A Novel Plant Resistance Inducer for the Protection of European Ash (Fraxinus excelsior L.) against Hymenoscyphus fraxineus—Preliminary Studies

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Abstract: Ash tree disease is caused by an ascomycete fungus Hymenoscyphus fraxineus, which first emerged in 1992, eastern Poland. Site factors, genetic predispositions, and resistance to the pathogen have not been fully described yet. The general aim of the study undertaken was to check the effect of using a new active substance representing benzothiadiazoles, a BTH derivative, namely, N-methyl-N-methoxyamide-7-carboxybenzo(1.2.3)thiadiazole (BTHWA), on ash saplings. A total of 41 ash saplings, aged three to five years, were subjected to this experiment in six variants of treatment. The results of the inoculation with H. fraxineus indicated that the treatment with BTHWA resulted in the limitation of the size of necrotic phloem lesions. Although the lesions were detectable in the cross section, the plants showed no visible signs of infection. The results suggest that H. fraxineus development in ash saplings can be slowed down or even completely stopped through triggering plant resistance by BTHWA.

Keywords: ash dieback; ash saplings; inoculation; Hymenoscyphus fraxineus; systemic acquired resistance (SAR); BTHWA

1. Introduction

Novel diseases, especially when unknown in the particular ecosystem, often cause mortality to newly infected host species. This can change, for example, the structure of forest communities, including the extinction of the most vulnerable species [1]. European ash dieback (ADB) is one of the examples of native tree species under the attack of an ascomycete fungus H. fraxineus (HF) [2]. The pathogen is associated with Fraxinus mandshurica Rupr and F. chinensis Roxb. [3] and was introduced to Europe with their saplings. Since 1992, the disease had spread to more than 25 European countries, with mortality rates of up to 85% and 69% recorded in plantations and woodlands, respectively [4]. ADB affects ash retreat, especially in riparian and ash-dominated forests [5], whereas the lowest mortalities of ash renewal occur in mesic habitats [6,7]. Recent studies revealed that the high mortality rate of ash saplings affects both forest nurseries and natural regenerations, even those with low ash regeneration density [7,8]. Each of the impacted host populations may have different susceptibility to ADB, as trees can evolve resistance mechanisms that allow them to defend themselves against pathogens [9]. Some ash individuals can resist ADB, which is associated with their genetics [10] or non-favorable site conditions for HF development [5]. Therefore, the number of less vulnerable individuals will have implications for the dynamics of forest and remaining populations [11].
One of the possibilities to overcome the issue with the negative effect of HF is the use of a natural defense mechanism, allowing plants to respond to different types of pathogens by the induction of their immune system. A systemic acquired resistance (SAR) works through inducing resistance in one tissue and then transferring the resistance signal, systematically, to the entire plant [12–15]. To trigger the SAR in plants, the infectious agent or resistance-inducing molecules such as salicylic acid [16], benzo[1.2.3]thiadiazole-7-carbothioic acid S-methyl ester (BTH) [17], and their derivatives [18–21] must be present. Our previous studies have shown that a group of ionic derivatives of benzothiadiazole can act as effective plant resistance inducers [22,23], with lower (eco)toxicity and higher biodegradability than the commercially available BTH [24]. The most effective plant resistance inducer discovered by us, a water-based formulation of N-methyl, N-methoxy-7-carboxybenzo[1–3]thiadiazole (BTHWA), the experimental product by Innosil company [25,26], in addition to high efficiency, has shown the highest level of SAR marker genes associated with pathogen defense response [27]. Considering the universal character of SAR phenomena and the high potential of the discovered BTHWA inducer, we decided to examine its utility in ash trees’ protection against HF. To our knowledge, this is the first report describing the use of BTHWA plant resistance inducer in ash tree protection. Thus far, only the studies on the use of fungicides such as thiabendazole, propiconazole, prochloraz, allicin, copper sulfate, and phosphate fertilizers against ADB have been performed [28,29].

We hypothesized that BTHWA will modify and strengthen the ash natural defense mechanisms in response to fungus inoculation and consequently will trigger ash resistance against HF.

2. Materials and Methods

2.1. Material Collection

In Spring 2019, 41 ash saplings, aged three to five years, were collected from the alder-ash forest (52°26′1.955″ N 16°52′39.371″ E, Poland). The stand species composition included a 70% share of F. excelsior (66 years old; similar to other species), Ulmus laevis L. (20%), and Populus alba L. (10%). Within the chosen stand, characterized by fertile, moist, and pH-neutral soil (Hyperhumic Gleyic Phaeozem) mature ash trees were highly infected with ADB—45% of mean crown defoliation [7].

