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ORIGINAL ARTICLE

Assessment of lesion development in *Fraxinus excelsior* cultivars Altena, Atlas and Westhof's Glorie inoculated with different isolates of *Hymenoscyphus fraxineus*

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Abstract

Ash dieback caused by the invasive fungus *Hymenoscyphus fraxineus* leads to massive mortality among common ash (*Fraxinus excelsior*) in Europe. To select tolerant genotypes, inoculation experiments are frequently conducted with isolates of the fungus. The aim of this study was to improve the inoculation methodology for evaluating susceptibility of ash genotypes to ash dieback through (i) testing the virulence of different isolates of *Hymenoscyphus fraxineus* and (ii) investigating temporal lesion development using three widely applied cultivars: Altena, Atlas and Westhof's Glorie. First, an experiment was conducted with a repeated measure design in which virulence of five different isolates was tested. Second, a subset of two isolates that induced the longest downward lesion length in experiment one was used in combination with a slightly adjusted inoculum protocol. Significant differences were found between isolates for downward lesion length, but a significant interaction effect of isolates and cultivars was absent. Also, the inoculation position within the stem affected lesion length; the largest lesions were found on the highest inoculation position within the stem. Furthermore, we found that cryopreserved isolates can remain virulent over years. The timing of inoculation at the end of the growing season was effective as large lesions already occurred during winter dormancy. For future inoculation studies, we propose to use: (i) isolates that induce large lesions, as less virulent isolates induced not only shorter but also fewer lesions; (ii) a similar inoculation position to better compare inoculations within and between experiments; (iii) cryopreserved isolates for testing over years; (iv) mycelial suspensions for inoculum preparation to cover wood chips more evenly; (v) reference clone—like the studied cultivars—to standardize research outcomes between years and research groups.

KEYWORDS
cultivar susceptibility, *Fraxinus excelsior*, *Hymenoscyphus fraxineus*, inoculation experiments, lesion development, virulence

1 | INTRODUCTION

Ash dieback caused by the invasive fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz and Hosoya most likely originating from Asia has been devastating populations of common ash (*Fraxinus excelsior* L.) in Europe since the late 1990s (Coker et al., 2019; Díaz-Yáñez et al., 2020; McKinney et al., 2014). Release of airborne ascospores facilitates the spread of *H. fraxineus* over Europe, primarily causing foliar infections resulting in lesions that may extent in twigs and stems thereby weakening trees leading to massive mortality (Coker et al., 2019; Díaz-Yáñez et al., 2020; Grosdidier, Ioos, Husson et al., 2018). This has a significant ecologic and economic impact on European forests as *Fraxinus excelsior* is an important keystone tree species also providing valuable timber (Hill et al., 2019; Mitchell et al., 2014).

To select tolerant genotypes for breeding purposes, efficient and reproducible testing methods are needed. Stem wound inoculation tests are a common method to assess the degree of susceptibility in ash plant material (Adamčíková et al., 2018; Kjaer et al., 2017; Lobo et al., 2015; McKinney et al., 2012). For instance, McKinney et al. (2012) showed that susceptibility of clones in long-term field trials from natural infections can be predicted by lesion development based on controlled inoculations with the fungus. In addition, lesion development also depends on the selected isolates of *Hymenoscyphus fraxineus* due to variability in virulence (Adamčíková et al., 2018; Kosawang et al., 2020; Kowalski & Bartnik, 2010; Kowalski & Holdenrieder, 2009; Lygis et al., 2017). In the study of Adamčíková et al. (2018), clones were inoculated with different isolates and significant differences were found in lesion development between clones and isolates. To select tolerant ash trees based on inoculation studies, it is important to have insight in differences in virulence between isolates, especially as studies show high variability in lesion length and also in percentages of inoculations resulting in lesions (Adamčíková et al., 2018; Bakys et al., 2009; Kosawang et al., 2020). It is also important to verify whether long-term storage is possible so that particular isolates can act as references to standardize research outcomes. This has been hampered by several reports suggesting that long-term storage might cause degeneration of isolates leading to reduced virulence (Gross & Sieber, 2016; Kowalski & Holdenrieder, 2009). Moreover, research outcomes are difficult to compare as a wide variety of ash genetic material—that differs in susceptibility to ash dieback—is used for experiments. Next to the virulence of isolates, also temporal aspects can influence lesion development. Nielsen et al. (2017) and Kosawang et al. (2020) found that lesion growth continued during winter, yet studies on this topic are still limited.

