*forests*

*Review*

# *Verticillium* Species as an Ecofriendly Alternative to Manage the Invasive Tree *Ailanthus altissima* (Mill.) Swingle

**Claudia Pisuttu**

Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy; claudia.pisuttu@agr.unipi.it; Tel.: +39-050-2210561

**Abstract:** Environmental pollution, unintended harm to beneficial organisms, and the development of herbicide resistance among weeds are the main consequences of the massive and consistent use of chemical herbicides in recent decades. The growing need for alternative solutions has been reinforced by restrictive policies, leading to a search for natural herbicidal candidates. Mycoherbicides, formulations containing plant pathogenic fungi, are viewed as promising substitutes for chemical herbicides. In the case of *Ailanthus altissima* (Mill.) Swingle, one of the worst invasive alien tree species in the world, *Verticillium*-based mycoherbicides offer a viable method for control, inducing a lethal wilt disease and leading plants to death within a few years. The demonstrated significant effectiveness enables addressing challenges posed by other—conventional—approaches. The current analysis matches key internal (strengths and weaknesses) and external factors (opportunities and threats) of *Verticillium* Nees isolates as environmentally-friendly control agents against the invasive *A. altissima*, by listing each singularly and then crossing them among the categories, drawing from the collaborative efforts of American, Austrian, and Italian research teams.

**Keywords:** agrochemicals; (bio)herbicide; Tree of Heaven; IAPS; vascular disease

check for updates

**Citation:** Pisuttu, C. *Verticillium* Species as an Ecofriendly Alternative to Manage the Invasive Tree *Ailanthus altissima* (Mill.) Swingle. *Forests* **2024**, *15*, 462. https://doi.org/10.3390/f15030462

Academic Editors: Luigi De Bellis, Genuario Belmonte, Massimiliano Renna, Elena Ciani, Monica Marilena Miazzi and Andrea Pieroni

Received: 1 February 2024
Revised: 27 February 2024
Accepted: 28 February 2024
Published: 1 March 2024



## 1. Introduction

Invasive alien plant species (IAPS) are a major threat to biodiversity loss [1] and their presence may also be a serious problem in agriculture. They commonly induce relevant crop losses by decreasing yields, competing for nutrients, water and sunlight, interfering with plant physiology and harvest operations, lowering product quality, and being potential vectors of noxious organisms, thus acting as an important biotic constraint to food production [2]. They are part of plants commonly called "weeds", of which the most appropriate definition is "plants that are objectionable or interfere with the activities or welfare of man" (https://wssa.net/wssa/wssa-glossary/, accessed on 18 December 2023), representing a critical factor in agricultural, rural and urban areas, and issues at archeological sites [3], trails and transportation corridors [4]. Also considering the role of climate change in inducing significant transformations in the weed flora of ecosystems (e.g., unbalanced presence of certain species as thermophile, late-emerging and opportunistic weeds) [5], weed management is one of the major challenges in crop production and has been of primary concern since ancient times, when humans undertook agricultural practices [6]. The European Commission is indeed increasingly asking for a consistent reduction in the use of conventional agrochemicals, as stated by the "Farm to Fork" strategy [7], also led by the recent upsurge in environmental awareness of the public interest in organic food production, as well as in some specific issues related to the use of herbicides [8,9], consequently biological control is one of the favorable management practices to limit IAPS diffusion.

The biological control of IAPS may be defined as the intentional use of a natural enemy or a complex of natural enemies—"biological control agents"—to bring about weed suppression or to limit weed abundance as a consequence of a reduction in their reproduction, competitive ability, and growth rate [10]. These agents can be insects and mites (phytophagous arthropods), fungi, oomycetes, bacteria and viruses (plant pathogens), fishes, birds, and mammals (e.g., grass carp, geese and sheep, respectively). However, generally speaking, the science of weed control using biological agents is still at the beginning. Nature-based eco-friendly potential regulators of weed species may represent a solution to limit the employment of chemical herbicides, but even though extensive research has been conducted on weed biocontrol, few products with such characteristics have actually been launched on the market.

Biofungicides and bioinsecticides have showed much higher commercial success [11]. Conversely, in the literature, microbial bioherbicides are rarely cited among the biopesticides [12]. Unlike classical biological control, bioherbicides use formulations of plant pathogens that are manipulated to produce large amounts of infectious material, which is then delivered ("augmentative biological control") to provide a temporary/permanent boost to the background level of natural enemies of the target weed. This material is commonly inoculated into the target organism, sprayed or applied as solid granules [13]. Specifically, mycoherbicides are formulations containing plant pathogenic fungi that are applied directly to target weeds. They are mainly based on living fungi, some of them producing host-specific phytotoxic secondary metabolites (i.e., phytotoxins), but no microbial molecule-based product is on the market thus far. This could be due to industrial production difficulties, formulation process, ecological fitness, duration of herbicidal effects, assessment of potential non-target effects and costly and time-consuming registration procedures. Indeed, a good bioherbicide should possess specific requirements and should have favorable environmental profiles, expecting to (i) reduce the risk of herbicide resistance among weeds thanks to new modes of action, (ii) be biodegradable, (iii) have a broad spectrum of action, and (iv) be usable in both conventional and organic farming [14].

The concept of mycoherbicide has roots that extend almost as far back as the field of the plant pathology itself, as documented by the observations of the impact of diseases on weeds dating back to the 19th century [15]. A milestone in this field dates back to the 1950s, when Russian scientists mass-produced and formulated the spores of *Alternaria cuscutacidae* Rudakov to control holoparasite *Cuscuta* species [16]. Although more than half a century had passed since the first mycoherbicide was registered, mycoherbicides have been used operationally only since the 1980s [17]. There are several noteworthy genera of fungi that have been proved to be effective against weeds in experimental conditions, and numerous products are in various stages of development throughout the world. A number of scientific and educational articles have been regularly published for several years with the aim to propose potential candidates or the so-called innovative strategies to control weeds belonging to various botanical families and affecting different crops. The analysis of fungus–plant interactions provides a fundamental source for bioherbicides assessment, posing the basis for the application of appropriate cropping systems for environmentally-friendly and sustainable weed control [18].

Key features of a "perfect" mycoherbicide are host specificity, non-target crop tolerance, efficacy (possibly it should kill the plant), environmental fate, ecological adaptation, mode of action and toxicology. In the present paper, we describe the potential of a few fungal strains to counteract the invasive tree *Ailanthus altissima* (Mill.) Swingle.

Specifically, this review aims to (i) describe the specific *Verticillium* Nees species/*A. altissima* challenge, considering the three main worldwide cases, and in the light of this first analysis, and (ii) highlight the strengths, weaknesses, opportunities and threats (SWOT) of the application of *Verticillium*-based products as biological control agents for the invasive tree.

