The assessment of edible chestnut forest stands in West Georgia and recommendations for their restoration

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Expertise based on the assessment of edible chestnut forest stands in West Georgia and elaboration of recommendations for their restoration

Report on a fact-finding mission to Georgia (February 5-9, 2018)

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1. Background

Edible chestnut (*Castanea sativa* Mill.) is a natural tree species in Georgia covering 3.8% of the countries forest territories uniting high ecological, economic and aesthetic values (Gigauri 2000). The species provides wood of high quality and fruits of high nutritional value for humans and animals. It is an essential factor for biodiversity, since the Caucasian chestnut stands are assumed as the origin of the species *Castanea sativa,* and they nourish and shelter many organisms (f.i. >130 insect species).

Edible chestnuts are endangered by a disease of high impact: edible chestnut blight, caused by the fungal species *Cryphonectria parasitica* (*C.p.*). This disease is listed worldwide among the most devastating plant epidemics. In Northern America, it nearly caused the extinction of the indigenous species *Castanea dentata* within 40 years after its introduction at the beginning of the 20th century. In Europe, it appeared in 1938 spreading rapidly within European edible chestnut stands. In Georgia, the disease was reported for the first time in 1938 as associated with *Cryphonectria* (*Endothia*) *parasitica*. However, descriptions of the symptoms were recorded much earlier (Protsenko 1939, Shavliashvilie 1953). The disease subsequently spread into all Georgian edible chestnut forest stands seriously affecting them.

Another disease, caused by the root pathogenic genus *Phytophthora* (water-moulds, fungal-like organisms), has been gaining increasing importance during the past 30 years worldwide. The disease in edible chestnut, known as ink-disease, is caused by two different species (*P*. *cambivora* and *P.cinnamomi*) killing trees within a few years and spreading via soil. Especially the latter is a typical follower of climate change, affecting a huge number of different hosts, both woody and herbaceous. Reports on ink disease from Georgia date back to the mid of the 19th century.

Other agents mentioned as causal for edible chestnut-problems in Georgia are Cylindrosporium leaf-spot disease (*Cylindrosporium castaneae*) and drought (Issinski 1968, Tavadze 1982, 2003, Shavliashvili 1953).

Following the independence of the Georgian Republic, increasing economic demand from both the domestic and the international market, resulted in illegal harvesting of trees causing the selective removal of the most vigorous trees and consequently the increase in the share of damaged chestnut trees in the stands. Nowadays edible chestnut is still widely used by the Georgian population, particularly wood provides a preferred material for many households. Thus, economic value, administrative/management constraints, lack of knowledge and experience of the restoration of chestnut forest ecosystems create a combination of severely endangering impacts and demonstrate the urgent need for effective strategies to save edible chestnut in Georgia.

According to the available literature and the information BFW received during the mission, edible chestnut blight is the predominant factor leading to a decrease in the amount of this tree species in Georgian forests thus strategies ensuring the survival of chestnut have to focus on management of this disease. Edible chestnut blight is one of few forest tree diseases, which can be managed by a biological control mechanism. This is based on the existence of a virus-infection of the pathogen *Cryphonectria parasitica* transmitted through mycelial contact (Rigling and Prospero 2018). By this, the aggressiveness of the fungus is markedly reduced enabling survival of the diseased trees. However, the phenomenon, known as hypovirulence (hv), is limited by vegetative incompatibility among strains of the fungus blocking a free transmission of the virus (Fig.1). To overcome this barrier, for each of the virulent (v) strains of the fungus present in a stand there must be a suitable compatible non-aggressive (hv) strain able to convert the aggressive one. Consequently, any efficiency in releasing virus-infected fungal strains in edible chestnut-forests requires a careful analysis of all strains present in a stand and the corresponding vegetative compatibility (vc) groups. To assess the composition of haplotypes of *C.p*. present in Georgian forests and to track mutual existing natural hypovirulence, two scientific projects lead by the Swiss Forest Research Institute WSL were conducted from 2009 to 2013 and 2014 to 2017 respectively. The outcomes of these and related projects on *C.p*. have to be considered as fundamental requirements for recommendations regarding management of the edible chestnut-stands problems in Georgia.

