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Fraxinus sogdiana, a Central Asian Ash Species, Is Susceptible to Hymenoscyphus fraxineus

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Abstract

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Susceptibility of new host trees is an essential prerequisite for the alien pathogens. Today, an acute problem in Europe is ash dieback, caused by *Hymenoscyphus fraxineus*. Among the possible invasion routes of this fungus to Europe from its natural range in easternmost Asia, an arrival alongside the unbroken, passing the whole Eurasia chain of ranges of ash species should not be ignored, at least not before the determination of the western extent of the natural range of the pathogen. We established that in Estonia the ash species *Fraxinus sogdiana*, growing naturally in the "bottleneck" of that belt, in Central Asia, is susceptible to *H. fraxineus*. It is the first record of *H. fraxineus* on a Central Asian ash species.

Keywords: "Krüssmann's ash belt"; invasion routs; invasion pathways; introduction; exotic trees

When discussing possible routes and pathways for the arrival of the ash dieback fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya (syn. *H. pseudoalbidus* Queloz et al.) to Europe from its natural range in East Asia, DRENKHAN *et al.* (2014a) considered, as one of the possible routes, the arrival of the fungus alongside the "Krüssmann's ash belt" (original term), i.e. an arc-shaped narrow in the middle belt, formed by the natural ranges of ca. 20 different ash species, which passes unbroken through the whole of Eurasia from east to west and is represented on Figure 55 of the Krüssmann's handbook (KRÜSSMANN 1965).

The obvious "bottleneck", i.e. the narrowest and apparently ecologically hardest for fungi zone in that belt, is situated in Central Asia.

In the earlier paper (DRENKHAN *et al.* 2014a) we also referred to the possible introduction cases of *F. mandshurica* Rupr. to the Central Asian countries Kazakhstan, Uzbekistan, and Turkmenistan, i.e. into the ranges of Central Asian ash species, as described in the Russian silvicultural scientific literature. Comprehensibly, these introductions might create immediate contacts between the local native ash species and the introduced *F. mandshurica*, at least through botanical gardens, and thereby support to the transmission of the pathogen directly to Central Asia – midway between easternmost Asia and Europe.

For both possible variants, i.e. for the movement of the fungus to the west, (1) consistently, step by step, from its natural range in easternmost Asia, or (2) beginning from the midway, i.e. from Central Asia, first it must be shown, are the Central Asian ash species susceptible to *H. fraxineus*.

MATERIAL AND METHODS

Among the Central Asian ash species, only *F. sogdiana* Bunge (syn. *F. potamophila* Herder) was found to grow in Estonia, more precisely in the Tallinn Botanical Garden (northern Estonia). At the sampling day (September 2013, leg. R. Drenkhan), it had symptoms

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typical for infectious ash dieback, generally attributed to *H. fraxineus* (cf. EPPO standard PM 7/117; see Anonymous 2013), including dead branches inside necrotic lesions on the bark of bush-like trees.

Both, pure culture and molecular investigations of *H. fraxineus* on *F. sogdiana* were carried out in the IFRE Laboratory of Forest Pathology and Genetics, Estonian University of Life Sciences, Tartu.

H. fraxineus was isolated (culture No. 145 725) from a symptomatic shoot as described by DRENKHAN & HANSO (2010). DNA extraction from mycelia, ITS-PCR reactions and sequences were carried out as described by DRENKHAN *et al.* (2014b). The sequence was edited using the BioEdit program, Version 7.2.5 (HALL 1999). BLAST searches for the fungal taxa confirmation were performed at GenBank (NCBI).

RESULTS AND DISCUSSION

Molecular identification of the fungus resulted in 100% *H. fraxineus*. ITS sequence of *H. fraxineus* from *F. sogdiana* in Estonia was deposited in GenBank (accession No. KM655828).

Still, nobody knows the western extent of the natural range of *H. fraxineus* in Asia. This range may end or not end at the western border of the ranges of the known hosts of the fungus there, *F. mandshurica* and *F. chinensis* Roxb. (syn. *F. rhynchophylla* Hance). Also, the range of *H. fraxineus* may enfold only the eastern part of the ranges of its hosts, where by today this fungus has been recorded (see Figure 24 in BARAL & BEMMANN 2014). Conversely, in Europe the still easternmost point of occurrence of *H. fraxineus* was established in eastern Ukraine (DAVYDENKO *et al.* 2013).

