Fungal pathogens of *Schinus terebinthifolius* from Brazil as potential classical biological control agents

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Summary

The Brazilian peppertree, *Schinus terebinthifolius* Raddi, is a shrub or small tree, native to Brazil, Paraguay and Argentina. It has been introduced into other regions of the world as an ornamental or as a source of condiment. It became an aggressive invader of several exotic ecosystems, particularly in oceanic islands such as Hawaii and Mauritius, as well as in Florida, USA, and Australasia. Although research involving the use of insect natural enemies from the plant's centre of origin as biological control agents for *S. terebinthifolius* has been going on for some time, no systematic survey had been undertaken for fungal pathogens until recently. A study of the mycobiota associated with this plant was initiated in 2001, which concentrated on the southeastern states of Brazil. Eleven fungal taxa have been found thus far, several of which are new to science, namely *Hainesia lythri* (Desmaz.) Höhn., *Irenopsis* sp., *Meliola* sp., *Oidium* sp., *Phyllosticta* sp. nov., *Pleomassaria* sp. nov., *Pilidium concavum* (Desmaz.) Höhn., *Pseudocercospora* sp. nov., *Septoria* sp. nov., *Stenella* sp. and one new coelomycete genus. These fungi were associated with various symptoms, viz. leaf spots, black mildew and powdery mildew. The *Septoria* leaf-spot fungus appears to have particularly good potential as a classical biological control agent. Pathogenicity has been demonstrated to biotypes of *S. terebinthifolius* from Brazil, Florida and Hawaii. Its host range is now being tested in Florida and Hawaii with the goal of possibly introducing the fungus into those areas.

Keywords: classical biological control, Brazilian peppertree, surveys.

Introduction

The Brazilian peppertree, *Schinus terebinthifolius* Raddi, known in Brazil as 'aroeira', is a small tree in the family Anacardiaceae native to Brazil, Argentina and Paraguay (Elfers, 1988; Binggeli, 1997; Taylor, 1998; Cuda *et al.*, 2006). In Brazil, *S. terebinthifolius* is found along the east coast from the state of Pernambuco in the north to Rio Grande do Sul in the south (Lorenzi and Matos, 2002). It occurs in habitats ranging from sand dunes to rainforests and semi-deciduous highland forests, often growing on river margins and in swampy areas (Binggeli, 1997).

Schinus terebinthifolius is generally regarded as a valuable plant in Brazil where it is used for medicinal purposes (Lorenzi and Matos, 2002) and as a source of tannin for treating fishing nets and fishing lines. The wood is used for fencing, firewood and charcoal, and the plant is also used as an ornamental and as a source of forage for goats and is valued by beekeepers (Baggio, 1988; Lorenzi, 1992). In several parts of the world, its fruits are used as a spice (pepper rosé), and there are also several other medicinal uses listed for the plant (Cuda *et al.*, 2006). However, *S. terebinthifolius* has become a noxious weed in many regions of the world where it has been introduced, such as Samoa, Fiji, French Polynesia, the Marshall Islands, New Caledonia Mauritius, and the USA, particularly Hawaii and Florida (Cronk and Fuller, 1995). It was probably introduced into Florida before 1850 as an ornamental plant (Mack, 1991). By the 1920s, it had already become widely distributed, and in the 1960s, it became recognized as an important component of the natural vegetation forming dense monocultures (Morton, 1978; Elfers, 1988; Binggeli, 1997; Anon, 2000, 2001a,b; Cuda *et al.*, 2006). Presently, the distribution of *S. terebinthifolius* in the USA includes central and

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southern Florida, southern Arizona, southern California, Texas, Louisiana, Hawaii and Puerto Rico (Anon, 2001a,b; Cuda *et al.*, 2006); it also occurs in the Bahamas (Elfers, 1988; Anon, 2001a,b). In Florida, *S. terebinthifolius* is common in areas where the soil is dry to moderately well-drained along roadsides and in the vicinity of lakes, and it is invading private and public gardens (Anon, 2002). Although it is a common pioneer of disturbed sites, such as abandoned farmland and waste areas, it is also capable of invading well-preserved natural areas, such as the drier areas of the Everglades and the coastline of peninsular Florida. Once established, *S. terebinthifolius* displaces native herbaceous communities due to its dense shading habit and the alleopathic substances it produces (Gogue *et al.*, 1974; Elfers, 1988; Binggeli, 1997; Anon, 2002; Morgan and Overholt, 2005; Cuda *et al.*, 2006). The plant is readily dispersed by birds (Lorenzi, 1992; Panetta and McKee, 1997) and is capable of vigorous regeneration after fire, cutting or frost, making its control particularly difficult (Binggeli, 1997). Invasions by *S. terebinthifolius* can result in the loss of local biodiversity (Anon, 2002; Cuda *et al.*, 2006).