Fig. 1: Bark cankers with virulent phenology (left) and hypovirulent phenology (right)

2. The situation of edible chestnut blight according to the two projects conducted by WSL

One study was conducted in 9 regions of Georgia distributed over the whole Georgian area harboring edible chestnut (Prospero et al. 2013). In each region, the population of *C.p*. was assessed in several forest sites of app. 0.5 ha size each. For isolation of *C.p.*, samples from chestnut blight bark cankers were taken. The isolated strains, 427 in total, comprised of 100 different haplotypes, up to 30 per region. Number of haplotypes per site was varying between 2 (Axalsopeli) to 30 (Satsire), but 3 haplotypes were most common: of these, CPGeo20 was the main haplotype (50.1% of all isolates) found in all 9 populations with prevalences of 22.9% (Tkilnazi) to 93.1% (Axalsopeli) of the isolates. Two further haplotypes, CpGeo97, present in 7 populations and CpGeo75, present in 5 populations showed prevalences of 5.4% and 8.9% of all isolates. Seventy-three haplotypes were restricted to a single population and comprised at maximum 1.4% of all isolates.

Vegetative compatibility tests showed that the *C.p*. isolates from Georgia were dominated by three VC types, which corresponded basically to the haplotypes mentioned above. Thus, 45% of the isolates of haplotype CpGeo97 corresponded to the European VC-group EU-1, the others to VC-groups Geo1 and Geo2, which were not among the 74 EU-VC groups (Aghayeva et al. 2017, Prospero et al. 2013). Haplotype CpGeo97 revealed the highest share in Tkilnazi, east of Batumi. Sites with the lowest amount of haplotypes were Axalsopeli (2), Khecili (5) and Tskalthashua (6) (Prospero et al. 2013).

3. Report on the mission in February 2018: Meetings, discussions, visited sites, collected information, assessment of laboratory facilities from the Adjara Forest Agency Batumi in Shuakhevi, and final debriefing workshop with stakeholders

On **Monday, Feb 5** two meetings were attended at **CENN and NFA Offices in Tbilisi** before departure to Western Georgia. A third planned meeting with the Agroforestry Service joined by experts from Vasil Guliashvili Forest Institute was canceled.

The first meeting was held at the office of CENN (Caucasus Environmental NGO Network). Information was given on the general situation of edible chestnut in the country and the awareness of an urgent need to stop or slow down the decline of this tree species in Georgia. The two Swiss projects were mentioned as the basic work on the identity of the main pathogen *Cryphonectria parasitica*. The expectations of CENN, represented by Nana Janashia, and National Forest Agency, represented by Merab Machavariani, were discussed and plans for the mission were finalized. The stakeholder workshop to be held at the end of the week was planned. The second meeting at the National Forest Agency (NFA) with Natia Iordanishvili and Marina Sujashvili confirmed the endangered status of Castanea in Georgia and the need for improving the situation. From the discussions in both meetings, it became evident, that the main problem of edible chestnut is chestnut blight, the other agents (for instance ink disease) were not considered as of similar importance. Generally, there is a need for further international collaboration not only on the research level, but also on the level of practical measures. In addition, national networks uniting science applied research and forest management should be improved. This should be the start of a long-term restoration project.

On the trip to Western Georgia (Kutaisi), first edible chestnuts were seen west of Surami on steep slopes exposed to the South, some of them showing symptoms of dieback. The following persons participated in the three-day field trip: Thomas Cech and Gernot Hoch (BFW), Iryna Matsiakh (Ukrainian Nat. Forestry Univ.), Merab Machavariani and Giorgi Mamadashvili (NFA), and Lado Basilidze (CENN).

On **Tuesday, Feb 6**, three sites with edible chestnuts in different stage and intensity of decline were visited in the area east of **Zestafoni (Shorapani)**.

The first site was a stand of edible chestnut mixed with *Alnus*, *Carpinus,* and *Corylus* on moderately steep slope exposed to the West, close to a river with stands of *Alnus*. Understory vegetation showed mainly *Rubus*; other shrubs were almost lacking. The stand was thin, the edible chestnuts were old, showing severe dieback or were dead; coppices were not abundant. Trees showed numerous bark necroses (cankers), most of them in late stages, i.e., with broad cracks exposing the wood, however, showing intense callus formation. Fresh bark cankers with orange colored surface were relatively rare and confined to branches with smooth bark. Fruiting bodies of *C.p*. were abundant, but not frequent; both asexual and sexual stages were present. Dry leaves on the twigs were missing. Some (few) necroses revealed an indistinct hypovirulent symptom expression (blackish-grey surface, no cracks and no associated dieback of tissues above - see Rigling and Prospero 2018).

The second site was an old aged poor stand of edible chestnut on a crest and distinctly drier. The stand was additionally used as pasture for cattle. It was obvious, that this stand had been used for food production in former times. Many of the chestnuts were dying but showed intense production of coppices, which were not much infected (approximately 15-20%). Fresh orange bark cankers were very rare. Both types of fruiting structures were present. Occasionally, cankers with hypovirulent phenology were present. Virulent cankers were mostly large (up to 1 m in length) associated with dieback of distal parts and with bark cracking open (Fig.2). Samples taken from both cankers with virulent and hypovirulent phenology yielded virulent and hypovirulent strains (Dr. Iryna Matsiakh, Fig.3).