All chosen saplings were approximately 20 cm in height and showed no visible symptoms of ADB. The saplings were collected together with soil and placed directly into pots with a capacity of four liters. The plants were incubated in garden tents and watered once or twice a week, to keep the soil moist.

2.2. Experimental Design of BTHWA Treatment

The experiment included six variants of treatment (Table 1). Every 10 days, each sapling was watered with 200 mL of BTHWA solution directly to the soil (W1; W2) or by spraying the whole plant with 10 mL of BTHWA solution (S1; S2). The total amount of BTHWA per plant applied in one treatment was 4 mg (W1; W2) and 0.2 mg (S1; S2).

The experiment was preceded by testing the BTHWA solution on 10 ash saplings in 2018. The regime of treatment referred to as the W1 variant of treatment gave promising results as all of the infected individuals by HF managed to survive till the next year.
Table 1. The variants of treatment in the experiment and simplified schedule of treatment.

| Variant of Treatment | Treatment Prior to Inoculation | Fungus Inoculation | Treatment Post Inoculation |
|----------------------|-------------------------------|--------------------|---------------------------|
|                      | 10 Treatments                 | 4 Treatments        |                           |
| W1                   | ✓                             | ✓                  | ✓                         |
| W2                   | ✓                             | ✓                  |                           |
| S1                   | ✓                             | ✓                  |                           |
| S2                   | ✓                             | ✓                  |                           |
| C (+)                |                               | ✓                  |                           |
| C (−)                |                               |                    |                           |

Abbreviations: W—watered; S—sprayed; C—control; ✓—activity performed.

2.3. Saplings Inoculation

HF, sourced from an infected ash stand in Serbia, was subcultured in Petri dishes (90 mm diameter) containing 2% malt extract agar and was incubated at room temperature under natural diffuse light for 7 weeks. Thereafter, small ash twig segments were autoclave sterilized (121 °C, 20 min) and placed onto the surface of the cultures of each HF isolate. The cultures were incubated at 20 °C under natural diffuse light for an additional 8 weeks according to the methodology by Madigan et al. [30]. Saplings were inoculated with HF after 7 days counting from the day of the tenth BTHWA application. The inoculation was carried out by cutting the bark approx. 10 cm above the ground to expose the underlying phloem and placing the inoculum onto the wound. The stem was later wrapped with Parafilm™ to secure the inoculum position. Control plants were mock-inoculated onto wounds with PDA (5 × 5 mm). In sapling of all variants of treatment, one shoot was inoculated per plant. The shoots were harvested 6 weeks after the inoculation. All 41 samples were inspected for symptoms of lesions. Shoot samples were later collected and cut to check for the presence of necrotic tissues. The tissue samples were collected from all inoculated saplings and from the control plants. Samples were surface sterilized, and the inocula were placed on PDA for the reisolation of HF. Optimal growth conditions for plants and the pathogen were provided; hence, the environmental factors are unlikely to limit the ability of HF to infect plants. For reisolation of the pathogen, shoots were washed in tap water, bark, and wood fragments were cut into fragments of 5 × 3 − 4 mm. Bark fragments were surface sterilized in 96% ethanol for 30 s, 0.5% sodium hypochlorite for 60 s, 96% ethanol for 30 s, and finally rinsed in distilled water (three times). The time of sterilization was shortened to 10 s in each disinfection agent for wood fragments. The inocula were placed in 90 mm Petri dishes with 2% malt extract agar (MEA). Petri dishes were incubated in the dark at 20 °C and checked weekly for the growth of fungi. The emerging colonies were subcultured on MEA for subsequent morphological identification.

3. Results

Necrotic phloem lesions were observed on all of the control individuals inoculated with the pathogen (variant C (+)). No lesions were observed on the negative control and W1 variant (Table 2, Figure 1). The lesions were more severe in the plants treated with BTHWA for a limited period of time prior to inoculation than in those treated and after the inoculation (Table 2, Figures 2–4). BTHWA was more effective when applied directly to the soil, W1, and W2 variants (Table 2). In the sprayed individuals, the necrotic tissues were present over a larger area (Figure 4). The largest lesions (2.3 × 1.9 cm) were noted in C (+) variant, followed by in W2 and S1 variants (Table 2). As observed before (the study in 2018), and confirmed in this experiment, plants treated with BTHWA in 10 days intervals before and after the inoculation (W1, S1 variants) sealed their wounds (Figure 4). Despite the necrosis, visible in the cross section of two individuals, the plants...
looked healthy (Figure 5). Moreover, during the whole experiment, we did not observe any symptoms of other pathogen infections.

