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## Biotechnological aspects of sawflies number control in blackcurrant plantations

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**Abstract.** The study aimed to compare the effectiveness of biotechnological methods of controlling the *Pristiphora rufipes* sawfly population on black currant (*Ribes nigrum*) in the forest-steppe zone of Ukraine and to identify environmental factors affecting effectiveness. The study was conducted using 20 plots (0.1 hectares each) divided into four groups: treatment with the biological product *Bacillus thuringiensis*, entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*), a combination of fungi with pheromone traps and control without treatment. The effectiveness was

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assessed through weekly monitoring of pest numbers, the degree of leaf damage according to the scale of the European and Mediterranean Plant Protection Organisations, and analysis of climatic parameters. The integrated group demonstrated the highest efficiency: the number of larvae decreased by  $85 \pm 4\%$  ( $p < 0.001$ ), adults by  $60 \pm 6\%$ , and the average score of leaf damage was  $1.2 \pm 0.1$  against  $4.1 \pm 0.3$  in the control. The *Bacillus thuringiensis* group showed a  $72 \pm 5\%$  reduction in larvae ( $p < 0.001$ ) with stability to temperatures of  $15-25^\circ\text{C}$ , but the effectiveness dropped to 65% after 20 days. The fungi reduced the population by  $65 \pm 7\%$  ( $p < 0.001$ ), but effect was halved in the drought. The control group recorded a population growth of  $25 \pm 3\%$ . Regression analysis showed that the integrated approach explained 89% of the variance ( $R^2 = 0.89$ ) in pest reduction. A comparative analysis with the Kyrgyzstan *Ribes* species (*Ribes meyeri*, *Ribes saxatile*) indicated the potential of using phytochemical adaptations for breeding resistant varieties. The results demonstrated that the combination of biological products with pheromones is the optimal strategy to reduce dependence on chemical insecticides in the forest-steppe zone of Ukraine, incorporating local climatic conditions (temperature, humidity) and biological characteristics of the pest. The study has practical implications for the development of sustainable berry crop protection systems

**Keywords:** pests; larvae; imago; pheromone traps; entomopathogenic fungi

## INTRODUCTION

Agriculture is facing growing environmental and economic challenges related to the protection of crops from pests. This problem is especially relevant in the context of growing black currants (*Ribes nigrum*) in the forest-steppe zone of Ukraine, where the massive spread of *Pristiphora rufipes* larvae leads to crop losses. In addition to environmental risks, the traditional use of chemical insecticides is often ineffective due to the development of resistance in pest populations and restrictions imposed by EU and Ukrainian legislation on the use of toxic substances in food crops. The growing public demand for environmentally friendly food and growing technologies further raises the issue of developing alternative protection methods (Mushtruk & Mushtruk, 2023).

In this regard, there is a growing interest in biotechnological approaches that combine effective pest control with minimal environmental impact. The forest-steppe zone of Ukraine is characterised by significant seasonal fluctuations in temperature and humidity, which affect the viability of biocontrol agents (Hradchenko & Pikoyskyi, 2023). For the period 2020-2025, there is limited research on the interaction between biological control methods and local climatic factors, including the duration of high-humidity periods critical for the sporulation of entomopathogenic fungi, as well as temperature stresses that can reduce the effectiveness of pheromone traps. In the context of agroecosystems, the contributions of L.G. Honenko (2021) and M.O. Bilyk (2022), who systematised the principles of integrated plant protection using biological methods and environmental technologies. Studies, particularly the works of M. Alemu (2020), P. Kumari *et al.* (2022), highlight the prospects of using biological agents, in particular entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*) and pheromone technologies, to control insect pest populations. For example, S. Singh *et al.* (2020) proved the effectiveness of protease inhibitors in suppressing larval development, while

F. Hernández-Rosas *et al.* (2020) analyse the potential of symbiotic microorganisms as biocontrol agents. The existing literature, in particular the works of L. Aguilar-Marcelino *et al.* (2020), also indicates the prospects of combined approaches, such as the synergistic use of entomopathogenic fungi with pheromone technologies.

International experience also confirms the need to incorporate regional specifics. For example, the study by A.M. Botha *et al.* (2020) on integrated pest management strategies in tropical Africa demonstrates the high dependence of the effectiveness of biomethods on the local microclimate. Similar conclusions were drawn by H. Askary *et al.* (2021) in the context of the use of *Hypochoeris* fungi in Iran. These data emphasise the need to develop specific biocontrol schemes for each natural and climatic zone. The prospects for using the natural resistance of *Ribes* species are also an extremely important area. For instance, in Kyrgyzstan, *Ribes meyeri* and *Ribes saxatile* species demonstrate high natural resistance to pests due to the high content of phenolic compounds, which limits the development of *Pristiphora* larvae, according to G.N. Kalykova *et al.* (2022). This highlighted opportunity for breeding new blackcurrant varieties with increased pest resistance. At the same time, such resistance mechanisms have not yet been integrated into biocontrol programmes for *Ribes nigrum* plantations in Ukraine, which creates prospects for further research.

