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Chestnut blight in Azerbaijan: current situation and prospects for disease control

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Abstract: *Cryphonectria parasitica*, the causal agent of chestnut blight, was officially found in Azerbaijan in 2004. Currently, the disease is widespread and causes severe damage in seven out of eight forest districts where sweet chestnut (*Castanea sativa*) is native. Studies conducted in 2015 and 2023 showed that the local fungal population is dominated by a single vegetative compatibility type which is also widespread in neighboring Georgia. All 296 sampled and analyzed bark cankers were caused by hypovirus-free *C. parasitica* strains suggesting that the hypovirulence-inducing virus CHV1 is still not present in Azerbaijan. Here we discuss the possibility of artificially introducing this virus into Azerbaijani *C. parasitica* populations by treating virulent cankers with CHV1-infected fungal strains.

Keywords: *biological control*, *Cryphonectria parasitica*, *disease incidence*, *hypovirulence*, *mycovirus*

INTRODUCTION

Biological invasions resulting mainly from anthropogenic disturbances have major impacts on forest ecosystems in terms of biodiversity and productivity [Evans et al., 2010; Ghelardini et al., 2017; Marsberg et al., 2017; Wingfield et al., 2017]. In recent years, new forest pathogens and new hosts have been regularly identified and reported worldwide [Cornejo et al., 2021; Gao et al., 2021; Heluta & Korytnianska, 2021]. Well known examples of invasive fungi and fungal-like pathogens include *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, the causal agents of Dutch elm disease [Brasier, 1991], *Fusarium circinatum* Nirenberg & O'Donnell causing pine pitch canker [Drenkhan et al., 2020], *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya causing ash dieback [Gross et al., 2014], several *Phytophthora* species [Hansen, 2015], and, last but not least, *Cryphonectria parasitica* (Murrill) M.E. Barr, responsible of chestnut blight [Rigling, Prospero, 2018].

Chestnut blight was first officially reported at the beginning of the 20th century in eastern North America on American chestnut (*Castanea dentata* Borkh.) [Anderson, Anderson, 1912]. Within a few decades the disease spread throughout the natural distribution range of this species, which was almost brought to the extinction [Roane et al., 1986]. The ascomycete fungus *C. parasitica* (Diaporthales, Valsaceae) is a bark pathogen native to East Asia. Symptoms of an infection consist of perennial necrotic lesions (so-called bark cankers) that develop on the bark of stems, branches, and twigs after fungal spores penetrated the bark via wounds [Rigling, Prospero, 2018]. The pathogen kills the bark and the cambium and once cankers have girdled the affected tree part, the stem/branch part distal to the canker dies. On the surface of the cankers, the fungus may form asexual (conidiomata) and sexual (ascomata) fruiting bodies. Infected trees may react by producing epicormic shoots at the basis of the bark cankers.

Since the appearance of chestnut blight, several different approaches to control the disease have been tested, including covering bark cankers with mud [Weidlich, 1978] or burning them [Trapiello et al., 2015; Cheradil et al., 2022]. Although the application of chemicals can help to control the disease in the greenhouse [Trapiello et al., 2015], their use is unfavorable given their phytotoxic potential and possible induction of resistance. In North America, breeding programs aiming at developing chestnut plants resistant to the disease have also been started [Powell et al., 2019].

However, a big, unexpected help has arrived from the nature. Following the detection of chestnut blight on sweet chestnut (*Castanea sativa* Mill.) in 1938 in Italy,

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the disease in Europe took the same dramatic course as in North America. Surprisingly, in the 1950s abnormal bark cankers started to be observed, first in Italy and then also in France [Grente, 1965]. These cankers were superficial and did not kill the infected trees. Subsequent investigations showed that the *C. parasitica* strains in non-lethal cankers were infected with Cryphonectria hypovirus 1 (CHV1) that induces a hypovirulent phenotype in the pathogen by reducing its growth and sporulation ability [for a review see Heiniger, Rigling, 1994]. This phenomenon has been called hypovirulence and mycovirus-induced hypovirulence is now successfully used against chestnut blight in several European countries [Rigling, Prospero, 2018]. Where hypovirulence has not occurred naturally, individual bark cankers can be treated therapeutically with hypovirus-infected *C. parasitica* strains [Heiniger, Rigling, 2009]. Besides saving infected trees, such treatments may result in the establishment and spread of CHV1 in the target area.