This research aims at improving the inoculation methodology for evaluating susceptibility of ash genotypes to ash dieback through (i) testing virulence of different isolates of *Hymenoscyphus fraxineus* and (ii) studying temporal aspects of lesion development. Two inoculation experiments were conducted, for which three *Fraxinus excelsior* cultivars were used: Altena, Atlas and Westhof's Glorie. These cultivars are widely used as roadside trees in the Netherlands and are reported to be moderately to highly susceptible to ash dieback (de Vries & Kopinga, 2017; Hiemstra & Meulenbelt, 2019).

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental site

For the two inoculation experiments, three *Fraxinus excelsior* cultivars were used: Altena, Atlas and Westhof's Glorie. Buds of these cultivars were grafted on one-year-old seedling rootstocks. After the first growing season, trees with a stem height between 150 and 175 cm were potted in 20 L containers in a commercial shade tree substrate (RHP Legro laanbomen, pH normal, multi-coat HK 8 months 14 + 8 + 20, micro-max premium CRF). The trees were then placed in rows, with no space between pots within the row and 2 m between the rows (Figure 1a) in an experimental site in Randwijk, the Netherlands (51.937672°, 5.704548°). The trees were frequently watered using a semi-automated irrigation system.

2.2 | Experimental set-up

In the first experiment, five isolates of *Hymenoscyphus fraxineus* were tested (CBS139782 Austria (Kirisits & Schwanda, 2015), CBS 122504 Poland, CBS 145275 the Netherlands, NL 31215 the Netherlands and MeU 1732 Czech Republic). CBS 139782 Austria and CBS 122504 Poland were obtained from the collection of Westerdijk Fungal Biodiversity Institute (the Netherlands), and MeU 1732 was obtained from the Mendel University (Czech Republic). CBS 139782 and CBS122504 were isolated on 11-11-2014 and 6-12-2000 respectively and cryopreserved in liquid nitrogen in the collections of Westerdijk Fungal Biodiversity Institute since 2015 and 2008 respectively. MeU1732 was isolated in 2013 and was stored as small pieces of culture on MEA in water at 5°C. In contrast, CBS 145275 and NL 31215 were freshly isolated from lesion margins using the protocol in Kowalski and Bartnik (2010). The inoculum production entailed sterilized wood chips made of fresh sapwood of *Fraxinus excelsior* that were colonized with the five isolates on ash leaf malt extract agar (AMEA) plates (Kirisits et al., 2013) for approximately 1 month (Figure 1b). Hereafter, six different positions per ramet (15 ramets per cultivar) were marked at fixed 20 cm intervals between 20 and 120 cm stem height. Inoculation positions were rotated around the stems with each position located at a 90 degrees angle from the previous position, to avoid overlapping lesions. Stem wound inoculations were performed at the end of the growing season when leaf abscission had started, on 30 October 2017. The five isolates and a negative control—a similar yet untreated sterilized wood chip—were randomly assigned to one of the six positions per ramet. Stem wound inoculations consisted of making a downward longitudinal incision of approximately 1 cm in the bark of the stem without removing the bark (Figure 1d), placing the inoculum on the xylem surface with forceps and sealing the wound with Parafilm™. In total, 15 ramets per clone