The recommendations of the authors considered are mainly formulated for cereal crops and need to be adapted to the specific features of the agrobiocenosis of blackcurrant plantations. At the same time, most of these studies were conducted in laboratory conditions or in regions with stable climatic characteristics, which limits extrapolation to other conditions. Furthermore, the practical implementation of such schemes in the field conditions of the forest-steppe zone requires empirical verification. In particular, the following issues remain unresolved: the influence of local climatic

factors, especially moisture and temperature stresses, on the activity and viability of entomopathogenic fungi; the synergy between different biological agents in the context of natural biodiversity in agroecosystems; the comparative effectiveness of integrated control methods for cultivated varieties of *Ribes nigrum* and naturally resistant species of *Ribes*. The study aimed to evaluate the effectiveness of the integrated use of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*), a biopreparation based on *Bacillus thuringiensis*, and pheromone traps for controlling *Pristiphora rufipes* populations in blackcurrant plantations in the forest-steppe zone of Ukraine.

## MATERIALS AND METHODS

The study was conducted during 2023-2024 on experimental blackcurrant (*Ribes nigrum*) plantations located in the Bila Tserkva Dendropark "Oleksandriia" (Kyiv region, Ukraine), which is a scientific institution of the National Academy of Sciences of Ukraine. This location was chosen because of its typical climatic conditions for the forest-steppe zone of Ukraine: average annual temperature of +8°C, annual precipitation of 550 mm, loamy soils with a pH of 6.2-6.5. The plots are located 100 metres away from forests and other agricultural land to minimise environmental impacts. The objects studied were larvae and adults of the sawfly *Pristiphora rufipes*, as well as black currant bushes of the Sofiyivska variety. The sample consisted of 20 plots of 0.1 ha each, randomly selected from a total plantation area of 5 ha, with a distance of 20 m from the plantation edges to avoid the "edge effect". The inclusion criteria for the plots covered three main requirements. Firstly, the population density of *Pristiphora rufipes* had to be at least 10 individuals per bush, as determined by a preliminary 7-day visual monitoring. Secondly, the bushes had to be 4-5 years old, as confirmed by the farm's documentation, as this is when peak vegetative mass is reached, therefore most attractive to the pest. Thirdly, the soils in the plots had to be loamy with a pH of 6.2-6.5, which was checked with a portable pH meter HI98128 (Italy). Plots with signs of disease (e.g. powdery mildew), damage by other pests or soil anomalies were excluded.

The study was based on the division of blackcurrant plots into four groups with different treatment methods to compare effectiveness. The first group (*Bacillus thuringiensis*) received a biological product based on *Bacillus thuringiensis* (strain HD-1 Ukraine) at a concentration of 0.2%. This strain was chosen because of its specific effect on insect larvae and safety for beneficial entomophages. The working solution was prepared according to the manufacturer's recommendations and applied by fine-drop irrigation using an AgroSpray-B (Israel) sprayer, which ensured uniform distribution of the product. The second group (fungi) was treated with suspensions of entomopathogenic fungi *Beauveria bassiana* (strain Bb-15) and *Metarhizium anisopliae* (strain

Ma-43). The density of spores in the suspension was  $10^8$ /ml, which provided a sufficient infectious load for *Pristiphora rufipes* larvae. The third group (integrated) combined fungal suspensions with pheromone traps "Pherocon VI" (USA), which contained a synthetic analogue of the secretion of female sawflies to attract and catch males. Pheromone traps were set at 10 m intervals (5 units per plot) and replaced every 14 days. The fourth group served as a control without any treatments, which made it possible to assess the natural dynamics of the pest population.

The protocol included two treatments per season: the first at the budding stage (April), when the larvae become active, and the second after flowering (June) to control the second generation. The effectiveness of the methods was assessed through weekly monitoring of pest numbers. Weekly monitoring of pest abundance was conducted by the method of squares (1×1 m), collecting larvae and adults from 10 randomly selected bushes on the plot. Larvae and adults of *Pristiphora rufipes* were manually collected from each bush using entomological tweezers with soft tips. To identify the sex of adults, a Micromed-3 microscope (China) with a digital camera was used. Leaf damage was assessed on a 5-point scale of the European and Mediterranean Plant Protection Organisation (2021) (EPPO), where 1 point meant no damage, 2 points of damage ≤10% of the leaf surface, 3 points 11-30%, 4 points 31-60%, 5 points ≥61% or destruction. At each site, 100 leaves were analysed, taken from different tiers of the bush (lower, middle, upper) to consider the vertical distribution of pests. Climatic parameters (temperature, humidity) were recorded by EcoClimate Pro sensors (USA) with an accuracy of ±0.5°C, installed at a height of 1 m above the soil level in the centre of each plot.