Both chemical and mechanical controls of *S. terebinthifolius* have been adopted with some success in Florida and Hawaii but only in areas that are cultivated or otherwise intensively managed. Neither of those strategies is appropriate for control in environmentally sensitive natural areas, such as the Florida Everglades (Anon, 1998; Anon, 2000; Cuda *et al.*, 2006). For such areas, biological control was recognized early on as the ideal strategy for managing *S. terebithifolius*. The initial search for insect natural enemies was led by entomologists who conducted surveys in Brazil and Argentina (Hight *et al.*, 2002). Several promising insects that were found in association with *S. terebinthifolius*, including a seed-feeding bruchid beetle, a stem-boring moth and a leaf-rolling tortricid, were eventually introduced into Hawaii (Yoshioka and Markin, 1991). Later, additional natural enemies were discovered by Bennett *et al.* (1990) for possible introduction into Florida. A stem-feeding thrips, *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae), was regarded as a particularly promising candidate for biological control of *S. terebinthifolius* because it was found to be highly host-specific and damaging to the flowers and young shoots (Cuda *et al.*, 2006). Its release in Florida was approved by the US Government's Technical Advisory Group for Biological Control Agents of Weeds (TAG) in May 2007.

There are now numerous examples of the successful use of fungal pathogens as classical weed biological control agents (Charudattan, 1991; Watson, 1991; Julien and White, 1997). Surveys for fungi associated with important weeds native to Brazil, which were initiated in the mid-1990s, have yielded a plethora of fungi (e.g. Barreto and Evans, 1994, 1995a,b,c, 1998; Barreto *et al.*, 1995; Pereira and Barreto, 2005; Monteiro *et al.*, 2003; Pereira and Barreto, 2005, Pereira *et al.*, 2007; Seixas *et al.*, 2007) including many that were new to science. Two such fungi have already been introduced as biological control agents into other parts of the world, namely *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *miconiae*, which was introduced into Hawaii and French Polynesia for the control of *Miconia calvescens* D.C. (Seixas *et al.*, 2007), and *Prospodium tuberculatum* (Speg.) Arthur, which was introduced into Australia for biological control of *Lantana camara* L. (Ellison *et al.*, 2006). Until recently, no surveys of the fungi-attacking *S. terebinthifolius* in Brazil were performed, and very little information exists in the literature about this plant's mycobiota. A list of all pathogenic fungi recorded from *S. terebinthifolius* is presented in Table 1. This paper presents a preliminary account of the first survey for fungal pathogens associated

Table 1. Fungi recorded in association with *Schinus terebinthifolius* worldwide.

Fungi	Distribution in association with. *S. terebinthifolius*
Anamorphic fungi	
Alternaria sp.	USA (Farr *et al.*, 1985)
Cercospora schini Syd.	Argentina (Chupp, 1953)
Corynespora sp.	USA (Farr *et al.*, 1985)
Diplodia sp.	USA (Farr *et al.*, 1985)
Helminthosporium sp.	USA (Farr *et al.*, 1985)
Macrophoma sp.	USA (Farr *et al.*, 1985)
Phyllosticta sp.	USA (Farr *et al.*, 1985)
Sphaeropsis tumefaciens Hedges	USA (Farr *et al.*, 1985)
Verticillium albo-atrum Reinke & Berthier	USA (Farr *et al.*, 1985)
Ascomycota	
Botryosphaeria ribis f. *achromogena* Gross. & Duggar	USA (Farr *et al.*, 1985)
Botryosphaeria ribis Gross. & Duggar	USA (Farr *et al.*, 1985)
Diaporthe sp.	USA (Farr *et al.*, 1985)
Irenopsis coronata (Speg.) F.L. Stevens	South America (Viégas, 1961)
Meliola brasiliensis Speg.	South America (Viégas, 1961; Hansford, 1961)
Meliola coronata Speg.	USA (Farr *et al.*, 1985)
Nectria cinnabarina (Tode) Fr.	USA (Farr *et al.*, 1985)
Seuratia millardetii (Racib.) Meeker	USA (Farr *et al.*,1985)
Basidiomycota	
Armillaria mellea (Vahl) P. Kumm	USA (Farr *et al.*, 1985)
Armillaria tabescens (Scop.) Emel	USA (Farr *et al.*, 1985)
Ganoderma orbiforme (Fr.) Ryvarden	South America (Viégas, 1961)

with this plant in Brazil and an assessment of the potential of each species as a biocontrol agent for *S. terebinthifolius.*

