Biological efficiency of *Pseudomonas fluorescens* strains against pathogens of Brassicaceae black rot under controlled conditions

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Abstract. The bacterium *Xanthomonas campestris* pv. *campestris* causes black rot of cruciferous plants, the most harmful bacterial disease of a wide range of cole crops. Breeding for resistance to the pathogen is carried out considering the racial structure of the pathogen population discovered in 1992 and the race-specific resistance reaction in plants of the Brassicaceae family. The model describing the gene-to-gene relationship between the pathogen and plants does not consider biometric indicators associated with the pathogen's effect on the suction, conducting, and photosynthetic systems of plants. In this paper, the effect of infestation of spring rape plants with *Xanthomonas campestris* races 1 and 6 on biometric indicators of plants important for crop formation is studied. A significant positive effect of the use of a preparation based on antagonistic bacteria *Pseudomonas fluorescens* (Binoram preparation) on the growth and stability of spring rape has been shown.

1 Introduction

Cabbage black rot (caus. *Xanthomonas campestris* pv. *campestris* (Dowson) Dye, et al. (1980) is a disease of cole (cruciferous) crops, such as cauliflower, white cabbage, bok choy, turnips, rapeseed, and mustard. Black rot not only damages the growth and development during plant growing season, but also dramatically (up to 10 times) increases the development of other diseases - soft rot (*Pectobacterium carotovorum, Pectobacterium* sp., *Pseudomonas marginalis*), white and gray rot (*Sclerotinia sclerotiorum., Botrytis cinerea*), Alternaria blight (*Alternaria brassicicola, A. brassicae*) during the growing season, storage, and transportation of products [1, 2]. The pathogen especially quickly affects plants in the initial phase of development (up to 5-7 true leaves), when the manifestation of age-related genetic resistance does not yet restrain the spread of the disease [1]. Bacteria penetrate into the vascular system of the plant (xylem) through hydatodes, stomata, and mechanical damage to the roots, leaves, stem. Spreading in the xylem, the bacteria provoke the synthesis by the plant of a lignin-like

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substance that closes the vessels, which causes chlorosis and necrosis of the affected tissues, wilt and death of the plant [2]. The systemic spread of the pathogen makes it difficult to fight the disease. In addition to preventive measures, only the use of resistant varieties and biological preparations that are pathogen antagonists or inducers of the immune response of plants are effective.

A biopreparation with fungicidal and growth-stimulating action based on a live culture of *Pseudomonas fluorescens* soil bacteria (strains 7G, 7G2K, 17-2) was developed by the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences of the city of Novosibirsk, tested and registered by ALSIKO-AGROPROM LLC, city of Moscow under the trade name Binoram [3].

The Binoram preparation is registered for use on cereals, potato, cabbage, table and fodder sugar beet, and some other crops [4].

For a detailed study of Binoram action mechanism and its effect on the growth and development of plants, we conducted studies on spring rape crop in the field [5]. To study the preparation effect on plant growth and the development of black rot on spring rape in greenhouse conditions, three varieties contrasting in resistance to the races of black rot causative agent were used [1, 6].

2 Materials and Methods

Plants of spring rapeseed of the resistant Griffin variety (resistance to race 4 *Xanthomonas campestris*, Syngenta LLC, Russia), medium-susceptible Forum variety (Branch of the Federal State Budgetary Research Center VNIIMK Lipetsk Rapeseed Research Institute, Russia), and susceptible Hanna variety (Sweden) were grown in greenhouse conditions in 3 l pots filled with compost, with full mineral fertilizer corresponding to the NPK norm recommended for rapeseed. The plants were grown in natural light (May-July), at a temperature of 28/20°C (day/night) and watered as necessary, maintaining soil moisture corresponding to 60% of the total moisture capacity. In phase 4 of real leaves, plants were inoculated with strains of bacteria *Xanthomonas campestris* B-32 (race 6) and PHW231 (race 1). The pathogen race was determined in preliminary experiments by infecting varieties of differentiators described by S. Kamoun [2] and J. Vicente with colleagues [6]. The bacterial inoculum was grown for 36-48 hours on an agarized medium based on yeast extract, dextrose, and calcium carbonate (YDC) [7], washed off with sterile water and brought to a concentration of 10^6 CFU/ml. Inoculation of plants was carried out with injury to the edges of the leaves with tweezers [6, 7]. In the experiment, a scheme of a complete three-factor experiment was used with three levels for the "Variety" factor, three levels for the "Strain" factor, and 4 levels for the "Preparation treatment" factor.

Treatment of plants with Binoram as a protection was carried out a day before infection (option 1), a day after infection (option 2) and when the first symptoms of the disease appeared (option 3). Records of the development of disease symptoms and the condition of plants (stem height, weight of the aboveground part) were carried out at the end of the experiment on the 40th day after infection. The disease development was considered by the percentage of the affected area on the leaves. The analysis of the obtained data was carried out by the method of multivariate variance and correlation analysis using the Statistica software package (ver. 12.5, StatSoft) [9].

