Prospects for biological control of field horsetail *Equisetum* arvense L. in New Zealand

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Summary

Project and Client

• The prospects for biological control of field horsetail *Equisetum arvense* in New Zealand were investigated by Landcare Research and Jane Barton, on behalf of Landcare Research, for Horizons Regional Council, funded by an Envirolink Medium Advice Grant (HZLC49).

Objectives

- Review the literature to identify potential biological control agents for field horsetail and to assess the feasibility of their release in New Zealand.
- Assess the prospects of achieving successful biocontrol of field horsetail in New Zealand.
- Estimate the total cost of the programme for the release of the agents.

Results

- No horsetail (*Equisetum*) species are native to New Zealand.
- Recent molecular analyses place the horsetails within, but only distantly related to, other ferns (Pteridophyta), having diverged by the end of the Devonian (~354 million years ago). The most closely related New Zealand native plants to *Equisetum* are believed to be the Marattoid ferns, of which there is one species, *Marattia salicina* (Para, King Fern). More distantly related native fern groups are the Ophioglossoid (5 species in two genera), Whisk (5 species in 2 genera), and Osmundaceous ferns (3 species in 2 genera).
- New Zealand Plantfinder (http://www.plantfinder.co.nz/, accessed 21/2/2008), listed no additional exotic Horsetail, Marattoid, Ophioglossoid, Whisk or Osmundaceous ferns that are sold commercially in New Zealand. All *Equisetum* species have been banned from sale now in New Zealand.
- Taxonomic isolation of *Equisetum* from native or valued exotic species in New Zealand indicates that relatively few test plant species should be required to demonstrate the host-range and environmental safety of candidate agents.
- Numerous arthropod and pathogens of field horsetail are present in the native range and many are apparently specific to *Equisetum*, such that there is a large pool of candidate agents likely to be suitable for use against field horsetail in New Zealand, including a flea beetle *Hippuriphila modeeri*, a weevil *Grypus equiseti*, numerous sawflies and several fungal plant pathogens, such as *Stamnaria persoonii* and *Ascochyta equiseti*.
- Biological control programmes have not been conducted against field horsetail overseas, and little information exists on the impact of these natural enemies on field horsetail in its native range.

Conclusions and Recommendations

- A biocontrol programme is proposed based on the premise that the project should be approached from first principles. A survey should be undertaken to clarify what organisms are already present on field horsetail in New Zealand (estimated cost ~\$50,000–80,000, depending on how comprehensive). If nothing of significance is found, the control agents available in the native range of field horsetail should be assessed to develop a prioritised shortlist of agents for host-range testing. Several tasks have been identified that would narrow the search for effective control agents, and clarify the prospects for effective biological control of field horsetail in New Zealand:
 - Contact weed research organisations in other countries where field horsetail is invasive (e.g., Australia, South Africa) to investigate the possibility of collaboration on biological control of field horsetail.
 - Survey areas within the natural range of field horsetail that climatically most resemble New Zealand and where candidate agents that appear to have biocontrol potential are known to occur (e.g., Western Europe and the USA). The estimated cost of three 1-

- week surveys (spring, summer and autumn) in Europe range from ~\$40,000 (for a limited survey in southern England; CABI Europe-UK) to \$80,000 (CSIRO European Laboratory, France). Both estimates assume samples would be sent to New Zealand for identification, which would be an additional cost.
- Ship the most promising arthropod agents into New Zealand quarantine for host-range testing &/or sub-contract an overseas agency (e.g., CSIRO or CABI) to conduct host-range testing on most promising pathogens (or send a pathologist overseas to test in USA, or Australia, or South Africa).
- The costs of host-range testing and preparing an application to ERMA for the release of a biocontrol agent (including ERMA fees that are currently \$30,000) are estimated at ~\$200,000–250,000 per agent (the cost of testing pathogens is likely to be greater than arthropods, because host-range testing would have to be conducted overseas). Note: this figure does not include the cost of mass-rearing and release of agents and is based on a purely operational programme (i.e. it does not include underpinning science, for example, to predict the impacts of biocontrol agents or to monitor agent establishment and impact, post-release). The cost of the programme will also be greater if molecular techniques are required to determine the origin of field horsetail in New Zealand or if it becomes necessary to plant out 'trap plants' in the native range, to ensure that populations of natural enemies that are capable of attacking field horsetail plants growing in New Zealand are identified. It is unusual for a single agent to provide sufficient control and it is likely that several would be required.