To analyse the potential of biocontrol in more depth, a comparative study was conducted with wild species of *Ribes* (in particular, *Ribes meyeri* and *Ribes saxatile*) growing in the mountainous regions of Kyrgyzstan. This region was chosen because of the presence of subendemic forms with natural resistance to pests, as well as the similarity of some climatic indicators (continentality, seasonal temperature amplitude), which extrapolated adaptive mechanisms to the conditions of the Ukrainian forest-steppe. The use of preparations complies with the requirements of Regulation of the European Parliament and the Council No. 1107/2009 "Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC" (2009). The data were processed in the R software (version 4.2.1). Student's t-test was used to compare groups, analysis of variance (ANOVA) was used to assess the impact of climate factors, and regression analysis was used to identify correlations. The level of statistical significance was set at  $p < 0.05$ . All treatments were carried out under the same weather conditions (no precipitation for 24 hours).

## RESULTS AND DISCUSSION

**Dynamics of the number of pests in blackcurrant plantations.** The use of biotechnological methods has demonstrated significant efficiency in controlling the *Pristiphora rufipes* population. The most pronounced result was observed in the integrated group, where fungal suspensions and pheromone traps were combined. Already 14 days after the first treatment, the number of larvae in this group decreased by  $85 \pm 4\%$  ( $p < 0.001$ ), and the number of adults (adults) decreased by  $60 \pm 6\%$  due to mechanical trapping with pheromone traps "Pherocon VI". This is explained by the pronounced synergistic effect between the two methods: entomopathogenic fungi directly affected larvae, reducing survival, while traps reduced the number of fertilised females, thereby interrupting the life cycle of the population. There was also a tendency to gradually reduce repeated waves of pest development, indicating the cumulative effect of the integrated strategy.

In the group treated with the biological product based on *Bacillus thuringiensis*, the number of larvae decreased by  $72 \pm 5\%$  ( $p < 0.001$ ) during the same observation period. The effectiveness of *Bacillus thuringiensis* was highest in the early stages of larval development, once in particularly vulnerable to bacterial toxins. The effect of the preparation remained stable even under conditions of air temperature fluctuations within  $15-25^{\circ}\text{C}$ , which makes it a reliable component of the protection system in the conditions of climatic variability of the forest-steppe. However, 20 days after application, the effectiveness gradually decreased (up to 65%), due to the natural decomposition of toxic proteins under the influence of ultraviolet radiation, as well as leaching by precipitation. This fact indicates the need for repeated application of the product to maintain a high level of population control in the later stages of the crop's vegetation.

The group using entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) showed a decrease in the number of larvae by  $65 \pm 7\%$  ( $p < 0.01$ ). The study determined that the maximum effectiveness of fungi is achieved under conditions of air humidity above 70%, as moisture stimulates spore germination and increases the probability of pest infection. Additionally, the study noted that the fungi had a prolonged effect: 30 days after the initial treatment, larval mortality increased to 75% due to the development of secondary infections transmitted through contact between infected individuals. However, during dry periods, when the air humidity dropped below 50%, the effectiveness of biological products dropped to 40%, indicating that use in conditions of unstable humidity is limited. Thus, the use of fungi requires careful consideration of weather conditions to achieve optimal results. The control group, which did not use any control methods, showed the opposite dynamics: the population of *Pristiphora rufipes* increased by  $25 \pm 3\%$

during the season. This growth confirms the high reproductive capacity of the pest under favourable conditions and the importance of active biocontrol. It was found that the peak in the number of adults coincided with the flowering period of currant, when the plants provided optimal conditions for feeding and reproduction of pests. The microclimate of the forest-steppe (temperature  $18-22^{\circ}\text{C}$ , humidity 60-75%) contributed to the active generation of new generations of pests, which made it impossible to regulate numbers naturally without external intervention.

The integrated approach proved to be the most effective among all tested methods, reducing the total pest population by 82% (including larvae and adults). Compared to other biocontrol options, *Bacillus thuringiensis* showed a fast but less long-lasting effect, which is optimal for "shock" control of the population in the early stages of larval development. Instead, entomopathogenic fungi provided a gradual but long-lasting reduction in numbers, especially in conditions of high humidity. Thus, the combination of methods made it possible to combine the advantages of each tool and compensate for disadvantages. The control observations also showed that in the integrated group, leaf damage was assessed at only 1.2 points on the EPPO scale, while in the control group, this indicator reached 4.1 points. These results demonstrate that the combination of biological methods with pheromone technologies is a promising and environmentally safe direction for long-term pest control in industrial currant plantations.

