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Apple tree, *Malus domestica* Borkh. alternaria and ways to reduce its harmfulness in the non-chernozem zone of Russia

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ABSTRACT

This study was conducted on the territory of the educational and experimental farm of the K.A. Timiryazev Russian State Agrarian University of Agriculture in Moscow during 2018-2020. The examination of apple-tree plantings, ornamental plantings, and weed vegetation was carried out for three years with determination of pathogens of the main diseases by microscopy method. The *Alternaria* fungi species membership were identified by morphological features of conidia and sporulation habitus, and the specific pathogen species was confirmed by PCR analysis. Two unspecified species of *Alternaria tenuissima* and *Alternaria infectoria* fungi were found to infect apple trees and other species of the surveyed plants with Alternaria blight. The fungus species, *Alternaria tenuissima* was predominantly recorded on apple trees. Out of 186 examined apple cultivars, the development of Alternaria blight was found in 85.5% of the cultivars with different ripening periods. The apple cultivars most resistant to Alternaria blight were identified. The apple cultivars, in which the damage of fruits by Alternaria blight was recorded, were identified. Infestation of apple seeds by *Alternaria* genus obtained from fruits affected by this pathogen was determined. The possibility of high Alternaria blight infestation degree in apple tree seedlings was detected. A high degree of *Alternaria tenuissima* and *Alternaria infectoria* fungi development was recorded in a number of orchard-protecting and ornamental plants, as well as in weeds, and their possible role as reservoirs and transmitters of infection to fruit crops was established. The efficacy of fungicides: Luna Tranquiliti, KS; Skor, KE; Medea, ME; Zato, VDG; Horus, VDG; Coside Super, VDG in suppressing Alternaria blight development on apple cultivars of different ripening dates was compared.

Keywords: Alternaria blight, fungicides, seedlings, apple tree, variety, diseases, fruit crops.
Article type: Research Article.

INTRODUCTION

Effective plant disease control can only be organised if the pathogen is accurately identified and its biology understood, since only then, the correct disease control methods can be selected. One of the key points in the organization of protective measures in orchards against fungal diseases is the selection of the most effective plant protection agents and using the cultivars most resistant to major diseases (Al-Masoodi *et al.* 2023). In recent years, many authors have noted an increase in the infestation of many crops by fungi of the genus *Alternaria* Nees. According to recent scientific publications, about 280 species of *Alternaria* genus fungi were described in the world, 50 of them cause economically important diseases; and about 20 such diseases have been recorded in Russia (Hannibal & Bilder 2008). Alternaria blight disease has been described in detail on field crops. In scientific works, the description of fruit crops affected by this disease begins with the description of a high degree of

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Alternaria blight development in Japan and the United States in 1962. Subsequently, the disease was reported in Yugoslavia and Korea (Hannibal & Bilder 2008). The description of fruit crops affected by Alternaria in Russia begins with the publications of 1999, where the development of Alternaria blight on apple trees in the south of European Russia was described (Gagkaeva & Levitin 1999; Kolesova & Chmyr 2005). Modern researchers reported Alternaria on apple trees, gooseberry, and currant (Delgado *et al.* 1998), as well as on forest crops (Hannibal & Bilder 2008; Yarmolovich *et al.* 2016). Due to the relatively recent description of this disease in Russia on pome fruits, a number of researchers noted the difficulty in identifying the specific pathogen species from this genus (Home 1920; Filajadic & Sutton 1991; Johnson *et al.* 2000; Gannibal; *et al.* 2007; Hannibal & Bilder 2008); many plant protection specialists have difficulties in its identification, often confusing it with other diseases; this leads to using ineffective means of protection against this disease. Notably, there is no information on the resistance of various apple cultivars to Alternaria blight, which also greatly complicates the organization of control of this disease. It is known that contamination of agricultural products with fungi of the genus *Alternaria* can pose a direct threat to human and animal health. The *Alternaria* spp. metabolites toxicity has been proved by many researchers, who pointed out their teratogenicity, embryo-toxicity, and ability to cause haematological diseases (Home 1920; Filajadic & Sutton 1991; Johnson *et al.* 2000). The following metabolites are the most frequently detected and toxic to humans: Alternariol, Alternariol monomethyl ester, Altenuene, Tenuazonic acid, and Altertoxin I. These metabolites are found both in apple fruits and in many fruit and vegetable crops (Home 1920; Filajadic & Sutton 1991; Johnson *et al.* 2000). One important and negative property of these metabolites is their resistance to degradation during processing agricultural products. For example, a number of researchers indicated that these toxins are found in apple and in tomato juice, as well as in red wine (Home 1920; Gagkaeva & Levitin 1999, Johnson *et al.* 2000). Researching the species composition of *Alternaria* fungi that cause the disease in apple trees, establishing the resistance of different apple cultivars, testing and isolating the most effective fungicides is important for proper organization of measures to control Alternaria blight. Also, it is important to establish the ways in which the *Alternaria* spp. can persist and spread in order to reduce the high development risk for fungi of this genus in fruit orchards.

