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Evaluation of European pathogens for the control of *Myriophyllum spicatum* in the United States of America

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Abstract. Surveys for *Myriophyllum spicatum* (Eurasian watermilfoil) have been carried out over a two-year period in the United Kingdom and parts of mainland Europe. Over 400 potential pathogens, 56 identified species from 39 genera, have been obtained from material collected, and to date 324 have been screened for activity against *M. spicatum*. Of the isolates tested, 15 show some potential to control the weed, by causing tissue senescence and reducing the growth of the plant. Twelve of these isolates were successfully reisolated from the plant tissue. Work is continuing to evaluate their biological control potential.

Introduction

Myriophyllum spicatum L. (Haloragidaceae) is a submerged aquatic plant that grows in a wide range of environmental conditions, in both fresh and brackish waters. In weedy situations, the plants are fast growing and form dense mats of foliage that interfere with the normal usage of water courses. Reproduction is by fragmentation of stems and the development of over-wintering buds; seed formation also occurs but may play little part in the spread of the weed.

Myriophyllum spicatum is widely distributed throughout the United Kingdom, particularly in still water in lime-rich areas, with records from Cornwall through to the Outer Hebrides. It occurs in most European countries from Scandinavia in the north to Sicily in the south (Kew Herbarium Records). It also occurs in most of Asia as well as in eastern Africa (Harley and Forno 1990).

Myriophyllum spicatum has been a problem in the United States of America since the 1930s (Harley and Forno 1990). In the 1950s and 1960s, it became an important ecological and economical weed in larger bodies of water in North America, with reports from the Potomac River, Chesapeake Bay and the Tennessee Valley Authority reservoirs (Bates *et al.* 1972; Reed 1977). As an ecological problem, *M. spicatum* can greatly reduce the numbers of naturally-occurring aquatic plant species. In Lake George (New York State) for example, *M. spicatum* cover increased from 2% in 1987 to 20-45% in 1989, over the same period the number of other plant species fell from 20 - 9 (Madsen *et al.* 1990).

Attempts to control *M. spicatum* have involved both mechanical and chemical methods. Mechanical clearance can be cheaper than chemical alternatives, but needs to be carried out twice during the summer to produce a reasonable reduction in plant biomass. Herbicide applications have been successful, both underwater applications made by boat and aerial applications can give good control. However, applications of chemical herbicides need careful consideration. Due to the dilution from a body of water, large amounts of herbicide need to be applied, and, if control is not sufficient, reinfestation can be rapid. The chemical applied needs to be specific, and to be persistent enough to control the weed with no residual activity.

Many of the early investigations into biological control agents for *M. spicatum* concentrated on insects. Species on other *Myriophyllum* sp. from within the USA have been identified as possible control agents. A pyralid moth, *Acentria nivea,* found in stands of *Myriophyllum exalbescens* in the St Lawrence River caused leaf loss and girdling of stems (Batra 1977). Surveys, predominantly for insect agents, have also been carried out in Pakistan, Bangladesh and much of eastern Europe and Asia (CIBC 1970; Harley and Forno 1990). However, many of the insects found were non-specific to the target weed and of limited potential as biological control agents.

The use of pathogens has long been regarded as a

good potential method of biological control for *M. spicatum* (Freeman and Charudattan 1981). Work has been undertaken on isolating and assessing fungal pathogens from within the USA; *Acremonium curvulum* and *Fusarium sporotrichoides* were tested at Wisconsin, but, although capable of causing lesions, both failed to control the weed in large-scale tests (Andrews and Hecht 1981; Andrews *et al.* 1982; Charudattan 1990). A fungal pathogen, *Colletotrichum gloeosporioides*, found on *M. spicatum* in Wisconsin, has been tested as a mycoherbicide, with three possible chemical herbicides at 10% of their recommended concentration (Sorsa *et al.* 1988). *Mycoleptodiscus terrestris*, has been tested against *M. spicatum* and a series of aquatic weeds and terrestrial crop plants, and has been shown to be virulent and reasonably specific (Verma and Charudattan 1993). Endophytic fungi have been reported in the literature on *Myriophyllum* sp., in both Europe and the USA (Sparrow 1974; Luther 1979) and appear to be very damaging.