The degree of leaf damage in the experimental plots. The use of biotechnological methods significantly affected the level of damage to blackcurrant leaves, assessed on a 5-point EPPO scale. In the control group, where no treatments were carried out, the average damage score was  $4.1 \pm 0.3$ , which corresponds to the destruction of 31-60% of the leaf surface. These results indicate the high activity of *Pristiphora rufipes* in the natural conditions of the forest-steppe, where the combination of moderate temperature and sufficient humidity created favourable conditions for the development of pest populations. The greatest damage was observed in the lower tiers of bushes, where the leaves were more accessible to larvae due to lower shoot density and lower ventilation, which contributed to the local accumulation of pests. In the group treated with the biological product based on *Bacillus thuringiensis*, the average damage score decreased to  $1.8 \pm 0.2$  ( $p < 0.001$ ), which corresponded to damage of  $\leq 10\%$ . This result demonstrates the high efficiency of *Bacillus thuringiensis* in the early stages of leaf development, when young tissues are most vulnerable to larval attacks. The application of the bacterial preparation led to rapid pest death, which was especially evident in the upper tiers of the bushes, where the average damage indicators varied within only 0.5-1 points. This is due to the uniform application of the



product, due to the open architecture of the upper parts of the plant, where the leaves were better accessible for processing and irrigation.

The group with entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) showed an average damage score of  $2.3 \pm 0.3$  ( $p < 0.01$ ). Although the fungi were less effective in reducing damage compared to *Bacillus thuringiensis* preparation, provided more uniform protection of different tiers of the bush. In particular, the middle tiers showed a significant reduction in damage: from 45% in the control group to about 15% after treatment with fungi. It is worth noting that due to the ability of entomopathogenic fungi to cause secondary infection through contact between individuals, the population was gradually suppressed even in those

areas of plants where the primary application of spores was insufficient. The best results were recorded in the integrated group, where the use of fungal biological products and pheromone traps was combined. The average damage score was  $1.2 \pm 0.1$  ( $p < .001$ ), indicating minimal damage: only 5-10% of the leaves were slightly damaged ( $\leq 5\%$  of the surface). Most of the damage was observed on the lower tiers of the bushes, where the availability of leaves for larvae was the highest, but even there, damage was limited due to effective control of adults and larvae (Table 1). The high effectiveness of the integrated approach is explained by a synergistic interaction: fungi reduced larval viability while reducing the number of adults by trapping limited new reproduction cycles.

**Table 1.** Comparison of leaf damage by treatment group

Group	Average score (EPPO)	Damage to the surface	Statistical significance (p)	Note
Management	$4.1 \pm 0.3$	31-60%	-	Most damage on the lower levels
<i>Bacillus thuringiensis</i>	$1.8 \pm 0.2$	$\leq 10\%$	$<0.001$	Maximum protection for the upper tiers
Mushrooms	$2.3 \pm 0.3$	11-30%	$<0.01$	Uniform distribution of effect
Integrated	$1.2 \pm 0.1$	$\leq 5\%$	$<0.001$	Minimal damage on all tiers

**Source:** compiled by the authors

The analysis of the distribution of damage by plant tiers revealed a clear dependence of protection efficiency on the spatial arrangement of leaves. Damage to the upper tiers in all treated groups was minimal (not exceeding 10%), which is explained by better lighting, ventilation and higher accessibility for treatment activities. In the middle tiers, fungal-based preparations proved to be more effective, reducing damage by up to 15%, while *Bacillus thuringiensis* showed a preservation of damage at around 10-12%. The lower tiers remained the most vulnerable, but even in these conditions, the integrated approach demonstrated a significant reduction in damage: from 60% in the control group to 8% in the combined protection group. Thus, the results obtained indicate that an integrated strategy of applying biotechnological methods not only effectively limits the development of the pest but also provides uniform spatial protection of the entire plant. This, in turn, is a critical factor for increasing the overall productivity of blackcurrants, improving the quality of berries and increasing the resistance of plantations to biotic stress factors.

**The impact of climatic factors on the effectiveness of methods.** The study revealed a significant impact of climatic conditions on the effectiveness of biotechnological methods, which emphasises the importance of considering local meteorological features when developing plant protection strategies. For entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*), relative humidity was the key factor in effectiveness. The ANOVA analysis showed a strong positive correlation between humidity and larval

mortality ( $r = 0.82$ ;  $p < 75\%$ ), with fungal efficacy reaching  $80 \pm 4\%$ , as high humidity created optimal conditions for spore germination, active penetration of hyphae into the larval body and development of mycosis. On the contrary, during dry periods (humidity  $< 50\%$ ), the effectiveness dropped to  $40 \pm 6\%$ , which limits the use of fungi in regions with unstable or insufficient moisture. This effect is explained by the fact that low humidity slows down the metabolic processes of fungi, reducing infectious activity.

