Resistance of carrots to Alternaria SP., Fusarium SP. and factors influencing it

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Abstract. The results of many years of work (2008–2018) in the Moscow region on the identification of genetic sources of resistance in wild species and varieties of the genus Daucus on natural and artificial infectious backgrounds to alternariosis and fusarium wilting are presented. When testing the breeding material for leaf and root diseases after storage, the most valuable breeding samples of sowing carrots were identified: Losinoostrovskaya 13, Marlinka, NIIOKH-336, Fakel, Moscow Winter A-75. Using the example of the medium susceptible variety of carrots Red cored and susceptible Nigel, a high correlation between precipitation, high soil moisture, and the development of Alternaria sp. and Fusarium sp. on different backgrounds. The prevalence of carrot diseases in the south of Russia in the Rostov region was less than in the central part of the RF NPP; in a wetter 2018 compared to 2017, the variety samples in both zones were more stable, except for individual hybrids.

1. Introduction
The strategic objective of modern vegetable growing is the creation and introduction into widespread production of varieties and hybrids of vegetable crops that are maximally adapted to specific soil and climatic conditions, capable of forming environmentally friendly products with high biochemical and technological qualities, with high disease resistance and, as a consequence, increased stamina.

The effectiveness of research to create new varieties of vegetable crops is largely due to the presence of forms of the source material as genetic sources of breeding valuable traits [1].

In many regions, an increase in the harmfulness of fungal diseases of root crops was noted, both during the growing season and during storage. Anamorphic fungi of the genera Fusarium and Alternaria are dominant in the pathogenic complexes of these cultures.

Fusarium plants can be affected at any age. In young plants, the disease manifests itself in the form of decay of the roots and root of the neck, the tissues of which turn brown, the stem becomes thinner, the leaves turn yellow, and die. During storage, dry rot of root crops often develops in combination with other pathogens. The most common pathogens are F. oxysporum species, which cause wilting diseases and affect the vascular system of plants [2]. Sources of infection can be infected seeds, plant debris, soil; fungus propagation occurs when infected soil is moved on cultivation tools, with wind and drops of water, with plant debris.
Fusarium and alternaria infections infect carrots of the 1st and 2nd year of life, reduce the shelf life of root crops during storage, cause lung attacks, worsen the sowing quality of seeds, which causes significant damage to commercial production and seed production of carrots. Depending on weather conditions and the phytosanitary condition of crops, the prevalence of diseases can reach 70–80 %, and the yield of root crops is reduced by 35–50 %. Alternaria radicina and A. dauci can cause crop losses of up to 90 % [3–5].

Modern approaches to solving the problem of protecting carrots from diseases are based on the accurate implementation of agricultural techniques for cultivating crops and the use of varieties and hybrids that combine valuable economic properties and high disease resistance to the complex of the most harmful pathogens [6].

In creating disease-resistant varieties and hybrids, great importance should be given to the selection of sources of resistance, one should not be limited to single donors, it is necessary to attract great genetic diversity [7]. Intraspecific hybridization within the genus Daucus L., including from 20 to 25 species, one of which is cultivated carrots (Daucus carota L. subsp. Sativus, D. sativus), is used to transfer genes that control, for example, resistance to pathogenic fungi, from wild to cultural species [2, 7–9].

Based on the aforementioned problem, the main research objectives were to assess the resistance of carrot varieties and isolate geniuses of resistance to phytopathogenic fungi of the genera Fusarium and Alternaria from wild species and varieties of the genus Daucus of different ecological and geographical zones.

Research Objectives:
1. To carry out a field assessment of the resistance of plants of the first year of life and root crops of carrots to Fusarium and Alternaria cultivars, wild species and varieties of the genus Daucus and distinguish resistant forms on artificial backgrounds of pathogens in the Moscow region.
2. To analyze the correlation between weather and climate conditions (precipitation, humidity of air and soil) and the development of diseases of hybrids of different stability.
3. To identify differences in the stability of varieties of carrots in different ecological and geographical zones.

2. Methods and materials
The research was carried out in the laboratory for the selection of root crops and onions at the All-Russian Research Institute of Vegetable Production in 2008–2018. Varieties and hybrids of planting carrots, samples of wild species and varieties of the genus Daucus from the collection of the All-Russian Research Institute of Vegetable Production, as well as pure cultures of fungi of the genera Fusarium and Alternaria were used as the starting material for research.