Materials and methods

The area surveyed for fungal pathogens of *S. terebinthifolius* covered only a small part of southeastern Brazil and was concentrated in the municipality of Viçosa and neighbouring regions in the state of Minas Gerais (MG), the municipality of Rio de Janeiro and northern coastal parts of the state of Rio de Janeiro (RJ) and parts of the valley of the Rio Paraíba do Sul in the state of São Paulo. Pathogen collections were conducted during the period from September 2001 to May 200. Although the botanical literature recognizes five varieties of *S. terebinthifolius* (Barkley, 1944), only *S. terebinthifolius* var. *acutifolius*, *S. terebinthifolius* var. *terebinthifolius*, and *S. terebinthifolius* var. *raddianus* are known to occur in the USA [only vars. *terebinthifolius* and *raddianus* occur in Florida (Cuda, 2002; Cuda *et al.*, 2006)]. Separation of such varieties in the field in Brazil was complicated by the common occurrence of intermediate forms. For practical reasons, all collections were simply referred as being from *S. terebinthifolius* without identification of host variety. Due to the difficulty in separating the varieties morphologically, the Florida populations of *S. terebinthifolius* were recently characterized genetically using microsatellites and chloroplast DNA sequence comparisons (Williams *et al.*, 2005).

Samples of diseased material were collected, dried in a plant press and taken to the lab for further examinations. Lesions were observed under a dissecting microscope and slides were prepared and mounted in lactophenol, lactophenol cotton blue or other mounting media, as required, and examined under a light microscope (Olympus BX 50) fitted with a camera and a drawing tube. Isolations were performed by either directly transferring fungal structures from sporulating lesions onto plates containing vegetable broth agar (VBA), as described by Pereira *et al.* (2003), or by transferring selected, surface-sterilized fragments of diseased tissues onto VBA plates. Pure cultures were kept in a refrigerator at 5°C in tubes containing potato–carrot agar until further use or permanent deposition in the culture collection at the Plant Pathology Department of the Universidade Federal de Viçosa (Brazil).

Pathogenicity was tested for selected fungal species either by brush-inoculating young (7-month old) healthy plants with a spore suspension adjusted to 2×10^6 spores per milliliter in a 0.05% Tween 80 solution or by depositing onto healthy plant parts 5-mm diameter culture disks cut from actively growing mycelium on VBA plates. For fungi suspected of being opportunistic wound pathogens, healthy plant parts were wounded with sterile scissors or needles before inoculation. Inoculated plants were kept in a dew chamber at 25°C for 48 h and then transferred to a greenhouse where they were examined daily for evidence of disease symptoms. Healthy plants treated in an equivalent manner but not exposed to fungal inoculum served as controls. *Schinus terebinthifolius* plants used in these tests were grown from seed originating either from Florida (Gainesville, Campus of the University of Florida), Hawaii or Brazil (Viçosa).

One fungal species collected and tested during these surveys was selected for further study. It was a species of the genus *Septoria*, which was associated with severe outbreaks of leaf spots in *S. terebinthifolius* populations, followed by extensive defoliation. A preliminary assessment of the host range of this *Septoria* sp. was performed with several local members of the Anacardiaceae: *Anacardium occidentale* L. (cashew); *Mangifera indica* L. (mango); *Schinus molle* L. (Peruvian peppertree); *Spondias lutea* L. (Brazilian plum) and *Tapirira guianensis* Aubl. (tatapiririca). Inoculations of the test plants were performed as described above.