For the biological product based on *Bacillus thuringiensis*, the temperature range of  $15-25^\circ\text{C}$  did not affect the results ( $p > 0.05$ ), which confirms the stability of bacterial toxins (Cry-proteins) to thermal fluctuations within the forest-steppe zone. However, at temperatures  $< 10^\circ\text{C}$ , the activity of the larvae was significantly reduced, which slowed down feeding and absorption of toxins. Despite this, the overall efficacy of *Bacillus thuringiensis* remained high ( $72 \pm 5\%$ ) due to the prolonged effect of the preparation, which provided long-term protection even under conditions of temporary cooling. Pheromone traps showed maximum efficiency at a temperature of  $18-22^\circ\text{C}$ , when the activity of *Pristiphora rufipes* adults reached its peak. Under such conditions, the traps attracted up to 80% of males, which led to a violation of the sex ratio of the population (1:4 in favour of females) and a decrease in the number of fertilised eggs by  $45 \pm 5\%$ . At a temperature of  $> 28^\circ\text{C}$ , the volatility of pheromones decreased due to the accelerated evaporation of active components, which reduced the radius of action of the traps by 30% (Table 2).

**Table 2.** Dependence of method efficiency on climatic factors

Method	Key factor	Optimum range	Efficiency	Note
Mushrooms	Humidity	>75%	80 ± 4%	Spore germination requires moisture
<i>Bacillus thuringiensis</i>	Temperature	15-25°C	72 ± 5%	Stability of Cry proteins
Pheromone traps	Temperature	18-22°C	60 ± 6%	Peak activity of adults

**Source:** compiled by the authors

The analysis of variance (ANOVA) revealed significant differences between the groups for all the parameters studied: the number of pests ( $F = 34.7$ ;  $p < 0.001$ ), the degree of leaf damage ( $F = 28.9$ ;  $p < 0.001$ ) and the influence of climatic factors ( $F = 19.4$ ;  $p < 0.01$ ). The highest level of variance ( $\eta^2 = 0.76$ ) was observed for the integrated group, which confirms the synergy between the methods. Regression analysis showed that the integrated approach explained 89% of the variance ( $R^2 = 0.89$ ) in the decline of the *Pristiphora rufipes* population. In comparison, the individual methods had a smaller contribution:

*Bacillus thuringiensis* 67% ( $R^2 = 0.67$ ), fungi 58% ( $R^2 = 0.58$ ). This indicates that the combination of methods does not just add effects but multiplies through the interaction of factors (e.g., reducing the number of adults reduces the pressure on fungal defence). Correlation analysis revealed a direct relationship between the number of adults and the degree of leaf damage ( $r = 0.91$ ;  $p < 0.001$ ). Each increase in the adult population by 10 adults/bush resulted in a 1.5-point increase in EPPO score (Table 3). This highlights the importance of controlling not only larvae but also adults to prevent re-infestation.

**Table 3.** Statistical indicators of method efficiency

Parameter	Integrated group	<i>Bacillus thuringiensis</i> group	Group of mushrooms	Management
$R^2$ (variance)	0.89	0.67	0.58	-
Correlation coefficient (r)	0.91	0.75	0.68	-
p-value	<0.001	<0.001	<0.01	-

**Source:** compiled by the authors

Thus, the integration of methods is a statistically optimal strategy ( $p < 0.001$ ). The effectiveness of individual methods is limited by biological mechanisms, which confirms the need for adaptation to climatic conditions. The high correlation coefficient between adults and damage indicates the priority of preventive measures before the start of oviposition. These results substantiate the feasibility of using biotechnology in a comprehensive current protection system, incorporating both biological and climatic features of the forest-steppe zone.

**Comparative analysis with the *Ribes* species of Kyrgyzstan and its implications for research.** Based on the results obtained on the effectiveness of biological products based on *Bacillus thuringiensis*, entomopathogenic fungi *Beauveria bassiana* and the combined use of fungal suspensions with pheromone traps against sawflies (*Pristiphora* spp.) in *Ribes nigrum* plantations in Ukraine, the search for additional sources of natural resistance capable of enhancing biocontrol becomes relevant. In this context, wild species of the genus *Ribes* growing in the mountainous regions of Kyrgyzstan deserve special attention. Endemic and subendemic taxa of Kyrgyzstan's mountain ecosystems have evolved effective mechanisms of resistance to biotic and abiotic stressors. This approach further demonstrates the adaptive potential of the genus and the identification of genetic resources that can be used to increase pest resistance in temperate climates.

The genus *Ribes* in Kyrgyzstan is represented by unique species that are evolutionarily adapted to the extreme conditions of mountain ecosystems, which determines high adaptability and resistance to abiotic and biotic stresses. In particular, *Ribes meyeri* (Meyer's currant), which is common in all mountainous regions of the country, demonstrates outstanding tolerance to low temperatures, drought and ultraviolet radiation. It has been established that these adaptations may be associated with the presence of specific alleles of genes that regulate the synthesis of antioxidant enzymes such as superoxide dismutase and catalase, as well as secondary phenolic and flavonoid metabolites that act as anti-feedants and limit pest feeding (Bate-Smith, 1976; Sun *et al.*, 2021). These molecular mechanisms are especially relevant in the conditions of high insolation and temperature fluctuations typical of the Kyrgyz mountains. In contrast, the cultivar *Ribes nigrum* (blackcurrant), which was studied in Ukraine, and *Ribes meyeri* in the Issyk-Kul Basin form dense, shrubby thickets with reduced internodes and a more branched shoot structure. This makes it difficult for insect pests to access the leaves, reducing the risk of mechanical damage and infectious processes, which is a morphological adaptation that is absent in most European varieties.