1. Introduction

Field horsetail *Equisetum arvense* L. is a vascular plant that reproduces by spores rather than seeds. The genus *Equisetum* includes ~15 species, commonly known as horsetails and scouring rushes, which are found throughout most of the world, but are not native to Australia and New Zealand (Webb et al. 1988). Traditionally, the horsetails and scouring rushes compose the entire class Equisetopsida, the sole member of the division Equisetophyta (Arthrophyta in older works), though a recent molecular analysis places the genus within the ferns (Pteridophyta), related to Marattiales (Pryer et al. 2004).

Three introduced *Equisetum* species became naturalised in New Zealand. According to Webb et al. (1995) two of these; *Equisetum fluviatile* L. and *Equisetum hyemale* L., were eliminated before they could become widely established, however, *E. hyemale* is still present, but rare in New Zealand (http://www.rnzih.org.nz/pages/nppa_075.pdf). In contrast, field horsetail, which is a Holarctic (i.e. native to temperate regions of Eurasia and North America) species, has become an aggressive weed in New Zealand, particularly where rainfall is moderate to high or in riparian sites.

Field horsetail develops extensive underground rhizomes which are resistant to herbicides so that, once established, it is extremely difficult and expensive to control (Webb et al. 1988). Biological control should, therefore, be a desirable control option. This report makes use of published literature reviews, internet web pages, and correspondence with botanical experts to provide information to allow decision-makers to decide if a biological control programme against field horsetail is feasible and should proceed, and to understand what such a programme would involve.

2. Background

Global distribution and biology of field horsetail

Field horsetail is native to Europe, Asia, and North America and has naturalised in Madagascar, South Africa, South America, Australia, and New Zealand (Large et al. 2006).

According to Doll (2001), field horsetail is a perennial with a spreading rhizome system that produces numerous shoots and tubers. The rhizomes are dark brown or blackish, 3–5 mm in diameter, covered with brownish hairs that give them a felt-like feel, and grow vertically to 1.8 m deep and horizontally to lengths of 25–50 cm. The horizontal rhizomes produce numerous shoots and form rounded tubers about 12 mm in diameter either singly or in pairs.

Plants produce two types of stems. Fertile (reproductive) stems (Fig. 1) appear in the early spring and are whitish to light brown, unbranched, hollow, c. 8 mm in diameter, cylindrical, leafless, jointed, and 15–30 cm long. The tips of fertile stems end in a yellowish to brownish spore-producing cone (called a strobilus), 12.5–31 mm long. Fertile stems wither and die once spores have been produced, usually by early summer.



Fig. 1. Sterile (left) and fertile (right) stems of

Equitesum arvense
Image: The UniProt Consortium
The Universal Protein Resource (UniProt)

Nucleic Acids Res. 36:D190-D195(2008). http://beta.uniprot.org/taxonomy/3258

Sterile (vegetative) stems (Fig. 1) emerge later than the fertile stems and look like miniature pine trees with their plume-like branches. This appearance also explains the plant's common name: horsetail. Sterile stems are green, either erect or somewhat prostrate, 15–60 cm tall and composed of slender, grooved, hollow joints, 1–1.5 mm in diameter.