To control the infection of mycoses under natural conditions, experiments were set up in the fields of the EXPERIMENTAL PRODUCTION FACILITIES in Bykovo, Ramensky District, Moscow Region, located in the central part of the floodplain of the Moscow River. The soil is alluvial-meadow, medium loamy. The plot area was 1.4 m2 with a sowing of 150 pcs. seed. In two different ecological and geographical zones (Moscow Region and Rostov), the field resistance of 3 varieties and 3 hybrids of carrot canteen was analyzed: Tavrida F1, Nante, Search 32 F1, Search 41 F1, Corsair, Royal Chantene [10].

Mushrooms were isolated from affected root crops, seedlings, leaf blades, umbrellas, seeds by the wet chamber method using Petri dishes and cellophane bags with sterile wet cotton wool. The mushroom coating appeared was examined under a microscope at a magnification of 16x40. Identification of the isolated fungi was carried out by analyzing their cultural and morphological characteristics visually and in the field of view of the microscope using reference guides. A pure culture of isolates of pathogenic fungi A. radicina M., D. et E. and F. oxysporum Schl., Including for artificial infection of plants, was obtained on artificial nutrient media (Capeka, potato and carrot agar) with the addition of the antibiotic gentamicin.

Experiments to assess the resistance of plants 1 year of life were placed on two artificial infectious backgrounds Fusarium and Alternaria, created by applying the inoculum to the soil and spraying the
plants with a spore suspension. 100 plots were sown on a plot of 0.25 m$^2$ seed. Infection of vegetative carrot plants with 1 year of life was carried out by spraying them with a suspension of spores, the viability of which was not less than 80% (for Fusarium $2 \times 10^7$ spores in ml, for Alternaria $2 \times 10^5$ spores in ml) with a manual spray gun. Samples were kept in film insulators at a humidity of 85% and a temperature of 20–25 °C for 15 days. The assessment was carried out using the scale of the eye measurement of the stability of breeding samples [11].

When creating a soil infectious background, the fungal inoculum propagated on a grain nutrient medium (oat grains or a 1:2 mixture of wheat and oats) was introduced (30–40 g/m) into the soil to a depth of 5–7 cm, immediately before sowing, carrot seeds were sown and sprinkled them with a small layer of soil.

On a natural infectious background during the growing season of plants (different varieties, varieties and species), phenological and phytopathological studies were carried out with a frequency of 7 days, starting from the second decade of June, in the phase of 4–6 true leaves.

Assessment of plant resistance to diseases during the growing season was carried out according to the defeat of leaf blades visually on the following scale: 5 – less than 20% of plants are affected (practically resistant); 4 – 21–40% of plants are affected (poorly susceptible); 3 – affected 41–60% of plants (medium susceptible); 2 – 61–80% of plants are affected (susceptible); 1 – 81–100% of plants (highly susceptible) are affected.

During the harvesting period, the resistance scale was used according to the weighted average root crop damage score: 0 – damage less than 0.8 points (practically stable); 1 – lesion 0.9–1.6 points (poorly susceptible); 2 – defeat 1.7–2.4 points (medium susceptible); 3 – defeat 2.5–3.2 points (susceptible); 4 – defeat 3.3–4.0 points (highly susceptible).

During storage, the resistance of carrot root crops to diseases was evaluated on a scale of: 5 – less than 20% of the root surface is affected (practically stable); 4 – 21–40% of the surface is affected (poorly susceptible); 3 – affected 41–60% of the surface (medium susceptible); 2 – 61–80% of the surface is affected (susceptible); 1 – 81–100% of the surface is affected (highly susceptible).

The prevalence and development of diseases was calculated according to generally accepted formulas.

3. Results
The breeding process to create resistant varieties and hybrids began with the formation of a collection of source material from various resistant genes and a comprehensive evaluation of samples using laboratory express methods and three artificial infectious backgrounds in the field. When seedlings appeared a month after sowing, the first examination was done, considering the manifestation of symptoms of damage on the roots (seedlings) on 3 backgrounds: natural infection (control) and on two artificial backgrounds Fusarium and Alternaria.

As a result of the evaluation, samples with signs of Fusarium wilting, which was manifested in yellowing, wilting of seedlings and decay of seedlings, were isolated on an artificial background of Fusarium. On the infectious background of Alternaria and on the natural background, when considering the seedling phase, the symptoms of the lesion did not appear.

When 6 true leaves appeared (July), the plants were sprayed with a spore suspension of Fusarium and Alternaria. After 2 weeks, on a fusarium infectious background, there were practically no signs of damage to the leaf plate of carrots, and on an alternarious background, there was a defeat of the leaf plate in the form of blackish point necrosis, which subsequently expanded and led to the complete death of the leaves.