## 2. Methodology

### *2.1. Database*

A database reporting the published literature on *Verticillium* species as potential biological control agents for *A. altissima* was created by examining the published peer-reviewed literature, searched in the Web of Science (Thompson-ISI, Philadelphia, PA, USA, http://apps.webofknowledge.com/, accessed on 13 January 2024) and Scopus (Elsevier, Amsterdam, Netherlands, http://www.scopus.com/, accessed on 14 January 2024) databases, using "*Ailanthus altissima*" and "*Verticillium*" as keywords. Database search was updated to January 2024 and performed without restrictions on publication year. The reference lists of any article identified by this literature search were cross-checked in order to include any other relevant reference, finally identifying around 35 relevant documents. Articles and their data were excluded if (i) they treated the topic approximatively and (ii) the topic was only cited in relation to another focus (e.g., insects commonly affecting *A. altissima* as potential vectors). In total, 25 studies were included in the review that met the proper criteria.

### *2.2. Strengths, Weaknesses, Opportunities and Threats Analysis*

SWOT analysis is one of the oldest and most widely adopted strategy tools worldwide [19], aimed at planning process that focuses on the four elements of the acronym, enabling identifying the forces influencing a strategy, action or initiative helping overcome challenges and determine what new leads to pursue [20]. The purpose of using SWOT is to identify the internal and external factors that are favorable and unfavorable to achieve the objectives of the venture or project. More in detail, strengths and weaknesses refer to internal factors, which are the resources and experience readily available; external factors are typically uncontrollable items, such as opportunities and threats. The SWOT matrix matches key internal and external factors by listing each singularly and then crossing them [21]. For the two macro-categories, the following questions were posed:

- Strengths and Weaknesses (internal factors)
    - Are *Verticillium* species effective in killing plants?
    - Which is the risk to release chemical residuals in the environment?
    - Which is the possibility that the tree becomes resistant to the pathogen?
    - Are *Verticillium*-based products applicable as a stable formulation?
    - Has the application of *Verticillium* species non-target effects?
- Opportunities and Threats (external factors)
    - Does the product match criteria for biological or sustainable management?
    - Are there limits for its application?
    - Which is the marketability of the product?
    - Is the formulation and inoculation protocol suitable for a wide use?
    - Is the formulation approved by national/international agencies?

Basically, according to Davis [21], eight steps have been involved during the analysis of *Verticillium*-based products: (1) list the key external opportunities, (2) list the key external threats, (3) list the key internal strengths, (4) list the key internal weaknesses, (5) match internal strengths with external opportunities and record the resultant strategies, (6) match internal weaknesses with external opportunities and record the resultant strategies, (7) match internal strengths with external threats and record the resultant strategies, and (8) match internal weaknesses with external threats and record the resultant strategies.

## **3. Results**

### *3.1. Potential Mycoherbicides for Ailanthus altissima*

#### 3.1.1. The Target

*Ailanthus altissima* is a deciduous tree in the family Simaroubaceae, native to China and commonly known as the "Tree of Heaven". Since its accidentally introduction to Europe and United States as ornamental species in the middle of the 18th century, *A. altissima* has now reached a subcosmopolitan distribution and colonized every continent except Antarctica [22,23]. Being a competitive invader, it causes relevant economic and ecological constrains due to several growth-promoting features: huge asexual reproduction, strong vegetative sprouting and formation of suckers, phenotypic plasticity, prolific production of wind dispersed seeds, extreme tolerance to unavoidable environmental conditions, ability to produce allelochemicals (e.g., quassinoids) that prevent the establishment of other plant species nearby, and low palatability for grazers [22,24]. It also may pose health concerns, causing allergenic reactions in some people and skin irritation.

Once established, *A. altissima* can grow over native vegetation by developing dense thickets of clonal trees. It can dominate colonized sites through indefinitely resprouting and root suckering. In the archeological sites (Figure 1) and more in general in the built environment, *A. altissima* poses hazards to the stability and conservation of the structures, represents visual obstacles, modifies the natural landscape, breaks/deforms walls, pavements and paths, and creates difficulties of all kinds, drawing attention to the need for continuous maintenance and removal [25,26]. For all these reasons, it is one of the worst IAPS worldwide, and it is included in the list of Invasive Alien Species of European Union (EU) concern [27] since 2019. Being subjected to several restrictions (i.e., keeping, importing, selling, breeding and growing), all EU Member States must address action on pathways of unintentional introduction and measures for the early detection and rapid eradication of these species, as well as to manage those that are already widely spread in their territory.

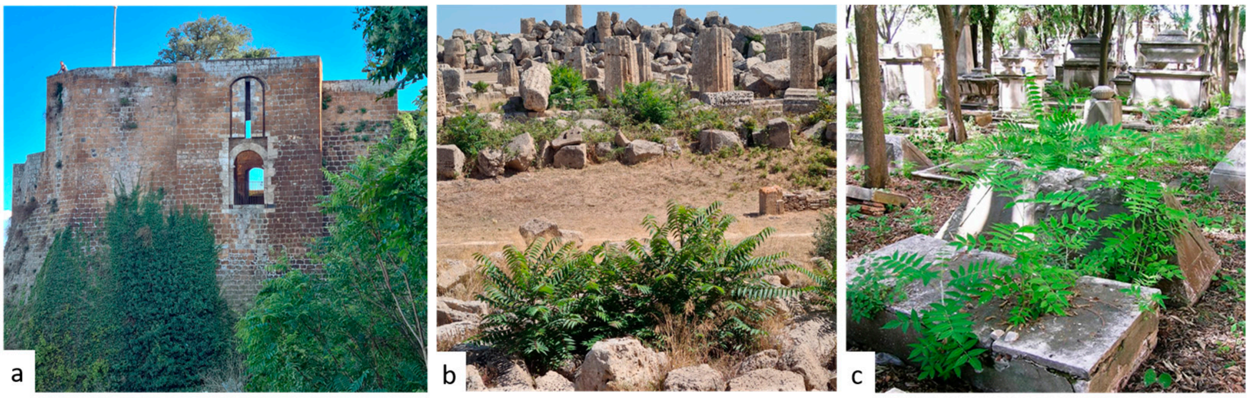


**Figure 1.** *Ailanthus altissima* colonization in Italian historical sites: (**a**) Albornoz fortress, Orvieto; (**b**) ancient Greek archaeological site, Selinunte; (**c**) English cemetery, Leghorn. Photo by Prof. Cristina Nali.

Elimination of *A. altissima* is not an easy task and requires diligence, especially due to its prompt and tremendous vegetative reproduction. Some studies pointed to describe the resistance/tolerance of *A. altissima* to several abiotic stresses, especially drought [28], and elucidate how this kind of species is important in the context of climate change in substituting those more sensitive, as for example in declining forests [29]. Conversely, many other studies focused on the fact that, in recent decades, *A. altissima* started to be affected by deteriorations and diseases. Despite the high resistance to sulfur dioxide and other major air pollutants [30,31], *A. altissima* is considered one of the most ozone-sensitive trees in southern Europe [32–34]. Gravano et al. [35] described the onset of symptoms consisting in whitish (ivory) stipples on the adaxial surface that turn quickly into brown necrotic spots after the exposure. Moreover, detrimental effects of sea aerosol on *A. altissima* were observed along the coast of Elba island, in Tuscany (Figure 2).

