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Can pruning help maintain vitality of ash trees affected by ash dieback in urban landscapes?

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ABSTRACT

Hymenoscyphus fraxineus causes a destructive invasive tree disease known as ash dieback threatening the survival of common ash not only in the forests, but also in urban and landscape settings. Pruning is a potential management practice that could help maintain tree vitality and aesthetics in parks, gardens, alleys and recreation areas, as well as maintaining veteran trees having high heritage or cultural value, or trees with high genetic importance (e.g. located in clonal seed orchards). In this study we investigated the maximum distance proximal to the lesion margin at which *H. fraxineus* can be detected on individual branches infected by the fungus in order to provide recommendations for pruning. Pruning of branches was carried out on 38 trees in southern Sweden. Tissue samples including bark and wood were collected from the margin of the lesion and at 5 cm intervals proximal to the lesion. Molecular analysis revealed presence of *H. fraxineus* in 91.3% of the investigated lesions. The proportion of lesions at which *H. fraxineus* could be detected declined with increasing distance from the lesion margin, with a significant reduction in the number of positive samples at 10 cm proximal to the margin. At 30 cm from the lesion edge the pathogen was never detected. Our results suggest that routine pruning may help maintain the vitality of younger trees. Pruning branches at least 35 cm from visible, active lesions in the bark should exclude the fungus and therefore reduce the probability of stem infection by *H. fraxineus*, however this cultural control tactic may only be economically feasible for high value amenity trees.

1. Introduction

Alien invasive forest pests and pathogens are increasingly recognized as a major threat to biodiversity and ecosystem functioning including forest tree productivity. During the last 30 years, the number of invasive pathogens introduced to Europe has increased exponentially (Desprez-Loustau et al., 2010; Santini et al., 2013). This trend is expected to continue with the increase in global transportation and trade of plants and plant products, and climate change (Pautasso et al., 2010; Boyd et al., 2013; Stenlid and Oliva, 2016). Though scientists and stakeholders agree that more efforts should be directed towards prevention, early detection and management of alien invasive species (Buckley, 2008), once they become established in nature and start to spread, management becomes increasingly difficult (Prospero and Cleary, 2017).

Dieback of European ash (*Fraxinus excelsior* L.) was initially reported in Eastern Europe in the early 1990s and has since been found throughout much of the natural distribution range of ash across Europe (Gross et al., 2014, and references therein), seriously threatening the existence of this important noble broadleaved species (Pautasso et al., 2013; McKinney et al., 2014). Ash dieback causes damage not only in forests and tree nurseries (Kowalski and Łukomska, 2005; Kirisits et al., 2009; Schumacher, 2011), but also in urban and landscape settings where ash is planted as an amenity tree species in parks, gardens, alleys and in recreation areas.

The disease is caused by an invasive ascomycete fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (2014). Recent studies suggest that the pathogen was introduced to Europe from Eastern Asia where it is likely a harmless endophyte/saprophyte on local ash species (Cleary et al., 2016a; Zhao et al., 2013; Gross et al., 2014). *Hymenoscyphus fraxineus* reproduces sexually mainly on blackened pseudosclerotial rachises from the previous year in the leaf litter (Cleary et al., 2013; Gross et al., 2014). Occasionally apothecia have been noted also on small ligneous stems (Kowalski and Holdenrieder, 2009; Kirisits et al., 2012). Following spore dispersal during several weeks in the summer (Hietala et al., 2013), germ tube formation and

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Fig. 1. Characteristic symptoms of ash dieback on *Fraxinus excelsior* trees. a) Twig, shoot, branch dieback; b) Bark cankers; c) Discoloration of xylem; d) Leaf spots and necrosis along leaf vein.

development of appressorium facilitate penetration of the ash leaf cuticle (Cleary et al., 2013) and necrosis expands proximally, preferentially along leaf veins to rachises and subsequently twigs and branches leading to a wide range of symptoms including wilting of shoots, bark cankers, wood discoloration and eventual dieback of twigs, branches and the crown (Bakys et al., 2009a,b) (Fig. 1). Epicormic shoots are often formed as a stress response on affected trees (Kirisits et al., 2009) and result in a bushy appearance in the crown. Recently, evidence suggests that necrotic lesions on the root collar and stem base are also primary symptoms of ash dieback (Husson et al., 2011; Chandelier et al., 2016). The presence of collar lesions can accelerate degradation of trees, and in many cases predispose trees to attack by *Armillaria* species (Lygis et al., 2005; Skovsgaard et al., 2010; Enderle et al., 2013), increasing the severity of root collar girdling (Marçais et al., 2016).

