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Short communication

Will a fungus save us from the *Ailanthus* invasion?

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Abstract

Rapid growth rate, prolific fruiting and vegetative reproduction from root sprouts, ready germination and extended root system, allelopathic effects, resistance to herbivory combined to tolerance to environmentally stressful conditions such as infertile sites, make *Ailanthus altissima* (Mill.) Swingle (also known as Heaven tree, *Simaroubaceae*) a noxious and highly invasive tree in all countries where it has been introduced and is become naturalized. After a brief historical trait of the importance of the presence of this plant and the complexity of eradication campaigns based on manual cutting and herbicide treatments, the note illustrates the possible role as a candidate mycoherbicide of a soilborne fungal wilt agent (*Verticillium nonalfalfae* H.W. Platt, R.M. Bostock, R.M. Davis & Subbarao) recently described in the USA and also reported from Austria.

Keywords: Verticillium nonalfalfae; mycoherbicide; biocontrol; Ailanthus altissima; tree-of-heaven; wilt disease.

Riassunto

Molti sono i caratteri di *Ailanthus altissima* (Mill.) Swingle (noto come "Albero del paradiso", *Simaroubaceae*) che lo rendono un albero invasivo che provoca gravi problemi in tutte le aree ove è stato introdotto e si è naturalizzato: velocità di accrescimento, abbondante fruttificazione e capacità di riproduzione vegetativa, facilità di germinazione dei semi e presenza di apparato radicale irruente, proprietà allelopatiche, resistenza all'erbivoria combinata a straordinaria capacità di tolleranza nei confronti di situazioni ambientali estreme. Dopo una breve descrizione delle difficoltà insite nelle campagne di eradicazione basate sul taglio meccanico seguito da trattamenti erbicidi, la nota illustra il possibile ruolo, come micoerbicida,o di un agente patogeno tracheomicotico (*Verticillium nonalfalfae* H.W. Platt, R.M. Bostock, R.M. Davis & Subbarao) recentemente descritto negli Stati Uniti e segnalato anche in Austria.

Parole chiave: Verticillium; micoerbicida; difesa biologica; Ailanthus altissima; tracheomicosi.

Imagine everything happened merely because of a mistake when (around 1750) the French clergyman Pierre d'Incarville brought to Paris from faraway China a stock of seeds that he thought were those of the prized lacquer tree [*Rhus verniciflua, Sin. Toxicodendron vernicifluum* (Stokes) F. Barkley] but were instead ailanthus seeds [*Ailanthus altissima* (Mill.) Swingle, sin. *A. glandulosa*, also known as Heaven tree, *Simaroubaceae*] (FERET, 1985). Thus begins one of the most important biological invasions of the plant

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world in the modern times. This neophyte was valued in Europe for its beautiful appearance (it can reach over 20-25 m in height with an elegant straight habit, and a trunk diameter of more than 1 m; its foliage is pleasing to the eye and is reminiscent of walnut and ash trees). Its hardiness is incomparable (it has no acclimatization problems as regards climate and edaphic factors). It is easy to cultivate and grows exceptionally fast. It is perfect for beautifying streets and parks, reinforcing slopes, and colonizing difficult, unstable terrain. One episode that contributed to its quick dissemination was when the breeding of the classical silk worm (*Bombyx mori* L.) underwent a severe health crisis and another lepidopteran was introduced from the East by the zoologist Paolo Savi in Pisa in 1856: the ailanthus silkworm, *Samia cynthia* Drury, which indeed feeds on the ailanthus. Thus, an alternative sericulture industrial chain was set up, with this tree becoming even more popular. In Florence, a "Società Ailantina Italiana" was set up to encourage the spread of this tree species (RIDOLFI, 1861). However, this situation did not last for long. While Louis Pasteur solved in 1864 the *Bombyx* infection problem – which was pebrine, a disease caused by the protozoan *Nosema bombycis* Nageli – the industrial quality of the cocoons produced by the *Samia* butterfly was quite scarce. However, by then ailanthus had established itself and conquered ever more new areas. In the meanwhile, it had also reached the USA (starting from Pennsylvania in 1784 – KASSON et al., 2013), imported by English settlers and even there it was appreciated. A second line of introduction also occurred in California in the mid-19th century at the hands of the Chinese who emigrated to work on the transcontinental railway (but also to search gold!). The ailanthus tree is a true cult for the Chinese, who use almost all its parts in their traditional medicine. The list of health problems that find a speedy and satisfying solution in extracts of its bark, leaves, fruit, and wood is endless (KUNDU and LASCAR, 2010). Strangely enough, this topic has never been tackled in real terms elsewhere on the planet even if by now the complex pool of secondary metabolites present in the various organs of the plant is well characterized. Within a few decades, this species has been naturalized on all the continents, and become a problem everywhere (KOWARICK and SAUMEL, 2007). Its favorite habitat is represented by such open areas as railway lines and communication routes, abandoned settings, and marginal stations with a high anthropic disturbance.