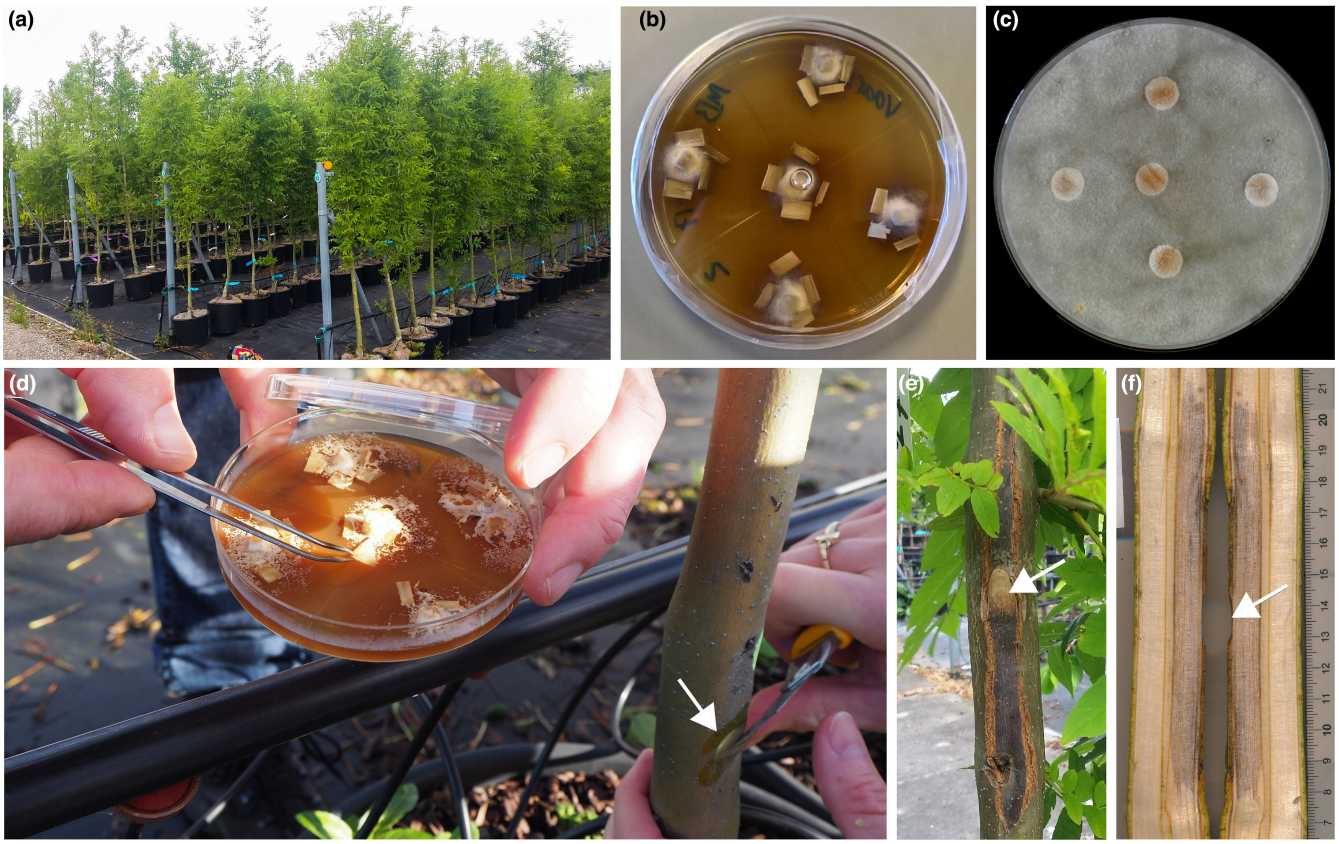

FIGURE 1 Experimental set-up of the inoculation experiments. (a) Experimental plot with the trees of experiment 2. (b) Inoculum preparation for experiment 1, sterilized ash wood chips placed around the isolate growing on AMEA plates. (c) Inoculum preparation for experiment 2, circular wood chips placed on MA plates inoculated by spreading a mycelial suspension of the isolate evenly over the plate. (d) Conducting stem wound inoculations by making a longitudinal incision in the stem using tweezers to insert the inoculum in the incision. (e) Exterior downward lesion (EDL) after 42 weeks for experiment 2. (f) Interior downward lesion (IDL) after 27 weeks for experiment 1, the arrow indicates the inoculation position.

were inoculated at six different positions leading to a total of 270 inoculations in 45 trees.

In the second experiment, two isolates (CBS 122504 and CBS 145275) were used that induced the longest mean lesion length and for which all inoculations resulted in lesions in the first experiment. Since in the first experiment, not all wood chips were covered by the fungus, inoculum preparation was modified by (1) using 5 mm diameter circular sterilized wood chips cut out of *Fraxinus excelsior* veneer (0.6 mm in thickness) using a hollow punch and (2) spreading a mycelial suspension of the fungus evenly over malt agar (MA) plates after which 5 wood chips were added to every plate (Figure 1c). In this experiment, 75 trees (25 ramets per cultivar) were inoculated at a stem height of 75 cm (1 inoculation per tree) on 12 September 2018, whereby the different isolates were inoculated into 10 trees per cultivar and the control treatment was performed on five trees per cultivar.

