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Towards the biocontrol of bindweeds with a mycoherbicide

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Abstract. Within the framework of the European COST Action 816, a five-year collaboration between scientists from five European countries has made an important contribution to biological control of field and hedge bindweeds (*Convolvulus arvensis* and *Calystegia sepium*, respectively). A fungus *Stagonospora convolvuli* strain LA39, able to infect both field and hedge bindweed, was found in the UK and its biocontrol efficacy improved by optimising mass production, formulation and storage techniques. This fungus controlled bindweeds in both a cemetery and in maize crops. Its use fits best in an integrated pest management system where a green cover controls most of the weeds except the bindweeds. DNA marker analyses indicate that the fungus reproduces sexually, which could be used to further improve this mycoherbicide. In addition, the insect *Melanagromyza albocilia*, which itself exhibits biocontrol potential against bindweeds, may be used in combination with LA39 to improve the ability of the fungus to penetrate the stem of bindweeds. Overall, the results suggest that *S. convolvuli* LA39 has promising potential as a bioherbicide for control of field and hedge bindweed.

Key words: calystegines, DNA marker, integrated weed management system, living mulch, phytotoxins, *Calystegia sepium*, *Convolvulus arvensis*

Introduction

Hedge and field bindweeds (*Calystegia sepium* [L.] R. Br. and *Convolvulus arvensis* L., respectively) are very successful weeds in agriculture and

amenity areas. They are deep-rooted perennials and thus can escape many chemical and mechanical weed control methods (Weaver and Riley, 1982; Westra et al., 1992). Chemical control is also restricted by the fact that chemicals effective against bindweeds can often affect many other plants, not to mention current public concern regarding environmental problems associated with chemical pesticide usage. In addition, the abundant reserves stored in the rhizomes of hedge bindweed and in the root of field bindweed enable the plants to survive repeated defoliations (Klimeš and Klimešová, 1994). Moreover, fragmentation and dispersal of underground parts leads to vegetative propagation (Maillet, 1988). In this context, biocontrol appears as a promising alternative against bindweeds (Table 1), both on farmland and in non-cropped areas (e.g. gardens and parks).

A five-year collaboration on biocontrol of bindweeds has been established between scientists from five European countries, within the framework of the European COST Action 816, and here we review the results of the project. We started with the collection of diseased field and hedge bindweed plants throughout Europe to search for a suitable control agent, which resulted in the selection of *Stagonospora convolvuli* Dearness and House strain LA39. The assessment of strain LA39 as a potential biocontrol agent included the determination of key genetic and pathogenic characteristics (i.e., phytotoxin production), dew requirement, spore concentration required for effective biocontrol, as well as the development of formulation, mass production and storage protocols. In addition, the impacts of LA39 on growth, biomass allocation and carbohydrate reserves of hedge bindweed were assessed before application in the field. Efforts were made to improve hedge and field bindweed biocontrol by combining the mycoherbicide with plant competitors, both in the greenhouse and the field. In addition, a survey of the phytophagous entomofauna associated with field bindweed was carried out to assess whether insects could act as vectors for pathogens and increase the effect of strain LA39. Lastly, the possible contribution to bindweed control by rhizosphere microorganisms capable of degrading calystegines, which are secondary metabolites of bindweeds (Tepfer et al., 1988b), was studied.

The biocontrol agent

Stagonospora convolvuli LA39, found in Long Ashton (UK) in 1994, was selected from approximately 600 fungal isolates as a possible biological control agent of field bindweed (Pfirter et al., 1997). Field bindweed was susceptible to the pathogen at all growth stages tested (Pfirter and Défago, 1998), showing brown lesions followed by defoliation and reduced plant growth. Several phytotoxins, with potential as herbicides, are produced in