Myriophyllum spicatum constitutes part of the natural aquatic flora throughout most of Europe and rarely reaches weed status. However, some of these ecosystems (in central western Europe) have recently been invaded by the North American exotic species *Myriophyllum heterophyllum* (Spangehl and Scharrenberg 1986). Domination in these areas by the introduced species would tend to indicate that a different spectrum of natural enemies occurs in Europe and that a search for a fungal biological control agent for *M. spicatum* within Europe would be beneficial.

Materials and methods

Surveys

From plant records (Kew Herbarium, National Water Boards and the Terrestrial Ecological Surveys), sites of *M. spicatum* were selected to give a range of locations and environmental conditions. Sites were sampled over a two-year period (1994-1995) during the growing season (May-October). *Myriophyllum spicatum* and other *Myriophyllum* species were collected. Samples of water and soil were also taken in some cases.

Isolations

Isolations from diseased tissues of *M. spicatum* collected during the surveys, were carried out following standard procedures, plants were washed under running tap water for two hours and rinsed in sterile distilled water before being placed on tap water agar (TWA). Samples of soil and water were also plated onto media selective for *Fusarium* species (Komada 1975), and specific baits were employed for Oomycetes and aquatic fungi. Cultures were forwarded to the International Mycological Institute (IMI) for identification.

Screening

Isolates of species that are commonly pathogenic to plants, and those species that were isolated constantly from several sites were screened against *M. spicatum*.

Sections of plants (with two nodes) were cut, weighed (after excess surface water was removed), and placed in 100 ml of sterile distilled water in a jar. These were inoculated with either two 9 mm agar plugs or 10^4 or 10^6 spores per ml of suspension (dependent upon sporulation of the isolate) and kept at a constant 25°C with 12 h light. Two uninoculated controls were included. After three weeks the plant was visually assessed for any indication of infection. After a further two weeks samples were again visually assessed, reweighed (after excess surface water was removed), and plated onto TWA with antibiotics for identification and proof of pathogenicity (Koch's postulate). Comparisons of initial and final weights were made to give an indication of any effects in the absence of physical signs of infection (it was noted during field collecting that the plants showed few lesions or other signs of infection).

Results and discussion

Surveys

Over the two seasons of the project, surveys have been carried out at nearly two-hundred sites in twelve European countries covering most of England, Wales and Scotland, as well as eastern France, northern Italy, northern Spain, northern Switzerland, southern Germany, central Austria, central Ireland, Portugal and Slovenia. Sites from which *M. spicatum* was collected varied in character from ponds and drainage ditches to large lakes, rivers and canals. Plants were found in both still- and fast-flowing water, and at depths from 5-8 cm to 4-5 m (in the clear waters of some of the southern European lakes). Though normally found in water of a neutral to alkaline pH, in a few sites in Scotland *M. spicatum* was found in water which, due to the surrounding peat, was mildly acidic. As the acidity increased *M. spicatum* was replaced by *M. alternifolium*.

The growth characteristics of the plants often varied, depending upon site features: in fast-flowing, shallow rivers, plants had noticeably red stems which trailed up to 1 m downstream, rooted at several points. In slower-moving rivers and canals, plants had more branched stems, larger leaves (up to 3 cm in the Royal Canal, Ireland) and more surface detritus. In lakes, the major change in character was dependent upon the depth at which the plant was growing: at shallow edges, stems could be only a few centimetres long increasing to several metres in deeper water. Plants grew deeper in the clearer and warmer southern European lakes compared to the more cloudy, colder northern lakes in England and Scotland. When returned to the standard laboratory conditions all plant samples grew in similar fashion indicating that these are ecotypes rather than biotypes.

Isolations

From the plant material (*M. spicatum* and related species), and from the water and soil samples that were collected, over 400 isolates (from normally pathogenic genera) have been isolated, 56 identified species from 39 genera (Table 1). There was no correlation between the species isolated and the collection site, either environmentally or geographically. The majority of isolates are common; *Fusarium* sp. and *Acremonium* sp. have been routinely isolated from all types of locations. Significantly, *Gliocladium roseum* has only been isolated from lakes and ponds and not from rivers. A few isolates are specific aquatic fungi; e.g. *Cylindrocarpon aquaticum* and *Nectria lugdunensis* from the Crinnean Canal in Scotland. Several isolates have been unusual records, such as the two *Embellisia* sp. isolated from Texel in Holland and Slapton Ley in

Table 1. Fungal species isolated from *Myriophyllum spicatum* during two years of surveys in Europe.