Another example of natural defence is the properties of *Ribes saxatile* (stone currant), common in the Central Tien Shan and Northern Kyrgyzstan. The leaves of this species contain high concentrations of phenolic

compounds, in particular tannins and flavonols, which effectively inhibit the development of fungal infections (*Botrytis cinerea*, *Alternaria spp.*) and reduce the attractiveness to leaf-eating insects (Kalykova et al., 2022). The high content of these compounds may explain the low population density of *Pristiphora spp.* in the natural habitats of *Ribes saxatile* compared to introduced cultivars in Ukraine. The biogeographical context is also essential in explaining differences in pest densities (Kushnir et al., 2023). In the Inner Tien Shan and Alai Valley, where daily temperature fluctuations reach 30°C, *Pristiphora spp.* Populations are minimal due to the instability of the environment, which makes it impossible for these insects to complete life cycle. This is in sharp contrast to the forest-steppe zones of Ukraine, where a favourable temperate climate (average annual temperature +8°C, average annual precipitation of about 550 mm) creates conditions for the massive development of phytophages (Skliar et al., 2024).

Notable is the case of *Ribes janczewskii*, a sub-endemic of the Western Tien Shan, which has evolved unique mechanisms of resistance to pests. Among them, the synthesis of volatile organic compounds with repellent properties, such as monoterpenes and sesquiterpenes, which repel adult pests and reduce feeding intensity, is significant (Meng et al., 2015). The aromatic profile of these compounds indicates the potential for biotechnological application in the development of natural insecticidal products. Practical implications for the study are as follows. Breeding of resistant varieties: identification and introgression of genes responsible for the synthesis of phenolic compounds in *Ribes saxatile* into cultivars of *Ribes nigrum* can significantly increase resistance to major pests without the need for synthetic pesticides. Development of biotechnological products: extracts from *Ribes meyeri* leaves, rich in alkaloids and polyphenols, can develop new bioinsecticides. The products can be used as enhancers of the effectiveness of biocontrol agents such as *Beauveria bassiana* through combined use. Adaptation of biocontrol methods: the experience of using the natural soil microbiota of the Tien Shan, where *Metarhizium anisopliae* fungi effectively suppress pest populations, can be adapted to Ukrainian conditions by introducing strains adapted to local climatic conditions. In addition, the results of the analysis indicate the feasibility of multidisciplinary approaches that combine breeding programmes, the development of bioactive products and ecosystem-based pest management.

Thus, the *Ribes* species of Kyrgyzstan, especially the sub-endemic mountainous regions, represent a valuable source of genetic and ecological adaptations for improving biocontrol methods in Europe. Natural resistance, formed under the influence of harsh climatic and biotic factors, can be the basis for the development of integrated plant protection strategies (Shahini et al., 2023; 2024). In particular, the combination of

entomopathogenic fungi with *Ribes saxatile* extracts has the potential to reduce dependence on chemical insecticides in the forest-steppe zone of Ukraine by 40-50%, which is consistent with the principles of sustainable agriculture, biodiversity and environmental protection. The results of the study confirm that the integrated use of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*) and pheromone traps is an effective strategy for controlling *Pristiphora rufipes* in blackcurrant plantations. These findings correlate with the study by E. Quesada-Moraga et al. (2020), who demonstrated that the combination of biological methods increases efficiency due to synergy between mechanisms of action. For instance, a  $60 \pm 6\%$  reduction in adult numbers due to pheromones reduced pressure on fungal defences, which is consistent with the “double hit” concept described by A. Chakravarthy (2020). A. Chakravarthy analysed innovative approaches to pest control, emphasising the importance of adapting methods to local conditions, which confirmed the results obtained on the impact of climate on the effectiveness of biological products.

A study by G. Uma et al. (2025) on biotechnological innovations in forest ecosystems highlights the role of entomopathogens in reducing chemical dependency, which is consistent with the reported 40-50% reduction in insecticide use. N. Tumenbayeva et al. (2024), in a study of biological protection of currants in Kazakhstan, found similar trends in the effectiveness of fungi, but the results were for other pest species, which highlights the need for species-specific approaches. S. Arthurs and L. Lacey (2024) analysed the use of microbial agents in orchards, highlighting the stability of *Bacillus thuringiensis* toxins, which confirms the findings of this study on effectiveness at different temperatures. The results of the study confirm the effectiveness of the integrated use of entomopathogenic fungi and pheromone traps for the control of *Pristiphora rufipes*. These findings correlate with the study by E. Quesada-Moraga et al. (2020), who proved the possibility of integrating biological methods into biocontrol systems. However, the experiments of these authors were conducted under controlled conditions, while the data obtained in this study indicate the criticality of adapting methods to open ground, where climatic factors such as humidity and temperature significantly affect efficiency.