2.3 | Measurements of lesion development

In experiment one, lesion development in the three cultivars was assessed by measuring exterior downward lesion length in the bark (EDL) and interior downward lesion length in the sapwood (IDL) respectively in May, 27 weeks after the inoculation (WAI). The IDL length was measured after dividing each stem in six sections through cuts made in between the inoculation positions. Then the stem sections were split longitudinally in such a way that the lesion visible on the bark of the stem was split in half (Figure 1f). In cases where the IDL was equal or longer than the stem section, the length up till the end of the section was used as lesion length. For experiment two, the EDL length in the bark (Figure 1e) was measured 4 (October), 10 (November), 28 (March) and 42 (July) WAI. In addition, the IDL length was measured 42 WAI. For the IDL length, small parts of the stem sections were cut from both ends until the lesion became visible. For both experiments, only the downward lesion (DL) length was analysed, as trees can be girdled by the fungus after which the upward lesion length can no longer be distinguished (Lygis et al., 2017). The number of girdled trees was noted.

2.4 | Statistical analyses

To test for differences in virulence between isolates and in susceptibility between the three cultivars in the first experiment, an

analysis of variance (ANOVA) could not be conducted, as this does not take into account that there were 21 inoculations with no lesion and that the lesion size may depend on the position within the stem. Therefore, a restricted maximum likelihood (REML) analysis was used, with a random term 'tree/position' and a fixed model 'position + cultivar*isolate'. Inoculations that did not result in visible lesions were omitted from analysis. Yet, in a separate analysis, the Fishers' exact test was used to test for differences between probabilities of absence of lesions for both the cultivars and the isolates.

The second experiment was analysed by ANOVA as each tree was inoculated once on a single position, and all inoculations resulted in lesions. A repeated measures ANOVA was employed to analyse the measurements at three assessment dates (November, March and July) simultaneously (using EDL length). An extra tree*-time stratum was added to the model in which differences between assessment dates and its interactions with cultivar and isolates are tested. Furthermore, a Pearson's correlation coefficient (with corresponding *p*-value) was calculated for both experiments between the EDL and IDL length. A log transformation of the data was not seen necessary based on the means of plots of residuals versus fitted values; therefore, all analyses were done on the original scale.

3 | RESULTS

3.1 | Experiment one

No lesions developed in the negative control treatment. For CBS 122504 and CBS 145275, all inoculations induced lesions, which was significantly higher ($p < .05$) than for NL 31215, MeU 1732 and CBS 139782 for which lesions were absent in 13 (28.9%), 7 (15.6%) and 1 (2.2%) out of 45 inoculations respectively.

A significant ($p < .001$) isolate effect was found both for the EDL and IDL length 27 WAI (Figure 2). The mean DL length was largest for CBS 145275, followed by CBS 122504 for both EDL (7.1 and 5.8 cm respectively) and IDL (8.1 and 6.5 cm respectively) (Table S1). Pairwise comparisons showed that all isolates significantly ($p < .05$) differed from each other in mean EDL length. For the mean IDL length, all isolates significantly ($p < .05$) differed from each other except CBS 139782 and NL 31215 (Table S1). No interaction effect between cultivars and isolates was found for mean EDL and IDL length ($p = .86$ and $p = .60$ respectively). A small but significant ($p < .01$) difference was found in mean EDL and IDL length between cultivars, whereby Atlas and Westhof's Glorie had smaller lesions compared with Altena (Figure 2, Table S1). Note that in 12 cases, IDL length might have been underestimated as lesions were present in the entire stem section. A significant effect ($p < .01$) of inoculation position was observed with inoculations at the highest position within the stem showing largest EDL and IDL lengths of 5.3 and 6.2 cm respectively and at the lowest position smallest lesions of 3.9 and 4.6 cm respectively, averaged over all isolates. Pearson's correlation coefficient between the EDL and IDL length was 0.87 ($p < .001$). The mean IDL length (5.3 cm) was 0.6 cm (11.7%) longer than the mean EDL length (4.8 cm) averaged over all measurements.

