A cost-effective evaluation of carrot resistance to *Alternaria* sp. and *Fusarium* sp.

L M Sokolova¹, M V Shatilov¹ and O A Razin²

¹ All-Russian Scientific Research Institute of Vegetable Growing – Branch of the Federal Scientific Center for Vegetable Growing, Vereya, Ramenskoye district, Moscow region 140153 Russia

² Federal Scientific Center for Vegetable Growing, 14 Selektssionnaya str., VNIISSOK Village 143080 Russia

E-mail: vniissok@mail.ru

Abstract. Creation of forms, varieties, and hybrids of plants resistant to biotic factors is an important national economic task. It is known that the defeat of agricultural plants by fungal, bacterial, and viral diseases, as well as pests leads to a loss of 38.8% to 60.4% of the crop. The most promising way to protect plants from phytopathogens is selection, which allows to obtain varieties and hybrids of crops with complex resistance. However, the process of creating varieties and hybrids remains long and reaches ten years or more. At the same time, the degree of plant resistance to harmful organisms is often insufficient and does not always meet the requirements of production. The efficiency of the breeding process can be improved and accelerated through the use of rapid methods based on the selection in the laboratory of samples resistant to selective factor. The study was carried out in the Laboratory of Immunity at the VNII – Branch of the Federal State Budgetary FNCO in the Moscow region (Russia). As source material used, three samples of carrot breeding in VNII characterized by different resistance to *Fusarium* and *Alternaria*. The results of the experimental work showed the possibility of rapid assessment of carrots for resistance to PP fungal diseases. *Alternaria* and *Fusarium* seeds on the culture liquid filtrate at a concentration of 50% when germinating. This allows in the laboratory in a short time to evaluate a large number of breeding samples. The method of rapid assessment allows in 3.4 times to reduce the time required for the evaluation of breeding material and to reduce the consumption of seed.

1. Introduction

In recent years, the demand for sustainable hybrids and varieties of vegetable crops has increased significantly. Sustainable hybrids and varieties should become the basis of integrated protection, which is especially important during the period of application of new agricultural production technology in connection with the threat of increased damage to crops.

The susceptibility of carrot root crops to disease is the main difficulty in obtaining a consistently high crops of garden carrots, preserving marketability, especially complicates the solution to the problem of growing healthy seeds [4; 11].

Defeating of plants by wreckers occurs at all stages of growth and development; therefore, a timely identification of the first symptoms of a disease and their correct diagnostics are highly important [1].
The genus *Fusarium* includes a number of species that are the causes of various diseases in a number of important crops, such as cereals, vegetables, etc. Some of the important species in this regard are *F. oxysporum* (Fo), *F. avenaceum* (Fa) and *F. poae* (Fp). The most common are the fungus species of *F. oxysporum*, causing wilting disease and affecting the vascular system of plants [2]. *F. avenaceum* is a widespread species that may exist, including as a saprophyte. *F. poae* belongs to the *Sporotrichiella Wollenw.* section [7; 12].

About 10 p. *Alternaria* species are causative agents of the most harmful diseases, significantly differing in pathogenicity, degree of specialization, harmfulness, sensitivity to fungicides, etc. The main problems of monitoring alternariosis in Russia are associated with the lack of modern determinants, poor use of microscopy and molecular methods to identify pathogens [8; 6]. Crop losses can reach even 40-99% [3; 14].

The main purpose of the research was to increase the efficiency of the selection process of garden carrots for resistance to *Fusarium* and *Alternaria*, based on research on the evaluation of carrot plants by various methods: germination of seeds on the filtrate of the culture medium of the causative agent of *Fusarium and Alternaria*, evaluation based on an artificial infectious backgrounds.

2. Research Tasks
1. To develop elements of the express-evaluation methodology of carrots for resistance to *Fusarium* and *Alternaria*, based on the germination of seeds on a filtrate of the culture medium of fungus *F. oxysporum* and *A. radicina*.
2. To evaluate carrot samples for resistance to *Fusarium* and *Alternaria* against an artificial infectious background using traditional methods.
3. To determine the economic efficiency of express-evaluation in laboratory conditions and traditional evaluation on an artificial infectious background.