In Azerbaijan, forests cover less than 20% of the country and are distributed mainly in the mountainous regions of the Greater, Lesser Caucasus and Hyrcan. In recent years, new forest areas have been created on the plains and foothills. Sweet chestnut occurs mainly along the southern part of the Greater Caucasus both in natural stands and plantations at 500-1700 m above sea level. Chestnut production has a favorable market in Azerbaijan and sweet chestnut is a prominent tree for subsistence of the local population [Wall, Aghayeva, 2014]. Although it was known that several fungal species can infect chestnut trees [Agayeva et al., 2018], chestnut blight was discovered relatively late. After first detection of the disease in 2004 [Aghayeva, Harrington, 2008], distribution, genetic diversity, and reproduction mode of *C. parasitica* were investigated [Aghayeva et al., 2017]. Here, we summarize the current knowledge about chestnut blight in Azerbaijan and discuss the potential of hypovirus-induced hypovirulence to control the disease in the country.

MATERIAL AND METHODS

Sampling and isolation. Bark cankers caused by *C. parasitica* were sampled in 2015 (seven chestnut growing districts) and 2023 (three districts) in chestnut stands affected by the disease (Tab. 1). Sampling was conducted by removing bark pieces (ca. 2 × 2 cm in size) with a knife from the cankers [Aghayeva et al., 2017]. In the laboratory, *C. parasitica* was isolated from the bark samples as described by S. Prospero and D. Rigling [2012]. Briefly, three fragments (ca. 0.5 × 0.5 cm in size) of each bark sample were dipped in 70% ethanol, briefly flamed, and placed on 1.5% water agar. After 5 to 7 days of incubation at room temperature in the dark, outgrowing mycelia were transferred onto potato dextrose agar (PDA).

Assessment of culture type. Hypovirus-infected *C. parasitica* isolates were identified by their culture morphology on PDA after incubation of the plates in the dark at room temperature for 7 days followed by an additional incubation under daylight for another 7 days [Bissegger et al., 1997]. Under this incubation regime, hypovirus-infected isolates become white with sparse sporulation, whereas hypovirus-free isolates become a yellow-orange pigmentation and produce abundant sporulation. The eventual presence of CHV1 in isolates with an unclear morphology was verified by RT-PCR as described by J. B. Meyer et al. [2019].

Determination of vegetative compatibility type. The vegetative compatibility (vc) type of the *C. parasitica* isolates was assessed according to the merging/barrage response [Bissegger et al., 1997]. Cultures (one isolate per bark canker) were paired on PDA with a tester strain of the most common vc type in neighboring Georgia [Prospero et al., 2013]. Isolates that were not compatible with this vc type were then paired with each other in 2015, but not in 2023.

RESULTS

In the laboratory, *C. parasitica* was isolated from 296 out of 389 sampled bark cankers, which corresponds to an isolation rate of 73.5%. The number of positive cankers pro district ranged from 27 (Oghuz) to 75 (Gabala) (Tab. 1, Fig. 1A, B). From two bark cankers (district of Zagatala), *Cryphonectria radicalis* (Schwein. ex Fr.) M.E. Barr was recovered [Hoegger et al., 2002]. After incubation as described above, all isolates but 10 from 9 cankers (districts of Balakan, Gabala, Zagatala) showed the typical morphology of virus-free isolates (orange pigmentation with sporulation). The 10 non-orange isolates were characterized by a debilitated appearance (lack of sporulation, whitish color, growth into the agar), but molecular analysis did not reveal an infection by CHV1. Pairing tests showed that a total of 92.9% of the bark cankers from which *C. parasitica* was isolated were caused by a fungal strain of the vc type AZ1 (Tab. 1). A clear predominance of this vc type was observed in all forest districts and ranged from 85.2% (Oghuz) to 97.8% (Zagatala) of the cankers. In 2015, the 14 isolates that did not belong to AZ1 belonged to 6 different vc types [Aghayeva et al., 2017].

Table 1. Summary statistics of the bark cankers caused by the chestnut blight fungus *Cryphonectria parasitica* analyzed (CHV1-infection and vegetative compatibility type) in Azerbaijan up to the end of 2023.

Forest district	Bark cankers[1] (N)	CHV1-infected cankers (N)	AZ1 vc type (% cankers)
Balakan	46	0	95.7
Zagatala	46	0	97.8
Gakh	45	0	93.3
Shaki	29	0	86.2
Oghuz	27	0	85.2
Gabala	75	0	92.0
Ismailli	28	0	96.4
Total	296	0	92.9

[1]Corresponds to the number of bark cankers from which *Cryphonectria parasitica* could be isolated.

