

# Pathocomplex of root rot of apple tree in nurseries and young orchards of the South of Russia

Irina Astapchuk\*, Galina Yakuba, and Andrei Nasonov

Federal State Budget Scientific Institution «North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making», 39 str. 40 Let Pobedy, Krasnodar, 350901, Russia

**Abstract.** Root rot poses a serious threat to the main fruit crop - domestic apple in nurseries and young orchards. Obtaining healthy planting material, free from root pathogens and root rot, is an especially priority task in conditions of intensive production. In the process of analyzing the affected plants from nurseries and young orchards at four sampling points, pathogens of apple root rot from 11 genera were identified and 431 isolates were extracted. The most common causative agents of apple root rot seedlings in the entire studied sample were species of the genus *Fusarium* spp. Soil micromycetes such as *Rhizoctonia* spp., *Cladosporium* spp., *Cylindrocarpon* spp., and oomycetes from the genus *Pythium* were rare. The composition and occurrence of species were heterogeneous at different points in the studied region. *Phomopsis mali* (Schulzer & Sacc.) Died., *Cytospora* spp., *Alternaria alternata* (Fr.) Keissl., and *Aspergillus niger* Tiegh. have been associated with root rot of seedlings and young apple trees.

## 1 Introduction

Among the diseases of woody plants, root rot is the most dangerous. This disease affects many fruit and berry crops: apple, pear, plum, peach, apricot, cherry, strawberry, etc. [1-8]. The causative agents of root rot are obligate and facultative pathogens, some of which become xylophages when the tree dies; as a result of pathogenesis, roots rot, the wood turns brown and cracks, the bark turns black, the branches weaken, the leaves turn yellow and crumble, the plant stops fruiting and eventually dies [9-10].

The spread of fungi-causative agents of rot and infection of plants occurs with spores when they get on the roots, the root neck of the stock, as well as mycelium and its various modifications (membranes, rhizomorphs) when the roots of diseased trees come into contact with healthy ones. The mycelium penetrates into the tree trunk through the wounds at the base of the trunk, then with the sap flow system it quickly spreads to its upper parts. Root rot that affects the tree above the graft site is called root collar rot. [1, 5, 9, 10].

In forest and artificial plantations, root rot can become widespread, covering large areas. The disease is widespread in plantations experiencing high recreational pressure,

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\* Corresponding author: [irina\\_astapchuk@mail.ru](mailto:irina_astapchuk@mail.ru)

which creates conditions conducive to the weakening of plants, a decrease in their biological resistance, an increase in the probability of mechanical damage to the roots and trunks and infection with root rot. Not only trees older than 10 years are susceptible to infection with pathogenic fungi, but also often weakened by improper care or unfavorable conditions, including weather, young seedlings and rootstocks in nurseries [1, 10].

The mother plants of clonal rootstocks and mother-cuttings orchards belong to specific plantings. Their areas are often limited, they are used for multiple cutting of cuttings, a huge number of cut wounds are applied during the period of planting exploitation. Such wounds form weakened tissues that are subsequently colonized by various pathogens; when planted in the garden, seedlings and young plants sometimes die during 1-2 growing seasons. When obtaining clonal rootstocks by rooting green cuttings in a greenhouse, it becomes necessary to protect against soil fungal infection [1, 10].