*Verticillium* (Ascomycota, Plectosphaerellaceae) is a well-characterized and well-studied genus of soil-borne fungal pathogens occurring worldwide [36]. Interestingly, some *A. altissima* decays caused by *Verticillium* species have been reported in recent years in several areas of United States and in Europe, so proposing biological control as a possible strategy to successfully counteract the apparently irrepressible spread of this invasive species. Additionally, recent studies have been focused on cases of *A. altissima* damages caused by *Aculus mosoniensis* (Ripka) (Acari: Eriophyidae), which is endemic in most European regions. This mite seems to be a pest only of the Tree of Heaven and no injuries have been observed on non-target species, leading to further studies to take in consideration also this organism as a potential candidate for biological control [37,38].

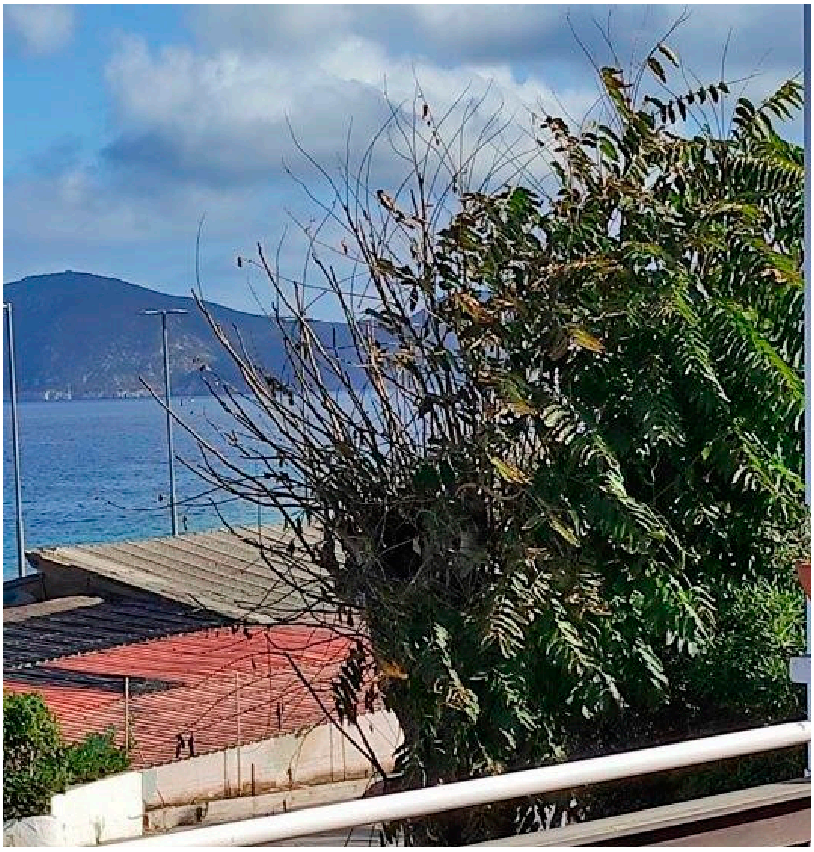

**Figure 2.** *Ailanthus altissima* grown on a village on the coast of Elba island (Tuscany, Italy) affected by sea aerosol. Photo by Prof. Giacomo Lorenzini, October 2021.

3.1.2. *Verticillium nonalfalfae*: United States and Austria

A research article published in 2009, reported the (extraordinary) *A. altissima* dieback event observed since 2002, affecting in the years more than 8000 trees diffused in the mixed hardwood (mainly oak-dominated) forests of south-central Pennsylvania [39]. From those areas in which the mortality was extremely high, the soilborne fungus recognized as *V. alboatrum* Reinke and Berthold was isolated, lately identified as the newborn *V. nonalfalfae* Inderb., while *V. dahliae* Kleb. was only isolated from a few infected trees [40]. The species was subsequently detected in the nearby states as Virginia [41,42] and Ohio [43].

*Verticillium nonalfalfae* is morphologically indistinguishable from *V. alfalfae* Inderb. and *V. alboatrum*. It develops slower on solid substrates in comparison to other species and the mycelium generally is abundant, floccose to pruinose, appearing white at first, later darkening due to the formation of the resting mycelium immersed in the agar. This last consists of brown pigmented hyphae, up to 9 mm wide and thick-walled. Conidia are hyaline and smooth-walled, cylindrical with rounded apices to oval, extremely small ($6 \pm 1.0 \times 3 \pm 0.5$ μm) [39]. Common hosts of *V. nonalfalfae* are cotton, hop, petunia, potato, spinach, tomato and wild celery and it is often reported in temperate climates (Belgium, Canada, China, Japan, Middle Asia, Netherlands, Slovenia, United States and United Kingdom) [44]. The isolated strains showed a greater aggressiveness, rapidity of infection and effectiveness [45–47], and due to the short life-span of resting mycelium and a rapid host mortality, the opportunities to infect susceptible hosts are very low [48]. Protocols for an easy and effective inoculation were developed and studies to improve the diffusion from diseased plants to healthy ones in inoculated stands were performed, considering that *V. nonalfalfae* can be transmitted through intraspecific root graphs [49–51].

In Austria, scientists studied a similar dieback related to *V. dahliae* and *V. nonalfalfae* [52]. The first survey was conducted in eastern and southern Austria in 2011 and identified more than 20 sites of *A. altissima* potentially affected by *Verticillium* wilt, showing foliar wilt symptoms, yellowish vascular discoloration, epicormic shoots on the stem, dieback, and premature death in certain cases. Similar symptoms on *A. altissima* were previously reported by Cech [53] in southern Styria in autumn 1997, attributed to *Verticillium* spp. and other fungi causing bark canker. From two of the 22 sites (Bad Radkersburg, province Styria, and Gänserndorf, province Lower Austria), *V. nonalfalfae* was isolated, while *V. dahliae* was occasionally found. Considering the high effectiveness of the *V. nonalfalfae* isolate Vert56 strain G1/5 and the development of a rapid inoculation protocol through the injection of a concentrated spore solution ($1 \times 10^8$ conidia mL$^{-1}$) [54,55], a bioherbicide based on this strain has been temporarily approved for use in Austria (for a maximum of 120-days; last period-use was from 15 April to 13 October 2023) under Emergency Authorization according to article 53 of Regulation (EU) No 1107/2009 [56]. The manufacturing protocol indicates to apply a single injection of a few mL of the concentrated formulation and to use it within 2 months. Pest risk assessment analyses performed on several plants potentially susceptible to *Verticillium* species revealed none or only a very limited impact on non-target tree species [57].

#### 3.1.3. *Verticillium dahliae*: Italy

Pisuttu et al. [58] reported and investigated some cases in Tuscany, finding the causal agent of *A. altissima* decay in *V. dahliae* strain VdGL16, after having fulfilled the four criteria of Koch's postulates. Pest risk assessment conducted on *V. dahliae* strain VdGL16 enabled identifying no more than eight herbaceous species potentially susceptible in controlled conditions. *Verticillium dahliae* can be easily and rapidly grown on agar medium (e.g., on potato dextrose agar it needs 15 days) and conidia (Figure 3) can be resuspended in sucrose-based broth, being stable and vital for a few days.