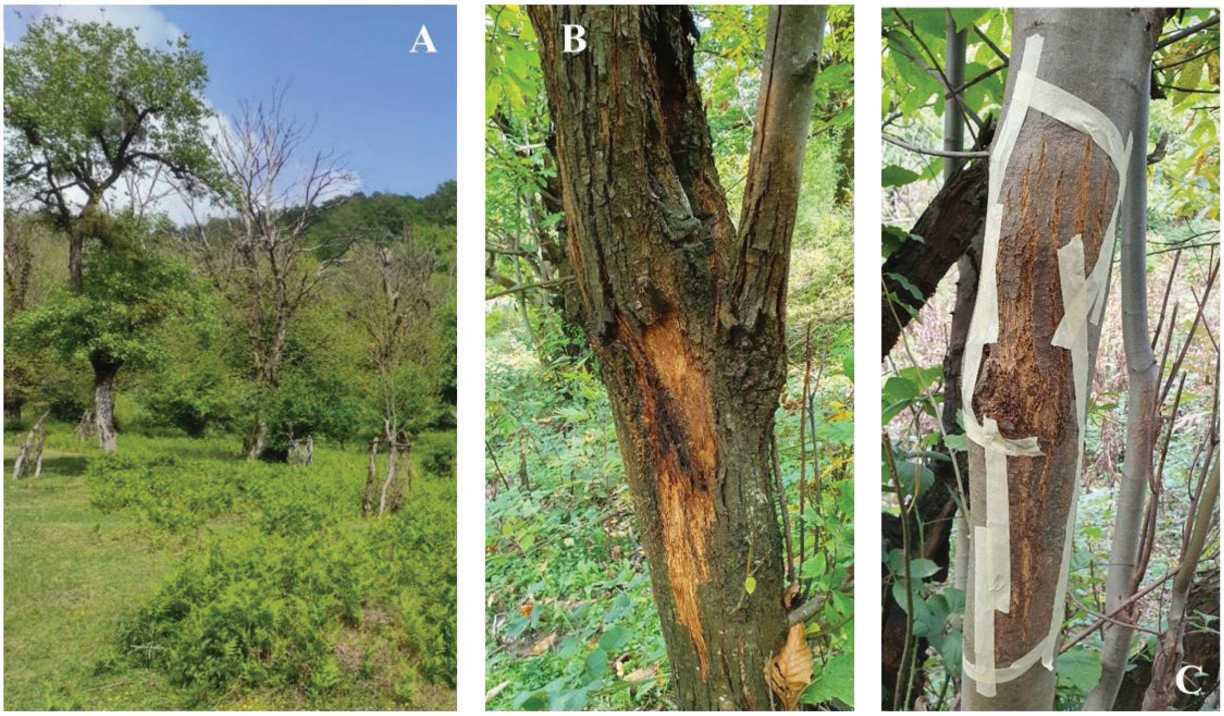

Figure 1. Chestnut blight in Azerbaijan: heavily infected chestnut stand in the district of Gabala (A); active bark canker (B); simulation of the treatment of an active bark canker with a hypovirulent *Cryphonectria parasitica* strain.

DISCUSSION

The studies conducted in the past years have shown that chestnut blight in Azerbaijan has been spreading rapidly in wild and cultivated chestnut areas since its first detection in 2004. The disease is now present in all chestnut growing forest districts and its severity is generally high. Felling and removal of symptomatic trees, which was locally conducted, could not successfully control chestnut blight. Thus, the future of the red-listed *Castanea sativa* seems even more uncertain.

More than 90% of the *C. parasitica* isolates recovered from the sampled cankers belong to the same vc type (AZ1). This dominant vc type corresponds to one of the vc types frequently recorded in Georgia, namely Geo1 [Prospero et al., 2013]. This suggests that chestnut blight arrived in Azerbaijan from neighboring Georgia where the disease had been present since long time. This hypothesis is supported by the fact that vc type AZ1 has so far not been found in Turkish populations or in other European populations of the pathogen.

Based on the phenotypic features of the *C. parasitica* isolates, natural hypovirulence caused by the hypovirus CHV1 is currently not present in Azerbaijan although being reported in Georgia [Rigling et al., 2018]. A delay between the first observation of the virulent form of chestnut blight and the arrival of hypovirulence was also reported in several European countries, including France, Switzerland, and Italy [Heiniger, Rigling, 1994]. Thus, it cannot be ruled out that CHV1 will also appear in Azerbaijan in the future, together with its fungal carrier from Georgia. Recent analyses of the total RNA in *C. parasitica* isolates using high-throughput sequencing approach have confirmed the absence of CHV1 [Wall, Aghayeva, 2014; Aghayeva et al., 2017], but also the presence of two new viruses, namely Cryphonectria parasitica sclerotimonavirus 1 (CpSV1) (family *Mymonaviridae*) and Cryphonectria parasitica ambivirus 1 (CpAV1) [Forgia et al., 2021]. CpSV1, which is relatively widespread in Azerbaijan, is the first negative-sense ssRNA virus known to infect *C. parasitica* naturally, whereas CpAV1 belongs to the only recently described *Ambiviricota*, a new phylum of the Ribovira with circular genomes [Kuhn et al., 2024]. Although in other fungal species viruses of the family *Mymonaviridae* have been associated to hypovirulence [Liu et al., 2014], it is still unknown which effect CpSV1 may have on *C. parasitica*. It would be of particular interest to test if these viruses may interact with CHV1, the successful biocontrol agent against chestnut blight.