Table 2. Treatment variants with the number of treated plants and lesions observed.

| Treatment  | W1 (cm) | W2 (cm) | S1 (cm) | S2 (cm) | C+ (cm) | C− (cm) |
|------------|---------|---------|---------|---------|---------|---------|
| No. of plants | 8       | 8       | 8       | 8       | 5       | 4       |
| No. of plants with lesions | 0       | 2       | 2       | 4       | 5       | 0       |
| Average length of lesion | 0       | 0.42 × 0.32 | 0.33 × 0.26 | 0.5 × 0.35 | 2.3 × 1.9 | 0       |

Figure 1. Samples of variant C (+) with visible necrotic lesion (a) and variant C (−) with no lesions observed (b).

Figure 2. Samples of saplings watered with BTHWA prior to inoculation (W2) in general view (a) and cross section (b).

Figure 3. Samples of saplings sprayed with BTHWA prior to inoculation (S2) in general view (a) and cross section (b).

Figure 4. Samples of saplings sprayed with BTHWA prior to and post inoculation (S1): sealed wound (a); lesion (b).
After the inocula were taken and placed on Petri dishes containing MEA, no HF culture was obtained from the plants that were treated with BTHWA for the whole period of the experiment. HF cultures were isolated from the plants with visible lesions.

4. Discussion

Trees, as long-living organisms, can evolve resistance mechanisms that allow them to defend against pathogens [9]. Nielsen et al. have indicated that ash through the genetic resistance of individuals seems to reduce the susceptibility to ADB [10]. Analyses indicate that most of the total European ash population presents some level of resistance against HF [31]. Other studies have indicated also that soil may indirectly affect ADB by the promotion of fungus inoculation because of high soil moisture and soil reaction variability [4, 6, 32]. Among the soil factors affecting the mortality rates of ash are soil pH, calcium carbonate content, high air humidity, and shallow groundwater table level [5, 33]. These soil conditions occur in alder-ash forest chosen to collect ash saplings for our experiment. Thus, a recent study revealed a high ash saplings mortality within the studied forest compartment [7]. However, the results of our experiment indicate that HF presence in ash individuals can be slowed down or even stopped.

Currently, there are no effective methods to protect ash trees. Different physical and chemical methods of treatment have been used to restrict the production and spread of ascospores, including the removal of plant debris from infected sites, preventing movement of infected ash saplings to new sites, or using fungicides and biocides [32, 34, 35]. A recent study has shown that the application of phosphites could improve the health of treated saplings after artificial inoculation with HF [29]. The same study indicated the importance of interactions between H. fraxineus and Phytophthora spp. inoculation. It turned out that when soil inoculation with Phytophthora was performed 2 weeks before H. fraxineus shoot inoculation, more than 40% of the seedlings survived. In the variant of inoculation with H. fraxineus only, the mortality rate was 100% [29]. This implies that in forest ecosystems, the interaction between plants and different pathogens, i.e., Phytophthora spp. plays an important role [36]. Another study has concerned the examination of the effectiveness of six fungicides against ADB. Among them, only thiabendazole and allicin slowed down significantly the rate of lesions development [28]. Our results suggest that BTHWA can trigger the natural defense mechanism in ash saplings. The induction works by inducing the resistance in tissue and then transferring the resistance signal, systematically, to the entire plant [12, 13]. Ash saplings treated with BTHWA were characterized by limited sizes and the number of necrotic phloem lesions. Interestingly, BTHWA was more effective when