Table 1. Potential biological control agents for field bindweed

Agent and taxonomic reference	Status and degree of control
EXOTIC MITES AND INSECTS[a]	
Aceria malherbae Nuzzaci (Acarina: Eriophyidae)	Canada. Ex Italy. Released in British Columbia and Alberta (1994), where the insect overwintered successfully, but establishment not confirmed. Not established in Manitoba or Saskatchewan. USA. Ex Greece. Released in Colorado, Maryland, Montana, New Jersey, Oklahoma, South Dakota, Texas and Washington (1989). Established in Montana, Texas[b] and Washington. South Africa. Ex Greece. Released in 1994.[c]
Tyta luctuosa (Denis and Schiffermüller) (Lepidoptera: Noctuidae)	Canada. Ex Italy. Released in Alberta and Saskatchewan (1989). Not established. USA. Ex Italy. Released in Arkansas, Iowa, Missouri, Oklahoma and Texas (1987). Not established. Released in Maryland (1991) and Washington (1996). Establishment not confirmed.
NATIVE MITES AND INSECTS[a]	
Chelymorpha cassidea (Fabricius) (Coleoptera: Chrysomelidae)	Canada. Native organism collected in 1979 in Saskatchewan and released in Alberta in an attempt to extend its range. Not established.
Chirida guttata (Olivier) (Coleoptera: Chrysomelidae)	Canada. Native organism collected in 1979 in Saskatchewan and released in Alberta in an attempt to extend its range. Not established.
Metriona purpurata (Boheman) (Coleoptera: Chrysomelidae)	Canada. Native organism collected in 1979 in Saskatchewan and released in Alberta in an attempt to extend its range. Established.
FUNGI	
Phomopsis convolvulus Ormeno[d] (Sphaeropsidales: Coelomycetes)	95% reduction in foliage biomass and up to 55% mortality on seedlings with 10^9 conidia/ml and 18 h dew. Up to 100% biomass reduction in pre-emergence application.
Phoma proboscis Heiny[e] (Sphaeropsidales: Coelomycetes)	Tested in the field during 1990–1993. Up to 90% seedling mortality.

[a] Julien and Griffiths (1998)
[b] Boldt and Sobhian (1993)
[c] Craemer (1995)
[d] Morin et al. (1990a) and Vogelgsang et al. (1999)
[e] Heiny (1994)

liquid media by strain LA39, one being identified as leptosphaerodione (Nicolet, 1999; Nicolet and Tabacchi, 1999).

The genus *Stagonospora* belongs to the Deuteromycota (Fungi imperfecti) in the class Coelomycetes, order Sphaeropsidales and family Sphaeropsidicaceae. So far, this genus has been neglected as a source of potential mycoherbicides, as the biocontrol literature describes only two other *Stagonospora*, i.e. *Stagonospora* sp. against bracken (*Pteridium aquilinum*

[L.] Kuhn) (Petrini et al., 1992) and *Stagonospora apocyni* (Peck) von Arx against hemp dogbane (*Apocynum cannabinum* L.) (Venkatasubbaiah et al., 1992). In 1991, Charudattan listed more than 100 potential agents for biological weed control, and most of them belonged to *Colletotrichum* spp., *Fusarium* spp., and *Alternaria* spp.