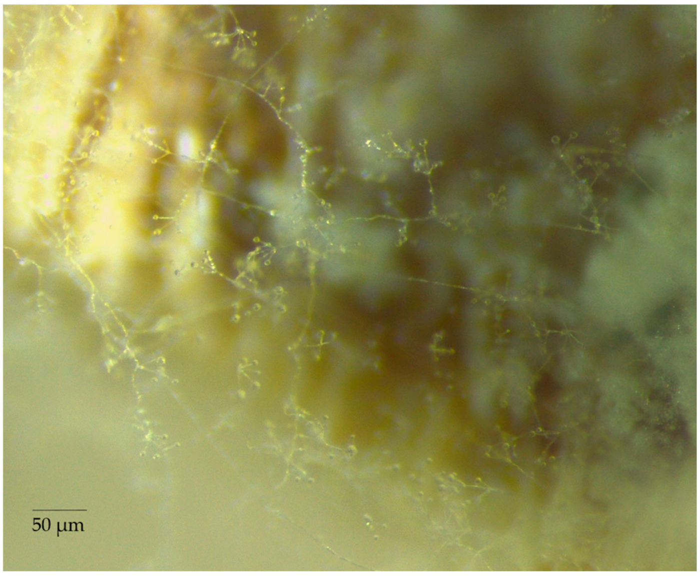


**Figure 3.** Conidiophores of *Verticillium dahliae* observed under a stereomicroscope.

Spore suspensions, prepared at high concentration ($1 \times 10^7$ conidia mL$^{-1}$) were used for inoculation protocols tested in controlled environment through typical root-deep inoculation on *A. altissima* seedlings and stem-inoculation on pot-grown mature trees [59] (Figure 4), in urban environment [60] and open field (data unpublished). In general, macroscopic responses are those characteristic of a wilt disease, including defoliation, abundant production of epicormic sprouts along the stem and vigorous sprouting.

**Figure 4.** *Ailanthus altissima* trees grown in pot and stem-inoculated with *Verticillium dahliae* strain VdGL16. Photo by Dr. Claudia Pisuttu.

Leaves wilt, turn yellow and fall in a short time after inoculation, even if the visual detection of the disease is difficultly recognizable since the absence of pathognomonic symptoms [59]. In cross sections of branches, a continuous discolored ring or arc is present in the outermost sapwood. The first symptom (i.e., yellowing) can occur already after two weeks post inoculation (wpi), preceding the decline of physiological parameters. After 4 wpi, stomatal and mesophyll limitations induced the fall of net photosynthesis. Water status imbalance occurred irreversibly after the occlusion of xylem vessels due to (i) physical blockage by the pathogen itself and (ii) host defense responses that are aimed at vessel plugging, such as gums and tyloses [59,60]. Osmolytes (i.e., abscisic acid and proline) accumulated as effect of *V. dahliae* in view of the redistribution of solutes in plant cells and the limited translocation of micro- and macronutrient translocation, as confirmed by the decrease in soluble sugars and ions imbalance. Overall, the primary metabolism in *V. dahliae*-inoculated plants is strongly delayed by the disease in less than 10 weeks. Despite the onset of water stress symptoms-like, they did not set-in motion the series of physiological events and osmotic adjustments analogous to those occurring in drought-resistant plants and already during the first vegetative season of inoculation, *V. dahliae*-infected *A. altissima* individuals are not able to avoid the premature senescence. Trees may die from 1 to 3 years depending on their age. Despite the high genetic variability existing in European and Italian *A. altissima* population, as typical of invasive species subjected to high rates of gene exchange due to the continuous multiple propagules introduction and constant movement, *A. altissima* plants showed a very high susceptibility to *V. dahliae*, independently to the provenances [61]. Consequently, other factors as environmental ones (e.g., changing temperatures, humidity, drought or rainfall, soils and nutrients, air pollutants, insects, nematodes in addition to other pathogens) at which trees or the pathogen itself could be exposed, should be considered compromising the success of *V. dahliae* inoculation [62]. Zero non-target effects were observed until now during the different experimental activities in uncontrolled conditions. However, considering the risks related to the pathogen diffusion and the possibility that the fungus, producing resisting structures called microsclerotia, remains vital in the soil or plant residues for several years (more than 400 known host plants of *Verticillium* species are reported) several tests must be conducted taking into account all possible favorable scenarios for *Verticillium* infection in susceptible hosts [36,44].

Differently to the other European case, the use of *V. dahliae* strain VdGL16 is permitted in a few areas of Tuscany Region and only for research purposes. Inoculation protocols and new formulations are currently under evaluation to make the product expendable to wider use.

### 3.2. SWOT Analysis of Verticillium-Based Mycoherbicides

The three research lines addressed similar objectives and deposited the genome sequence of the main strain used in each country as biological control agents [63–65]. The abundant useful information available concerning the three principle experiences (the American, the Austrian and the Italian one) now involved in attempts to develop a mycoherbicide against *A. altissima* enables drafting a preliminary SWOT matrix (Table 1).

**Table 1.** Summary of the factors characterizing strengths, weaknesses, opportunities and threats (SWOT) of *Verticillium*-based products as biological control agents for the invasive tree *Ailanthus altissima*. A green and orange scale has been attributed for positive (strengths and opportunities) and negative (weaknesses and threats) categories, respectively. Dark colors indicate solid outcomes, while lighter ones refer to less strong responses.

| | | Strengths | Weaknesses | Opportunities | Threats |
|---|---|---|---|---|---|
| *Are* Verticillium *species effective in killing plants?* | Yes | | | | |
| *Which is the risk to release chemical residuals in the environment?* | Zero | | | | |
| *Which is the possibility that the tree becomes resistant to the pathogen?* | Low | | | | |
| *Are* Verticillium*-based products applicable as a stable formulation?* | Yes | | | | |
| *Has the application of* Verticillium *species non-target effects?* | Yes | | | | |
| *Does the product match criteria for biological or sustainable management?* | Yes | | | | |
| *Are there limits for its application?* | Yes | | | | |
| *Which is the marketability of the product?* | Limited | | | | |
| *Is the formulation and inoculation protocol suitable for a wide use?* | Partially | | | | |
| *Is the formulation approved by national/international agencies?* | Partially | | | | |

#### 3.2.1. Strengths

In contrast to traditional chemical herbicides, the evaluated fungal strains present a significantly reduced, nearly eliminated, risk of human and animal toxicity. Concerns related to the presence of chemical residues and pre-harvest intervals for treatments on edible plants are irrelevant in this case, since *Verticillium*-based products are mainly prepared by suspending spores in sucrose broths. This eco-friendly and trendy approach enjoys widespread acceptance among the general public and decision makers. There is no risk of developing acquired resistance to these fungal strains. Additionally, they exhibit a prolonged efficacy, affecting not only above-ground vegetation but also the root system. With the capability to colonize clonal populations from a single inoculation, these strains do not require specialized expertise for use, making them easily learnable. Furthermore, precision application on a plant-to-plant basis ensures no drift. While a comprehensive cost analysis is yet to be available in the literature, a laboratory assessment suggests lower economical and time-consuming costs compared to conventional chemical herbicides.