Field horsetail thrives in many habitats, and is as at home in wet, poorly drained areas of fields and grasslands, wet meadows, streams and other sites with high water tables as it is in well-drained sites in farm fields, orchards and nursery crops, and sites with sandy or gravel soil such as along roadsides, railway tracks and beaches. In general, field horsetail appears most commonly in acidic and wet soil conditions.

In its native range, field horsetail can become a weed of field and vegetable crops (e.g., Cloutier & Watson 1985). Movement of rhizomes or tubers on tillage implements is a common means of starting new populations. Simulations done to predict the rate of spread estimated that six years after introducing horsetail into an agricultural field, the weed will infest 2.5 acres (Cloutier & Watson, 1985).

Since the time of ancient Rome, field horsetail has been used in traditional medicine in Europe. Its main uses are as a diuretic (something that increases urine output), for osteoporosis (it contains silica deposits, which may be beneficial for bone thickening), and for wound healing (http://www.nlm.nih.gov/medlineplus/). Modern studies have shown that field horsetail produces compounds with antimicrobial activity, and that these compounds are active against a wide range of organisms including bacteria and fungi (Radulovic et al. 2005).

While the weed may be useful in small doses, in larger quantities it can be toxic (http://www.nlm.nih.gov/medlineplus/). The coarse stems are not often eaten by mammalian herbivores because of their silica deposits (the origin of the alternative name of "scouring rush" is due to the abrasiveness of these silica deposits). If they are eaten, acute thiamine deficiency can result in "equisetosis". Horses are primarily affected, sheep to a lesser degree, and equisetosis is rarely fatal for cattle. Horses can be killed if large amounts of horsetail are consumed. Hay containing 20% or more horsetail produces symptoms in horses in 2–5 weeks. Symptoms include unthriftiness followed by weakness, "staggers", nervousness, faulty vision, and difficulty in turning. In advanced stages, horses may "go down" and not be able to rise. Such animals are nervous and make frantic efforts to stand (Hill & Foland 1986). In late stages, muscular rigidity and constipation may be observed. In fatal cases, death is preceded by quiescence and coma.

Distribution and status of field horsetail in New Zealand

Field horsetail was first recorded in New Zealand in 1922 (Webb et al. 1988). Healy (1966) stated that field horsetail stem fragments were unintentionally imported into New Zealand as a contaminant in the roots of *Iris* sp. imported from Japan, although neither Roy et al. (1998) nor Webb et al. (1988) identify the origin of New Zealand field horsetail populations. It may be that the material from Japan was detected subsequent to an earlier introduction of unknown origin.

Webb et al. (1988) described field horsetail as an aggressive weed that is becoming well established in some areas, particularly where rainfall is moderate to high or where it can grow in riparian sites. According to Roy et al. (1998), field horsetail is of limited distribution in New Zealand, but has the potential to become more widespread. Nevertheless, infestations have been recorded from Kawhia, Havelock North, New Plymouth, Wanganui, and Lower Rangitikei in the North Island and from Marlborough, Nelson (including a 200-ha infestation near Karamea), the West Coast, Christchurch, and Dunedin in the South Island. In New Zealand, field horsetail now forms pure stands in a wide range of damp habitats, preventing the seedlings of native species from establishing and in some areas blocking and altering watercourses, causing flooding.

In the Manawatu-Wanganui Region, field horsetail is commonly spread by rhizome and root fragments (fragments as small as 2–3 mm are viable) in gravel and sand used in landscaping and road works (Craig Davey, Horizons Regional Council, pers. comm.). Furthermore, random discoveries from areas where no building mix has been deposited (e.g., from the tops of some catchments) implies long-distance spore dispersal is also occurring. Indeed, the recent rate of field horsetail spread has been described as "phenomenal and unstoppable" and some very large infestations already occur on the Wanganui River system, with the potential for further spread considered to be "vast" (Craig Davey, Horizons Regional Council, pers. comm.).