Results and discussion

In total, 11 fungal taxa were found attacking *S. terebinthifolius* during the surveys: three ascomycetes (*Pleomassaria* sp. possibly associated with stem dieback, *Irenopsis* sp. and *Meliola* sp. associated with black mildew symptoms); four anamorphic coelomycetes, all of which were associated with leaf spot symptoms [*H. lythri* (Desmaz.) Höhn. and *P concavum* (Desmaz.) Höhn. (synanamorphic species), *Phyllosticta* sp., *Septoria* sp. and a possible new genus of coelomycete] and four anamorphic hyphomycetes (*Oidium* sp., a powdery mildew, a *Pseudocercospora* sp. associated with leaf spots and a *Stenella* sp. associated with yellowing and premature leaf senescence; Table 2).

Eight of the 11 fungi found to be associated with *S. terebinthifolius* were isolated in pure culture. Some were extremely slow growing such as *Pleomassaria* sp., which took nearly a month to produce a visible colony, while others were relatively fast growing such as *H. lythri* and *P. concavum*. The ability of the fungi to sporulate also varied widely, but most fungi did not sporulate readily in culture. Pathogenic status was only preliminarily investigated for seven fungal species: coelomycete gen. nov., *H. lythri*, *P. concavum, Pseudocercospora* sp., *Septoria* sp. and *Stenella* sp. Specific details on each fungal taxon are provided below.

Coelomycete gen. nov.

This fungus was compared with a series of possible genera of other coelomycetes to which it resembled but did not match any of them. We concluded that it represents a new fungal genus that will be described in a separate publication on the taxonomy of the fungi on *S. terebinthifolius*. This new fungus was restricted

Table 2. Synopsis of observations on fungi collected on *Schinus terebinthifolius* during surveys in Brazil.

Fungus	Disease	Damage to host	Purported specificity	Culturability	Biocontrol potential
Coelomycete (gen nov.)	Leaf spot	Moderate	Uncertain	Cultivable	Uncertain
Hainesia lythri	Leaf spot	Insignificant	Non-specific	Cultivable	None
Irenopsis sp.	Black mildew	Insignificant	High	Not cultivable	None
Meliola sp.	Black mildew	Insignificant	High	Not cultivable	None
Oidium sp.	Powdery mildew	Moderate	High	Not cultivable	Moderate
Phyllosticta sp.	Leaf spot	Moderate	Uncertain	Cultivable	Uncertain
Pleomassaria sp.	Branch dieback	Uncertain	Uncertain	Cultivable	Uncertain
Pilidium concavum	Leaf spot	Insignificant	Non-specific	Cultivable	None
Pseudocercospora sp.	Leaf spot	Severe	High	Cultivable	None (see comments)
Septoria sp.	Leaf spot	Severe	High	Cultivable	Very high
Stenella sp.	Leaf spot	Moderate	High	Cultivable	Uncertain

in its distribution, being found in only one location on plants growing on a hill in a coastal sand dune area in the state of Rio de Janeiro. It was always associated with a very distinctive circular leaf spot, and the disease was regarded as severe on the plants it was attacking, resulting in significant defoliation. Its pathogenicity was proven by Koch's postulates, but disease levels obtained under controlled conditions were less severe than those observed in the field. Perhaps, the fungus requires particular environmental conditions, such as those occurring where it was repeatedly collected, to become epidemic and to cause severe disease levels. It is difficult to determine at this stage whether this fungus has any potential as a classical biological control agent.

H. lythri and *P. concavum*

These fungi are known to be opportunistic pathogens, depending on wounds for infection; this study confirmed this relationship. Only wounding of tissues before inoculation allowed for disease to develop during the pathogenicity tests. These two species are known to be generalists having been described from a wide range of hosts (Shear and Dodge, 1921; Palm, 1991). Among these are several species belonging to the Anacardiaceae, e.g., *Rhus glabra* L., *Rhus typhina* L., *Toxicodendrun radicans* (L.) Kuntze and *Rhus aromatica* Ait. (Greene, 1950). This information alone would make these fungi of little interest for classical biological control because they might pose a threat to native plants in the area where introduction would take place. *P. concavum* has been frequently reported in association with old lesions of *H. lythri*, but the two species are normally not found simultaneously on the same lesions or during the same season. However, this association is not observed in all hosts (Shear and Dodge, 1921; Palm, 1991). In one study, *H. lythri* and *P. concavum* were found occurring together during the summer, but it is interesting to note that *P. concavum* was associated with non-senescent leaves; this suggests that, in the case of *S. terebinthifolius*, *P. concavum* may act as a true pathogen. Isolations of *H. lythri* from diseased tissues resulted in typical *H. lythri* colonies in pure culture, while isolations of *P. concavum* also resulted in typical *Hainesia* colonies in culture. This has already been reported for isolates from other hosts (Shear and Dodge, 1921; Palm, 1991), and it is accepted that *H. lythri* and *P. concavum* are genetically connected by being synanamorphs of the same ascomycete species, *Discohainesia oenotherae* (Cooke & Ellis) Nannf. (Palm, 1991). Although interesting and a new report for *S. terebinthifolius*, these two records have no significance for biological control.