### 3.2.2. Weaknesses

*Verticillium* species are not totally target specific. They show great genetic plasticity and some of them are able to infect more than 400 plant species, including high-value crops (e.g., cotton, potato and tomato) as well as landscape, fruit, and ornamental trees and shrubs [66,67]. Specifically, in the case of *V. dahliae*, the list of the hosts infected is continuously expanding as disease outbreaks on new hosts are identified [36,68], even if it has been observed that some isolates of *V. dahliae* are rather specialized, such as some from peppermint, pepper, strawberry [69], included the target species *A. altissima* [60]. Additionally, it is important to consider that the artificial stem inoculations bypass root defenses (which are crucial in plant defense against pathogens in nature) and flood the xylem with millions of conidia (which is unreasonable in nature). This feature may be regarded as a critical factor in forest and agricultural contexts, especially taking into account the potential development of endemic insect vectors or the diffusion of exogenous ones, as in the case of ambrosia beetles [49].

### 3.2.3. Opportunities

The use of *Verticillium* species could be suitable for certified organic agriculture and public spaces where chemical usage is restricted (e.g., parks, urban areas, and protected environment). This method proves to be an ideal option, particularly on tough surfaces like industrial areas, archaeological sites, and railway lines, where *A. altissima* is particularly dangerous, mining the stability and conservation of manufacts. Indeed, *Verticillium*-based products are advantageous in situations where the consideration of crop tolerance is not a factor in selecting an effective weed control method, as highlighted by Rask and Kristoffersen [70].

### 3.2.4. Threats

Navigating regulatory changes for the registration of bioherbicides proves to be long and resource-intensive process, encompassing considerations of mass production, time to market, and the protection of intellectual property. These challenges are particularly notable, given the primary focus on niches and limited markets. The formulation demonstrates excellent stability from shelf to field, coupled with a considerable shelf life, making it suitable for industrial manufacturing and incorporation into established sales networks.

## 4. Discussion

The invasiveness of *A. altissima* is an unacceptable issue for biodiversity, traffic and industrial safety, safe fruition of green areas, manufact safety and many other daily human activities. As a perfect opportunistic invader, this tree has occupied huge spaces and its spread seems unstoppable. Its environmental, social and economic impact is no more sustainable. Commonly, weeds are managed by manual weed uprooting, cultural practices as crop rotation designs and cover crops [71], the use of low-tech mechanical devices (cultivators and finger and brush weeders), many physical methods including direct flaming and steaming, not to mention innovative approaches (e.g., laser radiations, microwaving, electrocution, robotics and optical sensors) [2]. However, all these practices are not so easily considerable for *A. altissima*. Infestations of (very) small plants can be pulled, dug, cut, or mowed, possibly when the ground is moist. Removal of the entire root apparatus is necessary for control of individual trees, but because none of these methods usually removes the entire tree (including roots), they must be repeated until carbon reserves are exhausted and the plant dies. Mechanical control is generally not the best choice when dealing with larger trees. If the top is removed or the stump is cut, new sprouts from lateral roots may occur up to 30 m from the parent tree renewal [72]. Girdling involves manual cutting away bark and cambial tissues around the trunks of undesirable trees. This is a relatively inexpensive method and is performed in the spring when the trees are actively growing. However, hardwoods are known to resprout below the girdle unless the cut is treated with herbicides, and, in the same way, *A. altissima* actively resprouts after heat-girdling spot treatment. These actions must be accompanied by the periodical use of systemic (and non-selective) herbicides that can be transported to the root system and compromise (but usually only partly) the root and future vegetative renewal even if sprouting may occur and require one or more follow-up treatments [68]. Failure to perform follow-up monitoring and treatment could result in a return to pretreatment density levels (and sometimes even worst) [73]. Actually, chemical herbicides are often regarded as the most effective way to kill the root system of mature *A. altissima* and to control regrowth from cut trees that occurs as sprouts or root suckers. There are several registered products that can be applied in a variety of ways including (i) foliage application, (ii) topical application to cut stems and stumps, (iii) injection into the trunk, and (iv) basal spraying. However, recent regulatory measures have limited the application of chemicals. For instance, the Tuscany Region [74] has forbidden all non-agricultural uses of crop protection agents based on glyphosate, the universally acknowledged active ingredient indicated as the most suitable for such weed killer treatments. On the other hand, the National (Italian) Action Plan for the sustainable use of plant protection products [75] is leading to serious limitations in the use of chemical pesticides along roads (Action A.5.5) and in population centers (Action A.5.6). More specifically, Point A.5.6.1 (Use of herbicide products) says that "weed-killer treatments are banned and have to be replaced with alternative methods in population centers... in the case of exemptions plant-protection products cannot be used if the label bears the following expressions of risk R41 (i.e., risk of severe eye lesions, precisely the one referring to glyphosate)" [76]. On 28 November 2023, the European Commission with the Regulation (EU) 2023/2660 renewed the approval for 10 years of the active substance glyphosate, in accordance with Regulation (EC) No 1107/2009 [56] of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) No 540/2011 [77]. Specifically, paragraph 26 promotes the development and introduction of more sustainable and alternative products to reduce the use of pesticides, while paragraph 28 draws attention to the limited use of glyphosate in public areas.

The potential of *Verticillium* species as biocontrol agents to counteract the highly invasive *A. altissima* might deserve attention, given the need for effective and affordable non-chemical control strategies, fulfilling the criteria to be considered an ecofriendly and biological product.

In temperate climates, *V. nonalfalfae* has a greater aggressiveness, rapidity of infection and effectiveness compared to *V. dahliae* [45–47], and due to the short life-span of resting mycelium and a rapid host mortality, there may be less opportunities to infect other susceptible hosts [48,52,54]. However, differences in detection frequency between the widely distributed *V. dahliae* and the rarely occurring *V. nonalfalfae* might explain the fact that this last has not been yet detected on *A. altissima* in Italy, leading to consider its inability to grow in warmer climates and, as a consequence, *V. dahliae* the only endemic deadly pest in the Mediterranean basin.

Although traditional SWOT is sometimes considered a very subjective analysis in the determination of factors and usually descriptive in terms of high-level perceptions [78], in this specific work, it highlighted some topics to deal with.

Concerning the weaknesses, the host range studies of the American and Austrian group for *V. nonalfalfae* reported limited susceptible non-target species, comprising ten woody species (among which *Aralia spinose* L. and *Acer pensylvanicum* L.; in field), a few horticultural (among which *Spinacia oleracea* L. and *Cucumis sativus* L.) and floral species [*Petunia* × hybrid (Hooker) Vilmorin] [51,53,79,80]. Additionally, even if plants were not negatively affected by the disease, the pathogen was reisolated from artificial inoculated hop, actually classified as one of the main host of *V. nonalfalfae*, with important economic consequences for brewery companies. A few Leguminosae, Asteraceae and Solanaceae species were susceptible (in controlled environment) to *V. dahliae* VdGL16 [58].

All these results indicate that the use of *Verticillium*-based products should take into account all safety precautions when the application occurs in proximity of potential susceptible plants. These factors can also have an impact on the threats identified, since they may slow the processes to recognize *Verticillium*-based products at the regulative level. To overcome these issues, guidelines for use with appropriated indications of safe spaces in which the biological control agent can be applied without risks could be provided. For instance, industrial and archeological areas, and also certain railways traits, can be suitable for *Verticillium* species application, since here the presence of *A. altissima* often creates mono-specific plant stands and potential hosts are absent or particularly far.