Field horsetail is designated an Unwanted Organism in New Zealand and all *Equisetum* species are listed under the National Pest Plant Accord. It is therefore an offence under Sections 52 and 53 of the Biosecurity Act to knowingly propagate, distribute, spread, sell, offer for sale or display (in a place where organisms are offered for sale or exhibited) field horsetail in New Zealand (http://www.biosecurity.govt.nz/pest-and-diseases-response/pests-and-diseases-watchlist/field-horsetail).

Current control methods

Field horsetail's extensive and deep rhizome system means that tillage and cultivation alone only destroy the top growth and delay reestablishment. Weed scientists in Canada handweeded an area with horsetail 16 times during one summer. The following year these plots looked identical to the control plot (Cloutier & Watson 1985). Furthermore, the extensive underground rhizomes are resistant to herbicides, making field horsetail extremely difficult to control (Webb et al. 1988). Field horsetail suppression (and possibly eradication) will only result if the appropriate mix of practices is done as a sustained effort for several seasons.

The best means of controlling this weed while it infests only small areas are through quarantine and mechanical and cultural methods. In New Zealand, the recommended control methods

(http://www.weedbusters.co.nz/weed_info/detail.asp?WeedID=110, accessed 6 November 2007) are:

1. Dig out and incinerate all parts and contaminated soil.

2. Spray (summer): metsulfuron-methyl 600g/kg (5g/10L) or Tordon Brushkiller (25ml/10L). Add penetrant.

Overseas, it has been noted that cultural practices such as improved drainage and adequate lime and fertilization programmes will help suppress horsetail infestations. Applying nitrogen fertilizer to grass crops is helpful because horsetail responds minimally to nitrogen while grass crops respond quickly and significantly, gaining a competitive advantage over field horsetail because the weed is shade sensitive (Andersson & Ludegardh 1999).

Potential advantages and disadvantages of biological control

Biological control could offer many advantages over current control methods for the management of field horsetail, and may be the only realistic option for large infestations given the difficulty of controlling this weed by conventional means. Classical biological control, if successful, is more cost-effective than other forms of control as it offers continuous action and self-dispersal to areas that are not likely to be targeted by other control programmes.

Despite its advantages, biological control may not be a "silver bullet"; although recent analyses have indicated the success rate of weed biocontrol programmes has been greater than previously supposed (Hoffmann 1995; McFadyen 1998; Fowler et al. 2000; Briese 2000), complete successes, where biological control is so dramatic that no other control methods are required, only account for approximately one-third of all completed programs (McFadyen 1998). Furthermore, although biological control is often perceived as an environmentally benign alternative to chemicals, there have been recently reported cases of damage to nontarget plants (e.g., Louda et al. 2003). Nevertheless, the risk of failure and the impacts of nontarget attack are likely to be minor, compared with the potential benefits. For example, Paynter and Flanagan (2004) showed that, of the weed biocontrol programmes that did not deliver 'complete' control, most resulted in 'substantial' or 'partial' control (i.e. where biological control contributes to management, but other control methods are still required to achieve adequate control). Examples of 'partial control' in New Zealand (listed by Fowler et al. 2000) include alligator weed (where biocontrol of floating weed mats is successful, but agents do not attack terrestrial infestations) and ragwort (excellent control in many regions, but not in high rainfall areas such as the west coast of the South Island). 'Failure' (i.e. an inability to find or establish control organisms, or an absence of agent impact) is a rare outcome of weed biocontrol programmes (Paynter & Flanagan 2004).

Furthermore, data on host use of 112 insects, three fungi, and one mite indicated that virtually all risk of non-target attack is borne by native plant species closely related to the target weeds and that the risk to native flora can be judged reliably before introduction (Pemberton 2000). Similarly, a survey in New Zealand indicated that the overall reliability of host-range testing in past weed biocontrol programmes was high. Only two cases of non-target attack were recorded on native plant species: both examples were of plants very closely related to the target weeds, both examples were predictable from host-range testing and in both cases the impacts of non-target attack were minor (Paynter et al. 2004; 2008).