The "bottleneck" in Central Asia consists of the natural ranges of ash species belonging to the (1) section *Fraxinus: Fraxinus syriaca* Boiss. and *F. sogdiana* Bunge, and (2) section *Ornus: F. raibocarpa* Regel (all the three: after NIKOLAYEV 1981) and *F. micrantha* Lingelsh (HINSINGER *et al.* 2013). WALLANDER (2008), and HINSINGER *et al.* (2013) consider several Central Asian ash species (*F. syriaca, F. potamophila,* and *F. sogdiana*) as belonging to the species *F. angustifolia* s.l., but growing in the easternmost area of the huge range of this collective species, which was long time treated as mainly a southern European ash species, but having a restricted range also in western Africa and western Asia. Still, taxonomy of the hosts (*Fraxinus* spp.) in the ash dieback syndrome seems to be also

intricate, even more than the complex of the pathogen (*Hymenoscyphus* spp.). Therefore it is not surprising that, when growing in Central Asia (in Tashkent), some identical glycosides and coumarins were found in *F. mandshurica* and *F. sogdiana* (ARTEMYEVA *et al.* 1973a,b), both belonging to the same section of the genus *Fraxinus*. By that, the fungus had to meet not very different nourishment on its way from east to west alongside that ash belt. Above all, in its wider sense (i.e. *F. angustifolia* s.l.), the narrow-leafed ash had to restrict to the minimum the needful transfers of the fungus from one host species to another on this hypothetical natural east-west route alongside that "Krüssmann's ash belt" – from the easternmost Asia to Europe.

The fungus could reach Central Asia naturally beforehand the introduction of F. mandshurica, but concerning the introduction by humans, we are not sure that *H. fraxineus* is able to persistently accompany its natural host (F. mandshurica) at the introductions. It is doubtful, if to consider the long history of introduction of F. mandshurica to Estonia without any direct coincidences of the fungus or the disease symptoms on the highly susceptible European ash (F. excelsior L.) over more than a century (DRENKHAN et al. 2014a). It seems also of doubt that the fungus could accompany its host already at the last phases of the phylogeny and biogeographic history of ashes, i.e. in the course of the transcontinental expansion of that lineage of ash genus from the eastern Asia towards Europe, resulting in the modern geographical diversity of the section Fraxinus (HINSINGER et al. 2013), and survived until now in latent phase in a single (eastern Poland?) or several places in Europe.

Our investigation has thus assured the susceptibility of *F. sogdiana* to *H. fraxineus*.

In Europe, the narrow-leafed ash has also been found susceptible (KIRISITS *et al.* 2009). True enough, in the forest pathological literature, there is still no information about ash species that could not be successfully inoculated by *H. fraxineus* (regardless of pathogenicity, i.e. considering solely the potential of the tree as a subsequent vector for the fungus, as acted also *F. mandshurica*). The susceptibility of other ash species, by that, apparently should not stop transmission of the fungus through that "bottleneck" in Central Asia. However, little is known about the other obvious obstacles, including the harsh continental climate in Central Asia with hardly cold winters and dry and warm summers for the environmental

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requirements of the fungus on the leaves, twigs, and branches of ash trees. DAVYDENKO *et al.* (2013) do not exclude that already in eastern Ukraine namely the continental climate might create less favourable conditions for the fungus. In our opinion it reduces even more the probability of the arrival of *H. fraxineus* in Europe alongside that "Krüssmann's ash belt".

Still, *H. fraxineus* has not been found on moraine Balkan ash (*F. holotricha* Koehne, syn. *F. pallisae* Wilmott ex Pallis), which has a range west of the natural ranges of *F. mandshurica* and other East Asian ash species. Presumably, the susceptibility also of that ash species would not be an obstacle for the fungus, the rather that also *F. holotricha* belongs to the section Fraxinus (HINSINGER *et al.* 2013). Anyway, only the certain determination of the western extent of the natural range of *H. fraxineus* in Asia may finally close this variant of movement of the fungus to Europe, but also point to some other environmental requirements of the fungus, other than susceptibility of the hosts.

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