Additionally, independently to the efficacy, the development of a stable and durable formulation over time may also enhance the opportunities, popularity and marketability of the mycoherbicide, being the more favorable easy-to-use products approved by different stakeholders.

## 5. Conclusions

Now, biological control performed by a naturally occurring killing fungal wilt pathogen such as *Verticillium* seems to represent the best opportunity to counteract the Tree of Heaven, especially considering the abovementioned limitations on the use of chemical and physical approaches. Current limiting factors represented by technological implications and registration hurdles should still be overcome. Certainly, some ten years of research are required to piece together an authorization file and to obtain marketing authorization, whether for biological or chemical products [81]. Another possible limiting factor may be the limited market potential. However, the next steps in this research aim to conduct an active communicative campaign to promote (to the different stakeholders) the roles and advantages of *Verticillium* species.

**Funding:** This research received no external funding.

**Data Availability Statement:** No new data were created or analyzed in this study. Data sharing is not applicable to this article.

**Acknowledgments:** Thanks are due to Giacomo Lorenzini and Cristina Nali for their constant encouraging and input.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Jaggi, D.; Varun, M.; Pagare, S.; Tripathi, N.; Thore, M.; Singh, R.; Mhumesh, K. Invasive alien weed species: A threat to plant biodiversity. In *Plant Biodiversity: Monitoring, Assessment and Conservation*; Ansari, A.A., Gill, S.S., Abbas, Z.K., Naeem, M., Eds.; CAB International: Wallingford, UK, 2017.
2. Chauhan, B.S. Grand challenges in weed management. *Front. Agron.* **2020**, *1*, 3. [CrossRef]
3. Fracchiolla, M.; Lasorella, C.; Cazzato, E.; Vurro, M. Weeds in non-agricultural areas: How to evaluate the impact? A preliminary case study in archaeological sites. *Agronomy* **2022**, *12*, 1079. [CrossRef]
4. Adhikari, A.; Rew, L.J.; Mainali, K.P.; Adhikari, S.; Maxwell, B.D. Future distribution of invasive weed species across the major road network in the state of Montana, USA. *Reg. Environ. Chang.* **2020**, *20*, 60. [CrossRef]
5. Peters, K.; Breitsameter, L.; Gerowitt, B. Impact of climate change on weeds in agriculture: A review. *Agron. Sustain. Dev.* **2014**, *34*, 707–721. [CrossRef]
6. Bajwa, A.; Mahajan, G.; Chauhan, B.S. Nonconventional weed management strategies for modern agriculture. *Weed Sci.* **2015**, *63*, 723–747. [CrossRef]
7. Westwood, J.H.; Charudattan, R.; Duke, S.O.; Fennimore, S.A.; Marrone, P.; Slaughter, D.C.; Swanton, C.; Zollinger, R. Weed management in 2050: Perspectives on the future of weed science. *Weed Sci.* **2018**, *66*, 275–285. [CrossRef]
8. Farm to Fork Strategy. Available online: https://food.ec.europa.eu/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf (accessed on 5 January 2024).
9. Dayan, F.E. Current status and future prospects in herbicide discovery. *Plants* **2019**, *8*, 341. [CrossRef]
10. Pitcairn, M.J. Biological control of plants. In *Encyclopedia of Biological Invasions*; Simberloff, D., Rejmánek, M., Eds.; University of California Press: Berkeley, CA, USA; Los Angeles, SC, USA, 2011.
11. Berestetskiy, A.; Sokornova, S. Production and stabilization of mycoherbicides. In *Biological Approaches for Controlling Weeds*; Radhakrishnan, R., Ed.; IntechOpen: London, UK, 2018.
12. Duke, S.O.; Pan, Z.; Bajsa-Hirschel, J.; Boyette, C.D. The potential future roles of natural compounds and microbial bioherbicides in weed management in crops. *Adv. Weed Sci.* **2022**, *40*, e020210054. [CrossRef]