Absidia cylindrospora Hagem.
Acremonium strictum W. Gams.
Acremonium persicinum (Nicot.) W. Gams.
Acrophialophora levis Samson & T. Mahmood.
Alternaria infectoria E.G. Simmons. Agg.
Ascochyta sp. Lib.
Aureobasidium sp.Viola & Boyer.
Byssochlamys nivea Westling.
Botrytis cinerea Pers.
Chrysosporium sp. Corda
Cladobotryum sp. Corda
Colletotrichum dematium (Pers.: Fr.) Grove.
Coniothyrium fuckelii Sacc.
Coniothyrium sporulosum (W. Gams & Domsch) Aa.
Corynascus sepedonium (Emm.) Arx.
Cryptosporiopsis sp. Bub. & Kabat.
Cylindrocarpon destructans (Zinssm.) Scholten.
Cylindrocarpon aquaticum (Sv. Nilsson) Maranova & Descals
Cylindrocladium sp. Morgan
Embellisia sp. *Embellisia* cf. *telluster* E.G. Simmons.

Emericellopsis minima Stolk.
Fusarium acuminatum Ellis and Everhart
Fusarium avenaceum (Fr.) Sacc.
Fusarium crookwellense Burgess, P.E. Nelson and Touss.
Fusarium culmorum (W.G. Sm.) Sacc.
Fusarium equisitii (Corda) Sacc.
Fusarium flocciferum Corda.
Fusarium graminearum Schwabe.
Fusarium oxysporum Schlecht.
Fusarium poae (Peck) Wollenweber.
Fusarium sambucinum Fuckel
Fusarium solani (Martius) Sacc.
Fusarium sporotrichiodes Sherbak.
Fusarium sp. Link.
Geotrichum candidum Link.
Gliocladium catenulatum J.C. Gilman & E.V. Abbott.
Gliocladium roseum Banier.
Gliomastix murorum var. *felina* (Marchal) S. Hughes.
Glomerella cingulata (Stoneman) Spauld.& H. Schrenk.
Microdochium tabacinum (T.H. Beyma) Arx.
Microsphaeropsis sp. Höhn.
Mycocentrospora acerina (Hartig) Deighton.
Myrothecium cinctum (Corda) Sacc.
Myrothecium roridum Tode.
Nectria discophora (Mont.) Mont.
Nectria lugdunensis J.Webster
Phaeoseptoria sp. Speg.
Phoma complanata (Tode) Desm.
Phoma dennisii Boerema
Phoma eupyrena Sacc.
Phoma exigua Desm.
Phoma hedericola (Dur. & Mont.) Boerema.
Phoma leveillei Boer. & G.J. Bollen.
Phoma macrostroma Mont.
Phoma nebulosa (Pers.:Fr.) Berk.
Phoma tropica R. Schneid.& Boerema.
Phoma sect. Paraphoma (Morgan-Jones & White) Boerema
Phoma sp. Desm.
Phomopsis sp. Sacc.
Pithomyces chartarum (Berk. & M.A. Curtis) M.B. Ellis
Plectosphaerella cucumerina (Lindf.) Gams.
Pythium sp. Pringsh.
Pythium sp. group F
Pythium sp. group HS
Pythium sp. group T
Pythium aquatile Höhnk.
Pythium acanthophoron Sideris.
Pythium periplocum Drechsler.
Pythium scleroteichum Drechsler.
Sclerotium hydrophilum Sacc.
Stagonospora sp. Sacc.
Saprolegnia parasitica Coker.
Trichosporiella sporotrichoides Oorschot.
Verticillium nigrescens Pethybr.

Table 2. Isolates which show a degree of control of *Myriophyllum spicatum*. * - no identifiable spore types present. # -screened twice and produced consistent results.