MATERIALS AND METHODS

The research was conducted in the Educational and Experimental Farm of the Federal State Budgetary Educational Institution of Higher Professional Education, "K.A. Timiryazev Russian Academy of Agricultural Sciences", Moscow. The soil in the surveyed area was sod-podzolic, medium-loam, pH 6.2 and humus 2.6%. Plantings of mature trees and seedlings of pome fruits were represented with apple trees, *Malus domestica* Borkh. The tree plantations were surveyed. In addition, its fungal diseases were recorded and diagnosed on various fruit and berry crops (more than 27 species in total), ornamental plantations, and weed vegetation. The phytosanitary condition was monitored on the basis of route surveys of the orchard area, taking into account at least five plants of each variety between 12 and 15 years of age. Disease development surveys in the orchard were conducted between May and September 2018-2020. Disease diagnosis was made by collecting diseased tissues, plant shoots and fruit slices, and depositing them in a damp chamber for 3 days for further identification of the pathogen species by microscopy. Additional confirmation of the *Alternaria* species was carried out by PCR analysis with specific primers. Determination of the *Alternaria* genus was performed using the recommendations made by G. Simmons (Simmons 1999; Simmons 1999) and studies of the Federal State Budgetary Scientific Institution All-Russian Research Plant Protection Institute (FSBNU ARRPPI) (Hannibal & Bilder 2008). The method was based on determining the morphological characters of fungal conidia (size and colour of conidia, size and shape of the apical outgrowth, size, shape, and number of septa of conidia bodies) and determining the fungus' sporulation habitus. Assessment of plant infestation was carried out according to the conventional five-point phytopathological scale with calculation of prevalence (P), and disease development index (DI) (Dolzhenko 2009). The study examined the application on apple trees of the following preparations having State registration in the territory of the Russian Federation [14]: Luna Tranquiliti, KS (125 g L^{-1} fluopyram + 375 g L^{-1} pyrimethanil) at a rate of 1.2 L ha^{-1}, Skor, KE (250 g L^{-1} difenoconazole) at a rate of 0.35 L ha^{-1}, Medea, ME (50 g L^{-1} difenoconazole + 30 g L^{-1} flutriafol) at a rate of 1.2 L ha^{-1}, Zato, VDG (500 g kg^{-1} trifloxystrobin) at a rate of 0.14 kg ha^{-1}, Horus, VDG (750 g kg^{-1} ciprodinil) at a rate of 0.7 L ha^{-1}. The preparations were applied twice during the season: at the phase of "rosebud" and petal fall at 14 day intervals. The application of Koside Super VDG fungicide (350 g kg^{-1}copper hydroxide) at a rate of 2.0 L ha^{-1} three times during the season, starting at the "green cone"

phase at intervals of 12 days. The efficacy of fungicide application on apple-trees was evaluated in accordance with the guidelines for registration trials of fungicides in agriculture, using five plants of each variety for each experimental variant (Dolzhenko 2009). The fungicides were evaluated for their ability to reduce the degree of Alternaria blight development on three cultivars of apple trees differing in maturity including Bely naliv (Braeburn, summer kind of apples), Korichnoye polosatoye (autumn kind of apples), and Lobo (winter kind of apples).

RESULTS AND DISCUSSION

During the three years of the study, it was observed that the development of apple tree's Alternaria blight was stronger on the insect-damaged leaves. There was also a correlation between the affection of individual apple-trees and their location on the plots. So, plants located in the outermost rows and close to roads (more likely to be damaged by machinery) were more affected, which may be related both to wind transport of the pathogen conidia and to mechanical damage of fruit trees. It was found that a higher degree of Alternaria blight infestation was in plots where the action of orchard protective structures was weakened. No correlation could be found between the occurrence of apple-tree Alternaria blight disease and the development of other diseases on the same trees during the study. The onset of Alternaria blight development on apple trees was recorded from the 2nd - 3rd decades of June in the form of small rounded brown spots, which, with strong development of the disease, can merge and cover most (up to 70%) of the affected leaf area (Fig. 1). Severe development of Alternaria blight on apple leaves was found to cause premature leaf drop (at 2nd - 3rd weeks). The Alternaria blight development was detected on apple fruits from the beginning of ripening as rounded, slightly depressed red spots with a yellow border (Fig. 2). However, not all apple variety fruits were affected.