13. Roberts, J.; Florentine, S.; Fernando, W.G.D.; Tennakoon, K.U. Achievements, developments and future challenges in the field of bioherbicides for weed control: A global review. *Plants* **2022**, *11*, 2242. [CrossRef]
14. Cordeau, S.; Triolet, M.; Wayman, S.; Steinberg, C.; Guillemin, J.P. Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. *Crop Prot.* **2016**, *87*, 44–49. [CrossRef]
15. Wilson, C.L. Use of plant pathogens in weed control. *Annu. Rev. Phytopathol.* **1969**, *7*, 411–434. [CrossRef]
16. Pacanoski, Z. Bioherbicides. In *Herbicides, Physiology of Action, and Safety*; IntechOpen: London, UK, 2015.
17. TeBeest, D.O.; Templeton, G.E. Mycoherbicides: Progress in the biological control of weeds. *Plant Dis.* **1985**, *69*, 6–10.
18. Xu, D.; Xue, M.; Shen, Z.; Jia, X.; Hou, X.; Lai, D.; Zhou, L. Phytotoxic secondary metabolites from fungi. *Toxins* **2021**, *13*, 261. [CrossRef]
19. Puyt, R.W.; Lie, F.B.; Wilderom, C.P.M. The origins of SWOT analysis. *Long Range Plann.* **2023**, *56*, 102304. [CrossRef]
20. Friend, G.; Zehle, S. *Guide to Business Planning*; Bloomberg Press: New York, NY, USA, 2009.
21. David, F.R. *Strategic Management Concepts and Cases*, 11th ed.; Prentice Hall: New York, NY, USA, 2007.
22. Kowarik, I.; Säumel, I. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. *Perspect. Plant Ecol. Evol. Syst.* **2007**, *8*, 207–237. [CrossRef]
23. EPPO. PM 9/29 *Ailanthus altissima*. *OEPP/EPPO Bull.* **2020**, *50*, 148–155. [CrossRef]
24. Hu, S.Y. Ailanthus. *Arnoldia* **1979**, *39*, 29–50.
25. Celesti-Grapow, L.; Blasi, C. The role of alien and native weeds in the deterioration of archaeological remains in Italy. *Weed Technolog.* **2004**, *18*, 1508–1513. [CrossRef]
26. Trotta, G.; Savo, V.; Cicinelli, E.; Carboni, M. Colonization and damages of *Ailanthus altissima* (mill.) Swingle on archaeological structures: Evidence from the Aurelian walls in Rome (Italy). *Int. Biodeterior. Biodegrad.* **2020**, *153*, 105054. [CrossRef]
27. Invasive Species of European Union Documents. Available online: https://ec.europa.eu/environment/nature/invasivealien/list/index_en.htm (accessed on 4 January 2024).
28. Filippou, P.; Bouchagier, P.; Skotti, E.; Fotopoulos, V. Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species *Ailanthus altissima* to drought and salinity. *Environ. Exp. Bot.* **2014**, *97*, 1–10. [CrossRef]
29. Knüsel, S.; Conedera, M.; Zweifel, R.; Bugmann, H.; Etzold, S.; Wunder, J. High growth potential of *Ailanthus altissima* in warm and dry weather conditions in novel forests of southern Switzerland. *Trees* **2018**, *33*, 395–409. [CrossRef]
30. Ranft, H.; Dässler, H.G. Smoke-hardiness test carried out on woods in a $SO_2$-chamber. *Flora* **1970**, *159*, 573–588. [CrossRef]
31. Kovacs, M.; Opauszky, I.; Klincsek, P.K.; Podani, J. The leaves of city trees as accumulation indicators. In *Monitoring of Air Pollutants by Plants. Methods and Problems*; Steubing, L.S., Jäger, H.J., Eds.; Dr. W. Junk Publishers: The Hague, The Netherlands, 1982.
32. Gravano, E.; Giulietti, V.; Desotgiu, R.; Bussotti, F.; Grossoni, P.; Gerosa, G.; Tani, C. Foliar response of an *Ailanthus altissima* clone in two sites with different levels of ozone pollution. *Environ. Pollut.* **2003**, *121*, 137–146. [CrossRef]
33. Skelly, J.M.; Innes, J.L.; Savage, J.E.; Snyder, K.R.; Vanderheyden, D.; Zhang, J.; Sanz, M.J. Observation and confirmation of foliar ozone symptoms of native plant species of Switzerland and southern Spain. *Water Air Soil Pollut.* **1999**, *116*, 227–234. [CrossRef]
34. Cozzi, A.; Ferretti, M.; Innes, J.L. Sintomi fogliari attribuibili ad ozono sulla vegetazione spontanea in Valtellina. *Monti Boschi* **2000**, *51*, 42–50.
35. Gravano, E.; Ferretti, M.; Bussotti, F.; Grossoni, P. Foliar symptoms and growth reduction of Ailanthus altissima in an area with high ozone and acidic deposition in Italy. *Water Air Soil Pollut.* **1999**, *116*, 267–272. [CrossRef]
36. Pegg, G.F.; Brady, P.L. *Verticillium Wilts*; CABI: Wallingford, UK, 2002.
37. Marini, F.; Profeta, E.; Vidović, B.; Petanović, R.; de Lillo, E.; Weyl, P.; Hinz, H.L.; Moffat, C.E.; Bon, M.C.; Cvrković, T.; et al. Field assessment of the host range of *Aculus mosoniensis* (Acari: Eriophyidae), a biological control agent of the Tree of Heaven (*Ailanthus altissima*). *Insects* **2021**, *12*, 637. [CrossRef] [PubMed]
38. de Lillo, E.; Marini, F.; Cristofaro, M.; Valenzano, D.; Petanović, R.; Vidović, B.; Cvrković, T.; Bon, M.C. Integrative taxonomy and synonymization of *Aculus mosoniensis* (Acari: Eriophyidae), a potential biological control agent for Tree of Heaven (*Ailanthus altissima*). *Insects* **2022**, *13*, 489. [CrossRef]
39. Schall, M.J.; Davis, D.D. *Ailanthus altissima* wilt and mortality: Etiology. *Plant Dis.* **2009**, *93*, 747–751. [CrossRef] [PubMed]
40. Inderbitzin, P.; Bostock, R.M.; Davis, R.M.; Usami, T.; Platt, H.W.; Subbarao, K.V. Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the description of five new species. *PLoS ONE* **2011**, *6*, e28341. [CrossRef]
41. Snyder, A.L.; Kasson, M.T.; Salom, S.M.; Davis, D.D.; Griffin, G.J.; Kok, L.T. First report of Verticillium wilt of *Ailanthus altissima* in Virginia caused by *Verticillium nonalfalfae*. *Plant Dis.* **2013**, *97*, 837. [CrossRef]
42. Snyder, A.L.; Salom, S.M.; and Kok, L.T. Survey of *Verticillium nonalfalfae* (Phyllachorales) on tree-of-heaven in the southeastern USA. *Biocontrol Sci. Technol.* **2014**, *24*, 303–31442. [CrossRef]
43. Rebbeck, J.; Malone, M.A.; Short, D.P.G.; Kasson, M.T.; O'Neal, E.S.; Davis, D.D. First report of Verticillium wilt caused by *Verticillium nonalfalfae* on tree-of-heaven (*Ailanthus altissima*) in Ohio. *Plant Dis.* **2013**, *97*, 999. [CrossRef] [PubMed]
44. Inderbitzin, P.; Subbarao, K.V. *Verticillium* systematics and evolution: How confusion impedes Verticillium wilt management and how to resolve it. *Phytopathology* **2014**, *104*, 564–574. [CrossRef] [PubMed]
45. Heale, J.B.; Isaak, I. Wilt of lucerne caused by species of *Verticillium* IV. Pathogenicity of *V. alboatrum* and *V. dahliae* to lucerne and other crops; spread and survival of *V. alboatrum* in soil and in weeds, effect upon lucerne production. *Ann. Appl. Biol.* **1963**, *52*, 439–451. [CrossRef]

