Dissemination of *Cryphonectria parasicita* (Murr.) Barr fungus, and the possibilities of protection of a chestnut orchard in Romania

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Abstract

The chestnut plantation, which have been regularly visited for more than three years, located at the outskirt of Salard village. It lies less than 30 kilometers from the Hungarian-Romanian border. On the family farm initially 2 hectares of chestnut were planted, which has now expanded to 4 hectares. However, some of the propagation materials from Italy have already been infected with chestnut blight. Thanks to early intervention, the amount of treatments required has decreased considerably. Also in this plantation the presence of chestnut gall-wasp (*Dryocosmus kuriphilus* Yasumatsu) was first detected in Romania by our group. By this time its spread has been managed to stop. This plantation exemplifies well how diseases and pests can spread easily through the absence of plant protection control.

Keywords: chestnut, *Cryphonectria parasitica*, chestnut blight, chestnut plantation, *Dryocosmus kuriphilus*

Introduction

The most important disease of chestnut (*Castanea* spp.) is the *Cryphonectria parasitica* (Murr) Barr. fungus causing chestnut blight disease. Nothing shows its significance better than after its first observation in 1904 (Merkel, 1906) its spreading has not yet been stopped. *Castanea dentata* (American chestnut) is the most susceptible plant species to the pathogen. *Castanea sativa* (European chestnut) is also sensitive, while Asian chestnuts (due to coevolution relationships) have become tolerant to the pathogen. These tree species are also more tolerant to dieback disease. The dieback disease pathogens (*Phytophthora spp.)* attack the root network of the chestnut trees. To prevent from this scions were grafted on Asian rootstocks in European

plantations and nurseries at that time. Since the symptoms of the chestnut blight on these Asian tree species are also less noticeable, it has begun to spread rapidly first in Italy (Biraghi, 1946). This was also facilitated by two other infection paths. One of them was in the 1950s, led straight from America to France (Prospero, Rigling 2012), while the other was in the Middle-East region of Georgia (Dutech et al., 2012). The first appearance of the pathogen in Romania was detected by Florea and Popa in 1984. Chestnut is mainly found in the western part of the country, primarily in mixed forests, or in smaller plantations. As already described, the EU-12 vegetative compatibility group has been presented in this region (Görcsös 2012; Radócz 2001). However according to Adamčíková et al. (2015) the EU 2-es strain also has appeared.

The pathogen has many vegetative compatibility (VC) groups. Previously 31 VC groups were registered in Europe. This number has now increased to 74 (Peters et al. 2012) due to continuous mutations and genetic recombinations. Molecular-biology methods are also used for their identification.

The most important pest of chestnut has also been introduced from Asia. It has caused significant damage in Central-European chestnut plantations in recent years. The oriental chestnut gall-wasp (*Dryocosmus kuriphilus*) was first detected in Northern Italy in 2002 (Brussino et al, 2002). Its natural spread is 8 kilometers per year as an average (Melika, 2016). Following its first appearance in Italy, it spread rapidly with propagating material. Within ten years it has spread to chestnut producing areas of Switzerland, France, Slovenia, Croatia, Czech Republic, Spain and Portugal as well as Hungary and Turkey too. The presence of chestnut gall-wasp on saplings originated from Italy was also discovered in the examined plantation in Romania (Radócz et al., 2015).

Materials and methods

Against *Cryphonectria parasitica* fungus just few protection techniques are available. As the fungus damages the bark so fungicide spraying will not lead to results. The only known effective curative method is the use of hypovirulent strains of the pathogen. A mycovirus particle can be found in the cytoplasm of the hypovirulent fungal isolates, which reduces the virulence of the host fungus. This mycovirus can be transmitted through hyphae-anastomoses (with other cytoplasmic parts). However, the mycovirus is only be transmitted if the virulent and hypovirulent isolates are vegetative compatible to each other. This is the base of the biological protection strategy on the fields.

The appearance of the hypovirulent fungal strains is not always evident in an area, or presumably requires some time for its development. The blight disease was introduced into Europe firstly to Italy, in 1938 (Biraghi, 1946). Then 12 years later (in 1950) near Genova diseased trees that did not die due to the infection were observed. On the other hand even healthy, callused tissue started to grow on the damaged necroses (Biraghi, 1950).

In Romania some chestnut trees are located 10-15 kilometers east from the examined plantation (a former plantation) whose age (according to the locals) are more than 100 years. Although branch dying caused by chestnut blight are clearly visible on these trees, but we did not find any new cankers on them in the spring of 2018.

By contrast, south from the examined orchard, a former plantation was completely extinct, presumably due to the chestnut blight infection. In the ravaged, shrubby environment we found only a few rootstocks in 2016.

The planting time of the examined orchard in Salard was in 2012. Bouche di Bertizac and Marigoule cultivars were planted. The initial two-hectare plantation has now expanded to more than 4 hectares. As far as possible, every year, new saplings are planted and they usually bought from the same Italian distributor. In the family farm they had not previously been involved in fruit production, so the farmer did not immediately recognize the symptoms of chestnut blight infection.