When dealing with disease-causing biocontrol agents, it is important to characterise their host range, and if non-target plants can be affected there is also the need to assess (risk analysis) persistence patterns and spread of the inoculum after release (Bourdôt et al., 2000). In the case of *S. convolvuli* LA39, necrotic lesions formed on all *Convolvulaceae* species tested (i.e., *Convolvulus arvensis*, *Convolvulus scammonia* L., *Convolvulus siculus* subsp. *agrestis* [Hochst. ex Schweinf.] Verdcourt, *Convolvulus tricolor* L., *Convolvulus humilis* Jacq., *Calystegia sepium*, *Ipomea purpurea* (L.) Roth [= *Convolvulus purpureus* L.], *Ipomea quamoclit* L. [= *Quamoclit pinnata* (Desr.) Bojer], *Ipomea hederifolia* L., and *Ipomea versicolor* Meissner [= *Quamoclit lobata* House]) except sweet potato *Ipomoea batatas* var. *batatas* [L.] Lam. Necrosis observed on field and hedge bindweeds was much more severe than on the other *Convolvulaceae* species. The susceptible species of the *Convolvulaceae* are not common at the sites where biocontrol of bindweed would be of interest. The fungus had no effect on the crop plants (i.e., *Zea mays* L., *Triticum aestivum* L., *Lolium multiflorum* Lam., *Sinapis alba* L., *Medicago sativa* L., *Trifolium pratense* L., *Trifolium suaveolens* Willd., *Vitis vinifera* L.) that could be common at application sites (Pfirter and Défago, 1998). Therefore, the specificity of strain LA39 is better than that of *Phytophthora palmivora* (Butler) Butler, the active ingredient in the commercial product DeVine® used for control of stranglervine (*Morrenia odorata* L.). Indeed, the latter fungus is pathogenic to several crop plants such as onion (*Allium Cepa* L.), citrus (*Citrus limon* [L.] Burm.) and pea (*Pisum sativum* L.) (Ridings, 1986), but safety of the product with regards to non-target plants was achieved using site-specific application protocols (Ridings, 1986).

The genetic relationship between *Stagonospora convolvuli* LA39 and 37 *Stagonospora* isolates (taken from field and hedge bindweeds) and 10 *Septoria* isolates (from field bindweed) was assessed using Restriction Fragment Length Polymorphism (RFLP) analysis of PCR-amplified Internal Transcribed Spacer (ITS) region and the comparison of Randomly Amplified Polymorphic DNA (RAPD) markers. The genus *Septoria* was included in the study because of its close relatedness with *Stagonospora*. Little variation was found in the PCR-RFLP assay, where only three types of banding patterns were found. In contrast, 26 different groups could be distinguished based on cluster analysis of RAPD data (Pfirter et al., 1999a). PCR-RFLP data

Table 2. Main clusters ($n > 2$ isolates)[a] obtained by cluster analysis (UPGMA) of 38 isolates of *Stagonospora* sp. and 10 isolates of *Septoria* sp. based on RAPD markers (Pfirter et al., 1999a). LA39 was included in RAPD cluster C1

RAPD cluster	*n*	PCR-RFLP type[b]	Fungal genus	Geographic origin[c]	Plant host[d]
C1	11	A	*Stagonospora*	UK: Long Ashton ($n = 10$) or Yeorile ($n = 1$)	*C. arvensis* or *C. sepium*
C2	5	A	*Stagonospora*	UK: Long Ashton ($n = 1$) or Yeorile ($n = 4$)	*C. arvensis* or *C. sepium*
C3	3	B	*Stagonospora*	UK: Long Ashton	*C. arvensis*
C4	5	C	*Stagonospora*	CH, CzR, Fra	*C. sepium*
C5	7	C	*Septoria*	CH, CzR, Fra, You	*C. arvensis*

[a] RAPD clusters were defined using an arbitrary value of 0.96 for Dice similarity coefficient.
[b] PCR-RFLP analysis was carried out on the Internal Transcribed Spacer (ITS) region.
[c] CH, Switzerland; CzR, Czech Republic; Fra, France; UK, United Kingdom; You, Yougoslavia.
[d] In C1 and C2, all isolates from Yeorile were obtained from *C. sepium* and those from Long Ashton were obtained from *C. arvensis*.

and RAPD clusters were generally in agreement. RAPD clusters allowed classification of the fungi according to genus, collection site and host plant (Table 2), as well as year of sampling. Strain LA39 corresponded to PCR-RFLP type A, but could be distinguished from all other type-A isolates when PCR-RFLP patterns were compared in more detail. The variation between isolates collected from a same place in different years suggests that sexual reproduction occurs under natural conditions, but a sexual stage has not been observed yet. In *Stagonospora* nodorum (Berkeley) Castellini et Germano and S. avenae (Frank) Bissett, the two best-known species of the genus, the sexual stages are known as *Phaeosphaeria nodorum* (Müller) Hedjaroude and *P. avenaria* (Weber) Eriksson, respectively.