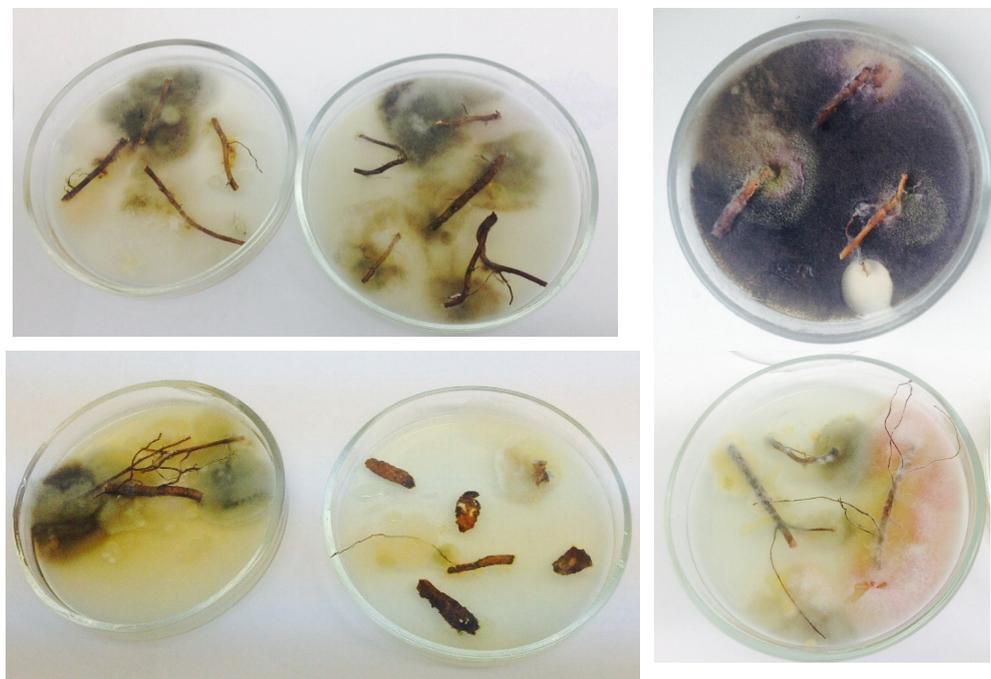
Depending on the conditions of humidity, air temperature, illumination and the predecessor in the crop rotation, seedlings and young plants of fruit crops are affected by such root rot pathogens as *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*) [11, 12], species from genus *Pythium* Pringsheim [6, 11, 13], *Cylindrocarpon* Wollenw [14], *Fusarium* Link [10, 11, 15] and *Phytophthora* de Bary [6, 7, 8, 9]. Pythiosis, late blight, fusarium, rhizoctonia, the prevalence of which can vary from 10 to 100 %, weaken the root system, affect the development of plants, and can cause their complete death [1]. Surviving clonal rootstocks, seedlings or grafted plants in nurseries and young gardens can later be affected by pathogens from the genera *Phomopsis* (Sacc & Roum), *Phoma* [11] in the area of the root collar and above, which later leads to drying out of plants, losses can reach 50-80 % [1].

In connection with the above, root rot poses a serious threat to the main fruit crop - domestic apple trees in nurseries and young orchards. To carry out a timely set of protective measures, as well as to create technologies to reduce stress for propagated plants, constant monitoring of the pathocomplex of the disease is required. Obtaining healthy planting material, free from pathogens of root and root rot, is an especially priority task in conditions of intensive production. Objective of the research: to study the species composition of causative agents of apple root rot in young seedlings of domestic apple trees in Southern Russia under changing environmental conditions.

## 2 Materials and methods

Research conducted in 2019-2020 in the laboratory of biotechnological control of phytopathogens and phytophages of FSBSI «North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making». The selection of one- and two-year-old grafted seedlings with symptoms of root rot damage was carried out in February-March 2019 by routine examinations according to generally accepted methods [15] in industrial gardens of the South of Russia: Karachay-Cherkess Republic (KCR), Rostov Region, Krasnodar Region and Belgorod Region.

The objects of research were causative agents of apple root rot. Isolation of micromycetes was carried out under sterile conditions from the central and lateral roots, root collar using a microbiological method [15] on potato-glucose agar (PGA) at a temperature 24–26 °C (Figure 1).



**Fig. 1.** Isolation of *in vitro* fungi from the roots, root neck of affected apple seedlings (original)

Determination of the species composition of mycopathogens was carried out in 2019-2020 using standard techniques and domestic and foreign definitive literature [15]. The names of species and genera, as well as their nomenclature, are given according to modern taxonomic systems and databases <http://www.mycobank.org/> and <http://www.indexfungorum.org/>.