3. Materials and Method
As an object of research, we used 3 samples of carrots from the VNIIO (Branch of the Federal Scientific Center for Vegetable Growing), characterized by different field resistance to *Fusarium* and *Alternaria*: stable to fungal diseases from the p. *Alternaria* and *Fusarium* variety “Vitaminnaya 6,” the medium susceptible variety “Amsterdamskaya,” and the susceptible variety “Tayfun-16.”

3.1. Method of preparation of laboratory experience
The laboratory research was conducted according to methodical recommendations [10]. The pathogen cultures were sown on an agarized nutrient medium with hyphae and mycelium spores, and on liquid nutrient media with a suspension of macro- and microconidia. Pathogen cultures were cultured at 26 °C under thermostat conditions in a nutrient medium using distilled water [5]. The concentration of sucrose in the nutrient medium was 30 g/l. Accounting for the development of colonies was carried out visually on 10, 20, 30, and 40 days by increasing their size in diameter.

The suspension culture of the pathogen strain was prepared according to the Bilay method and introduced into a liquid nutrient medium.

A culture medium was obtained by growing fungal isolates in 300 ml flasks in 100 ml of nutrient medium. To obtain a culture medium, a piece of agar measuring 0.5 cm x 0.5 cm, and containing about 10^6 conidia of the fungus was placed in each flask. The flasks were placed in a thermostat at the temperature of 25 °C, for 1 month with regular shaking in a shaker. A freshly grown culture medium (turbid liquid with a specific smell) was filtered through 4 layers of gauze fabric. The resulting filtrate of the culture medium) was kept in an autoclave for 30 minutes [10].

The degree of damage to plants was determined and calculated by the formula:
\[ Dp = (a \times 1) + (b \times 2) + (c \times 3) / (3 \times p) \times 100\% , \]
where Dp – a disease progression (%); a, b, c, d – a number of affected plants; 1, 2, 3 – a defeat score; p – total plants.

3.2. Preliminary research results and discussion

As a result of the studies, it was found that the germination of carrot seeds in a susceptible sample “Typhoon-16” on the filtrate culture medium of the fungus p. *Alternaria*, at a concentration of 70% seed germination was not observed. On the resistant variety “Vitaminnaya-6” and the medium-receptive variety “Amsterdamskaya,” when germinating seeds on 70% filtrate culture medium, the seed germination was 50% and 15%. The root length of the resistant variety “Vitaminnaya-6” and the medium susceptible variety “Amsterdamskaya” did not differ significantly, – it was 10.6% and 9.6%.

When germinating carrot seeds on the filtrate of the culture medium in concentrations of 5%, 10%, and 20%, in germination and root length in all three samples – “Vitaminnaya-6,” “Amsterdamskaya,” and “Typhoon-16,” no significant differences were revealed.

When germinating seeds on a 50% filtrate culture medium, there was a significant inhibition of germination. At the same time, differences were found in the germination of seeds of the studied varieties of garden carrots. In the susceptible variety “Typhoon-16,” the root length was 29.2% less than in the resistant variety “Vitaminnaya-6,” and 9.2% less than in the medium-susceptible variety “Amsterdamskaya.”

The best results were obtained when germinating seeds of garden carrots on the filtrate culture medium of the fungus p. *Alternaria* at a concentration of 50%, which allowed to obtain breeding material with increased resistance to *Alternaria*. An analysis of the data indicates the possibility of express-evaluation of carrots for resistance to *Alternaria* when using a 50% filtrate of the culture medium.

As a result of the studies, it was found that when germinating carrot seeds, in a susceptible sample of “Typhoon-16” on the filtrate of the culture medium of the fungus p. *Fusarium* at a concentration of 70%, seed germination was not observed. On the stable variety “Vitaminnaya-6” and the medium-receptive variety “Amsterdamskaya,” when germinating seeds on 70% filtrate culture medium, the seed germination was 60% and 35%. The root length of the resistant variety “Vitaminnaya-6” and the medium susceptible variety “Amsterdamskaya” did not differ significantly, – it was 12% and 10.7%.