Fig. 3: Virulent (left) and hypovirulent (right) strain from site 2

Fig. 2: Old virulent canker, cracks and callusing on site 2

The third site was a younger stand of edible chestnut showing many necroses (also with hv phenology, Fig.4) and intense dieback. Again, there were rather few fresh cankers on the coppices. The understory consisted of *Rhododendron* showing some leaf spots.

Fig. 4: Cankers with hv phenology, site 3

On none of the sites visited there were indications for a presence of the Oriental chestnut gall wasp Dryocosmus kuriphilus, an invasive species currently spreading in Europe, leading to a loss in fruit harvest and affecting the balance between virulent and hv strains of C.p. in favor of the virulent ones.

Both, the second and the third site could be suitable for management including the application of hypovirulent strains however under the assumptions listed below.

On Wednesday, Feb 7, the laboratory of the Adjara Forest Agency in Shuakhevi was visited to see the capacities of the production of hv strains of C.p. for a release in selected stands. The overall facilities in this lab are limited to the propagation of fungal cultures. The equipment is sufficient for the production of sterile agar plates and to transfer mycelia under sterile conditions (sterile bank). Both, dissection microscopes and transmission microscopes to check cultures and investigate fungal organs are present. Transforming virulent strains into hv ones is possible. However, it was not clear how far the staff of this lab that is involved in propagation and application of hv strains receives any scientific feedback and keeps contacts to plant pathologists; this is necessary for a steady quality of this work. The remote location of the laboratory will be practical for the release of hv strains in the area; however, is problematic concerning the above mentioned scientific exchange. Close cooperation with a research organization or university researchers is recommended.

On Friday, Feb 9, a Stakeholder Workshop took place in the Ministry of Environment Protection and Agriculture of Georgia on the assessment of degraded chestnut forest stands in West Georgia together with CENN, the National Forestry Agency of Georgia and ADC (represented by Gerhard Schaumberger). Archil Supatashvili and Bidzina Tavadze, the two scientists involved in the previous project with WSL were also present at the workshop. This allowed highlighting the importance of basing the planning of a chestnut restoration planning on knowledge gathered in this project. Gernot Hoch and Thomas Cech presented initial findings following the fact-finding-mission and suggestions (see file attached). The basic principles of using hv strains in the management of chestnut blight, its chances, and constraints, as well as supporting silvicultural approaches were discussed.

4. Recommendations for management strategies of Cryphonectria parasitica in Georgian edible chestnut forests

a) Artificial introduction and spread of hypovirulence

Success in the release of hv *Cryphonectria parasitica* means the establishment of strains apt to propagate naturally to result in a balance of v and hv cankers in a stand (Fig. 5) and consequently in survival of the stand with rather low mortality rates of trees. To reach this, several crucial requirements have to be met, which are listed as below. Furthermore it is strongly recommended to have persons trained by technicians and researchers experienced in the procedures. To achieve this, it would be best to send these persons to WSL (one of the leading research units in Europe) or another experienced centre for a couple of days to gain knowledge on details.

Fig. 5: Canker with virulent phenology (left) and hypovirulent phenology (right)

Selection of appropriate sites to establish experimental plots

Sites to be selected for the establishment of experimental plots should be easily accessible and should consist of a sufficient amount of edible chestnuts in different stages of dieback with actively growing cankers. A plot should be at minimum 1ha in the square. Furthermore, they should harbour a **low number of haplotypes and VC-groups dominant in share** (see the results of the WSL studies). The haplotypes have to be identified by isolation from virulent cankers; however, cankers with hv phenology should be sampled as well to get hv strains naturally present on the site. Cankers, where samples were taken for isolation, should be labelled so that they can found again if repeated attempts to isolate become necessary. To allow for a quick natural spread of the artificially established hypovirulence, experimental plots should be surrounded by further edible chestnut stands (distance to the next stand outside the plot at maximum 1km, preferably less).

Propagation of hv strains

If natural hv is detected on the site and VC groups are defined, the hv strains can be propagated in the lab and can be released. If hv strains of haplotypes originating from other areas belonging to suitable VC-groups should be released, it is essential to transfer the virus (in the laboratory) into virulent strains that were isolated at the site before releasing it. This is necessary to avoid introduction of a whole new genome into the population, which would facilitate sexual recombination and consequently the development of new haplotypes.

Identification of haplotypes, hypovirulence and vc-groups based on molecular methods should be done by an experienced lab with adequate facilities. During the visit in February, it was not possible to visit a national lab of that kind. However, such a national lab would have the advantage to be easier and faster available than labs in other countries. In addition, quarantine problems can be avoided by keeping cultures of aggressive pathogens in the country.