A complete description of the botanical and ecological features and distinguishing characters of the ailanthus is readily available (e.g. http://www.cabi.org/isc/datasheet/3889; HU, 1979). Ailanthus is a wonderful biological machine and by no means a "banal" tree, which exhibits rapid establishment and growth, with a high rate of sexual reproduction. The tree bears unisexual flowers on different trees, and a single female plant can produce up to 1 million wind-dispersed seeds in a year. Its true peculiarity lies in the capacity to grow side-shoots and root-suckers. Starting from the trunk, surface roots, sometimes tens of meters long, are differentiated and from them suckers easily branch off. Removing the trunk just triggers quick and stubborn differentiation reactions of buds from the stump and roots so that, due to the ability to resprout rapidly and form clonal stands after disturbance, paradoxically, "*the more it is cut back, the more the production of new biomass is stimulated*". The average lifespan is estimated at a hundred years maximum but, due to its capability to propagate via underground stems, suckers or stolons, it may be considered "immortal" in the sense that its vitality does not end with the death of the original trunk, as it has yielded a pure clonal colony that, if undisturbed, can even cover a 4,000 square meter area. It has a marked allelopathic activity, which grants it further advantages as regards competition. In can be found at a range of altitudes up to 2400 m. Its hardiness is proverbial, making use of an exceptional phenotypic plasticity that lets it adapt to a wide range of thermal and water regimes. It is a perfect example of a "water saving mechanism", through modifications of water conductance of the root system and evapotranspiration processes (TRIFILO' *et al.*, 2004). It easily deals with critical situations, such as those of simple cracks in stone materials. It can easily grow in extreme environmental conditions (**Fig. 1**), and is viewed by many as a symbol of dereliction and abandonment. It is resistant to urban environment and to herbivores and is actually free from attacks by pests, at least in Europe

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(the above-mentioned lepidopteran *Samia cynthia* has been a mere scientific curiosity for some time, a real "prize" for photo-naturalists).

Fig. 1. Severe colonization of ailanthus saplings in the Old English Cemetery of Livorno
Grave colonizzazione di giovani piante di ailanto nell'Antico cimitero degli Inglesi di Livorno

All these characteristics could make ailanthus an interesting candidate for the remediation of marginal and degraded areas. But, like few others, this is an invasive pioneer plant (it successfully rivals with black locust, *Robinia pseudo-acacia* L.). Not accepted by ungulates, it threatens the biodiversity of natural and anthropized ecosystems, easily establishing mono-specific plant formations. Once introduced into environments with a high natural importance (e.g. the Isle of Montecristo), it rapidly replaces the indigenous flora, jeopardizing the conservation of native biocenoses, and forcing difficult (and usually useless) eradication campaigns. The presence of stands along traffic routes and transportation corridors is unacceptable because of the problems linked to the reduced visibility and the invasiveness of the foliage and roots that damage road surfaces. It also poses human health problems, causing contact dermatitis and possibly immunoallergic respiratory issues and myocarditis. In Tuscany, the Regional Law 56/2000 forbids the use of this species for reforestation, re-vegetation, and reinforcement works.