M. Gołębowski et al. (2021), analysing the effect of fungi on the lipid composition of insect cuticles, confirmed the mechanisms of infection described in the study. However, the study did not address climatic variations, which were found to reduce the effectiveness of fungi at humidity below 50%. This fact highlights the need to consider local conditions when developing biocontrol strategies. Similar limitations were identified in the study by G. Devi (2020), where entomopathogenic nematodes showed a dependence of effectiveness on humidity, which confirms the universality of this factor

for various biological methods. T. Lampasona (2022), on the behaviour of pests in orchards, highlights the importance of environmental factors such as temperature in the development of control strategies. These results are in line with the optimal temperature range (18-22°C) for pheromone traps found in this study. In addition, J. Strand *et al.* (2025) determined that volatile compounds released during plant injury can attract parasitoids, which complements these observations on the role of pheromones in disrupting the sex balance of pests.

The study by Q. Guignard *et al.* (2020) on the pheromones of the wasp *Sirex noctilio* and the study by G. Svensson *et al.* (2023) on the identification of pheromones of *Euhyponomeutoides albithoracellus* confirmed the versatility of pheromone methods for different insect species. However, in contrast to these studies, the present study found that the volatility of synthetic pheromones significantly decreases at temperatures above 28°C, indicating the need to optimise formulation for sustainable use in the face of climate change. Innovative solutions proposed by C. Hellmann *et al.* (2024), such as polymeric carriers with controlled pheromone release, may be a promising way to overcome this limitation. L. Xu *et al.* (2020) on pheromones of gall midges and the study by A. Nagy *et al.* (2022) on the optimisation of baits for *Autographa gamma* highlighted the need for a species-specific approach, which confirms the relevance of the methodology applied in this study, focused on *Pristiphora rufipes*. However, in contrast to these studies, the present study found that the combination of methods (mushrooms + pheromones) provides a synergy that reduces the pest population by 85 ± 4%, which exceeds the results obtained using individual methods.

M. Knapp *et al.* (2020), analysing integrated pest management in greenhouses, demonstrated the potential of integrated approaches, but the recommendations of the authors need to be adapted to open agroecosystems where environmental factors are less predictable. The study by S. Pacheco *et al.* (2023) on the structural stability of *Bacillus thuringiensis* toxins confirmed effectiveness at different temperatures, which is consistent with the results obtained. However, in contrast to the study by T. Patyka and M.V. Patyka (2020), where *Bacillus thuringiensis* was used against mosquitoes, the data of this study emphasise its specific efficacy for leaf-eating pests such as *Pristiphora rufipes*. N. Thakur *et al.* (2021) on the interaction of soil microbes with bioagents opens prospects for further integration of entomopathogens into integrated protection systems. These results complement the findings of this study, indicating the potential of combining fungi with soil microbiota to increase the resilience of agroecosystems. S. Parajuli *et al.* (2022) described biopesticides as a basis for sustainable agriculture, which is consistent with the described recommendations for reducing chemical burden. T. Achari *et al.* (2022) studied the

effect of *Bacillus thuringiensis* on mosquitoes under heat stress, which highlights the need for further research to adapt methods to climate change.

The effectiveness of fungi dropped to 40 ± 6% at humidity <50%, which contradicts the study by G. Uma *et al.* (2025), where *Isaria fumosorosea* remained active in arid regions. This difference can be explained by the species specificity of fungi and the characteristics of forest-steppe soils. The synergy of methods in this study ( $R^2 = 0.89$ ) exceeds the results of T. Lampasona (2022), where combinations of methods had a lesser effect due to competition between agents, emphasising the importance of proper component selection. The results of the study directly impact agricultural practices: the integration of biopreparations can reduce insecticide use by 40-50%, which is in line with sustainable agriculture goals. Recommendations for humidity (>75%) and temperature (18-22°C) will optimise treatments, especially in the context of climate change. The results of the study prove that biotechnological methods can become an alternative to chemical insecticides in the forest-steppe zone of Ukraine. Effectiveness depends on climatic conditions, which highlights the need for adaptation to local conditions. A comparison with international experience shows that such approaches can be scaled up for other crops and regions.

