INVASIVE SPECIES CAUSING PLANT DISEASES (I)

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Invasive plant pathogenic agents are microorganisms that with high probability can enter territories of definite countries, spread there and cause big losses on agricultural crops or trees. This paper presents an information on three invasive plant disease agents including causes of the brown fruit rots of fruit trees, butternut canker and thousand canker disease of walnuts.

Currently, the list of plant diseases of quarantine significance for the Republic of Uzbekistan includes 40 objects, including 16 fungal, 4 nematode, 9 bacterial and 11 viral diseases. However, many new and dangerous plant diseases appear in different countries, the pathogens of which are considered as invasive species by representatives of plant protection service of these countries.

The aim of this article is to make an initial analysis of some dangerous plant diseases whose pathogens are invasive species in many countries, including our country. These species are potentially harmful to various branches of agriculture, including fruit, nut, forest trees, vegetable, field crops and other plants.

Causative agents of fruit-tree moniliosis

As is known, moniliosis of seed and stone fruit trees can be caused by several species of marsupial fungi whose development cycle is usually dominated by their anamorph, i.e., the sexless or conidial stage, and the sexual stage develops very rarely and usually does not appear in natural conditions (except for M. linhartiana). Of these, the following four species are best known [1]:

- Monilinia linhartiana (Prill. & Delacr.) N.F. Buchw. (synonym Monilinia cydoniae (Schellenb.) Whetzel), anamorph Monilia cydoniae Schellenb. (synonym Monilia linhartiana Sacc.) [2];

- three causative agents of flower scorch, shoot scorch and brown fruit rot of fruit trees, including Monilinia fructigena (Aderhold & Ruhland) Honey ex Whetzel, anamorph Monilia fructigena (Pers.ex Pers.) Eaton;

- Monilinia laxa (Aderhold & Ruhland) Honey (synonym Monilinia cinerea (Schröt.) Honey), anamorph Monilia laxa (Ehrenb.) Sacc. (synonym Monilia cinerea Bonorden);

- Monilinia fructicola (G. Winter) Honey, anamorph Monilia fructicola L.R. Batra.

Of these species, M. fructigena is the object of external quarantine in the United States [3, 4], and M. fructicola (list A2) in European Community countries [5].

M. linhartiana, M. fructigena and M. laxa are found in Uzbekistan. Here it should be noted that M. fructicola, called the causal agent of "American" fruit-tree brown rot, which is more aggressive than the other two species, a pathogen of stone fruit-trees, does not occur in our country. This species, previously absent in European countries, entered 13 countries (Austria, Czech Republic, France, Germany, Hungary, Italy, Poland, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland) between 2001 and 2015, and its spread to new countries continues every year (details are given in Khasanov and Bojigitova, 2018) [1].

It should be noted that in recent years, three more species of moniliosis pathogens have been discovered - Monilia mumecola Y. Harada, Y. Sasaki & T. Sano (marsupial stage unknown) and Monilinia polystroma (Leeuwen) L.M. Kohn with anamorph Monilia polystroma Leeuwen on material of sick plants from Japan, and Monilia yunnanensis M.J. Hu & C.X. Luo (teleomorph stage unknown), from China.

The latter two species are very close to M. fructigena in morphological and molecular characters, the range of affected host trees and pathogenicity. After the validation of M. polystroma species on Chinese material in 2002, it was registered in 2003-2016 in China, Serbia, Switzerland, Poland, Hungary, Italy, and Croatia. In Serbia, M. polystroma is the second dominant causative agent of apple brown rot during vegetation and storage after M. fructigena. Hence, it can be seen that the range of this species also expands every year (details are given in Khasanov and Bojigitova, 2018) [1].

Disease pathogens of walnut trees

The supreme bodies of our country adopted a number of documents aimed at increasing walnut production in 2017-2020, including the importation of seedlings and rootstock from other countries. In the course of this task it is required to take measures against penetration with planting materials of invasive agents - pathogens of dangerous diseases of this crop. Such dangerous diseases of walnut trees include gray walnut cancer (Juglans cinerea L.) and "disease of a thousand sores" of American eastern black walnut (Juglans nigra L.), whose causative agents belong to the species Ophiognomonia clavigignenti-juglandacearum and Geosmithia morbida of the Real Fungi Kingdom, respectively.

Gray Nut Cancer

The fungus Ophiognomonia clavigignenti-juglandacearum (V.M.G. Nair, Kostichka & J.E. Kuntz) Broders & G.J. Boland (synonym Sirococcus clavigignenti-juglandacearum V.M.G.Nair, Kostichka, & Kuntz.) is the causative agent of a devastating disease, gray nut cancer. The fungus is a member of the phylum Ascomycota, group of orders Pyrenomycetes, order Diaporthales. It occurs in nature only in the conidial stage; the marsupial stage has not been found [6, 7, 8, 9].

At present, O. clavigignenti-juglandacearum is distributed in 29 US states (in fact, in the entire range of the gray nut) and in three Canadian provinces. Its geographical origin is unknown; it is assumed that the fungus comes from Asia or South America.