3.2 | Experiment two

In this experiment, lesions developed in all inoculated trees, except for the control treatment. In five trees, the leader shoot died because the trees were girdled by the fungus at the inoculation point. At the

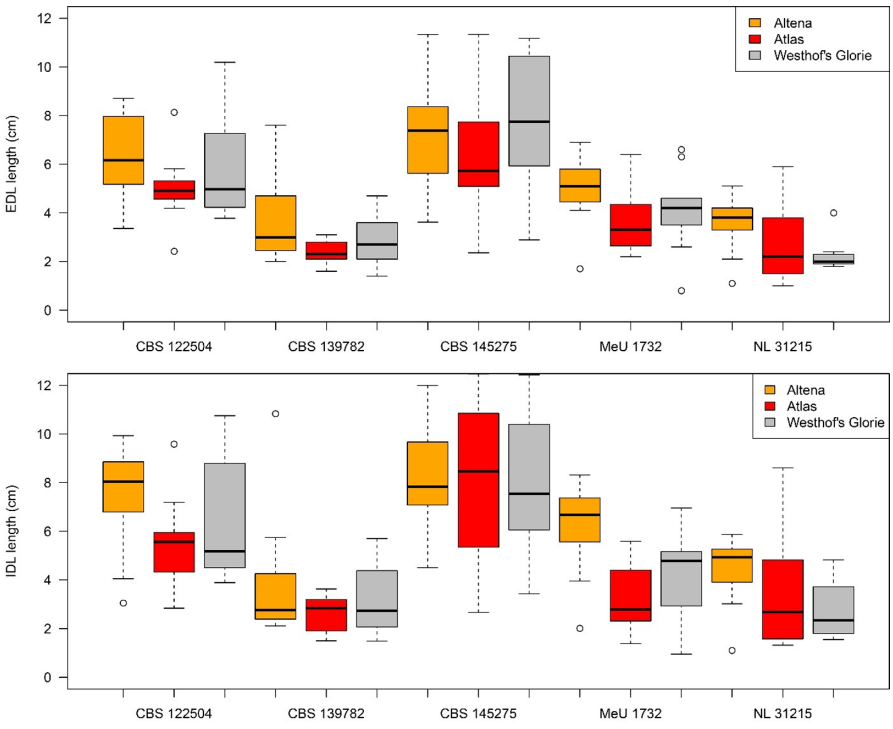

FIGURE 2 Exterior downward lesion (EDL) and interior downward lesion (IDL) length in three *Fraxinus excelsior* cultivars Altena, Atlas and Westhof's Glorie (control treatment not shown) inoculated with five isolates, 27 WAI. A significant ($p < .001$) isolate effect was found for both EDL and IDL length, and a small but significant ($p < .01$) difference in mean EDL and IDL length between cultivars was found. No interaction effect between cultivars and isolates was found for mean EDL and IDL length ($p = .86$ and $p = .60$ respectively).

first assessment date (4 WAI), no lesions had developed yet; therefore, only the assessments from 22 November (10 WAI) onward were analysed. A significant isolate effect for the EDL (10, 28 and 42 WAI) and IDL length was found, with $p < .001$ and $p < .05$, respectively. Similar to experiment one, CBS 145275 inoculation resulted in a significantly larger mean DL length than CBS 122504 for both EDL and IDL measurements, with $p < .001$ and $p < .05$ respectively (Figure 3). For the final lesion measurement in July (42 WAI), the mean EDL length for CBS 145275 was 18.3 cm (Figure 4, Table S2), which was 5.2 cm (39.5%) longer than for CBS 122504 (13.1 cm). For the mean IDL, the length for CBS 145275 (25.9 cm) was 4.3 cm (19.9%) longer than for CBS 122504 (21.6 cm). The three cultivars differed in mean EDL length for the three assessment dates ($p < .01$), with mean EDL length, after 42 weeks, ranging between 11.4 and 16.1 cm for CBS 122504 and 16.8 and 20.3 cm for CBS 145275, whereby Atlas and Altena had smaller lesions compared with Westhof's Glorie (Figure 3, Table S2). There was no significant interaction effect found between isolates and cultivars ($p = .690$, 42 WAI). Pearson's correlation coefficient between the EDL and IDL length was 0.68 ($p < .001$). The IDL length (23.8 cm) was 8.1 cm (51.6%) longer than the EDL length (15.7 cm) averaged over all measurements.

The development of the EDL from November to March and March to July was very similar for both isolates in all clones (Figure 3). There were no significant interactions between time*cultivar ($p = .651$), time*isolate ($p = .060$) and time*cultivar*isolate ($p = .231$).