When germinating carrot seeds on the filtrate culture medium in concentrations of 5%, 10% and 20%, the germination and root length in all three samples (“Vitaminnaya-6,” “Amsterdamskaya,” and “Typhoon-16”) did not reveal significant differences.

When germinating seeds on a 50% filtrate culture medium, there was a significant inhibition of germination. At the same time, differences were found in seed germination of the studied varieties of garden carrots. In the susceptible variety “Typhoon-16,” the root length was 33.1% less than in the resistant variety “Vitaminnaya-6,” as well as 8.7% less than in the medium-susceptible variety “Amsterdamskaya.”

The best results were obtained when germinating seeds of garden carrots on the filtrate culture medium of the fungus p. *Fusarium* at a concentration of 50%, which allowed to obtain the breeding material with increased resistance to *Fusarium*. An analysis of the data indicates the possibility of express-evaluation of carrots for resistance to phytopathogenic fungi from pp. *Alternaria* and *Fusarium*, when using a 50% filtrate culture medium.

The duration of express-evaluation of carrots for resistance to fusarium and alternariosis in laboratory conditions, taking into account the preparatory work, is 54 days. In particular, to obtain a pure culture fungus pp. *Alternaria* and *Fusarium*, a total of 14 days is required. It takes 30 days to get a culture medium. The period of seed germination on the filtrate of the culture medium of the mushroom pp. *Alternaria* and *Fusarium*, according to the requirements for carrot culture, is 10 days.

Let us analyze the cost of this experience (Table 1). In total, 22 m/hrs were spent for carrying out experience. Despite the fact that the experiments were carried out by full-time employees, to determine the total cost of conducting the experiment, it is necessary to take into account how much of
the payroll fund was paid for the time spent on the experiment. We divided the salary before deducting personal income tax, taking into account deductions by the number of working hours in the current year. Electricity costs were calculated according to the data indicated by the manufacturers of the devices, the current tariffs of the electricity supplier and the actual amount of the devices. Calculation of depreciation was carried out by a linear way. At the same time, the annual amount of deductions was divided by the number of experiments conducted per year.

| Table 1. Expenses for laboratory testing. |
|------------------------------------------|
| **Expenses** | **Expenses, rub.** | **Expenses, m/hrs** |
| Cooking medium (mixing components) | 546.12 | 1 m/hrs |
| Selective medium cooking | 546.12 | 1 m/hrs |
| Autoclave sterilization | 2,184.48 | 4 m/hrs |
| Experience development | 8,737.92 | 16 m/hrs |
| Seeds | 0.20 | |
| Preparations | 1,040 | |
| Electrical energy | 51,758.22 | |
| Depreciation | 20,600 | |
| Total | 85,413.06 | |

Our cost analysis showed that the fixed costs account for 24% of the total ones. In this case, payroll expenses were assigned to the workers as variables, since the costs were calculated on conducting experience for the general case, and the time spent by the employees could be used to solve other current tasks that are part of the employees’ duties. In the structure of variable costs, the costs of electrical energy occupy a large part. Most of the expenses are spent on the thermostat, since according to the methodology for conducting the experiment, it was activated for 10 days continuously.

3.3. The method of laying the field experience on two artificial infectious backgrounds

To create a soil infectious background, pathogens were propagated on a grain nutrient medium. The oat grain nutrient medium for reproduction of infectious material was prepared in the following way. The oat grains were poured into flasks, poured with water in a ratio of 1: 1, and sterilized with dry steam in an autoclave under a pressure of 1 atmosphere for 1 hour. The prepared cereal medium was seeded with a clean fungus culture (14 days old), using standard microbiological methods. The flasks were kept for 14 days in a thermostat at a temperature of 20-250C, and during this time they were shaken every day to evenly distribute the infection. After 14 days, when the substrate is uniformly overgrown with mycelium, it was removed from the flasks and dried to dry completely at the room temperature for 3 days [13].