Six *Stagonospora* isolates were tested for pathogenicity on three field bindweed ecotypes, which originated from the USA, England and Switzerland. Differences were found both in terms of pathogenicity and host susceptibility. Only strain LA39 was highly pathogenic on all ecotypes (Table 3) (Pfirter et al., 1999a). All *Stagonospora* isolates from the UK but LA39 showed a lower degree of pathogenicity on the UK ecotype compared with the Swiss ecotype. The US ecotype was as susceptible as the Swiss ecotype, except for one Swiss strain and one from the UK (Pfirter et al., 1999a).

Table 3. Susceptibility of field bindweed ecotypes to six isolates of *Stagonospora* sp. at two weeks after inoculation with 10^7 spores per ml sprayed until run off occurred (Pfirter et al. 1999a)

Stagonospora isolates	Susceptibility of the ecotypes (%)[a]		
	CH	USA	UK
LA39 (UK)[b]	100 (97)	97	102
LA30B (UK)	100 (97)	99	55
LA24 (UK)	100 (90)	96	36
LA31 (UK)	100 (99)	99	37
LA10A (UK)	100 (89)	34	60
92 Co a (CH)	100 (50)	38	51

[a] Susceptibility of the ecotypes from the USA and the UK is expressed by comparison with susceptibility of the Swiss (CH) ecotype, which is arbitrarily shown as 100%; in brackets: the percentage of the necrotic leaf area caused by the isolates on the Swiss ecotype.
[b] Country of origin of the isolates.

Formulation, mass production and storage of *Stagonospora convolvuli* LA39

The goal of a formulation is to facilitate storage, handling and application of an active ingredient. With a mycoherbicide, formulation may also enhance biocontrol efficacy (Greaves et al., 1998), for example by reducing dew requirement during infection. Formulation of conidia of *S. convolvuli* LA39 in a 10% vegetable oil emulsion (Potyka, 1995) significantly increased biocontrol effectiveness, reducing the conidial dose needed to cause 80% necrotic leaf area by a factor of 10 (i.e., from 10^8 to 10^7 spores per ml sprayed until run off). The formulation was developed for *Mycocentrospora acerina* (Hartig) Deighton against field pansy (*Viola arvensis* Murr.) and reduced the dew period needed to infect and kill field pansy from >36 h to 12 h (Potyka, 1995). Under field conditions, disease development took place even when exposure of LA39-treated bindweed plants to 100% relative humidity was delayed by up to 8 h after mycoherbicide application. Importantly, severe disease was found even without exposure to 100% relative humidity (Pfirter and Défago, 1998). As dew is irregular in the field, resistance to short-term desiccation greatly increases the suitability of a pathogen as a control agent. The favourable effect of the emulsion may be explained by extraction of water from leaf tissue cells and a better spore distribution in spray droplets (Lawrie et al., 1997; Greaves et al., 2000). Electron microscope examination of transverse sections of field pansy leaves showed that the oil phase had penetrated into the leaf tissue and contained many small droplets of water.

Table 4. Spore production of *Stagonospora convolvuli* strain LA39 on five different solid substrates 15 days after inoculation

Substrate	Spores ($\times 10^8$) per g substrate
Couscous	4.7 a
Maize semolina	2.8 b
Hard wheat semolina	1.1 c
Soya beans	0.7 c
Wheat bran	0.3 c

Values marked with the same letter are not significantly different ($p < 0.05$) (Pfirter et al. 1999b)

This was also observed in the deposits on the leaf (Greaves et al., 2000). Klein et al. (1995) reported that, compared to aqueous formulations, canola and soybean oil (0.5, 1, 5, and 10%) emulsions improved control of Bathurst burr (*Xanthium spinosum* L.) by *Colletotrichum orbiculare* (Berk. and Mont.) Arx in the field in 1991–1992, but not in 1992–1993. Oil-in-water formulations are relative inexpensive, easy to prepare and can be applied with standard equipment. This formulation was used in all our mycoherbicide experiments reported here.