Irenopsis sp. and *Meliola* sp.

These are two ascomycetes belonging to the family Meliolaceae, a family of fungi that is widely distributed in the tropics and contains highly host-specific, obligate parasites that cause diseases known as black mildews (Hansford, 1961; Kirk *et al.*, 2001). *Irenopsis* sp. and *Meliola* sp. often were found attacking the same *S. terebinthifolius* individuals in the field and sometimes occurring together on the same leaf. Their colonies were indistinguishable with the naked eye. A comparison of the morphology of these fungi with known black mildew species associated with the Anacardiaceae led to the conclusion that both species on *S. terebinthifolius* are new to science. These will be described in a separate publication. Although pathogenic, diseases caused by the Meliolaceae are generally considered to be too weak to be of any interest for use in weed biological control.

Oidium sp.

This fungus is an anamorphic form from the Erysiphaceae, an important family of ascomycetes that are obligate biotrophs causing powdery mildews. Fungi in this group can be highly host-specific and cause heavy losses in some crop plantations. Unfortunately, in the case of this fungus, it was found only on a few occasions, always during the dry and colder season of the year, and damage to *S. terebinthifolius* was regarded as only moderate. Perhaps, further studies will reveal more aggressive strains of this fungus that may be of interest to be used in classical introductions. The complete identity of this fungus remains obscure at this stage. A preliminary DNA analysis surprisingly placed this taxon close to *Oidium tuckeri* Berk., the aetiological agent of the powdery mildew of grapes, than to the Erysiphales known to attack the Anacardiaceae.

Phyllosticta sp.

Phyllosticta is a genus that contains some important pathogens of crop plants (Nag Raj, 1992), but there also are saprophytes and endophytes in this genus. The species of *Phyllosticta* recorded on the Anacardiaceae are difficult to separate taxonomically and are, in general, poorly described in earlier publications. These are *Phyllosticta schini* Thüm, *Phyllosticta rhois* West., *Phyllosticta rhoina* Kalch., *Phyllosticta toxicodendri* Thüm and *Phyllosticta toxica* Ell. & G. Martin (Saccardo, 1884, 1902). A precise comparison with the fungus found on *S. terebinthifolius*, based on such descriptions, was impossible. A review of the genus was published by van der Aa and Vanev (2002), and these authors considered that all the taxa listed above, except *P. toxica*, should be excluded from *Phyllosticta*. It is likely that the species collected in this survey represents a new taxon for the genus, but further investigation is required to confirm this. Obtaining an isolate of *Phyllosticta* sp. from *S. terebinthifolius* proved difficult. An isolate was finally obtained but in the latter stages of this work, which did not allow for confirmation of its pathogenicity. The leaf-spot disease on *S. terebinthifolius* with which *Phyllosticta* sp. was consistently associated was often severe. Further studies on this fungus as a possible biological control agent should be given high priority.

Pleomassaria sp.

This fungus is a new species for this ascomycete genus, which will be described separately. Its pathogenic status is still uncertain. The fungus was found only once during examinations of branches showing dieback symptoms (in Guaraciaba, state of Minas Gerais). Such diebacks are very commonly observed in the field in Brazil and are often very debilitating to the host plants. We have observed that most of the striking differences between healthy versus unhealthy plants when they are growing in exotic situations, such as in Florida, compared to plants from the native range, such as in Brazil, are the result of this dieback of a large proportion of branches, particularly the lower ones. The aetiology of this disease has thus far been elusive. Isolations from inner parts of diseased branches usually yield a range of sterile fungi, which do not yield disease symptoms when inoculated onto healthy plants. *Pleomassaria* was thought to perhaps be the aetiological agent involved in such diebacks. The fungus was isolated in pure culture but grew extremely slowly and did not sporulate. Nevertheless, work on this fungus should be continued to clarify its possible involvement in this spectacular disease.