46. Sinclair, W.A.; Lyon, H.H. *Diseases of Trees and Shrubs*, 2nd ed.; Cornell University Press: Ithaca, NY, USA, 2005.
47. Schall, M.J.; Davis, D.D. Verticillium Wilt of *Ailanthus altissima*: Susceptibility of associated tree species. *Plant Dis.* **2009**, *93*, 1158–1162. [CrossRef]
48. Brooks, R.K.; Baudoin, A.; Salom, S. The natural persistence and distribution of the proposed biological control agent *Verticillium nonalfalfae* on *Ailanthus altissima* in Virginia, USA. *For. Pathol.* **2020**, *50*, e12639. [CrossRef]
49. O'Neal, E.S.; Davis, D.D. Intraspecific root grafts and clonal growth within *Ailanthus altissima* stands influence *Verticillium nonalfalfae* transmission. *Plant Dis.* **2015**, *99*, 1070–1077. [CrossRef]
50. O'Neal, E.S.; Davis, D.D. Biocontrol of *Ailanthus altissima*: Inoculation protocol and risk assessment for *Verticillium nonalfalfae* (Plectosphaerellaceae: Phyllachorales). *Biocontrol Sci. Technol.* **2015**, *25*, 950–967. [CrossRef]
51. Knapp, L.P.; Rebbeck, J.; Hutchinson, T.; Fraser, J.; Pinchot, C.C. Controlling an invasive tree with a native fungus: Inoculating *Ailanthus altissima* (Tree-of-Heaven) with *Verticillium nonalfalfae* in highly disturbed Appalachian forests of Ohio. *J. For.* **2022**, *120*, 558–574.
52. Maschek, O.; Halmschlager, E. Effects of *Verticillium nonalfalfae* on *Ailanthus altissima* and associated indigenous and invasive tree species in eastern Austria. *Eur. J. For. Res.* **2018**, *137*, 197–209. [CrossRef]
53. Maschek, O.; Halmschlager, E. Natural distribution of *Verticillium* wilt on invasive *Ailanthus altissima* in eastern Austria and its potential for biocontrol. *For. Pathol.* **2017**, *47*, e12356. [CrossRef]
54. Maschek, O.; Halmschlager, E. A rapid, reliable and less-destructive method for stem inoculations on trees. *For. Pathol.* **2016**, *46*, 171–173. [CrossRef]
55. Cech, T.L. Absterben von götterbäumen (*Ailanthus altissima*) in der Südsteiermark [Cases of dying *Ailanthus altissima* in Southern Styria]. *Forstschutz Aktuell* **1998**, *22*, 16–18.
56. Regulation (EU) No. 1107/2009. Available online: https://www.baes.gv.at/en/admission/plant-protection-products (accessed on 29 December 2023).
57. Lechner, Y.; Maschek, O.; Kirisits, T.; Halmschlager, E. Further pathogenicity testing of *Verticillium nonalfalfae*, a biocontrol agent against the invasive Tree of Heaven (*Ailanthus altissima*), on non-target tree species in Europe. *Phytoparasitica* **2023**, *51*, 113–130. [CrossRef]
58. Pisuttu, C.; Marchica, A.; Bernardi, R.; Calzone, A.; Cotrozzi, L.; Nali, C.; Pellegrini, E.; Lorenzini, G. Verticillium wilt of *Ailanthus altissima* in Italy caused by *V. dahliae*: New outbreaks from Tuscany. *iForest* **2020**, *13*, 238–245. [CrossRef]
59. Pisuttu, C.; Lo Piccolo, E.; Paoli, L.; Cotrozzi, L.; Nali, C.; Pellegrini, E.; Lorenzini, G. Physiochemical responses of *Ailanthus altissima* under the challenge of *Verticillium dahliae*: Elucidating the decline of one of the world's worst invasive alien plant species. *Biol. Invasions* **2023**, *25*, 61–78. [CrossRef]
60. Pisuttu, C. How to Counteract the *Ailanthus altissima* Invasion: Could *Verticillium* Have a Role in the Biological Control of the "Tree of Heaven"? Ph.D. Thesis, Department of Agriculture, Food and Environment of the University of Pisa, Pisa, Italy, 2022.
61. Pisuttu, C.; Ganino, T.; Rodolfi, M.; Ricci, G.P.; Lorenzini, G.; Nali, C.; Pellegrini, E.; Cotrozzi, L. Genetic differences among *Ailanthus altissima* collections across and outside Italy by a Citizen science approach and their susceptibility to the candidate mycoherbicide *Verticillium dahliae* Kleb. *Biol. Control* **2023**, *185*, 105315. [CrossRef]
62. Scholthof, K.B.G. The disease triangle: Pathogens, the environment and society. *Nat. Rev. Microbiol.* **2007**, *5*, 152–156. [CrossRef]
63. Kasson, M.T.; Kasson, L.R.; Wickert, K.L.; Davis, D.D.; Stajich, J.E. Genome sequence of a lethal vascular wilt fungus, *Verticillium nonalfalfae*, a biological control used against the invasive *Ailanthus altissima*. *Microbiol. Resour. Announc.* **2019**, *8*, e01619-18. [CrossRef]
64. Berger, H.; Maschek, O.; Halmschlager, E. Draft genome sequences of three strains of *Verticillium nonalfalfae* exhibiting different levels of aggressiveness on *Ailanthus altissima*. *Microbiol. Resour. Announc.* **2020**, *9*, e01384-19. [CrossRef] [PubMed]
65. Pisuttu, C.; Sarrocco, S.; Cotrozzi, L.; Baroncelli, R.; Lorenzini, G. Genome resources of *Verticillium dahliae* VdGL16: The causal agent of vascular wilt on the invasive *species Ailanthus altissima*. *Plant Dis.* **2023**, *107*, 1207–1209. [CrossRef] [PubMed]
66. Klosterman, S.J.; Atallah, Z.K.; Vallad, G.E.; Subbarao, K.V. Diversity, pathogenicity, and management of *Verticillium* species. *Annu. Rev. Phytopathol.* **2009**, *47*, 39–62. [CrossRef]
67. Fradin, E.F.; Thomma, B.P.H.J. Physiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. alboatrum*. *Mol. Plant Pathol.* **2006**, *7*, 71–86. [CrossRef]
68. Da Lio, D.; De Martino, L.; Tavarini, S.; Passera, B.; Angelini, L.G.; Vannacci, G.; Sarrocco, S. First report of *Verticillium dahliae* causing Verticillium wilt on *Stevia rebaudiana* in Europe. *J. Plant Pathol.* **2019**, *101*, 1291. [CrossRef]
69. Resende, M.L.V.; Flood, J.; Cooper, R.M. Host specialization of *Verticillium dahliae*, with emphasis on isolates from cocoa (*Theobroma cacao*). *Plant Pathol.* **1994**, *43*, 104–111. [CrossRef]
70. Rask, A.M.; Kristoffersen, P. A review of non-chemical weed control on hard surfaces. *Weed Res.* **2007**, *47*, 370–380. [CrossRef]
71. Abou Chehade, L.; Antichi, D.; Frasconi, C.; Sbrana, M.; Tramacere, L.G.; Mazzoncini, M.; Peruzzi, A. Legume cover crop alleviates the negative impact of no-till on tomato productivity in a Mediterranean organic cropping system. *Agronomy* **2023**, *13*, 2027. [CrossRef]
72. Feret, P.P. *Ailanthus*: Variation, cultivation, and frustration. *J. Arboric.* **1985**, *11*, 361–368. [CrossRef]
73. DiTomaso, J.M.; Kyser, G.B. Control of *Ailanthus altissima* using stem herbicide application techniques. *Arboric. Urban For.* **2007**, *33*, 55–63. [CrossRef]

74. Resolution 821-2015. Available online: http://www.stopglifosato.it/download/Reg-Toscanadelibera-821-04-uso-agricolo-e-non-agricolo-glifosato.pdf (accessed on 12 December 2023).
75. Decreto Interministeriale 22 Gennaio 2014—Adozione del Piano di Azione Nazionale per L'uso Sostenibile dei Prodotti Fitosanitari, ai Sensi Dell'articolo 6 del Decreto Legislativo 14 Agosto 2012, n. 150. Available online: https://www.mite.gov.it/normative/decreto-interministeriale-22-gennaio-2014-adozione-del-piano-di-azione-nazionale-luso (accessed on 12 December 2023).
76. Lorenzini, G. Will a fungus save us from the *Ailanthus* invasion? *Ital. J. Mycol.* **2016**, *45*, 13–18. [CrossRef]
77. Regulation (EU) 2023/2660. Available online: https://eur-lex.europa.eu/eli/reg_impl/2023/2660/oj (accessed on 3 January 2024).
78. Hill, T.; Westbrook, R. SWOT analysis: It's time for a product recall. *Long Range Plan.* **1997**, *30*, 46–52. [CrossRef]
79. Kasson, M.T.; O'Neal, E.S.; Davis, D.D. Expanded host range testing for *Verticillium nonalfalfae*: Potential biocontrol agent against the invasive *Ailanthus altissima*. *Plant Dis.* **2015**, *99*, 823–835. [CrossRef]
80. Dauth, B.; Maschek, O.; Steinkellner, S.; Kirisits, K.; Halmschlager, E. Non-target effects of *Verticillium nonalfalfae* isolate Vert56 used for biological control of *Ailanthus altissima* on agricultural crops known to be generally susceptible to *Verticillium* spp. *Biol. Control* **2022**, *174*, 105030. [CrossRef]
81. Huber, L.; Lorenz, C.S. Registration or the Sword of Damocles over the development of biopesticides. In *Microbial Control Agents: Developing Effective Biopesticides*; Puopolo, G., Ed.; CAB International: Wallingford, UK, 2023.