Effective production of *S. convolvuli* LA39 on couscous (cracked hard wheat, *Triticum durum* Desf.) highly improved its potential to become a cost-effective bioherbicide (Pfirer et al., 1999b). Couscous was selected after comparison of 17 solid substrates (e.g. maize semolina, hard wheat semolina, wheat bran, soya beans, spaghetti, lentils, pieces of carrots, bean seeds) because it is relatively inexpensive and easy to purchase and handle. Compared with V8-juice agar, which is the standard substrate for the cultivation of *Stagonospora* spp., couscous enabled to produce 80 times more spore material per weight unit of substrate (i.e. up to 4×10^8 spores/g substrate; Table 4) (Pfirter et al., 1999b). Various solid substrates have been evaluated for spore production by *Phomopsis convolvulus*, a potential mycoherbicide for field bindweed (Morin et al., 1990b). Pot barley grains produced a similar yield of virulent conidia (5×10^8 conidia/g substrate) as we found with *S. convolvuli* strain LA39 on couscous.

A potential bioherbicide should have an appropriate shelf-life in order to pass through the market network and allow storage before application. Conidia dried on kaolin and stored at 3 °C stayed viable for 180 days, though germination declined to 70% and 50% after 140 and 175 days, respectively. The dried conidia were easily rehydrated and formulated in oil emulsion. No loss in pathogenicity was observed (Pfirter et al., 1999b). This strategy

seems, therefore, acceptable for commercial use. Nevertheless, further studies should be carried out to develop granule-based formulations, which afford physical protection, provide nutrients for the incorporated conidia and can be directly added to the soil. This approach has been developed with so-called 'Pesta' granules (Connick et al., 1991), containing fillers such as wheat flour and kaolin, which may confer advantages to *S. convolvuli* LA39 conidia. So far, solid carriers have mostly been used with mycoherbicides applied to soil (e.g. *Fusarium oxysporum*, *Phomopsis convolvulus*, Connick et al., 1998; Vogelgsand et al., 1998). The pathogen may either sporulate on the substrate or directly infect the host by mycelial growth, as described for *Sclerotinia sclerotiorum* (Lib.) deBary against Canada thistle (*Cirsium arvense* [L.] Scop.) (Brosten and Sands, 1986).

Field release and monitoring

The development of a bioherbicide must consider its application in the range of field conditions it would meet in commercial use. *S. convolvuli* LA39 was applied in the field using standard spraying technique (i.e. flat fan nozzles operating at 2 bars; 300–600 l per ha; 3×10^{11} to 10^{15} spores per ha) (Guntli et al., 1998, 1999a; Pfirter et al., 1997).

In 1995, *S. convolvuli* LA39 was applied in a field trial with maize (Pfirter et al., 1997). Ground cover with bindweed in the *Stagonospora*-treated plots did not increase and remained at 14.6%; the necrotic leaf area reached 78% (45.4% of leaves dead). In the control plots (treated with the fungicide benomyl), ground cover increased by 115%, and necrotic leaf area was 13.8% (6% of leaves dead) (Pfirter et al., 1997). Field trials with *S. convolvuli* LA39 in maize were continued in 1996 and 1997 using ground cover with red clover (*Trifolium pratense* L.) to compete with hedge bindweed (Guntli et al., 1999a). In both field trials, the fungus caused severe epidemics. Disease severity increased throughout the observation periods. Humid, cool weather increased disease severity. In 1997, up to 79% of the leaf surface was necrotic at 10 weeks after the application, but only 34% in 1996. Thus, ground cover by bindweed was reduced by 83% in 1997 and 70% in 1996 (Table 5). Undersowing with red clover had no positive effect on bindweed control (Guntli et al., 1999a). Living green cover used in maize crops (the so-called 'maize meadow'; Burgos and Talbert, 1996; Garibay, 1996; Garibay et al., 1997; Hall and Hartwig, 1990) can suppress many weeds but not bindweeds (Garibay et al., 1997). Using *S. convolvuli* LA39 to control the escaping bindweed would fit well in an integrated pest management system. Application of a bioherbicide to a broad weed spectrum, where bindweed would be replaced by another weed, seems unwise. A mixture of various mycoherbicides is a