4 | DISCUSSION

In this research, the aim was to improve the inoculation methodology for evaluating susceptibility of ash genotypes to ash through (i) testing virulence of different isolates of *Hymenoscyphus fraxineus* and (ii) studying temporal aspects of lesion development in three widely used cultivars.

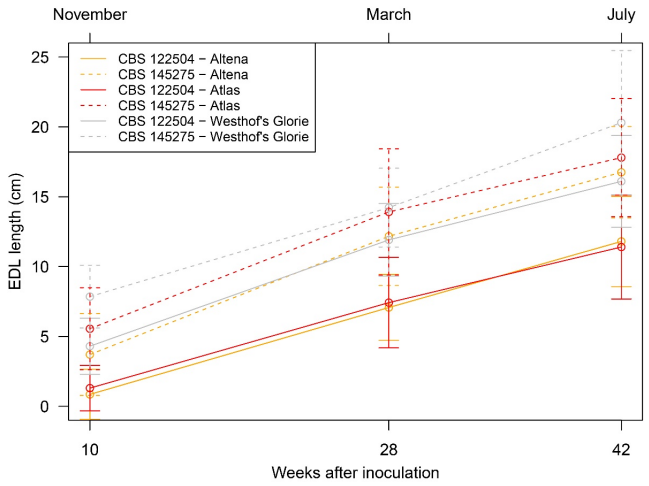

FIGURE 3 Mean exterior downward lesion (EDL ± SD) length in three *Fraxinus excelsior* cultivars Altena, Atlas and Westhof's Glorie (control treatment not shown) inoculated with two isolates, 10, 28 and 42 WAI. A significant isolate effect ($p < .001$) and significant cultivar differences ($p < .01$) for EDL length (10, 28 and 42 weeks after inoculation) were found.

4.1 | Percentage of inoculations inducing lesions and isolate virulence

In line with other studies, we found significant differences between isolates in lesion length (Kosawang et al., 2020; Kowalski & Holdenrieder, 2009; Lygis et al., 2017) and also in the percentage of inoculations resulting in lesions (Adamčíková et al., 2018). We found that the isolates CBS 145275 and CBS 122504 always induced lesions in both experiments. In contrast, inoculation with the other three isolates—with shorter mean lesion lengths—showed no lesion in up to 28.9% of the inoculated trees. To the best of our knowledge,

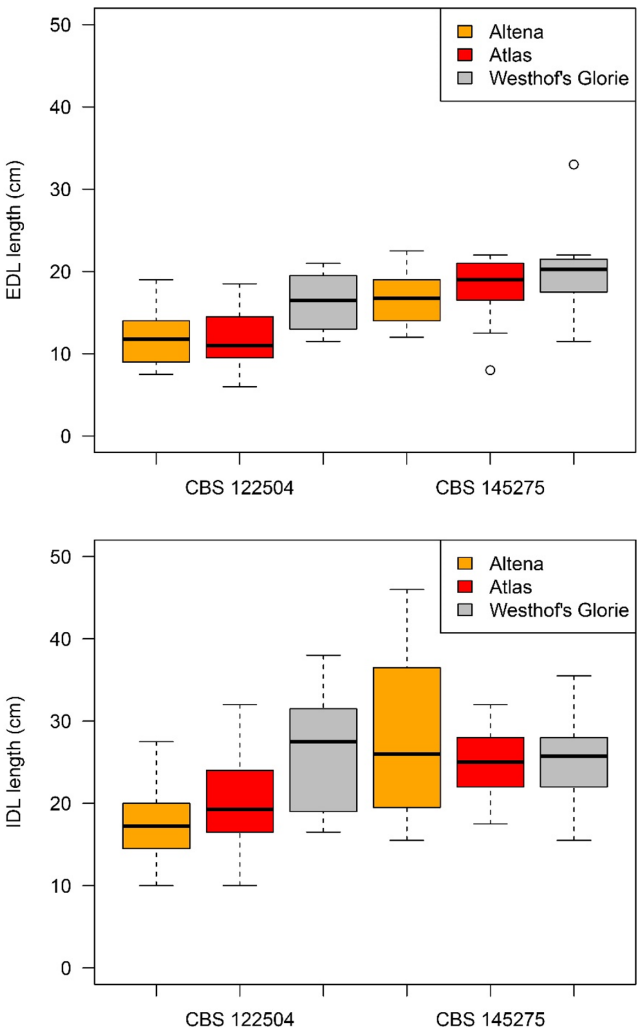

FIGURE 4 Exterior downward lesion (EDL) and interior downward lesion (IDL) length in three *Fraxinus excelsior* cultivars Altena, Atlas and Westhof's Glorie (control treatment not shown) inoculated with two isolates, 42 WAI. A significant isolate effect for EDL ($p < .001$) and IDL ($p < .05$) length, and significant cultivar differences ($p < .01$) were found. There was no significant interaction effect found between isolates and cultivars ($p = .690$).