Researchers have focused attention on finding strategies to counter the growth of this invasive plant in such sensitive areas as archeological sites, railway lines, roadways, natural parks, and urban settlements. There are ecological, economic, and cultural reasons urging the adoption of effective measures of eradication. But how

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can it be done? Some key points: the ailanthus is like an iceberg, since most of the problem is underground! A simple (manual) eradication and/or a mechanical cut at the base of the trunk or girdling are totally ineffective (even if they are useful for neutralizing highly prolific seed-bearing mother plants). On the contrary, they stimulate the tree to rapidly produce an abundance of vigorous and aggressive young runners and suckers. The above-mentioned actions must be accompanied by the periodical use of systemic (and nonselective) herbicides that can be transported to the root system and compromise (but usually only partly) future vegetative renewal (DITOMASO and KYSER, 2007). However, recent regulatory measures have made these interventions almost impossible. On the one hand, the Tuscan Region (Resolution 821 of August 4, 2015) 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, the National Action Plan for the sustainable use of plant protection products (D.M. January 22, 2014) is leading to serious limitations in the use of chemical pesticides on 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). What can be done, then?

A careful examination of the abundant literature on this subject points out an interesting novelty. In the USA, for some years now, a case of the ailanthus naturally decline and dying-off has been under investigation (tens of thousands of dead trees in Pennsylvania and in some neighboring States). It has been attributed to the attacks of a soilborne microfungus, new to the sciences, and described as an (almost) host-specific strain of *Verticillium nonalfalfae* (INDERBITZIN et al., 2011), originally referred to *V. albo-atrum* Reinke & Berthold (SCHALL and DAVIS, 2009). This pathogen is a wilt agent, able to move within the vascular system to reach the suckers and to transfer from an infected individual to a nearby healthy one by intraspecific root grafting (O'NEAL and DAVIS, 2015a) and is specialized on the ailanthus, causing little or no adverse effects on non-target forest species (KASSON et al., 2014). It leads to the rapid death of the affected trees and seems a perfect mycoherbicide candidate. *In vitro* culturing techniques and artificial inoculation protocols based on an efficient and stable form of *V. nonalfalfae* are now ready (O'NEAL and DAVIS, 2015b). Following seedling artificial inoculations, the first visible symptom is wilt of leaflets, commonly followed by chlorosis and/or necrosis of the leaflets, or proceeding directly into defoliation, and eventually death of the plant in 9-11 weeks (SNYDER et al., 2014) (**Fig. 2a, b**). Once the *Verticillium* infections have spread in a stand, the disease can progress independently, starting from the inoculation produced by diseased plants. Since *Verticillium*-resting structures can remain dormant for years in soil depending on conditions, new ailanthus seedling and sprouts could eventually succumb to the resting pathogen. This would potentially allow the sites to be free from ailanthus reintrusion for years and allow native plants to begin re-establishment (SNYDER et al., 2014). An important role of insects in the spread of propagules has been verified. Recently, KASSON *et al.* (2015) tested 71 woody species for their susceptibility to *V. nonalfalfae* infection and found that only *Aralia spinosa* L. (devil's walkingstick) and *Acer pennsylvanicum* L. (striped maple) acquired infections through natural spread from infected *Ailanthus altissima*. When purposefully injected with *V. nonalfalfae*, numerous woody species showed minor wilting and the fungus was able to be reisolated from the woody tissue; however, these other species were able to survive the infection after six years. This research confirms that *V. nonalfalfae* is host adapted to tree of heaven and that other woody species are tolerant of the fungus. Recently (MASCHEK and HALMSCHLAGER, 2016) this pathogen has been recorded in Austria causing typical symptoms on ailanthus.

Fig. 2 (Photos courtesy of Prof. Donald D. Davis, The Pennsylvania State University).
a: *on the left*, ailanthus healthy plant (control); *on the right*: ailanthus plant artificially inoculated with *Verticillium nonalfalfae*. **b**: wilting of a twig of an ailanthus tree naturally infected by the pathogen.

a: a sinistra: *pianta di ailanto sana (controllo);* a destra: *pianta di ailanto inoculata artificialmente con* Verticillium nonalfalfae. **b**: *avvizzimento di un rametto di una pianta di ailanto infettata naturalmente dal patogeno.*

It is easy to dream about the possibility of actively introducing this microorganism and using it for effective, economical, and eco-sustainable actions to finally counter the ailanthus's otherwise unrestrainable advance. The idea of using plant pathogens as contrast agents of infesting and invasive plants is as old as the science of plant pathology itself (WILSON, 1969). Unfortunately, practical applications are rather limited, given the remarkable difficulties that the technique entails. Obviously, a rigorous risk assessment would be indispensable, aimed first of all at ensuring that the pathogen does not also infect the non-target plants. However, have we perhaps found a solution?

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