Table 5. Effect of the mycoherbicide *S. convolvuli* strain LA39 on ground coverage of hedge bindweed in a maize field in 1996 and 1997 (Guntli et al., 1999a)

		Ground coverage with hedge bindweed (%) Weeks after application					
Year	LA39[a]	0	2	4	6	8	10
1996	−	26	70	95	93	90	71
	+	26	49	64*	55*	34*	21*
1997	−	19	67	68	64	63	58
	+	19	36*	21*	19*	12*	10*

[a] LA39 was applied at 3×10^{12} spores per ha.
* Statistically different from the untreated control according to Bonferroni's protected test ($p < 0.05$).

possible answer to this problem but they should all be formulated and applied in the same way to become a real alternative to broad-spectrum chemical herbicides. Careful integration of bioherbicides into any pest management system is essential for successful product promotion (Müller-Schärer and Scheepens, 1997). LA39 offers potential for such integration.

The potential of *S. convolvuli* LA39 as a bioherbicide was also demonstrated in an amenity area (a cemetery), where cotoneaster (*Cotoneaster dammeri* CK. Schneider) was heavily infested by field bindweed (Guntli et al., 1998). Within 20 days after application of 3×10^{12} spores per ha, 60% of the bindweed leaf area was necrotic, this increasing to over 80% after 40 days. Cover of the cotoneaster plants by bindweed decreased from 40% to 17% in the plots treated with *S. convolvuli* LA39.

Effects of *Staganospora convolvuli* LA39 on bindweed growth

The effects of *S. convolvuli* LA39 on hedge bindweed were studied in pots placed outside to mimic field conditions (Guntli, 1998). Pots were used to allow observation of the entire root systems of the plants and to eliminate possible interference by other plants (Klimeš and Klimešová, 1994). However, this approach does restrict the development of the underground plant parts. Hedge bindweeds were grown from seeds or rhizomes to represent both modes of propagation. In agricultural fields, transmittable seeds usually cause new infestations while rhizomes are responsible for bindweed multiplication in an already infested field. This experiment included two levels of nutrient (i.e. high level: 262.5 mg/l N, 225 mg/l P_20_5, 300 mg/l

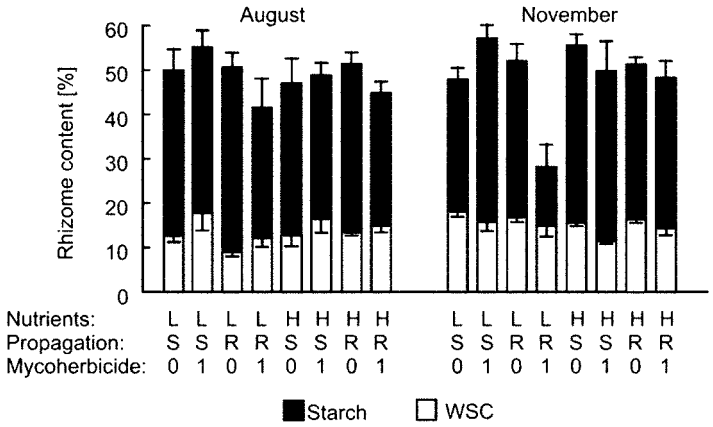

Figure 1. Effect of mycoherbicide LA39 on contents in starch and water-soluble carbohydrates (WSC) in rhizomes of hedge bindweed in August and November. Factors studied included nutrition level (L: low; H: high; see text), bindweed propagation method (S: from seeds; R: from rhizomes) and mycoherbicide (0: not applied; 1: applied at 10^7 spores per ml sprayed until run off). Error bars indicate standard error (Guntli, 1998).