Pseudocercospora sp.

Until this study, there were no members of the genus *Pseudocercospora* known to infect *S. terebinthifolius* or any other species in this genus. We found two kinds of *Pseudocercospora* spp. associated with leaf spots on *S. terebinthifolius*. They have distinct morphological features and may deserve to be treated as separate species, to be named and described in a separate publication. In terms of disease symptoms produced by these two species on *S. terebinthifolius*, they were essentially indistinguishable. Pathogenicity to *S. terebinthifolius* was demonstrated for one isolate. Because isolates of the two fungi did not sporulate in culture, Koch's postulates were performed with culture disks serving as inoculum. Disease symptoms produced with this method were not very severe and were different from those observed in the field. *Pseudocercospora* leaf spot was one of the most common diseases of *S. terebinthifolius* in the surveyed areas in Brazil and sometimes led to significant levels of defoliation. Unfortunately, it appears that *Pseudocercospora* spp. have no potential for classical biological control, as on two separate visits to Florida, one of us (RWB) collected leaf spots in the Everglades and near the town of Plantation, bearing *Pseudocercospora* colonies. Unless an especially virulent *Pseudocercospora* strain is obtained from Brazil, introductions of this fungus would probably be superfluous and innocuous, at least in Florida, as the fungus is already present in areas where S. *terebinthifolius* is a problem but is not reducing infestations.

Septoria sp.

This fungus was compared with other members of the genus *Septoria* described in the literature on members of the Anacardiaceae and is clearly distinct. It represents another new taxon discovered during this survey to be fully described in a separate publication. Although it grew slowly, *Septoria* sp. sporulated abundantly on VBA. Its pathogenicity to *S. terebinthifolius* was demonstrated, and abundant lesions formed and coalesced leading to substantial defoliation of inoculated plants. A preliminary host-range study performed with this fungus indicated that it is highly host-specific, which is often observed for other members of

Septoria as discussed in the review by Priest (2006). Fungi in this genus already have been used for classical biological control of weeds. Three examples involved introductions of species of *Septoria* into the USA, all coincidentally in Hawaii. *Septoria passiflorae* Syd. was introduced from Colombia in 1995 for biological control of *Passiflora tarminiana* Coopens (=*Passiflora mollissima*, *Passiflora tripartita*, *P. tripartita* var. *tripartita*) (Norman, 1995). Another *Septoria* sp. was introduced from Ecuador as a biological control agent against *L. camara* L. in 1993 (Trujillo and Norman, 1995). *Septoria hodgesii* Gardner was regarded by Gardner (1999) to have potential for biological control of *Myrica faya* (Ailton) Wilbur. It was also introduced in Hawaii in 1997 but did not establish, probably due to unsuitable environmental conditions at the release sites (E. Killore, personal communication). Excellent control of *L. camara* and *P. tarminiana* was reported after the introductions of *Septoria* spp. against these weeds (Trujillo, 2005). Likewise, *Septoria* sp. collected on *S. terebinthifolius* appears to have good potential as a classical biological control agent. It not only caused considerable damage through defoliation of infected plants in the field in Brazil but was found to be pathogenic to plants grown from seeds of *S. terebinthifolius* from Hawaii and Florida. More importantly, it appears to be host-specific, as it did not infect any of the other five species of Anacardiaceae (i.e. cashew, mango, Peruvian peppertree, Brazilian plum and tatapiririca) included in the preliminary host-range test performed during this study. Isolates of this fungus are now under additional evaluation in approved quarantine laboratories located in Hawaii (HDOA-Biological Control Labs, Honolulu) and in Florida (FLDACS, DPI Pathogen Containment Laboratory, Gainesville).

Stenella sp.

Most members of the genus *Stenella* are plant pathogens causing leaf-spot diseases. There are over 20 species described in the literature (Kirk *et al.*, 2001), but none was described in association with *S. terebinthifolius* or any other member of the Anacardiaceae. This fungus appears to be a new taxon of cercosporoid fungus, also to be described later. Observations in the field strongly indicated that this is a pathogenic fungus that forms extensive brown colonies on adaxial leaf surfaces, accompanied by abaxial yellowing and premature dropping of infected leaves. Unfortunately, pathogenicity was not proven during attempts to fulfil Koch's postulates. One possibility is that the use of culture disks of this fungus as inoculum was inadequate for that purpose or that an incompatible combination of fungal isolate and host genotype led to such a failure. This is, therefore, still considered in this study as an unresolved issue, and the subject of its potential for biological control of *S. terebinthifolius* will be pursued in a subsequent study.