In the autumn of 2015, we sampled the bark of infected trees during the field examination. Then it was cultured on Potato Dextrose Agar (PDA) media at the Plant Protection Institute of the University of Debrecen. The EU-12 strain, which is features the Romanian chestnut blight population is presented in the plantation. This is also a strain, which is typical in Italy and damages the saplings. However, isolated virulent strains were converted with the adequate hypovirulent strains.

After the selection of the appropriate strain, field treatments were performed as follows: at the edge of the healthy and infected bark inoculation holes are drilled into the bark to block the canker. Than culture medium (crossed with hypovirulent strain) was placed into the inoculation holes (Fig.1). The hypovirulent fungus strain contacts the virulent fungal hyphae following penetration into the bark of the treated tree. Through hyphae-anastomoses, it passes the mycovirus, so the virulence of the fungus decreases and the healing process (plant-callus formation) begins.

Figure 1. Inoculation holes at the edge of the healthy and infected part of the bark

Every year the farmer performs the necessary plant care, so after leaf fall the fallen foliage is rotated into the ground or removed from the area.

In the autumn of 2015, however, the presence of the chestnut gall-wasp was detected (Radócz et al., 2015) on 3 trees (GPS coordinates: N47° 13' 11,15", E22° 06' 38,25"). It is highly possible that the chestnut gall-wasp comes with the newly purchased tree saplings to the region (Fig. 2.).

Figure 2. Chestnut gall-wasps on chestnut leaves

Other pests have also appeared in the plantation. In 2017, due to the damage of the bark beetles (*Scolytus* spp.) two previously healthy and already cultivated chestnut trees had to be removed, while in the spring of 2018, we found a number of holes indicating the presence of wood moths (*Zeuzera pyrina*) on the tree trunk (Fig. 3.).

Figure 3. Damage of *Zeuzea pyrina* and *Scolytus spp.*

Results

From the spring of 2015, the orchard was visited by our group twice a year. After the sampling, we completed the necessary field treatments in October 2015. These were young trees so the protection was urgent, since the initial development is extremely important for later fructification. In addition young, thin branches are quickly destroyed by *Cryphonectria parasitica* fungus. The farmer also pays great attention to prevention. The injuries caused by the various cultural tools has been treating and disinfecting. The wounds on the bark that indicates chestnut blight infection were marked, thus the treatment process speeding up. Due to the quite a few years of biological protection any new mortality caused by the chestnut blight fungus is very rare. There are approximately 600 trees in the orchard. The half of them are 7-8 years old. Only 5% of them required treatment by the hypovirulent strain in the spring of 2018, while earlier this need was about 10% (Fig. 4.).

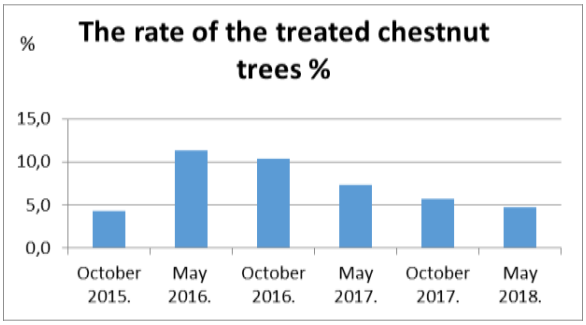

Figure 4. The frequency of chestnut blight infections in Salard between 2015 and 2018

The diagram clearly shows that we did only some treatment in the first year. The reason of this was to test whether the hypovirulent fungal cultural was really appropriate, so we only treated some of the necroses. Next spring the signs of healing cankers were visible, the virulent fungus did not spread further, so in May 2016 we performed the intervention for each damages.

Not a negligible fact that in 2017 the famer created a seedling garden with seed sowing, where at present more than 300 seedlings are propagated. He wants to graft his existing varieties on these seedlings. That is while it excludes the distributors the potential source of infection.

To counteract the damage of *Cryphonectria parasitica* fungus, in 2015 it was possible to stop the spread of chestnut gall-wasp, which was introduced from Italy with saplings, into the plantation reaching the western region of Romania. The infected leaves and buds were removed from the trees and destroyed.

We also prevented the trees from further destruction of other pests, only a few trees were damaged by the bark beetles and wood moths.

Conclusions

By field treatments with hypovirulent fungal strains spreading of virulent chestnut blight fungus subpopulation was successfully stopped in the examined orchard.

In the constantly expanding plantation, regular plant protection monitoring is especially important as well as the sampling and treatments. Should be focus on prevention and improvement the conditions of saplings. Imported plant propagation material can carry several hazards – pathogens, pests. Prior to this, the farmer intends to expand his stock this year by seed sowing and later by grafting. And there is continuous cooperation also needed between the

farmer and the professional extension service (Plant Protection Institute of University of Debrecen).

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