## CONCLUSIONS

The study confirmed the high efficiency of biotechnological methods in controlling the number of *Pristiphora rufipes* on black currant in the forest-steppe zone. The best results were achieved through an integrated approach that combined the use of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*) and pheromone traps (*Pherocon VI*), which reduced the total number of pests by 82% and minimised leaf damage to the level of 1.2 points on the EPPO scale. A pronounced synergistic effect was found between the direct action of biological products on larvae and mechanical control of adults, which led to the interruption of the pest's life cycle and a gradual decrease in its population during the season. The biopreparation based on *Bacillus thuringiensis* demonstrated high initial efficacy (72 ± 5% reduction in larval numbers), especially in the early stages of pest development and under moderate air temperature conditions (15-25°C). However, its efficacy gradually decreased after 20 days due to natural abiotic factors (ultraviolet radiation, precipitation), which requires regular updating of treatments to maintain control. Entomopathogenic fungi showed a relatively slower but longer effect: larval mortality reached 75% 30 days after treatment due to the development of secondary infections. At the same time, a significant dependence of fungal effectiveness on air humidity was found at humidity above 75%, the maximum effectiveness (80 ± 4%) was observed, while in dry periods (<50% humidity), the effectiveness was halved.



The analysis of the degree of leaf damage confirmed the effectiveness of the applied protection methods: in the integrated group, the damage was only 5-10% of the leaf surface, which is significantly less compared to the control (31-60%). The uniform distribution of the protective effect by plant tiers was best with the integrated approach, which ensured the preservation of the photosynthetic potential of plants and optimal conditions for fruiting. The study also revealed the importance of adapting the protection system to climatic conditions. High air humidity is critical for entomopathogenic fungi, while *Bacillus thuringiensis* preparations showed high stability to temperature fluctuations in the range of 15-25°C, but effect decreased at temperatures below 10°C due to a decrease in larval activity. Comparative analysis showed that wild *Ribes* species from the mountainous regions of Kyrgyzstan are characterised by higher natural resistance to pests compared to cultivars, due to specific morphological traits, biochemical profiles and ecological adaptability to extreme environmental conditions. The obtained

results confirm that the integrated use of biotechnological means is the most promising direction for environmentally safe, stable and highly effective pest control in industrial blackcurrant plantations. The limitation of the study was that the size of the plots (0.1 ha) did not incorporate the migration of adults from neighbouring areas, which may have underestimated the effectiveness of the methods. Further research should be aimed at optimising the modes of application of biological products, incorporating weather forecasts and the possibility of increasing the stability of the products to abiotic factors.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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## Біотехнологічні аспекти контролю чисельності пильщиків в насадженнях чорної смородини

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**Анотація.** Метою дослідження було порівняти ефективність біотехнологічних методів контролю популяції пильщика *Pristiphora rufipes* на чорній смородині (*Ribes nigrum*) в умовах лісостепової зони України та виявити екологічні фактори, що впливають на їхню результативність. Дослідження проводилося з використанням 20 ділянок (0,1 гектарів кожна), розподілених на чотири групи: обробка біопрепаратом *Bacillus thuringiensis*, ентомопатогенними грибами (*Beauveria bassiana*, *Metarhizium anisopliae*), комбінацією грибів із феромонними пастками та контроль без обробок. Ефективність оцінювали через щотижневий моніторинг чисельності шкідників, ступінь пошкодження листя за шкалою Європейської та Середземноморської організацій захисту рослин та аналіз кліматичних параметрів. Інтегрована група продемонструвала найвищу ефективність: чисельність личинок знизилася на  $85 \pm 4$  % ( $p < 0,001$ ), імаго – на  $60 \pm 6$  %, середній бал пошкоджень листя склав  $1,2 \pm 0,1$  проти  $4,1 \pm 0,3$  у контролі. Група *Bacillus thuringiensis* показала зниження личинок на  $72 \pm 5$  % ( $p < 0,001$ ) зі стабільністю до температур 15-25°C, але ефективність падала до 65 % через 20 діб. Гриби знизили популяцію на  $65 \pm 7$  % ( $p < 0,01$ ) із максимальною ефективністю  $80 \pm 4$  % при вологості  $>75$  %, проте їх дія зменшувалася вдвічі за посухи. Контрольна група зафіксувала зростання популяції на  $25 \pm 3$  %. Регресійний аналіз виявив, що інтегрований підхід пояснює 89 % дисперсії ( $R^2 = 0,89$ ) у зниженні шкідника. Порівняльний аналіз з видами *Ribes* Киргизстану (*Ribes meyeri*, *Ribes saxatile*) вказав на потенціал використання їхніх фітохімічних адаптацій для селекції стійких сортів. Результати доводять, що комбінація біопрепаратів із феромонами є оптимальною стратегією для зменшення залежності від хімічних інсектицидів у лісостеповій зоні України, враховуючи локальні кліматичні умови (температура, вологість) та біологічні особливості шкідника. Дослідження має практичне значення для розробки сталих систем захисту ягідних культур

**Ключові слова:** шкідники; личинки; імаго; феромонні пастки; ентомопатогенні гриби

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