The calculation of similarity coefficients, or commonality indices, using lists of species of fungi communities was performed using the Jaccard index  $K_j$  (equation 1). These coefficients are equal to 1 in the case of complete coincidence of community species and equal to 0 if the samples do not include common species.

$$K_j = \frac{N_{ab}}{(N_a + N_b - N_{ab})} \quad (1),$$

where  $N_{ab}$  – is the number of common species in communities A and B;

$N_a$  – is the number of species in community A;  $N_b$  – is the number of species in community B.

### 3 Results and discussion

We studied 115 samples of young plants from four surveyed regions of the South of Russia. During the analysis of the affected plants at all four sampling points, causative agents of apple root rot from 11 genera were identified and 431 isolates were isolated (Table 1).

The most widespread species in the South of Russia were *Fusarium* spp., *Alternaria alternata* (Fr.) Keissl. and *Aspergillus niger* Tiegh., which were found in all studied points (Table 1). Moreover, in terms of the total number of isolated isolates, micromycetes of the genus *Fusarium* are in the lead, whose share is 61 % of the entire sample.

**Table 1.** Pathocomplex of causative agents of the main rot of the domestic apple tree in the South of Russia (2019)

Genera	The frequency of occurrence of pathogens, %				The number of isolates, pieces
	Rostov region	Krasnodar region	Belgorod region	Karachay-Cherkess Republic	
<i>Fusarium</i> spp.	112	98	34	20	264
<i>Alternaria alternata</i>	29	28	13	1	71
<i>Aspergillus niger</i>	25	25	6	3	59
<i>Agrobacterium tumefaciens</i>	-	12	-	-	12
<i>Cytospora</i> spp.	-	-	9	-	9
<i>Cladosporium</i> spp.	-	6	-	-	6
<i>Cylindrocarpon</i> spp.	-	3	-	1	4
<i>Rhizoctonia</i> spp.	-	2	-	-	2
<i>Pythium</i> spp.	-	-	-	2	2
<i>Phomopsis mali</i>	-	1	-	-	1
<i>Coryneum microstictum</i>	-	-	-	1	1
The number of examined seedlings affected by root rot, pcs.	48	38	17	12	

The next in frequency of occurrence is the genus *Cylindrocarpon* spp., the isolates of which were isolated from the roots of young plants of industrial gardens in the Krasnodar Territory and KCR; other root rot pathogens were unique for each sampling point.

The broadest pathocomplex of root rot pathogens was observed in the Krasnodar Territory - 8 genus, 175 isolates were isolated. Among the isolated isolates, 56 % of the sample were represented by the genus *Fusarium*, 16 % by the species *A. alternata*, and 14 % by *A. niger* and 7 % *Agrobacterium tumefaciens* Beijerinck and van Delden; other representatives from different genus were isolated with a frequency of less than 5 %.

In the Rostov region, 67 % of the sample of root rot pathogens were represented by the genus *Fusarium*, 17 % of the sample - by *A. alternata*, and 16 % - by *A. niger*.

In the Belgorod region, the same diversity of dominant species was found, among which 55 % of the sample is represented by species of the genus *Fusarium*, *A. alternata* is represented in 21 % of cases, *Cytospora* spp. Ouellette at 14 % and *A. niger* at 10 %.

Among the isolates of root rot pathogens from industrial gardens of the KCR, the genus *Fusarium* prevailed (71 %); *A. niger* was found with a frequency of 11 %, *Pythium* spp. represented in 7,5 % of the sample, the share of other isolates was less than 5 %.

As a result of calculating the Jaccard index, it was revealed that the highest coefficient of similarity of the pathocomplex communities of pathogens of root rot in apple young plants was noted at the sampling points from Rostov and Belgorod regions, while between the same gardens and the gardens of Krasnodar region there was a minimum degree of similarity of the studied pathogenic communities, the community similarity between the rest of the sampling points was average (Table 2).