Moreover, a recent economic analysis indicated that the overall weed biocontrol effort provided a strongly positive return on investment, with benefits provided by successful programmes far outweighing the total costs: for every dollar invested in weed biocontrol in Australia, a benefit of \$23.10 is generated (Page & Lacey 2006)

Predicting establishment of biocontrol agents

Reliably predicting the likelihood of establishment and impacts of introduced arthropods and pathogens on plant populations has long been a goal of weed biological control programmes

and research has been conducted to determine the importance of both release size (e.g., Memmott et al. 1998; 2005) and climate matching (e.g., van Klinken et al. 2003) in relation to biological control agent success.

The best predictor of establishment success of new organisms is the number and size of releases. In New Zealand, weed biological control agent establishment rates are now very high, largely due to the increased effort put into multiplication, release and monitoring in the extensive technology transfer programme that Landcare Research operates with regional councils, Landcare groups, Landcorp, local farmers' groups, the Department of Conservation, and forestry companies (Fowler et al. 2000).

Furthermore, in a broad sense, climate matching should rarely be problematic because coevolved weed biological control agents should, generally, be adapted to the same climatic
conditions as those to which their host-plants are adapted. However, van Klinken et al. (2003)
noted that climate became an issue when agents were collected from a restricted part of the
range of a plant species that established over a wide range of climatic and ecological
conditions (a similar scenario could also occur if the native distribution of a plant was
restricted, compared with its introduced range). Problems may also arise if the target country
has an unusual climate that the plant can tolerate but in which the agent struggles. Given the
vast native range of field horsetail, it should be prudent to choose agents that are adapted to
regions where the climate is most similar to New Zealand. Therefore, for both climatic and
logistical reasons, the relatively mild climatic regions of Western Europe would appear to be a
more promising area in which to conduct native range surveys than, for example,
Scandinavia, Siberia or Canada. Climatic matching could be further refined by, for example,
using climate matching software (e.g., CLIMEX), but this may still require a detailed
understanding of agent biology to be able to climate-match in detail.

Predicting impact of biocontrol agents

Estimating the likely impact of an exotic herbivorous organism in a new environment is more challenging than predicting the likelihood of establishment. In addition to climatic matching, an organism will face a host of other factors, such as predation, parasitism and competition, that might affect its ability to thrive in a new environment, as well as the ability of its host plant to compensate for attack. Furthermore, information regarding the growth of field horsetail in New Zealand compared with the native range is lacking, as is information regarding the impact of pathogens and invertebrate herbivory on field horsetail in the native range. Note, however, that this is not unusual before beginning a weed biological control programme.

Denoth et al. (2002) found that the success of biological control against weeds increased with the number of agents released, although they argued that this result might be because of the likelihood that the right control species is released increases with the number of agents released (lottery model), rather than because of the cumulative impact of multiple natural enemies. Certainly, spectacular biocontrol successes have been achieved with only one agent (Denoth et al. 2002) and a challenge for biocontrol practitioners is to identify the agent(s) that are most likely to impact on weed populations.

Crawley (1989) and Charudattan (2005) reviewed, respectively, the use of insect and plant pathogenic fungi in weed biological control. Crawley (1989) showed that certain insect groups have proved more successful at reducing host plant abundance than others: for example, ~50% of releases of insects in the *Dactylopiidae* (cochineal insects), *Curculionidae* (weevils) and *Chrysomelidae* (leaf-beetles) that established resulted in marked or complete control of the target weed, whereas all the programmes that successfully established insects in the *Cerambycidae* (longhorn beetles; 17 examples), *Coreidae* (leaf-footed bugs; 9 examples)