absence of lesion development after inoculation among ramets is seldomly reported (Adamčíková et al., 2018) but important to understanding inoculation success as the absence of a lesion could also indicate a false negative. Adamčíková et al. (2018) reported absence of lesions among ramets (~15 WAI), in an inoculation study with four isolates that induced relatively low lesion lengths. Compared with our study, Adamčíková et al. (2018) found a higher percentage (52%, 31 out of 60) of inoculations that did not induce lesions, which is mostly related to one isolate that hardly induced lesions and did not differ significantly from the control treatment.

The mean total lesion lengths for isolates CBS 145275 and CBS 122504 were 29.5 and 19.9 cm respectively, 42 WAI (data not shown). The mean total lesion length for CBS 145275 in experiment two is actually an underestimation, since five trees were excluded as they were girdled by the fungus after which the upward lesion length could no longer be distinguished. These lesion lengths indicate high virulence of these isolates as in other studies—albeit with a different experimental set-up, duration and other isolates and planting material—smaller lesions were generally observed (Lobo et al., 2015; Kjaer et al., 2017; Nielsen et al., 2017, Lygis et al., 2017). For example, Lygis et al. (2017) inoculated three-year-old seedlings—also at the end of the growing season (October)—with 200 isolates from Switzerland and Lithuania and found smaller mean total lesion lengths—ranging between 0 and 12 cm—with a slightly longer duration of the experiment (~44 weeks). Two studies reported longer lesion lengths compared with our study (Kosawang et al., 2020; Kowalski & Holdenrieder, 2009). Kosawang et al. (2020) studied 19 single-spore isolates—instead of isolates derived from lesion margins—and found high variation in virulence with observed lesions ranging between no lesion (0 cm) and 39.5 cm, 36 WAI. Kowalski and Holdenrieder (2009) used 10 fresh isolates—to prevent degeneration in vitro—and reported median EDL length up to 40 cm (52 WAI) in six-year-old plantation trees. In our study, we found that even an 18-year-old isolate (CBS 122504) induced long lesions and thus remained virulent. This isolate was cryopreserved indicating that for long-term storage cryo-preservation in liquid nitrogen might be a good option. This is in contrast to other studies that suggested that long-term storage might cause a degeneration of isolates, which could result in decreased virulence (Gross & Sieber, 2016; Kowalski & Holdenrieder, 2009). It is important to report and determine the best long-term storage conditions for isolates of *H. fraxineus*.

4.2 | Temporal lesion development

Exterior lesion development became visible between four and 10 weeks after inoculation and continued during winter dormancy. The results confirm previous studies that lesion development continues during winter dormancy (Bakys et al., 2009; Kosawang et al., 2020; McKinney et al., 2012). For example, McKinney et al. (2012) found similar results when inoculation was done in September in a field trial in Denmark—with snow until March—where lesions over 20 cm in length could be observed during the first assessment in March. Bengtsson et al. (2014) found that lesion development even occurred in winter months in which the temperature did not rise above the freezing point. This is in agreement with Hauptman et al. (2013) and Kowalski and Bartnik (2010), who found that *Hymenoscyphus fraxineus* can be classified as a mesophile and cold tolerant microorganism able to cause necrosis at low (5°C) temperature. These studies support our results that inoculation at the end of the growing season can be effective as large lesions already occurred during winter dormancy. Moreover, inoculating trees at the end of the growing season has the advantage that high temperatures—that may occur during the growing season—are avoided during which growth and survival of the fungus is limited (Grosdidier et al., 2018; Hauptman et al., 2013; Kowalski & Bartnik, 2010).