K_20, pH 6.0; low level: one third of the high level) representative of levels likely to be met in the field, as fertiliser levels are known to affect the growth of hedge bindweed (Klimeš and Klimešová, 1994).

Application of *S. convolvuli* LA39 resulted in infected hedge bindweeds with fewer leaves, taller climbing stems with greater number of nodes and flowers and rhizomes containing less carbohydrate reserves (Figure 1). However, total plant biomass was not affected. Necrosis of leaves and defoliation caused by the fungus probably reduce the photosynthesis rate, which is known to increase leaf production in bindweeds (Bakke and Gaessler, 1945). However, we observed a reduction in the number of leaves. This suggests that the effect of the fungus on the bindweed was important, particularly when considering the reduction in rhizome carbohydrate reserves, and could not be completely compensated for. Possibly, the new leaf surface was produced at the cost of rhizome carbohydrate reserves. The amounts of these largely influences winter survival and subsequent emergence of the weeds (Frazier, 1943; van Ast and van Groenendael, 1993). Therefore, *S. convolvuli* LA39 might have the potential to control bindweeds in the long term by depleting the carbohydrate reserves stored in the rhizomes (Figure 1).

The interaction of bindweeds with microorganisms able to degrade bindweed alkaloids

Calystegines (tropane alkaloids) are produced by hedge and field bindweeds and a few other plants species (Tepfer et al., 1988b; Fellows et al., 1992; Dräger, 1995). In bindweeds, they are present in high quantities in roots and rhizomes (Tepfer et al., 1988b). Calystegines can be poisonous to arthropods (Nash et al., 1993) and mammals (Todd et al., 1995), presumably as a result of glycosidase inhibition (Asano et al., 1996; Molyneux et al., 1993). They may also be allelopathic to plants (Goldmann et al., 1996).

Calystegine-degrading microorganisms are present in the rhizosphere of many plant species (Tepfer et al., 1988a; Goldmann et al., 1996; Guntli et al., 1999b). Although they can be found also in the rhizosphere of calystegine-negative plants, their prevalence is higher in the rhizosphere of calystegine-positive plants, such as hedge bindweed (Guntli et al., 1999b). Moreover, calystegine-degrading rhizobacteria are selectively favoured for the colonisation of bindweed rhizosphere, as shown with *Sinorhizobium meliloti* (Dangeard) De Lajudie strain Rm41, which harbours the genes for calystegine catabolism (cac genes) on the non-symbiotic plasmid pRme41 (Guntli et al., 1999c; Tepfer et al., 1988a). Similarly, plants producing opines have been shown specifically to favour rhizosphere microorganisms capable of using these compounds as carbon sources (Guyon et al., 1993; Hartwig et al., 1990; Oger et al., 1997; Savka and Farrand, 1997).

Bindweed development and health were not affected by the presence of calystegine-degrading rhizobacteria in the rhizosphere, even when the bacteria were provided experimentally (gnotobiotic conditions) with the opportunity to grow and colonise the roots of bindweed at unusually-high population levels (in excess of 10^7 CFU/g root) (Guntli et al., 1999c). Furthermore, inoculation of calystegine-degrader strain Rm41 had no effect on the biocontrol efficacy of the mycoherbicide LA39 (Guntli, 1998). However, because of its specific advantage for rhizosphere colonisation, the ability to use calystegines as nutrient source could be a useful trait to search for in rhizobacteria that are deleterious to bindweed. This would ensure that screening programmes designed to find novel biocontrol agents against bindweeds do not yield strains restricted by poor root-colonising ability. Alternatively, the genes for calystegine degradation could be inserted in bacteria deleterious to bindweeds. Attempts to mobilise the catabolic plasmid pRme41a into other bacterial species have failed so far, but other genetic strategies could be considered since the cac genes have been cloned (Boivin et al., 1990).