During harvesting (mid-September), final counts were made, and stable samples and genotypes were identified, which were stored for further breeding. As a result of a comprehensive assessment of wild species, the following samples were included into the practically stable group: No. 10. Daucus carota subsp. maximus (Desf.) Ball.; No. 11. Daucus broteri Ten.; No. 13. Daucus halophilus Brot.; No. 16. Daucus carota L.; No. 17. – 70-13 Daucus maximus Desf.; No. 19. Daucus carota L.; No. 20., Daucus setifolius Desf.; No. 21. Daucus carota L.; No. 22. Daucus muricatus (L.) L.; No. 23. Daucus
setifolius Desf.; No. 26. Daucus carota L.; No. 27. Daucus carota L. Isolated species and subspecies are genetic sources of resistance to fungal diseases [11].

In 2011–2013, more than 20 carrot samples were evaluated for resistance to alternariosis, of which this article presents data on promising domestic varieties: Losinoostrovskaya 13, Marlinka, Moscow Winter A-75, NIIOKH-336, Fakel, on which selection was made.

The first symptoms began to appear only in early August. The average lesion score for the samples ranged from 1 on the Marlink variety to 1.2 points on the Torch. The final sustainability survey was carried out at the time of harvesting on September 25th. For winter storage, root crops without signs of damage and up to 1 point of damage on the leaf plate were laid for 7 months.

The effectiveness of the use of selection selection (2011–2013) by genotypes (resistant plants) is shown in figure 1.

| 2011 sowing = selection of resistant plants |
| 2012 planting plants of the second year of life = selection of resistant seed plants, obtaining seeds |
| 2013 sowing = selection of resistant plants |

**Figure 1. One-year selection**

In 2011, on the Marlink variety, the damage score on the leaf plate was 2.5, the safety of root crops was 81.6 %, after selection in 2013, the damage score was 1, and the safety was 88.9 %. The Moscow Winter variety in 2011 had 1.5 points of defeat, in 2013 – up to 0.8 points and was included in the stable group.

Thus, by a single selection, resistance to varieties of carrot of the dining room is increased, and it is possible to select promising material (genotypes) for further breeding work.

The determination of the correlation dependence makes it possible to determine stable and indirect relationships between characters, such information is of particular interest when performing selection work [12].

To study the variation in the prevalence of diseases from pp. Fusarium and Alternaria, depending on weather and climate conditions, carrot crops of different resistance groups were placed on two infectious backgrounds. The control was crops in natural field conditions. The following were used in the experiment: Maestro F1 – steady, Red cored – medium susceptible, and Nigel – susceptible variety specimens. The experience data are presented in table 1.

**Table 1. Correlation dependence between agro-climate conditions and development of diseases on different infectious background and variety on variety of varieties (average for 2011–2015)**

| Agroclimatic indicators | Varieties / Hybrids |
|-------------------------|---------------------|
|                         | Maestro (steady)    | Red cored (medium susceptible) | Nigel (susceptible) |
| Temperature, t          | A*( -0.15)          | A -0.15                         | A 0.24              |
|                         | F* -0.23            | F -0.15                         | F 0.22              |
|                         | E* -0.14            | E 0.50                          | E -0.12             |
| Humidity %              | A 0.46              | A 0.46                          | A 0.13              |
|                         | F 0.39              | F 0.50                          | F 0.14              |
|                         | E0.19               | E 0.61                          | E 0.12              |
| Precipitation, mm.      | A 0.91              | A 0.92                          | A 0.79              |
|                         | F 0.82              | F 0.94                          | F 0.79              |
|                         | E 0.78              | E 0.87                          | E 0.60              |

Note: * A – artificial background Alternaria, F – artificial background Fusarium, E – natural infectious background.

In 2011, increased humidity prevailed – 72.6 %, precipitation – 42.2 mm and an average temperature of 17.5 °C. In 2013, air humidity is 72.8 %, moderate precipitation is 55.1 mm and
average temperature is 16 °C. In 2015, the humidity is 69.2 %, the minimum rainfall is 21.9 mm, and the temperature is 16.4 °C.

The highest correlation for Maestro’s stable hybrid is between the precipitation: for Alternaria – 0.91, Fusarium – 0.82, for the natural background – 0.78. Moderate correlation with respect to air humidity: for Alternaria – 0.46, Fusarium – 0.39, for the natural background – 0.19.

When analyzing the correlation dependence on the Red cored medium-sensitive control, it was found that a high correlation between precipitation and the development of diseases on the backgrounds from 0.87 on the natural and from 0.92 to 0.94 on the infectious backgrounds of Alternaria and Fusarium.