However, it is not registered in the countries of these continents, as well as in the EU region; in the EU, it is included in the List A1 of quarantine objects [6, 8].

This fungus is the causative agent of gray walnut cancer, which is the main host of the pathogen. Under natural conditions, two other North American species, eastern black walnut (J. nigra) and heartnut (J. ailantifolia Carr. var. cordiformis Max.), are also affected by the fungus, but the disease occurs infrequently on them and only when heavily infested gray walnut trees are found around them. In experiments with artificial infestation, some other species in the Nut family (Juglandaceae), including walnut (Juglans regia L.), Japanese walnut Zibold (J. ailantifolia Carr.), and some hybrids were also affected. Studies have shown that this fungus can survive on species of another genus of this family (Carya spp.), and even on species of other families (Corylus, Quercus, Prunus) [6, 7, 8, 9].

Symptoms and development of the disease. Seedlings, young and adult trees are affected by the fungus. The fungus causes occurrence of sores on all woody parts - shoots, branches, trunks and supporting roots. Cracks appear on the surface of the sores on the bark. On the trunk (especially in its lower part), ulcers are numerous, located separately or merging. Young ulcers are oblong, depressed, often appear in places of leaf attachment to branches, lateral buds, stomata, natural holes, sometimes directly on healthy tissue. In spring, a black exudate emerges from the ulcers. In summer, black, sooty areas with whitish edges remain in place of exudates. If you remove the bark, you can see elliptical-shaped dead cambium areas under it.

Young shoots and branches die quickly after infestation. On the stems of mature trees, ulcers become chronic, oblong and open under the cracked bark. Around old ulcers, callus grows. When the ulcers ring the trunk, the trees die.

The pathogen persists and sporulates on living and fallen dead trees for at least 20 months. Conidia emerge from pycnidia and are spread by raindrops and wind. The fungus penetrates shoots and branches through wounds and natural openings, causing numerous canker sores to appear, first in the lower and then in the upper parts of the crown.

The fungus is transmitted by seeds (this has been proven in gray and eastern black walnut) - sprouts grown from infected seeds quickly die. Healthy sprouts after infection also quickly die off.

It was established that three beetle species are vectors of the fungus, which can spread conidia within 16 days [7].

Signs of the fungus. Pycnidia of O. clavigignenti-juglandacearum are formed in thin, dark brown to black stroma under the bark. The stromas consist of single or multilocular pycnidia. Bundles of hyphae, 1.55-1.9 x 0.45-0.51 mm in size, grow from stroma, visible to the naked eye. Pycnidia are formed under infected bark; they are smooth, without a neck, rounded or flat, 100-375x90-320 microns in size, sometimes larger when merged. Conidiophores are 5-24 μm long, septate, conidiogenic monophyletic cells. Conidia from pycnidia emerge in wet weather in the form of

filaments consisting of a viscid, slimy, colorless liquid. They are bicellular, hyaline, spindle-shaped, 9-17x1.0-1.5 μm, the filaments are beige to light brown in mass. The fungus is easily isolated in common agarized nutrient media, developing beige or light brown colonies with jagged edges at 24-28°C. Substrate mycelium darkens to black over time. In culture, the fungus forms bundles of hyphae and abundant pycnidia, especially in aging colonies [6].

The secondary fungus Melanconis juglandis (Ellis & Everh.) A. H. Graves (anamorph Melanconium oblongum Berk.), which is sometimes confused with O. clavigignenti-juglandacearum, often settles on diseased parts of trees. M. juglandis does not cause cancer; its fruiting bodies appear as very small bulges on the bark, and black masses of spores, which are asymmetrical, ovoid, and unicellular, are emitted from them.

Harmfulness of the disease. In the USA and Canada, O. clavigignenti-juglandacearum is the greatest threat to gray nut trees, killing them almost completely (up to 90%) in many states. Between 1981 and 1996 in the United States, this fungus killed 58% of trees in Wisconsin and 84% in Michigan. In 1993, 91% of trees in Wisconsin were infested. In 1994, in North Carolina and Virginia, 77% of gray nut trees were killed by the disease (5 million trees out of 7.5 million were killed) [6, 7, 8, 10].

Preventive and other control measures. Against the disease of walnut trees caused by O. clavigignenti-juglandacearum there are no reliable control methods until now. Only breeding of resistant varieties, in particular, using Asiatic gray nut trees resistant to the disease in breeding, and preventive measures are considered promising in this respect [7]. Uprooting of recently killed trees and trees with obvious symptoms of the disease may locally reduce the number of inoculum, but this method is currently considered harmful, as it loses the possibility of selecting partially resistant specimens among less affected trees [10].

Walnut in the United States is grown almost exclusively in the state of California, where the pathogen does not occur naturally. To protect walnut plantations, seedlings of Juglans species from more eastern states of California are prohibited from being imported into the state.

For phytosanitary purposes, it has been recommended that EU countries prohibit importing seeds and planting materials of host plants from countries where O. clavigignenti-juglandacearum occurs, or ensuring that materials are obtained from places where this pathogen does not occur. In addition, it is recommended that EU countries limit the cultivation of gray walnut and avoid the cultivation of other species of the genus Juglans near J. cinerea trees [6, 8].