The list of fungi already recorded in association with *S. terebinthifolius* (Table 1) contained 16 records from the USA, but only four from the centre of origin of the plant in the Neotropics. This survey, although preliminary and covering a small part of the native distribution of *S. terebinthifolius*, raised the number of fungi known to attack this species in the Neotropics to 14. This type of result is not uncommon and was already observed for other weeds native to Brazil such as *L. camara* (Barreto *et al.*, 1995) and *Chromolaena odorata* (L.) R.M. King & H. Rob. (Barreto and Evans, 1994). After becoming invasive in new, exotic situations, these plants become ubiquitous and an abundant substrate for saprophytic or generalist fungi. Fungal collections become frequent, and many fungal–host associations are then described and published. The majority of the records from the USA are probably explained by this approach. For instance, fungal species such as *Armillaria mellea*, *Armillaria tabescens*, *Ganoderma orbiforme*, *Botryosphaeria ribis*, and *Nectria cinnabarina* (Table 1) are well-known generalist pathogens. Conversely, fungi already recorded from the Neotropics or those newly recorded in this study are (with the clear exception of *H. lythri* and *P. concavum*) likely to be specialized host-specific pathogens.

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Testing the efficacy of specialist herbivores to control *Lepidium draba* in combination with different management practices

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Summary

Lepidium draba L. [=*Cardaria draba* (L.) Desv.; Brassicaceae] is a perennial mustard, indigenous to Eurasia. In the 18th century, *L. draba* was introduced into North America, where it is now listed as noxious in 16 states and three Canadian provinces. In 2001, a biological control program was initiated investigating the possibilities for biological control of *L. draba* in North America. To determine whether specialist herbivores are actually limiting population growth of *L. draba* in its area of origin and how their effect might interact with soil nutrients and management regimes, we established manipulative field experiments in Spring 2006 in eastern Romania, where five of the currently studied biological control agents are present. Plots (3 × 3 m) were established in already existing *L. draba* stands, and four treatments applied in a split-plot design: (1) grazing (yes, no); (2) cultivation (none, shallow, shallow + sowing of grasses/legume mix; (3) pesticide application to exclude herbivores and/or augmentation of specialists (yes, no) and (4) carbon addition in the form of sawdust to reduce plant available soil nitrogen (yes, no). First results indicate that cultivation and cultivation + sowing reduced the number of *L. draba* plants, however only at the beginning of the field season. As expected, neither pesticide nor sawdust applications had an effect on plant numbers or plant vigor in the first year; they will probably need more time to become apparent. We expect that grazing treatments, which will start in May 2007, will probably have the largest effect on *L. draba* vigor and densities. Final results should allow us to develop recommendations for an integrated management strategy for *L. draba*.

Keywords: integrated weed management, top–down effects, hoary cress, grazing, carbon addition, insect exclusion.

Introduction

Hoary cresses or whitetops, *Lepidium* spp. (=*Cardaria* spp.), are perennial mustards of Eurasian origin (Hegi, 1987), which were introduced to the USA in the late 19th century and have since then spread throughout the western and the northeastern states. They are aggressive invaders of crops, rangelands and riparian areas, but they grow particularly well in disturbed and/or irrigated areas (Lyons, 1998). Because they are difficult to control sustainably using mechanical or chemical methods, a consortium was established in Spring 2001 to investigate the scope for classical biological control. As a result of literature and field surveys conducted at CABI Europe–Switzerland between 2001 and 2003, seven phytophagous insect species were prioritized as potential biological control agents based on records of their restricted host range, and five species (four weevils and one flea beetle) are currently being investigated (Cripps *et al.*, 2005). In addition, one gall-forming weevil and an eriophyid mite are being studied by the US Department of Agriculture Agricultural Research Service and European Biological Control Laboratory in Montpellier, France, and Montana State University, Montana, respectively.

One of the assumptions in biological weed control is that, in their area of origin, invasive plants are regulated by natural enemies. However, evidence for the top–down regulative ability of herbivores is equivocal (Crawley, 1989; Price, 1992; Maron and Vilà, 2001 and references therein). The outcome of studies depended

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