Isolate number	Isolation site	Isolate name	Symptoms
Mir 93b IMI 368670	Lac de Longemer, France	*Gliocladium roseum*	death or necrosis of tissues limited new growth (reisolated) x2#
Mir 49a IMI 368845	River Hart, England	Hyphomycete Indeterminate sp.*	death of old tissue and reduction in new growth (reisolated) x2#
Mir 80c IMI 368033	Grasmere, England	Hyphomycete Indeterminate sp.*	death of old tissue and reduction in new growth (reisolated) x2#
Mir 68c	Chantry Point Ditch, England	*Acremonium* sp.	death of old tissue and reduction in rate of growth (reisolated) x2#
Mir 80b IMI 364456	Grasmere, England	*Cylindrocarpon destructans*	necrosis of old tissues and reduction in rate of growth (reisolated) x2#
Mir 3iii IMI 359296	Slapton Ley, England	*Embellisia telluster*	death of old tissue and reduction in rate of growth (reisolated) x2#
Mir16	Wicken Fen, England	*Fusarium solani*	death of old tissue (reisolated)
Mir 59 IMI 368844	Dockens Water, England	*Geotrichum candidum*	death and necrosis of tissue and reduction in growth (reisolated)
Mir 35 IMI 362768	River Great Ouze, England	Coelomycete Indeterminate sp.*	death of old tissue and reduction new growth (reisolated)
Mir 36	Soham Lode, England	Coelomycete Indeterminate sp.*	death of old tissue and reduction new growth (reisolated)
Mir 34 IMI 362766	Harold and Odell Pond, England	*Gliocladium roseum*	death of old tissue no effect on new growth (reisolated)
Mir 64a IMI 368840	Llan Bwch-llyn Lake, Wales	*Coniothyrium fuckelii*	necrosis of old tissue no effect on new growth (reisolated)
Mir 96b	Canal du Rhône au Rhin, France	*Fusarium sporotrichoides*	death of whole sections
Mir 134a IMI 368660	Oversley Edge River, England	*Cryptosporiopsis* sp.	death of old tissue and reduction new growth
Mir 51 IMI 362886	Basingstoke Canal, England	*Glomerella cingulata*	reduction in rate of growth x2#

England, which previously had been recorded from desert soils in Wyoming only. *Sclerotium hydrophilum*, isolated from Afrilzer See in Austria has previously been recorded on *M. spicatum* in Yugoslavia (IMI Culture collection).

Screening

To date, 324 isolates have been tested, of these 15 have shown some degree of pathogenicity, or control, causing a reduction in growth (assessed by weight) and in more severe cases, loss of leaves, necrosis or death (Table 2). The majority of isolates damage the older tissue of the plant and have only a minimum affect on the newer growth. Of the isolates giving some degree of control, 12 of these were reisolated from the plant tissue.

Of these 12 isolates which satisfy Koch's postulate, two are still unidentified Hyphomycetes (Mir 49a and Mir 80c) and two Coelomycetes (Mir 35 and Mir 36). Identification has been hampered by the very low and sporadic sporulation, though this does not appear to hinder either infection or reisolation from plant tissues.

Three of the isolates showing some degree of control are similar to those already screened in the USA (Andrews and Hecht 1981; Andrews *et al.* 1982; Charudattan 1990; Verma and Charudattan 1993). *Acremonium* sp. (Mir 68c) has been screened twice giving good results and was reisolated both times. Results of reisolation of *Fusarium sporotrichoides* (Mir 96b) are still pending, but the isolate has been able to cause the death of sections of plants inoculated. The native American isolates screened; *Acremonium curvulum* and *Fusarium sporotrichoides* (Andrews and Hecht 1981; Andrews *et al.* 1982; Charudattan 1990), were successful in small-scale tests, but failed to control the weed in large trials. Though this may be the case with the European isolates, their closer evolution with the plants should allow for more consistent results.

An isolate of *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*), has been tested as a mycoherbicide in the USA (Sorsa *et al.* 1988), whilst the European strain (Mir 51), though not reisolated from plant tissue, has been screened twice, and reduced growth-rate in both tests. Significantly, *Mycoleptodiscus terrestris*, which has been isolated in both the USA and China, and shown to be virulent and reasonably specific to *M. spicatum* (Verma and Charudattan 1993), was not found during any of the European surveys.

Several of the isolates which have shown a degree of control (*Cylindrocarpon destructans*, *Fusarium solani*, *Coniothyrium fuckelii*, *Geotrichum candidum* and *Gliocladium roseum*) are generally not regarded as pathogenic or specific. Their ability to infect *M. spicatum* was probably opportunistic, aided by the small plant-sections used in the screen, and may not be repeatable with whole plants.

Conclusions

Though only an initial evaluation of European pathogens is presented, there is evidence to suggest that several of the screened isolates may have some potential as biological control agents of *Myriophyllum spicatum* in the USA. Clarification of this would be dependent upon further work to test the effectiveness of selected isolates in larger-scale trials and to establish their host specificity.

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