Calystegines are not only produced in underground structures of bindweeds but also in stems and leaves (Hoeke and Dräger, unpublished). There-

fore, interactions between bindweeds and microorganisms in the phyllosphere might be similar to those in the rhizosphere. Whether microorganisms present in the phyllosphere of bindweeds and/or fungal biocontrol agents of bindweeds (Table 1) display calystegine degradation ability is unknown (Heiny, 1994; Morin et al., 1990a; Ormeno-Nunez et al., 1998; Vogelgsang et al., 1998, 1999), even in the case of the mycoherbicide LA39. From the viewpoint of biocontrol however, this trait is probably of less interest in the phyllosphere where an effective microbial biocontrol agent (LA39) is already available.

Insects associated with field bindweed

An extensive survey of the entomofauna associated with field bindweed in Slovakia was done in 1997–1998 (Tóth, 2000; Tóth et al., 1998). Of 108 insect species belonging to 5 orders and 17 families (e.g., Coleoptera, Lepidoptera, Diptera), the fly *Melanagromyza albocilia* Hendel (Diptera: Agromyzidae), two flea beetles *Longitarsus pellucidus* Foudras and *L. longipennis* Kutschera (Coleoptera: Chrysomelidae) and one tortoise beetle, *Hypocassida subferuginea* Schrank (Coleoptera: Chrysomelidae) were selected as possible biocontrol agents. *Aceria malherbae* and *Typha lactuosa* used by others to control bindweeds (Table 1) (Boldt and Sobhian, 1993; Craemer, 1995; Julien and Griffiths, 1998; McClay et al., 1999; Rosenthal and Platts, 1990) were also found in the study.

M. albocilia, a native European stemborer of *C. arvensis*, was considered by Rosenthal and Buckingham (1982) and Tóth et al. (1998) as promising candidate for biological control of bindweed. Larvae are boring a tunnel in bindweed stem and crown of roots, causing death of infested shoots (Spencer, 1973). Also, the fly can promote secondary damage due to infection by pathogenic microorganisms. During 1998–2000, thirty *C. arvensis* plants were checked every week, starting at mid May at the locality Tehla (48°12′ N 18°23′ E; 175 m) in Slovakia. For each selected plant, number of healthy, dried and infested sprouts were recorded. The total amount of infested and dried bindweed shoots ranged from 38% in August to 50% in October. At the end of the autumn, 100% of the plants were infested. Infestation effects were more evident on plants growing in wild areas and dry fields than in irrigated fields and other moist areas (Tóth, 2000).

Conclusions and outlook

The fungus *Stagonospora convolvuli* effectively controlled bindweeds in both amenity and crop situations. It is best fitted for use in an integrated

pest management system where a green cover controls most of the weeds except the bindweeds. Its commercial potential needs to be enhanced through improved mass production, formulation and storage techniques. Recent discoveries of secondary metabolites of the fungus (potential phytotoxins) and the weed (calystegines) might be of key importance. DNA marker analyses indicate that the fungus reproduces sexually, which might be useful to further improve the strain. Also, the insect *M. albocilia* might improve the ability of the fungus to penetrate the stems of bindweed.

A study of the genetic variation among isolates of *Stagonospora* sp. collected in different European countries suggested that identification and tracking of the strain applied as a mycoherbicide is possible. The data obtained, along with further research on disease development under different environmental conditions, would allow epidemics of strain LA39 to be modelled to optimise timing of application for effective disease development and control of bindweeds. The development of DNA markers for the host plants, analogous to those described for the pathogens, will allow studies on the impact of the pathogens on bindweed population structure. The use of molecular methods will help with clarification of pathogen taxonomy, tracking of released bioherbicide and assessment of its impact on host population structure, thereby facilitating standardisation of protocols for the development and the release of biological control agents.

So far, *S. convolvuli* LA39 has been studied only where fungicides were not used. Application to other agricultural areas (e.g. vineyards) will necessitate compatibility with commonly used fungicides.

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