Figure 5. Inoculated saplings showing no symptoms of the disease shown in side view (a) and view from above (b). Saplings were watered by BTHWA prior to and post inoculation (W1 variant). The arrow indicates the place of inoculation.
applied directly to the soil than when sprayed on the plants. Higher watering efficiency can be explained by calculating the total amount of active substance applied to each plant. When plants were watered, the amount of BTHWA was 20 times higher than when plants were sprayed. Moreover, contact time between active substances applied to soil is longer in comparison to the spraying method. On the other hand, our substance can decompose in small amounts by the interaction of an active substance with soil component; however, its availability to plants is still higher, which has been confirmed by limited sizes and number of necrotic phloem lesions. Our previous studies have shown that a group of used ionic derivatives of benzothiadiazole can act as effective plant resistance inducers [22,23]. The experiments were carried out with *Nicotiana tabacum* var. *Xanthi*—tobacco mosaic virus (TMV) model has shown that application of the inducer 7 days before the pathogen attack decreased the level of the disease up to 100% in comparison to the control. Other studies indicated that treatment with BTHWA resulted in the changes in the level of expression of SAR marker genes *PAL*, *NPR1*, *PR-1b* that are associated with a pathogen defense response of the plant, compared to untreated control and other tested plant resistance inducers, including commercially available BTH [30]. The plants treated with BTHWA solution exhibited a lower level of viral RNA accumulation; thus, the viral replication was less efficient, even if the plant had been previously infected by the virus. Studies on the effectiveness of BTHWA in disease prevention are also carried out on other plants and have shown the significant potential of this treatment. The relevant reports will be published soon.

The main disadvantage of this experiment is the limited number of ash saplings and the collection of the plants only from one forest site. However, it is worth mentioning that the number of saplings is already limited by a low number of forests with ash natural regeneration. A crucial unanswered element is also that some individuals could be nonsymptomatic due to genetic resistance developed in natural conditions, or how the BTHWA influences SAR. Therefore, in the second stage of our research, we plan to examine gene expression before and after fungus inoculation to provide information about the number of genes used, including resistance ones producing protein resistance. Moreover, we assume that the survival rate of saplings after discontinuing the use of the substance and introducing trees from laboratory or nursery conditions into forest habitats is not certain. This problem will be the subject of further detailed research based on a higher number of plants.

5. Conclusions

The study reports the first use of BTHWA plant resistance inducer in the protection of ash saplings against *H. fraxineus*. The results suggest that the fungus presence in saplings can be slowed down if not completely stopped through triggering their natural resistance. In comparison to the control plants, the treatment with BTHWA resulted in the limitation of the size of necrotic phloem lesions. The lesions were more severe in the plants treated with BTHWA only until inoculation, than in the plants treated before and post inoculation. BTHWA was more effective when applied directly to the soil than when sprayed on the plants. The results are the core element of future research carried out on a larger number of ash individuals.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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References

1. Dukes, J.S.; Pontius, J.; Orwig, D.; Garnas, J.R.; Rodgers, V.L.; Brazee, N.; Cooke, B.; Theoharides, K.A.; Strange, E.E.; Harrington, R.; et al. Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America: What can we predict? Can. J. For. Res. 2009, 39, 231–248. [CrossRef]

2. Baral, H.O.; Quelo, V.; Hosoya, T. Hymenoscyphus fraxineus, the correct scientific name for the fungus causing ash dieback in Europe. IMA Fungus 2014, 5, 79–80. [CrossRef]

3. Timmermann, V.; Borja, I.; Hietala, A.M.; Kirisits, T.; Solheim, H. Ash dieback: Pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. EPPO Bull. 2011, 41, 14–20. [CrossRef]

4. Coker, T.; Rozsyphal, J.; Edwards, A.; Harwood, T.; Buttfoy, L.; Buggs, R. Estimating mortality rates of European ash (Fraxinus excelsior) under the ash dieback (Hymenoscyphus fraxineus) epidemic. Plants People Planet 2019, 1, 48–58. [CrossRef]

5. Turczatski, K.; Rutkowski, P.; Nowiński, M.; Zawieja, B. Health status of European ash (Fraxinus excelsior L.) in relation to the moisture of selected forest sites. Sylvan 2020, 164, 133–141. [CrossRef]

6. Koltay, A.; Szabó, I.; Janik, G. Chalara fraxinea incidence in Hungarian ash (Fraxinus excelsior) forest. J. Agric. Ext. Rural Dev. 2012, 4, 256–238. [CrossRef]

7. Turczatski, K.; Dyderski, M.; Rutkowski, P. Ash dieback, soil, and deer browsing influence natural regeneration of European ash (Fraxinus excelsior L.). Sci. Total Environ. 2021, 752, 141787. [CrossRef]

8. Pušpure, I.; Matisons, R.; Laivins, M.; Gaitnieks, T.; Jansons, J. Natural regeneration of common ash in young stands in Latvia. Balt. For. 2017, 23, 209–217.