Humidity in relation to the backgrounds: a noticeable correlation of 0.69 is against the natural background; for the infectious backgrounds of Alternaria and Fusarium, a moderate correlation is from 0.40 to 0.50, respectively.

A weak correlation between temperature and the development of the disease on a natural background is 0.17.

When analyzing the correlation dependence on the Nigel susceptible control, a high coefficient with respect to precipitation is 0.79 for Alternaria and Fusarium, and for the natural background – 0.60. A weak correlation with respect to air humidity is 0.13, 0.14 and 0.12, respectively.

Thus, the obtained results allow us to conclude that, with increased precipitation and high soil moisture, pathogens from r. Fusarium and Alternaria develop very well and affect the root system, root crops and leaf blade, often leading to the complete death of the plant.

In studies conducted in 2017–2018, simultaneously in two different areas: the Moscow region (All-Russian Research Institute of Vegetable Production, a branch of the Federal State Budget Scientific Institution, Federal Scientific Center, Ramensky District) and the Rostov Region (the Biryuchekut Vegetable Breeding Experimental Station, a branch of the Federal Scientific and Technical Center, Oktyabrsy District) assessed the sustainability carrots depending on the weather and climatic conditions of the growing region.

Ramensky district of the Moscow region belongs to the southern forest zone of the European province of the central part of the Russian Plain. The soil is alluvial, meadow, medium loamy. The humus content is 2.7–3.0 %, pH 6.4 6.7, the degree of provision with nutrients: phosphorus – medium, potassium – low.

The Oktyabrsy district of the Rostov region is located in the southern part of the East European Plain. The soils in the experiments are represented by the North Priazov variety of ordinary chernozem [13]. The humus content is 6.0–6.6 %, pH 7.0 7.5, the degree of provision with nutrients is high.

Meteorological conditions 2017. In the Moscow region, spring was cold, which increased the emergence period of seedlings by 2–3 days. Precipitation in May fell more than the mean annual value. The average daily temperature in June, 15.2 °C, coincided with the average annual temperature. Precipitation in June was more than the annual average, which was unfavorable for the development of carrots in the "fork" phase and the beginning of the formation of root crops. July and August were hot; there was a lot of rainfall. In September, during the harvesting of root crops, the weather was warm and humid. In the Rostov region, spring was warm, the time of emergence of seedlings was normal (8–12 days). Summer was arid and hot, in July and August the air temperature reached 40 °C in the shade, the relative humidity did not exceed 30–40 %. These factors adversely affected the development of root crops. In September, during the harvesting of root crops, the weather was warm and dry [10].

Meteorological conditions of 2018. In the Moscow region, spring was hot, rainfall in May fell at the level of long-term average values, but most fell in the form of rain showers immediately after sowing, forming a soil crust that caused the death of seedlings and delayed their appearance up to 28 days. June was warm and dry, July and August were hot and dry. In September, the weather was warm and dry, which favored the harvesting of root crops. In the Rostov region, spring was hot, the emergence of seedlings was delayed (14–19 days). Summer was arid and hot, in July and August the air temperature in the shade exceeded 40 °C, the relative air humidity was in the range of 20–40 %,
and the corresponding factors adversely affected the growth and development of root crops. In September, during the harvesting of root crops, the weather was warm and dry.

The results of the evaluation of the resistance of samples of carrots of the dining room to leaf diseases are presented in table 2.

| Grade F1      | Moscow region | Rostov region |
|---------------|---------------|---------------|
|               | 2017 | 2018 | 2017 | 2018 |
| Corsair       | 42.5 | 28.9 | 45.7 | 41.9 |
| Chantane Royal| 22.6 | 19.8 | 21.3 | 20.0 |
| Nantes        | 23.9 | 20.1 | 22.9 | 20.8 |
| Tauris F1     | 33.1 | 23.8 | 25.8 | 32.1 |
| Search 32 F1  | 14.9 | 11.8 | 20.3 | 18.7 |
| Search 41 F1  | 16.8 | 12.9 | 20.8 | 30.5 |

The prevalence of leaf diseases in the conditions of the Moscow and Rostov regions in 2018 was lower compared to 2017, and the samples are more stable, except for the Taurida F1 and Search 41 F1 hybrids. In 2017, the following samples could be considered practically stable: Search 32 F1 (14.9 %), Search 41 F1 (16.8 %), in 2018 – Royal Chantenay (19.8 %), Nantes (20.1 %), Search 32 F1 (11.8 %), Search 41 F1 (12.9 %). In the Rostov region in 2017, the samples were practically stable: Search 32 F1 (20.3 %), Search 41 F1 (20.8 %), in 2018 – Royal Chantenay (20.0 %), Nantes (20.8 %), Search 32 F1 (18.7 %)) The worst result was shown by the corsair variety (although it belonged to the medium-sensitive group) – 45.7 and 41.9 % (Rostov Region). In the Moscow region, the same variety in 2017 was moderately susceptible (42.5 %) to leaf diseases, in 2018 it was poorly susceptible (28.9 %). In general, the prevalence of diseases in the dining room carrots in the south over the years of research was less than in the central part of the RF NPP.

