation of microbial insecticides. Biotechnology (Bulgaria), 3: 62.

Shternshis, M.V. (1995a). Viral entomopathogenic formulations. Plant Protection (Moscow), 9: 29.
Shternshis, M.V. (1995b). Enhancement of Microbial Insect Control., State Agrarian University, Novosibirsk.
Shternshis, M.V., Tsvetkova, V.P. and Tomilova, O.G. (1995). Insect control on cabbage in West Siberia. In: The ecologically safe plant-growing output production (eds. M. S. Sokolov and E.P.Ugrjumov), Pushchino, 195.
Shternshis, M.V., Ermakova, N.I., and Tsvetkova, V.P. (1997). Natural ceolites as carriers for viral entomopathogenic formulations. Siberian Journal of Ecology, 6: 585.
Shternshis, M.V., Sorokoletov, O.V. and Bedin, D.P. (1999). Zoohumus as a substrate for preparations based on the predatory fungi. In: Biotechnology of organic waste processing and ecological problems (eds. I.I. Gudilin and A.F. Kondratov), Novosibirsk, 347.
Shternshis, M.V., and Gouli, V.V. (2000). Dimethylsulfoxide as an enhancer of *Bacillus thuringiensis.* In: XXXIII Society for Invertebrate Pathology. Annual Meeting, University Guanajuato, Mexico, 89.
Shternshis, M.V. (2001). Biological control of insect populations. In: Insect Pathogens: Structural and Functional Aspects (ed. V.V.Glupov), Publishing House All The Year Round, Moscow, 562.
Shternshis, M.V. (2002). Biopreparations based on microbial metabolites. Plant Protection and Quarantine (Moscow), 9: 18.
Shternshis, M.V., Beljaev, A.A.,Shpatova, T.V., Bokova, J.V., and Duzhak, A.B. (2002a). Field testing of Bacticide, Phytoverm and Chitinase for control of raspberry midge blight in Siberia. BioControl, 47: 697.
Shternshis, M.V., Ovchinnikova L.A., Duzhak, A.B., and Tomilova, O.G. (2002b). The efficiency of vial and bacterial entomopathogen formulated with chitinase for biocontrol of Lepidopteran cabbage pests. Archives of Phytopathology and Plant Protection, 35: 161.
Shternshis, M.V. (2004). Ecologically safe insect pest control: The past, the present and the future. In: Emerging Concepts in Plant Health Management (eds. R. Lartey and A. Caesar), Research Signpost, Kerala, 187.
Shternshi*s,* M.V.*,* Beljaev, A.A., Shpatova, T.V., Duzhak, A.A. and Panfilova, Z.I. (2004a). Chitinase as a control agent of *Didymella applanata* causing the raspberry spur blight. IOBC/WPRS Bulletin, 27: 67.
Shternshis, M., Shpatova, T, Tomilova, O., and Soytong, K. (2004b). Evaluation of Ketomium-mycofungicide on Siberian isolates of phytopathogenic fungi. In: Proceeding of the 1st KMITL International Conference on Integration of Science & Technology for Sustainable Development (eds. .K.Hyde and K.Soytong), Bangkok, Thailand, 25-26 August. 2004, 272.
Shternshis, M.V., Andreeva, I.V. and Tomilova, O.G. (2004c). The influence of plant on interaction between entomopathogens and their phytophagous hosts. In: Siberian Zoological Conference, Abstracts,15-22 September 2004, Novosibirsk, 417.
Shternshis, M.V., Shpatova, T.V., Borisov, B.A., and Beljaev, A.A. (2005). The effect of entomopathogenic hyphomycetes and Phytoverm on raspberry cane midge, and the spur blight causal agent. Mycology and Phytopathology , 39: 68.
Shulgina, O.A. and Shternshis, M.V. (2004). Application of Lepidocide for cabbage insect control in Kemerovo region. Siberian Bulletin of Agricultural Science 1: 39.
Sleptsov , S.S., Vlasenko, N.G, .and Stepanov, A.I. (2003). The efficiency of biological pest control on cabbage. Agro XXI, 7: 61.

Spichenko, L.A., Gouli, V.V., and Krupko, L.A. (1980). Application of bacterial and viral formulations against Lepidopteran. In: Biological Control of Pest Organisms, (ed. V.V.Gouli.), Siberian Branch of the Academy of Agricultural Sciences), Novosibirsk, 16.

Talalaev, E.V. (1959). Bacterial control of Siberian silkworm. In:Transactions of the First International Conference on Insect Pathology and Biological Control, Prague, 1958, 51.

Telenga, N.A. (1956). The enhancement of beet weevil muscardinosis with aid of hexachlorine. Proceedings of the Academy of Sciences of the USSR, 109: 665.

Tepljakova, T.V. (1977). Perspectives of predatory fungi usage in the middle Ob-river region. In: Microbial Pest Control (ed.V.V.Gouli), Novosibirsk, 43.

Thamthiankul, S., Moar, W.J., Miller M.E., and Panbangred, W. (2004). Improving the insecticidal activity of *Bacillus thuringiensis* subsp. *aizawa* against *Spodoptera exigua* by chromosomal expression of a chitinase gene. Applied Microbiology and Biotechnology, 65: 183.

Tulpanova,V.A., Gromovykh, T.I., Malinovsky, A.L., Kozlova, T.L., Arefjeva, I.A. and Shipilova, I.A. (1997). Biotechnology of new fungal formulation for plant protection. Siberian Journal of Ecology, 5: 501.

Vaskin, M.A. and Shternshis, M.V. (2004). Biological control of insect pests on black currant. In: Biological Plant Protection as a Basis for Stabilizing Agroecosystems (eds. V.Nadykta, V.Ismailov, G.Levashova and E.Sugonyaev), Krasnodar, 251.

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