Besides new viruses, the chestnut blight investigations conducted in Azerbaijan have also revealed the presence of two other fungal species of the genus *Cryphonectria* (Sacc.) Sacc. & D. Sacc., namely *C. radicalis* and *C. carpinicola* D. Rigling, T. Cech, Cornejo & L. Beenken. Both species are known to occur in Europe: while the saprotrophic *C. radicalis* is documented from at least the early 1900s, *C. carpinicola* was first described only in 2021 [Cornejo et al., 2021]. In Azerbaijan, *C. radicalis* and *C. carpinicola* were found during the sampling campaign 2023 in the forest district of Zagatala, the first on sweet chestnut and the latter on hornbeam (*Carpinus betulus* L.). Additional bark sampling and analyses would allow to better estimate the incidence of these other *Cryphonectria* species.

CONCLUSIONS AND OUTLOOK

Hypovirus-mediated hypovirulence has shown to be successful in controlling chestnut blight in several European countries [Rigling, Prospero, 2018]. Although CHV1 has so far not been detected in Azerbaijan, it may spread from neighboring Georgia where it naturally occurs. If this happens, the clonal structure of the local *C. parasitica* populations would be highly favorable for the spread of the hypovirus. To accelerate its spread, CHV1 could be artificially introduced into virulent cankers by treating them with hypovirulent *C. parasitica* strains. Such canker treatments have been conducted, for example, in Northern Switzerland where no natural hypovirulence has appeared and their results are encouraging [Heiniger, Rigling, 2009]. In order not to increase the genetic diversity of the Azerbaijani *C. parasitica* populations, CHV1 would be introduced from an infected Geo1 isolate into a virulent isolate of the dominant vc type AZ1, which in turn would be used to treat cankers. In this regard, FAO organized an initial training on canker treatment for the local forest service in the district of Gabala in 2023 as part of the project “Strengthen capacities and information base for the management of key forest pests and diseases” (TCP/AZE/3901/C1). A second training took place in 2024 in the district of Zagatala (Fig. 1), during which local forestry officials and chestnut growers were introduced to the method of biological control of chestnut blight in nature.

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Azərbaycanda şabalıd qabığının xərçəngi: mövcud vəziyyət və xəstəliklə mübarizə perspektivləri

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Azərbaycanda şabalıd xəstəliyinin törədicisi olan *Cryphonectria parasitica* rəsmi olaraq 2004-cü ildə aşkar edilib. Hazırda bu xəstəlik geniş yayılıb və adi şabalıdın (*Castanea sativa*) təbii bitdiyi səkkiz meşə rayonundan yeddisində ciddi xəstəlik törədir. 2015 və 2023-cü illərdə aparılan tədqiqatlar göstərdi ki, yerli göbələk populyasiyasında geniş yayılmış bir vegetativ uyğunluq tipi qonşu Gürcüstanda da üstünlük təşkil edir. Nümunə götürülmüş və təhlil edilmiş bütün 296 qabıq nümunələri virussuz *C. parasitica* ştammları tərəfindən törədilib ki, bu da hipovirulentliyə səbəb olan CHV1 virusunun Azərbaycanda hələ də mövcud olmadığını göstərir. Burada biz CHV1 ilə yoluxmuş göbələk ştammları ilə virulent qabıq yaralarını müalicə etməklə bu virusun Azərbaycan *C. parasitica* populyasiyasına süni şəkildə introduksiyasının mümkünlüyünü müzakirə edirik.

Açar sözlər: *bioloji mübarizə*, *Cryphonectria parasitica*, *xəstəlik*, *hipovirulentlik*, *mikovirus*

Рак коры каштана в Азербайджане: текущая ситуация и перспективы борьбы с заболеванием

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Возбудитель каштана *Cryphonectria parasitica* официально обнаружен в Азербайджане в 2004 году. В настоящее время заболевание широко распространено и наносит серьезный ущерб в семи из восьми лесничеств, где произрастает съедобный каштан (*Castanea sativa*). Исследования, проведенные в 2015 и 2023 годах, показали, что в местной грибной популяции преобладает один тип вегетативной совместимости, который также широко распространен в соседней Грузии. Все 296 отобранных и проанализированных язв коры были вызваны безвирусными штаммами *C. parasitica*, что позволяет предположить, что вызывающий гиповирулентность вирус CHV1 до сих пор не присутствует в Азербайджане. Здесь мы обсуждаем возможность искусственного введения этого вируса в азербайджанскую популяцию *C. parasitica* путем лечения вирулентных язв с помощью штаммов грибов, инфицированных CHV1.

Ключевые слова: *биологический контроль*, *Cryphonectria parasitica*, *заболеваемость*, *гиповирулентность*, *миковирус*