Fig. 1. Apple tree cultivar Bely naliv with leaf infestation by Alternaria blight (20. 07. 2020).

It was found that disease symptomatology on fruits was strongly dependent on cultivar, which may also contribute to difficulties in visual diagnosis of the disease. The development of *Alternaria* genus on apple-tree shoots during the study was not observed, but some authors noted the possibility of Alternaria blight development on apple-tree shoots (Kolesova & Chmyr 2005). Medea and ME preparations exhibited the greatest efficacy against Alternaria blight development on leaves and fruits: 82-88.4% (on leaves) and 85-91% (on fruits); Luna Tranquiliti and KS fungicides reduced Alternaria blight development on leaves by 78-85.5% and on fruits by 80-86.4%. The efficacy of Skor, KE and Zato, VDG fungicides was close to them and was 70-74.5% by the disease development on leaves and 71-74.5% on fruits. The preparations of Horus, VDG and Coside Super, VDG showed efficacy in reducing Alternaria blight development on leaves at 65.1-68.4% and 60-70% on fruits (Table 1).

Table 1. Average biological efficacy of fungicides on apple trees during 2019-2020.

Name of the drug	Biological efficacy in the suppression of Alternaria blight	
	Leaves (%)	Fruits (%)
Medea, ME	82-88.4	85-91.0
Luna Tranquiliti, KS	78-85.5	80-86.4
Skor, KE	72-74.5	71-74.5
Zato, VDG	70-75.2	72-74.0
Chorus, VDG	65.5-68.4	64.8-70

Koside Super, VDG	65.1	60-63.5

Fig. 2. An apple fruit of Antonovka cultivar with symptoms of Alternaria blight (20.08.2020).

The tested preparations retained their effectiveness for at least 14 days. The reduced development of Alternaria blight on leaves and fruits as a result of fungicide application contributed to the production of less-damaged fruits. During fruit removal, we noted that fruits affected by Alternaria blight, even to a moderate degree, exhibited a firmer flesh and thicker skin; they became angular and lost their marketable appearance; their palatability decreased. During storage of removed fruits, it was noted that in the place of Alternaria blight disease symptoms during the first month of storage, fruits of Spartan and Lobo cultivars began to develop storage rots, the main species of which were: Penicilliosis rot (*Penicillium digitatum* (Pers.) Sacc.), bitter rot (*Colletotrichum fructigenum* (Berk.) Wass.), mould rot (*Botrytis cinerea* Pers.), with Penicillosis rot predominating (up to 75%). In addition to reducing the quality of the apple crop, damage to apple fruits by Alternaria blight reduced the fruit preservation during their storage to a significant extent. In the course of three years, 186 apple cultivars were evaluated for their susceptibility to Alternaria blight (development of the disease on leaves); it was established that 27 cultivars were not affected by Alternaria blight, or 14.5% of their total number (Table 2). The development of Alternaria blight on leaves in 46.2% of the surveyed apple cultivars was less than 5%. The development of Alternaria blight on the leaf surface in 32.3% of the cultivars was rather high, ranging from 5 to 15%. A total of 11.3% of the cultivars with Alternaria blight development from 10 to 15% degree and 4.3% of the cultivars with the disease of 15 to 20% were recorded. More than 20% disease development was recorded in 6.5% of the surveyed apple cultivars. Ben Deviz, Druzhba Narodov, Orlovskoe zimnee, Pamyat Isayeva, Pamyat Michurina, Papirovka Yantarnaya, and Suvorovets were the most severely affected apple cultivars in relation to the disease under consideration. Alternaria blight development on fruits was detected in 14% of the apple cultivars. The cultivars with the highest incidence of Alternaria blight infestation were the following: Antonovka ordinary (Honeycrisp), Lobo, Sinap Severny, Sinap Orlovsky: the development of the disease ranged from 10 to 26% in these cultivars. Such cultivars as Aidared, Aromatnoe, Babushkino, Belorusskoye malinovoye, Belyy naliv, Bryanskoye, Doch' Mekintosha, Zimnyaya krasavitsa, Moskovskoye pozdneye, Rozmarin Russkiy, Saltykovskoye zimneye, Saule, Stepnaya krasavitsa, Spartan, Stroyevskoye, Tat'yanin den', Titovka exhibited the damage to fruits with index of Alternaria blight development from 3 to 5% (Table 3). A significant source of accumulation and an Alternaria blight apple reservation was the fruit drops (Fig. 3). Notably, the development of Alternaria blight to a considerable extent was recorded on apple-tree shoots of different cultivars. Apple fruits of Antonovka ordinary, Sinap Severny, and Spartan cultivars, when cut, showed that their seed pockets were affected by Alternaria blight. During germination of seeds extracted from such fruits under wet seed pocket conditions, the infestation of apple-tree seedlings with fungi of Alternaria blight genus was detected by microscopy. Thus, it was found that this disease in apple trees can be transmitted with seeds. This mode of infection spreading is the characteristic of all Alternaria blight genus fungi and was confirmed for tree crops by modern scientific works (Yarmolovich *et al.* 2015). In our studies, Alternaria blight infestation was also recorded on annual apple-tree seedlings. Typically, rapid development of this disease was recorded from the 3rd decade of June. High development of the seedlings' disease was recorded in the following apple-tree cultivars: Alesya, Antonovka ordinary, Korichnoye polosatoye, and Medunitsa. The index of disease development on them was more than 20% at a prevalence of 100% (Fig. 4).