Preparing the inoculum to create a soil infectious background began 1 month before sowing the seeds. The inoculum propagated by the grain mixture was placed directly in rows (a plot size of 1.50 cm, a row spacing of 30 cm) in the surface soil layer (5-7 cm), the seeds were leveled and sown with further planting.

4. Results and Discussion

After assessing resistance against an artificial infectious background of *Alternaria*, the resistant variety “Vitaminnaya-6” had 54% of resistant genotypes with a lesion score of 0 to 0.8. The variety “Amsterdamskaya” was included in the group of low-susceptible varieties, with a score of 0.9 to 1.6 and had 78% of genotypes. The “Typhoon-16” was included in the group of susceptible with a score of 3.3 to 4, and it had only 2% of stable genotypes.

According to the *Fusarium* infectious background, the distribution of the genotypes is as follows: (a) the resistant variety “Vitaminnaya-6” has 52% of the resistant genotypes; (b) the medium susceptible variety “Amsterdamskaya” has 78% of the genotypes; and (c) the susceptible variety “Typhoon-16” has 2% of the resistant genotypes.
Thus, combining both laboratory and field experience, it is possible to obtain a complete assessment of samples for disease resistance.

The traditional method’s duration for evaluating carrots for resistance to *Fusarium* and *Alternaria* in an infectious background is 181 days. To obtain a pure culture fungus pp. *Alternaria* and *Fusarium*, it is necessary to spend 14 days. Another 14 days are necessary for the germination of the mycelium on the substrate (oats), as well as 3 days are taken for drying the substrate. The period from sowing to final assessment for sustainability during harvesting is 150 days.

Similar to the previous analysis, we will analyze the second experiment (Table 2). In the field experiment, the working time of employees for conducting the experiment increases significantly, up to 955.5 m/hrs. This is due to the fact that it is necessary to prepare the soil for sowing and care for the plants throughout the duration of the experiment.

Comparison of the studied express method and the traditional method for evaluating carrots for resistance to alternariosis and fusariosis shows the possibility of accelerated evaluation and saving material for selection. The evaluation method based on germination, in laboratory conditions and on the filtrate of the culture medium, allows to reduce the duration and increase the productivity of the evaluation of samples compared to the traditional assessment on an artificial infectious background by 3.4 times.

![Figure 1](image)

*Figure 1.* The structure of operating costs for the analysis of 1 sample in the laboratory and the field, RUB, %.

After analyzing the costs, we got the prime cost of 1 experience at the full possible load during the year (6 laboratory experiments and 1 in the field). It is 854.13 rub. for the analysis of each sample by the laboratory method and 16,871.43 rub. by the field method. Unfortunately, the laboratory experiments do not replace field experiments, and some studies require both options. Let’s consider operating costs of each method of carrying out experience to understand what way would be less expensive, as well as to choose it in case both options are suitable for a research.

In a specific case, operating costs include all of the costs presented, other than depreciation. Operating costs for the analysis of 1 sample in the laboratory are 648.13 rub., it is 14492.51 rub. in the field experiment. Let's analyze their structure (Figure 1). The diagram shows that all indicators are significantly higher in the field experiment in absolute values. The payroll fund accounts for 77% of the costs, which means a large share of manual labor when conducting experience using this method. The multiple difference in the absolute values of the depreciation rate is due to the shorter downtime of the equipment during the laboratory experiment.

| Table 2. The costs of the field experience. |
|--------------------------------------------|
| **Expenditures** | **Expenses, RUB.** | **Expenses, man-hours** |
| Soil preparation and frame making | 5,461.20 | 10 |
| Reproduction and preparation of the substrate | 4,642.02 | 8.5 |
| Sowing, soil preparation | 8,737.92 | 16 |
| Care | 494,238.60 | 905 |
Cleaning, rating 8,737.92 16
Seeds 0.4
Preparation – Czapek's medium + alcohol 480
Film 5,646
Electrical energy 51,756.23
Depreciation: 95,157.34
Total 674,857.63

5. Conclusion
Our analysis of the effectiveness of the two variants of the experiments showed that the laboratory experience in conducting year-round studies demonstrated a significant reduction in costs compared with the field.

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