Protection of experimental plots from abuse or other disturbances

Most consequent documentation of the introduction and spread of the applied hv strains in the plots is essential. Therefore, the plot should be protected from access by nonauthorized persons (illegal harvesting!) preferably by fencing. The same refers to grazing animals (goats, sheep, cattle, deer), since additional damaging factors may retard or reduce the success of the hv strain establishment.

Selection of trees

Trees to be inoculated with hv strains should be moderately diseased, i.e., they should not show too large and too many cankers. The spread of hv takes time, and too many cankers can cause the death of the trunk before hv is established. For the classical method of inoculating cankers on branches and stems by use of a cork borer, younger trees are more appropriate since the “target” region, the border of the virulent canker is far more distinct on smooth bark. Selected trees should be permanently labelled for later observations and documentation. The number of trees to be selected should be at minimum 50 per ha.

Select suitable active cankers

Actively growing cankers should be selected for treatment: here the success is more easily to be checked. It is further recommended to document the cankers before and immediately after treatment by measuring length and width and photography.

Methods of inoculation

1. The classical method of inoculation is to remove a disk of the bark from the edge of an actively growing canker with a cork-borer and plug the hole with a disc from a hv culture. This procedure has to be repeated every 10 cm around the canker. To facilitate growth, the canker can be sealed with parafilm or tape. In Austria, this method was modified by removal of the bark of the whole canker and replacing it with a paste of the hv fungal culture. The disadvantage of this method is that it is rather time-consuming and that for a successful establishment of hypovirulence many cankers should be treated. The treatment should be performed in early summer. For further details see Heiniger & Rigling 1994.

Two new methods (2. and 3.) are currently being tested at WSL (submitted manuscript, more information: Dr. Joana-Beatrice Meyer, WSL). These methods look quite promising for a broad application of hv. Since hv strains usually show much lower sporulation of the conidial stage than v strains, both methods are targeted at an artificial increase of the sporulation intensity.

2. Stems of smooth-barked healthy chestnuts are cut into short segments, autoclaved and sealed with melted paraffin wax at their ends. Subsequently, the segments are inoculated with hv strains using the cork-borer method. Then the segments are stored in boxes in sterile distilled water for six weeks: by this time the hv strain has colonised the bark and produced numerous asexual fruiting bodies containing spores infected with the virus. The segments are then attached to trees on the stem or branches above virulent cankers, preferably in early autumn. The numerous spores effectively spread the hypovirulence on the tree.

3. For the second novel method, asexual spores from hv cultures are produced artificially in the lab on petridishes and sprayed or brushed over the virulent cankers in the field. This method turned out to be slightly more effective since hv spore are directly applied to the canker and not above it.

Documentation

The treatment of the cankers (regardless of method used) should be carefully documented. The development of the cankers should be checked at least one time per year. Isolates should be gained from cankers which do not change from v to hv phenology after treatment. These cultures should be re-checked for their identity. Generally, the composition of the vc-groups and haplotypes should be re-assessed from time to time.

b) Hygienic measures

According to Prospero et al. (2006), Cryphonectria parasitica can survive on dead tissues of Castanea sativa for more than one year. Therefore, removal of dying trees seems adequate as a method to reduce inoculum pressure in an infested stand. This refers to virulent strains. However, the actual study by WSL (as mentioned above) confirms a similar potential of hv strains to survive and sporulate on dead logs. Thus it can be recommended, that in stands, were hv is already established, thinning by removal of dead or declining trees is rather counterproductive for the spread of the hypovirulence.

c) Containment

If there are still stands of noninfected edible chestnut in Georgia, protection of these areas should be considered by removal of chestnut trees at the border of this area. However, this requires careful screening of the stands and an assessment of the health status of both the stands and the surrounding area.

d) Quarantine

Regarding edible chestnut blight, any introduction of new haplotypes (and VC-groups) should be avoided. In this sense trade with chestnut plants should be limited from countries, were other haplotypes of the pathogen exist.

Regarding Phytophthora, sites harbouring Rhododendron in the understory should be carefully checked for the presence of Phytophthora species both by baiting soil and isolating the pathogens from symptomatic tissues. Rhododendron is worldwide one of the main hosts of this pathogen.

e) International and national collaboration

In Europe, WSL can be regarded as one of the leading institutions regarding both scientific and applied research on edible chestnut blight. Given the knowledge and the experience gained by the projects conducted in Georgia and other countries, we strongly recommend increasing the contacts with WSL to support Georgian people responsible for forest health and its management in Georgia.

Similarly, a collaboration of researchers and forest managers in the management of chestnut should be intensified and improved on the national level.

5. Literature

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