Table 2. Alternaria blight infestation of apple leaves in different cultivars, 2018-2020.

Cultivars	Alternaria blight development on leaves (%)
Alonushkino, Amurskoye urozhaynoye, Arktik, Aromatnoye, Belorusskoye malinovoye, Belorusskoye sladkoye, Bryanskoye aloye, Bryanskoye zolotistoye, Vita, Gislop, Gulliver, Darunak, Kitayka vostochnaya, Pamyat' Kovalenko, Persikovoye, Pospekh, Pushkinskoye, Rodnikovaya, Rozhdestvenskoye, Syabryna, Talisman, Topaz, Fermer, Flagman, Florina, Fregat, Fridom, Tsvetayevskaya	0 (No infestation)
Bessemyanka Komsimska, Zaslavskoye, Kovalenkovskoye, Mayak Zagor'ya, Moskovskoye zelenoye, Prishvinskoye, Renet Kichunova, Slava Michurinska,	≤ 1
Abrikosovoye, Altayskiy golubok, Antey, Bel'fler Kitayka, Blagovest, Bolotovskoye, Ven'yaminovskoye, Vesyalina, Zimnyaya krasavitsa, Imant, Imrus, KV 1, Kaluzhanka, Kal'vil' nezhnyy, Kandil' novyy, Kitayka Dolgo, Konfetnoye, Korichnoye polosatoye, Krasavitsa Moskvy, Krasivaya, Krasulya, Kuban' spur, Lezhkoye, Letneye aloye, Letneye polosatoye, Ligol, Martovskoye, Mekanis, Melba, Morozovskoye, Moskovskoye zimneye, Moskovskoye pozdneye, Naydzeny, Olimpiyskoye, Osennyaya radost', Pinova, Pobeda Chernenko, Podarok detyam, Podsnezhnik, Rekord Michurina, Saltykovskoye zimneye, Sinap gornyy, Slavyanin, Solnyshko, Spartan, Tat'yanin den', Tolunay, Chashnikovskoye, Chistotel, Yubiley Biofaka, Yubilyar,	1-3
Altayskoye ranneye, Antonovka aportovaya, Apport krovavo-krasnyy, Borovinka krasnoye, Butuz, Vorob'yovskoye, Zimneye prevoskhodnoye, Kandil' Nikitina, Koreyanka, Krasnoye ranneye, Kurnakovskoye, Medunitsa, Mironchik, Noris, Paskhal'noye, Paula red, Pervoural'skaya, Rozmarin russkiy, Rossiyanka, Stepnaya krasavitsa, Tellisaare, Shafrannoye, Yablochnyy spas	3-5
Akayevskaya krasavitsa, Antonovka desertnaya, Antonovka 600 grammovaya, Afrodita, Beforest, Beforest krasnyy, Bogatyr', Bryanskoye, Verbnoye, Veteran, Vishnevoye, Voskhod, Desertnoye Petrova, Doch' Mekintosha, Zhemchuzhnaya, Zhigulevskoye, Zimneye naslazhdeniye, Kloz, Lobo, Nakhodka Lebedyanska, Obil'naya Vladimirova, Orlik, Orlovskoye polosatoye, Pamyat' Sikory, Pamyat' Syubarovoy, Podarok Grafskomu, Prima, Renet Ottsovskiy, Stroyevskoye, Uelsi, Favorit, Shtreyfling krasnyy,	5-10
Alesya, Anis polosatyy, Antonovka zolotaya, Antonovka obyknovennaya, Belyy naliv, Borovinka, Vargulek, Izbrannitsa, Zarya Alatau, Zolotaya korona, Kollet, Krasa Sverdlovska, Lyubetovskoye, Mechta, Pervinka, Pobeditel', Rubin, Sentyabr'skoye, Sinap orlovskiy, Sinap severnyy,	10-15
Babushkino, Bashkirskiy krasavets, Grushovka rannyaya, Mantet, Narodnoye, Pepin Shafrannyy, Skryzhapel', Shtreyfling	15-20
Vityaz', Zvozdochka, Oktyabrenok	20-25
Golub' mira, Charovnitsa,	25-30
Pamyat' Isayeva, Pamyat' Michurina, Papirovka yantarnaya,	30-35
Ben Deviz, Orlovskoye zimneye	40-45
Druzhba narodov	47.4
Suvorovets	54