In 2018, compared to 2017, the samples in both zones were more stable, except for the Taurida F1 and Search 41 F1 hybrids.

4. Conclusion

In the conditions of the Moscow Region, resistant and wild carrot species and subspecies were identified against Alternaria and Fusarium wilt on natural and artificial infectious backgrounds, including Daucus carota subsp. maximus (Desf.) Ball.; D. broteri Ten.; D. halophilus brot.; D. maximus Desf.; D. setifolius Desf.; D. carota Roth.; D. carota L.; D. muricatus (L.) L.; D. setifolius Desf., Which may be genetic sources of resistance to disease pathogens when involved in breeding work to create new resistant varieties and hybrids of canteen carrots.

When tested for resistance in a single selection of breeding material to leaf and root diseases after storage, the most valuable breeding samples of sowing carrots were identified: Losinoostrovskaya 13, Marlinka, NIIOKh-336, Fakel, Moscow Winter A-75. Good preservation of the uterine material is associated with the effectiveness of the selection for the resistance of carrot plants of the dining room of the first year of life.

Using the example of the medium susceptible variety of carrots Red cored and susceptible Nigel, a high correlation between precipitation, high soil moisture, and the development of Alternaria sp. and Fusarium sp. on natural and infectious backgrounds.

The prevalence of diseases in canteen carrots in southern Russia in the Rostov region over the years of research was less than in the central part of the Russian Federation's non-health care facilities; in a wetter 2018 compared to 2017, the variety samples in both zones were more stable, except for individual hybrids.
References

[1] Thymine N I and Timina L T 2015 Breeding and genetic identification of forms and inbred lines of carrot *Breed. and seed product. of vegetable crops* **46** 555–60

[2] Gagkaeva T A, Gavrilova O P, Levitin M M and Novozhilov K V 2011 Fusariosis of cereal crops *Suppl. to the J. Protect. and quarant. of plants* **5** 112

[3] Ben-Noon E, Shlaimeng D, Shlavin E et al 2001 Optimization of chemical suppression of Alternaria dauci, the causal agent of alternaria leaf blight in carrots *Plant Disease*

[4] Farrar J J, Pryor B M and Davis R M 2004 Alternaria diseases of carrot *Plant Disease*

[5] Akhatov A K, Hannibal F B, Meshkov Y I et al 2013 Diseases and pests of vegetable crops and *potatoes* (Moscow: Moscow Partnership of scientific publications KMK) 463 p

[6] Krivosheev S M 2001 Development and evaluation of forms of interspecific hybrids of carrot genetic sources of valuable breeding traits (Cand. Dissertation thesis) (Moscow)

[7] Nalobova V L 2015 Analysis of accessions of vegetable crops on the infestation of fungal, bacterial and viral diseases *Breed. and seed product. of vegetable crops* **46** 429–36

[8] Hannibal F B, Orina A S and Levitin M M 2010 Alternariosis crops in Russia *Protect. and quarant. of plants* **5** 30–2

[9] Semenov A N et al 2016 Comparative analysis of polymorphism of microsatellite markers in a number of Fusarium species *Proc. of the Timiryazev agricult. Acad.* **1** 40–50

[10] Yusupova L A, Sokolova L M, Kornev A V and Khovrin A N 2019 Variety testing of carrots canteen in the Moscow and Rostov regions *Potatoes and vegetables* **1** 37–40

[11] Leonov V I, Howren A H, Sokolov L M et al 2018 Genetic collection of wild carrot species and hybrids on mushroom resistance Alternaria SP. and Fusarium SP *Achievements of sci. and technol. of agricult.* **32**(7) 26–30

[12] Sokolova L M 2019 Effects of weather conditions on disease prevalence and resistance of table carrots *Bull. of Altai state Univer.* **4**(174) 21–6

[13] Agafonov E V and Poluektov E V 1999 *Soils and fertilizers in the Rostov region* Training manual (Persianovka) 90 p