The extent of ash population decline varies among European countries. For example, in Denmark up to 90% of ash trees are infected (van Opstal, 2011). In Lithuania, which has experienced one of the longest dieback histories, more than a half of ash population has been lost due to the disease and subsequent response with large-scale sanitation fellings (Anonymous, 2015). Self-regeneration of ash in diseased forests is poor and over time, has resulted in a major species conversion shift (Brunet et al., 2014; Lygis et al., 2014).

Common ash is recognized as an important keystone species in natural habitat and protected under European legislation (European Commission, 2007). The species influences the ecology of forests, e.g. affecting the flourishing of spring flowers (Meo, 2012) and hosts a large variety of associated organisms, many of which have obligate associations with ash (Pautasso et al., 2013; Mitchell et al., 2014). In Sweden, ash is now a Red-listed species whose conservation status has recently been elevated from 'vulnerable' to 'critically endangered', considered to have high risk of extinction in the wild (Artdatabanken, Swedish Species Information Centre, www.artdata.slu.se). Thus, ash dieback may have greater ecological consequences by indirectly affecting the biodiversity of organisms associated with ash.

There are no effective means to control spread of the disease and the prospects for growing common ash in Europe are gloomy. However, within *F. excelsior* populations, a small proportion of trees seem to exhibit increased and inheritable tolerance against *H. fraxineus* (Stener, 2013; McKinney et al., 2012; Kjær et al., 2012), showing very little disease expression in the crown. Hence, one option for disease management in the future is directed towards breeding for increased resistance in the host tree. While genetic control seems the most feasible, durable, and long-term solution for conserving ash, more immediate control tactics have been explored which offer varying success. In theory, chemical control using various fungicides could offer a solution. However, recent investigations (Cooke et al., 2013; Hejna et al., 2013; Dal Maso and Montecchio, 2014; Hauptman et al., 2015) so far indicate no feasible prophylactic or curative chemical treatment for *H. fraxineus*. In general, fungicides may only be desirable and economically feasible in nurseries, stool beds, and ornamentals, and in most cases, due to negative impacts of fungicides on the environment, these methods are rather discouraged.

Biological control which entails use of natural predators or antagonistic organisms may offer some promise in the future. Fungal endophytes (Schulz et al., 2015; Ogris et al., 2010) and mycoviruses (Schoebel et al., 2014) have been recognized as possible agents of control against *H. fraxineus*, however more research is needed to test their efficacy under natural field conditions.

Similarly, cultural control options such as burying or composting fallen leaves (Forestry Commission UK, 2015) and hot water treatment (Hauptman et al., 2013; McCartan et al., 2015) may prevent the spread of *H. fraxineus*, though none offer any reliable means by which the disease can be controlled on a large scale.

Pruning has been recognized as a cultural control method against tree stem diseases including rusts (Hunt, 1991; Zeglen et al., 2010; O'Hara et al., 2010) where branch infections typically precede the development of stem cankers. In the case of ash located in urban and landscape settings where the aesthetic value is high, e.g. trees located in parks and gardens, veteran trees having high heritage or cultural value, or those with high genetic importance (e.g. located in clonal seed orchards), pruning may be one option to maintain tree vitality. One obstacle to providing guidance to practitioners is the lack of knowledge on the internal spread of the pathogen, such that pruning will be effective to exclude the disease.

The objective of this study was to determine the extent to which *H. fraxineus* can be detected in advance of visible lesions on pruned branches towards the stem of the tree and to provide guidelines with respect to effective pruning practices that may help achieve better control against damage caused by *H. fraxineus*.

2. Materials and methods

2.1. Sites and sampling of material

In June 2013, 76 *F. excelsior* trees were examined along an alley in Alnarp, southern Sweden. The trees were initially planted in 1994 at a minimum spacing of 1.5 m. Source material originated from Bruns-Pflanzen in Bad Zwischenahn, Germany and was a mix of Westhof's Glorie varieties – a known cultivar of *F. excelsior* with moderate to high susceptibility to *H. fraxineus* (R. Kehr, personal communication; Anonymous, 2011). All trees were naturally infected with *H. fraxineus* but to varying degrees and most lesions were confined to outer branches (i.e. no visible stem infections). The crowns of all trees were assessed and assigned a disease severity score based on the extent of visual dieback according Kirisits and Freinschlag (2012). For the assessments, the crown of each tree was divided into thirds, and assigned to one of seven classes relating to the percent crown dieback.