**Table 2.** Similarity coefficients of the communities of apple root rot pathogens from different sampling points according to the Jaccard index (Kj)

Areas	Rostov region	Belgorod region	Karachay-Cherkess Republic
Krasnodar region	0.37	0.33	0.40
Rostov region	-	0.75	0.50
Belgorod region	-	-	0.42

Mycological analysis of the root and root parts of young home apple seedlings showed an identical pathocomplex of root rot pathogens in the Rostov and Belgorod regions, which may be occurs due to both the close location of sampling points and one source of planting material, including the transfer of pathogens from soil on the root system.

The study of root rot pathogens in the Krasnodar region showed a wide variety of the pathocomplex composition; *Cladosporium* spp., *Rhizoctonia* spp., *Phomopsis mali* turned out to be unique.

*Ph. mali* is the result of damage and partial death of the root system from root rot, which led to the weakening of plants. *Cylinrocarpon* spp., *A. alternata* and *A. niger* species were identified by us as wound pathogens: in places of root necrosis, caused, as a rule, by *Fusarium* root rot, that is, as secondary infecting fungi. The causative agent of cytosporosis *Cytospora* spp., which causes an infectious drying out of fruit crops and also affects the roots, was isolated from the sites of necrosis resulting from root rot.

Comparative analysis of our results with the data of S.E. Golovin [1] showed that changes in environmental conditions, including cultivation technology, weather and climatic parameters, as well as the use of various sources of planting material, influenced the species composition of the pathocomplex of pathogens of apple root rot. So, in the nursery of Krasnodar region in 1991, it included 5 species of the genus *Pythium*, which prevailed, as well as only 2 species of the genus *Fusarium*, while in our studies the genus *Fusarium* was dominated - 11 species [15].

To prevent the development of root rot, a complex of organizational, economic, agrotechnical and phytosanitary measures is carried out. The first is to ensure spatial isolation of at least 1 km of areas planned for mother plantings from industrial plantings [1]; selection of areas with light texture soils. Agrotechnical measures provide for the exclusion of mechanical damage to plants in the zone of the root collar, in young gardens when processing row spacings, and also after irrigation or excessive rainfall, the embedding and silting of the grafting sites with soil should not be allowed, especially with low grafting of seedlings.

The phytosanitary unit provides for the destruction of plant residues, which are sources of primary infection with root rot pathogens; regular monitoring of signs of root rot, culling and burning of dead plants along with the roots. There are no specialized fungicides for the protection of fruit crops against root rot on the territory of the Russian Federation. Researchers recommend soaking the bottom of the cuttings before planting green cuttings by immersing them in fungicide solutions for 2-3 minutes; to prolong the action of drugs, it is proposed to use film formers of new generation. When laying the first field of the nursery, high efficiency of protection against a complex of pathogens of root rot was obtained by soaking the root system and the area of the root collar of rootstocks in solutions of systemic fungicides [1].

We have begun *in vitro* studies to select drugs that are effective in controlling apple root rot. Fungicides with active ingredients difenoconazole and thiram were identified as promising.

## 4 Conclusion

According to the results of the study, a wide species diversity of representatives in the complex of pathogens that cause apple root rot in nurseries and young orchards in the South of Russia - 11 genera can be noted. The most common causative agents of root rot of apple seedlings in the entire studied sample were species of the genus *Fusarium* spp. Soil micromycetes such as *Rhizoctonia* spp., *Cladosporium* spp., *Cylindrocarpon* spp., and oomycetes from the genus *Pythium* were rare. The composition and occurrence of species were heterogeneous at different points in the studied region. *Phomopsis mali*, *Cytospora* spp., *Alternaria alternata*, and *Aspergillus niger* have been associated with root rot of seedlings and young apple trees. According to primary data, *in vitro* efficacy has been revealed against a complex of causative agents of apple root rot in some fungicides: with active ingredients difenoconazole and thiram; researches will continue.

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