3 Results and Discussion

The development of diseases in the options with inoculation and without protection was high due to favorable weather conditions for infection (the average daytime air temperature for

the entire period of the experiment (40 days) was 22.3°C, night - 16.9 °C, average humidity – 78.9%, precipitation - 128 mm.

Considering biometric indicators of rapeseed 40 days after inoculation showed leaf damage in unprotected control from 32 to 79%, depending on the variety. The conducted calculations showed a significant reduction in the harmfulness of black rot when treated with Binoram. A significant and high correlation between biometric indicators was observed for the average height – weight pair of the aboveground part of the plant (0.98), and the correlation between the degree of damage with *X. campestris* to plants and the height/weight of the aboveground part of the plants, as expected, was negative (-0.68-0.69).

According to the results of three-factor variance analysis and ranking of averages according to the Duncan criterion [10], the differences between the strains of *X. campestris* and the methods of using Binoram were significant at 95% significance level (Table 1). The differences between the varieties were not reliable in terms of leaf infestation (Table 2). Among the rapeseed varieties, Forum had the highest indicators of plant height and weight, on average 20% more than Griffin and Hanna, who did not differ from each other.

Table 1. The generalized result of a three-factor dispersion analysis of the effect of variety, strain *Xanthomonas campestris*, and protection scheme on the biometric indicators of rapeseed plants. The effect of A) the variety, B) the strain, C) the method of Binoram application.

A)

Effect	Multivariate significance test					
	Test	Value	F	Degree-of-freedom effect	Degree-of-freedom error	p
Total variability	Wilks	0.00924	4107.77	3	115	0.000
Variety	Wilks	0.53189	14.23	6	230	0.000

B)

Effect	Multivariate significance test					
	Test	Value	F	Degree-of-freedom effect	Degree-of-freedom error	p
Total variability	Wilks	0.0175	2146.36	3	115	0.00
Strain	Wilks	0.402	22.12	6	230	0.00

C)

Effect	Multivariate significance test					
	Test	Value	Test	Degree-of-freedom effect	Test	p
Total variability	Wilks	0.0127	2946.80	3	114.0	0.00
Binoram	Wilks	0.27	21.97	9	277.59	0.00

When plants were infected with the B-36 *X. campestris* strain, the average lesion reached 49%, while the PHW231 strain caused an average lesion equal to 38% of the leaf area. In the variant without the use of Binoram, leaf damage averaged 54%. The use of Binoram before infection of plants reduced leaf infestation to 23%, while its use the next day led to the defeat of 28% of the leaf area, and the use after the appearance of signs of infection – to the defeat of 31% of the area, which is 35% more than the first protection option. Thus, the biological efficiency of Binoram varied from 39 to 57% with a single application. The effect of treatments on plant biometric parameters differed from the protective effect against black rot. The highest plant height was obtained for the treatment option a day after infection, but in

any case, the use of the preparation against the infection background led to an increase in stem length by 66-75%, and stem mass by 50-57%.

Table 2. The result of the variance analysis by the variety factor according to the indicator of average leaf damage by *Xanthomonas campestris* strains (% of the area).

Effect	Average score of leaf damage				
	SS	Degree-of-freedom	MS	F	P
Total variability	1447.97	1	1447.97	193.67	0.000
Variety effect	12.18	2	6.09	0.81	0.445
Error	874.75	117	7.47		

Earlier field trials of the Binoram preparation on two rapeseed varieties (Forum and Griffin) when plants are infected with three different pathogens - causative agents of fusarium, gray rot, and black rot, showed a significant positive effect on the weight of the aboveground part and the weight of the root of rapeseed plants, and on the average plant height and leaf width [5].

On average, treatment with Binoram against the background of plant infection increased the height of the plant by 10.5%, the number of leaves by 14.4%, the length and width of the leaves by 7.4%, and 14.72%, respectively. The mass of the plant aboveground part and the root was respectively 37 and 42.7% more than in the option without protection.

4 Conclusions

The results obtained in greenhouse conditions show that the biometric indicators of rapeseed plants in conditions of infection with black rot causative agent when treated with Binoram did not show a variety-specific effect. This is probably due to the fact that *Xanthomonas campestris* strains used for infection were virulent with respect to the resistance gene *Rxcc4*, assumed in the Griffin variety. This may mean that the presence of a race-specific resistance gene does not generally increase the plant's resistance to virulent pathogen races. The role of race-specific genes in the formation of field resistance of plants was previously shown for white cabbage [11, 12], but the relationship between resistant rapeseed varieties and the pathogen has not been studied before.

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