"Disease of a thousand sores" of oriental black walnut

The causative agent is the fungus Geosmithia morbida M. Kolarik, E. Freeland, C. Utley & N. Tisserat. This invasive species belongs to the phylum Ascomycota, order Hypocreales, family Bionectriaceae [11, 12].

Symptoms and disease development. The complex "beetle Pityophthorus juglandis + fungus G. morbida" is the causative agent of the deadly disease of Oriental black walnut (J. nigra) widespread in the USA and Mexico. Due to the appearance of a very large number of canker sores on the affected branches and trunks, the disease of walnut trees caused by this fungus is called in English "a thousand cankers disease".

In 2004, observers for the first time noted the complex nature of the disease, linking its occurrence with the presence of the "beetle + fungus" complex on the affected tree organs. Tree death is caused by joint (synergistic) effect of the shoot woodworm beetle Pityophthorus juglandis Blackman and fungus G morbida . This beetle aggressively feeds on organs of a walnut tree, gnawing out passages in which cancerous lesions caused by the fungus rapidly develop. Each penetration of the beetle into the branches or trunks of the tree necessarily leads to the development of cancerous sores initially around the passages. It takes less than 30 days from the beetles' entry into the tree to the appearance of oval, black canker sores. The sores quickly grow and spread into the surrounding tissue and often coalesce, surrounding branches that quickly die off. On eastern black walnut branches, ulcers reach 3 cm in width in a short time. In contrast to open perennial, concentric black canker sores caused by the fungus Neonectria ditissima (Tul. & C. Tul.) Samuels & Rossman (synonym Nectria galligena Bres.), typical sores caused by Geosmithia morbida are more superficial, not open and tightly covered by bark (which makes them very difficult to find visually on trees).

Affected trees weaken strongly, which attracts huge numbers of new beetles, and more canker sores appear, leading to yellowing and wilting of leaves, then shoots and large branches, which is the last stage of disease development, followed by complete death of the whole tree [11, 12, 13].

Pityophthorus juglandis beetles can persist for 2-3 years in cut trees of oriental black walnut or even in small pieces of trees. The ingestion of such tree parts with the beetle containing mycelium or conidia of the pathogen can cause the disease to invade new habitats. Since the development of the disease may be latent at first, symptoms may not be visible to the naked eye for 10-20 years [13].

The causative fungus was first identified in Colorado in 2007. [11]. It was found only in the conidial stage and its marsupial stage is still unknown. Species of the genus Geosmithia are widely distributed in the world and are common saprotrophs found together with various beetles living on the bark of deciduous and coniferous trees. Geosmithia morbida is the first species of this genus described in the world as a tree pathogen and its phytopathogenic properties have been proved experimentally. Species of the genus Geosmithia have common features with representatives of the genus Penicillium, but differ from them by the cylindrical shape of conidia and their yellowish coloration in mass. In some species of the genus Geosmithia, a teleomorph stage was found in the genus Talaromyces [11, 12, 13]. The fungus does not grow on Chapek's medium [11].

Affected species of walnut trees. Besides Juglans nigra, which is the most susceptible species, walnut (Juglans regia L.) and under natural conditions American black nuts can also be affected: California (J. californica S. Wats.), Hinds nut (J. hindsii [Jeps.] R.E. Smith), Arizona (J. major [Torrey] Heller) and Texas, or lesser (J. microcarpa Berlandier). However, California and Texas black walnut are quite highly resistant to Geosmithia morbida, while walnut and Hinds black walnut show different degrees of intermediate susceptibility [13].

Harmfulness of the disease. The species epithet "morbida" (i.e., "deadly") in the name of the causative fungus indicates its ability to cause rapid death of the affected host plant (Geosmithia, 2018). Affected trees die within 3-4 years of infection, and highly susceptible trees die within 2-3 years of the first symptoms of leaf yellowing. Mass mortality of walnut trees, especially eastern black walnut in the western United States has been observed since the 1990s. In the 2000s, many reports of tree losses of this species began to appear. In 2004, the beetle + fungus complex caused the death of 60% of all J. nigra trees in Colorado [7, 11, 12, 13].

Disease spread. Since the 2000s, the disease has spread widely on eastern black walnut trees in the western United States (Arizona, California, Colorado, Idaho, Oregon, Utah, Washington, New Mexico.) and northern Mexico; its spread rapidly progressed, and by 2016, affected trees were already found in nine states, including Tennessee, Virginia, Pennsylvania, Maryland, Ohio, and Indiana. In 2014, the disease and its causative agents (beetle + fungus) were recorded for the first time in Europe, in northern Italy [7].

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The above data indicate the high invasiveness of the microscopic fungi Monilinia fructicola, Monilinia polystroma, Ophiognomonia clavigignenti-juglandacearum, Geosmithia morbida and the danger of their penetration into the CIS countries, including our country. Probably, these species should be included in the number of external quarantine objects in our country.

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