9. Jones, J.D.G.; Dangl, J.L. The plant immune system. Nature 2006, 444, 323–329. [CrossRef] [PubMed]

10. Nielsen, L.R.; McKinney, L.V.; Kjær, E.D. Host phenological stage potentially affects dieback severity after Hymenoscyphus fraxineus infection in Fraxinus excelsior seedlings. Balt. For. 2017, 23, 229–232.

11. Cavers, S. Evolution, ecology, and tree health: Finding ways to prepare Britain’s forests for future threats. Foresty 2015, 88, 1–2. [CrossRef]

12. Oostendorp, M.; Kunz, W.; Dietrich, B.; Staub, T. Induced disease resistance in plants by chemicals. Eur. J. Plant Pathol. 2001, 107, 19–28. [CrossRef]

13. Wani, M.Y.; Mehraj, S.; Rather, R.A.; Rani, S.; Hajam, O.A.; Ganie, N.A.; Mir, M.R.; Baqual, M.F.; Kamili, A.S. Systemic acquired resistance (SAR): A novel strategy for plant protection with reference to mulberry. Int. J. Chem. Stud. 2018, 6, 1184–1192.

14. Durrant, W.E.; Dong, X. Systemic acquired resistance. Annu. Rev. Phytopathol. 2004, 42, 185–209. [CrossRef]

15. Eyles, A.; Bonello, P.; Ganley, R.; Mohammed, C. Induced resistance to pests and pathogens in trees. New Phytol. 2010, 185, 893–898. [CrossRef] [PubMed]

16. Klessig, D.F.; Choi, H.W.; Dempsey, D.M.A. Systemic Acquired Resistance and Salicylic Acid: Past, Present, and Future. Mol Plant Microbe Interact 2018, 9, 871–888. [CrossRef] [PubMed]

17. Friedrich, L.; Lawton, K.; Russ, W.; Masner, P.; Specker, N.; Gut Rella, M.; Meier, B.; Dincher, S.; Staub, T.; Uknes, S.; et al. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J. 1996, 10, 61–70. [CrossRef]

18. Smiglak, M.; Kwakwa, R.; Lewandowski, P.; Pospieszny, H. Cationic derivatives of the plant resistance inducer benzo[1,2,3]thiadiazole-7-carbothioic Acid S-methyl Ester. Tetrahedron Lett. 2014, 55, 3565–3568. [CrossRef]

19. Kwakwa, R.; Czerwoniec, P.; Lewandowski, P.; Pospieszny, H.; Smiglak, M. New ionoic liquids based on systemic acquired resistance inducers combined with the phytotoxicity reducing cholinum cation. New J. Chem. 2018, 42, 11984–11990. [CrossRef]

20. Feder-Kubis, J.; Czerwoniec, P.; Lewandowski, P.; Pospieszny, H.; Smiglak, M. Ionic Liquids with Natural Origin Component: A Path to New Plant Protection Products. ACS Sustain. Chem. Eng. 2020, 8, 842–852. [CrossRef]

21. Zajac, A.; Kwakwa, R.; Pawlowska-Zygarowicz, A.; Stolarska, O.; Smiglak, M. Ionic liquids as bioactive chemical tools for use in agriculture and the preservation of agricultural products. Green Chem. 2020, 20, 4764–4789. [CrossRef]

22. Smiglak, M.; Kwakwa, R.; Lewandowski, P.; Budziszewska, M.; Obrębska-Stepińska, A.; Krawczyk, K.; Zwołinska, A.; Pospieszny, H. New Dual Functional Salts Based on Cationic Derivative of Plant Resistance Inducer Benzo[1,2,3]thiadiazole-7-carbothioic Acid, S-Methyl Ester. ACS Sustain. Chem. Eng. 2016, 4, 3344–3351. [CrossRef]

23. Smiglak, M.; Lewandowski, P.; Kwakwa, R.; Budziszewska, M.; Krawczyk, K.; Obrębska-Stepińska, A.; Pospieszny, H. Dual functional salts of benzo[1,2,3]thiadiazole-7-carboxylates as a highly efficient weapon against viral plant diseases. ACS Sustain. Chem. Eng. 2017, 5, 4197–4204. [CrossRef]
24. Markiewicz, M.; Lewandowski, P.; Spychalski, M.; Kukawka, R.; Feder-Kubis, J.; Beil, S.; Smiglak, M.; Stolte, S. New bifunctional ionic liquid-based plant systemic acquired resistance (SAR) inducers with improved environmental hazard profile. *Green Chem.* 2021, 23, 5138–5149. [CrossRef]