Fig. 4. One-year-old apple seedling of the Antonovka ordinary cultivar with an Alternaria blight-infected leaf (15.06.2020).

Table 3. Apple tree cultivars with Alternaria blight infestation on their fruits (2018-2020).

Cultivars	Alternaria blight development on fruits (%)
Malt Bagaevsky,	≤ 1
Altayskiy golubok, Zaslavskoye, Imant, Imrus, Kuban' spur, Lozhkoye, Morozovskoye	1-3
Aydared, Belorusskoye malinovoye,Titovka, Spartan, Babushkino, Korichnoye polosatoye, Saule, Stroyevskoye, Tat'yanin den', Bryanskoye, Moskovskoye pozdneye, Olesya, Rozmarin Russkiy.	3-5
Aromatnoye, Belyy naliv, Doch' Mekintosha, Zimnyaya krasavitsa, Saltykovskoye zimneye, Stepnaya krasavitsa.	5-10
Antonovka ordinary, Lobo, Sinap Orlovsky,	10-15
Sinap Severnyy	23-26

Laboratory tests showed that according to M.E. Barr & E. G. Simmons, *Alternaria tenuissima* Nees and *Alternaria infectoria* (Fuckel) were the two unspecified fungi species that caused Alternaria blight infestation in all apple cultivars. The majority (up to 85%) of the isolated specimens were of the fungus species *A. tenuissima*. As a result of route surveys on the territory of the educational and experimental farm, the development of Alternaria blight was recorded on weeds of the following species: meadowsweet (*Convolvulus arvensis* L.), round-leaved mallow (*Malva pusilla* Sm.), field thistle (*Sonchus arvensis* L.), common mugwort (*Aegopodium podagraria* L.). The development of *Alternaria* spp. on these weeds was high, ranging from 10 to 25%, with a prevalence of the disease close to 100%. Microscopy and correlation of morphological characters revealed that *A. tenuissima* and *A. infectoria* species were the causative agents of Alternaria blight on the weeds. Thus, there is every reason to assume that these species of dicotyledonous weeds may be an additional source of spread of the Alternaria blight pathogen in orchards. Infections of various species of weeds with fungi of the *Alternaria* genus have been previously recorded by researchers from various countries (Home 1920; Johnson *et al.* 2000)

Summary

1. Apple trees were affected by two unspecialized fungi species *Alternaria tenuissima* and *Alternaria infectoria*. The *Alternaria tenuissima* fungus species was responsible for 85% of the development of Alternaria blight in apple trees.
2. Alternaria blight infestation was recorded in 85.5% of 186 apple cultivars.
3. To organize the control of Alternaria blight in apple trees, the diseases should be diagnosed by microscopy in order to identify the pathogen accurately.
4. The following fungicides should be included in the system of protective measures on apple-tree in the phase of "rosebud" and petals falling at intervals of 14 days: Luna Tranquiliti, KS at a rate of 1.2 L ha^{-1}, Skor, KE at a rate of 0.35 L ha^{-1}, Medea, ME at a rate of 1.2 L ha^{-1}, Zato, VDG at a rate of 0.14 g ha^{-1}, Horus, VDG at a rate of 0.7 L ha^{-1}. It is also possible to use fungicide Koside Super, VDG at the rate of 2.0 L ha^{-1}, three times during the season, starting from the phase of "green cone" with an interval of 12 days.
5. Protective measures against Alternaria blight should include: elimination of dicotyledonous weeds, removal of wolfberry shoots, timely pest control, collection or incorporation of fallen leaves, collection of diseased fruits.

CONCLUSION

For effective control of apple tree's Alternaria blight, it is necessary to continue researching this disease. The research of developmental peculiarities, possibility of infection transfer from one plant species to another, and improvement of methods for Alternaria blight fungi identification are actual problems in plant protection. It is necessary to continue researching the degree of hazard of Alternaria blight mycotoxins for humans.

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