4.3 | Lesion length is related to inoculation height within the stem

A significant effect of inoculation height was found in our first experiment with larger lesions occurring higher up in the stem. This suggests that a similar inoculation position is important to make inoculations more comparable. To the best of our knowledge, this is the first study in which lesion development was studied in relation to the inoculation position within the stem. Stem structural differences may favour mycelial growth higher up in young stem parts as the proportion of pith, parenchyma and phloem—in which fungus easily spreads—is higher compared with lower stem positions where more xylem is present in which the fungus mostly grows in axial or ray parenchyma (Schumacher, 2011; Maso et al., 2012; Gross et al., 2014). Our study also confirms that there is a high correlation between the interior and exterior lesions, whereby the interior lesions in the xylem are larger than exterior lesions (Bakys et al., 2009; Schumacher, 2011).

4.4 | Cultivar differences

Small but significant differences were found between the cultivars for lesion length, whereas in experiment one, Atlas and Westhof's Glorie had significantly smaller lesions compared with Altena; in experiment two, Atlas and Altena had significantly smaller lesions compared with Westhof's Glorie. All three cultivars developed large lesions and therefore can be classified as susceptible to ash dieback. This is in line with later inoculation experiments in which one-year-old ramets of these cultivars were inoculated (data not published) and also small differences in lesion length between these cultivars were found, with on average Atlas showing the shortest exterior lesions. Comparable results were observed after natural infection in two Dutch clonal seed orchards where Atlas and Altena showed less twig dieback compared with Westhof's Glorie (de Vries & Kopinga, 2017) and in a clonal collection the mean twig dieback was estimated 55%, 70% and 75% for Atlas, Altena and Westhof's Glorie, respectively, in 2019 (data not published). Hiemstra and Meulenbelt (2019) reported these three cultivars to be among the moderately to highly susceptible cultivars based on a visual

assessment of natural infection of ash dieback in roadside trees in combination with an inoculation experiment with eight other cultivars. Thus, the results of lesion development of the three cultivars in our inoculation experiments are in agreement with the ranking for susceptibility for trees exposed to natural infection. Moreover, the results did not reveal substantial interaction between the cultivars and isolates used. In the Netherlands, these three cultivars are now used as susceptible reference clones in inoculation studies for selecting tolerant genotypes, whereby genotypes with a significantly smaller lesion length are seen as tolerant. Inclusion of susceptible and tolerant reference clones in further inoculation studies allows to compare results between experiments. It also helps to correct for other factors that cause variation in lesion length such as timing of inoculation, age of the plant material, inoculum and environmental conditions.

4.5 | Recommendations for inoculation studies

Our study shows the importance of using highly virulent isolates in testing genotypes for tolerance to ash dieback, as it showed that only for the two most virulent isolates lesions were always induced in inoculated trees. This is particularly important when single genotypes are screened for susceptibility, whereby the absence of a lesion may imply a false-negative or a highly tolerant individual. We showed that cryopreserved isolates may remain virulent over years. Also, no significant interaction between cultivars and isolates was found. Both results seem promising to develop standard isolates to be used for largescale screening tests over years. The timing of inoculation at the end of the growing season was effective as large lesions already occurred during winter dormancy. To save time, only EDL could be measured and the final assessment could be advanced, since the second experiment showed similar results for the assessments of 10, 28 and 42 WAI. To standardize the inoculation procedure, based on the inoculum production of the two experiments, we recommend using a mycelial suspension to start inoculum production as it resulted in wood chips that were more evenly covered with mycelium. As inoculation height in the stem was significantly affecting lesion length, it indicates that it is important to use a similar inoculation position. The studied cultivars may act as susceptible references in inoculation experiments to standardize research outcome between years and research groups.

Overall, stem wound inoculation used for susceptibility testing is time consuming and not appropriate for high throughput screening. Wound inoculation also has the drawback that it bypasses potentially important early barriers at the foliar level where the infection starts under natural conditions. Therefore, it is important to keep developing alternative methods like the ascospore inoculum that potentially allow for more natural and rapid screening of ash genotypes for tolerance (Mansfield et al., 2018) or the rachis inoculation method of Orton et al. (2019) that reduces the assay time from months to weeks. In addition, other screening alternatives, for example based on molecular or biochemical markers (Harper et al., 2016; Nemesio-Gorriz et al., 2020; Stocks et al., 2019) or near-infrared spectroscopy (Villari et al., 2018) might contribute to efficiently screening ash trees for susceptibility to ash dieback.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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