25. Spychalski, M.; Kukawka, R.; Krzesiński, W.; Śpiżewski, T.; Michalecka, M.; Poniatowska, A.; Puławska, J.; Mieszczakowska-Frać, M.; Panasiewicz, K.; Kocira, A.; et al. Use of New BTH Derivative as Supplement or Substitute of Standard Fungicidal Program in Strawberry Cultivation. *Agronomy* 2021, 11, 1031. [CrossRef]

26. Smiglak, M.; Pospieszny, H.; Kukawka, R.; Lewandowski, P.; Stolarska, O.; Maciejewski, H. Application of 7-Carboxybenzo(1,2,3)Thiadiazole Amides as Plant Stimulants. Patent Application No. WO/2017/017626, 2 February 2017.

27. Frackowiak, P.; Pospieszny, H.; Smiglak, M.; Obrepska-Steplowska, A. Assessment of the Efficacy and Mode of Action of Benzo(1,2,3)-Thiadiazole-7-Carbothioic Acid S-Methyl Ester (BTH) and Its Derivatives in Plant Protection Against Viral Disease. *Int. J. Mol. Sci.* 2019, 20, 1598. [CrossRef]

28. Dal Maso, E.; Cocking, J.; Montecchio, L. Efficacy tests on commercial fungicides against ash dieback in vitro and by trunk injection. *Urban For. Urban Green.* 2014, 13, 697–703. [CrossRef]

29. Keča, N.; Tkaczyk, M.; Žolciak, A.; Stocki, M.; Kalaji, H.M.; Nowakowska, J.A.; Oszako, T. Survival of European Ash Seedlings Treated with Phosphite after Infection with the *Hymenoscyphus fraxineus* and *Phytophthora* Species. *Forests* 2018, 9, 442. [CrossRef]

30. Madigan, A.; Bełka, M.; Taylor, A.F.S.; Krisits, T.; Cleary, M.; Nguyen, D.; Elfstrand, M.; Woodward, S. Can *Hymenoscyphus fraxineus* infect hardy members of the Oleaceae other than ash species? *For. Path.* 2015, 45, 426–429. [CrossRef]

31. Kjaer, E.D.; McKinney, L.V.; Nielsen, L.R.; Hansen, L.N.; Hansen, J.K. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evol. Appl.* 2012, 5, 219–228. [CrossRef]

32. Cooke, L.; Fleming, C.; McCracken, A. Sustainable Agri-Food Sciences Division, AFBI, 2013. DARD E&I Project 12/3/S7: Efficacy of Biocides, Disinfectants and Other Treatments to Limit the Spread of Ash Dieback Caused by *Chalara fraxinea*. AgriFood and Biosciences Institute. Available online: www.afbini.gov.uk (accessed on 30 March 2021).

33. Turczański, K.; Rutkowski, P.; Dyderski, M.K.; Wrońska-Pilarek, D.; Nowiński, M. Soil pH and organic matter content affects European ash (*Fraxinus excelsior*) crown defoliation and its impact on understory vegetation. *Forests* 2020, 11, 22. [CrossRef]

34. Hauptman, T.; Piskur, B.; de Groot, M.; Ogris, N.; Wrońska-Pilarek, D.; Nowiński, M. Soil pH and organic matter content affects European ash (*Fraxinus excelsior*) crown defoliation and its impact on understory vegetation. *Forests* 2020, 11, 22. [CrossRef]

35. Przybylski, P.; Sikora, K.; Mohytyh, V.; Włostowski, M. Effect of agrotechnical treatment on the health condition of the clonal seed ash plantation (*Fraxinus excelsior* L.) in the context of its infection by *Hymenoscyphus fraxineus* (T. Kowalski). *Sylwan* 2020, 164, 404–413. [CrossRef]

36. Tkaczyk, M.; Nowakowska, J.A.; Oszako, T. *Phytophthora* species isolated from ash stands in Białowieża Forest nature reserve. *For. Pathol.* 2016, 46, 660–662. [CrossRef]