and *Carposinidae* (fruitworm moths; 7 examples) failed to control their target plants (table 8, Crawley 1989). Charudattan (2005) noted that weeds with robust capacity for vegetative regeneration (such as field horsetail) are more difficult to control than those that lack this trait. Indeed, field horsetail produces shoots during the entire summer (Cloutier & Watson 1985), which implies that either a multivoltine (multiple generation) agent or a combination of agents, acting in succession, will be required to inflict sustained damage throughout the growing season. Furthermore, in its native range, field horsetail can be a weed of field and vegetable crops (e.g., Cloutier & Watson 1985). One might speculate, that reduced plant competition, or elimination of natural enemies by pesticide use might contribute to an absence of regulation of field horsetail in such habitats. If so, it follows that while biocontrol may be likely to succeed in a range of habitats, additional control methods may still be required should field horsetail become an invasive weed of cropping systems in New Zealand.

Weed biological control agents, especially rust fungi, can be so specialised that they show host specificity within a given plant species. Matching the target host's susceptibility with the candidate pathogen's virulence is of utmost importance for biocontrol success with some agents since, for example, host-pathogen interactions at the species and subspecies levels are often governed by single-gene differences in rusts (e.g., varietal specificity; Charudattan 2005). For example, efficacy of the skeletonweed rust fungus Puccinia chondrillina varies from low to high, depending on weed forms (Burdon et al. 1981). Eriophyid mites can show similar levels of specificity: at least one biotype of St John's wort Hypericum perforatum appears to be resistant to the eriophyid mite Aculus hyperici in Australia (Mayo & Roush 1997). For certain candidate agents, to facilitate searching for natural enemies from the correct biotype(s) it would be prudent to determine which subspecies and forms of a weed are present in the introduced range, thereby reducing the potential for host-plant resistance. This can involve the use of molecular techniques to determine the origin of a weed. Some studies have utilised a cheaper method of directly testing the susceptibility of a weed to candidate agent species by planting out 'trap plants' collected in the weeds' introduced range and monitoring damage to them in the native range.

Finally, Charudattan (2005) also concluded that the stakeholders' perceptions of the effectiveness of a biocontrol programme can be unpredictable, leading to conflicting views of 'success'. We believe the aims of any biocontrol programme against field horsetail should therefore be clearly defined from the outset, so that success or failure can be assessed objectively against a well-defined goal.

3. Objectives

- Review the taxonomy of field horsetail in relation to native plant species and economically important exotic plant species present in New Zealand, to develop a testplant list for host-range testing of biological control agents and to identify any potential barriers to the use of a biological control programme against field horsetail in New Zealand.
- Review existing literature on the natural enemies of field horsetail.
- Summarise the literature and current information available from researchers worldwide on the potential for biological control of field horsetail.

4. Methods

Selecting a test-plant list for host-range testing of biological control agents

Host-specificity testing is used to discard potential weed biological control agents that are likely to cause significant undesirable non-target damage to either native or valued exotic plants. The simplest tests are extremely robust 'no-choice' tests where arthropods are confined on a particular test plant and either feed or die, or, for pathogens, inoculation is attempted to see if infection can result. No-choice tests define the fundamental host-range of a particular species (all the plant species it can survive/complete development on; van Klinken & Heard 2000). However, for many herbivorous insects this fundamental host range is broader than the realised host range under field conditions, so more complex and costly tests, such as field cage oviposition tests, may be required.

A centrifugal phylogenetic method (Wapshere 1974) has long been used to determine the host-range of a potential biological control agent by sequentially testing plant taxa most closely related to the target weed followed by increasingly distantly related taxa until the host-range has been circumscribed. This approach is supported by recent advances in molecular techniques: host-shifts in lineages of specialist phytophagous insects are strongly linked to the evolution of host-plant lineages, and in particular plant chemistry. Such insects show a strong phylogenetic conservatism of host associations (see Briese 1996; Briese & Walker 2002). This pattern of strong phylogenetic conservatism in diet suggests the non-target plants of greatest risk are those closely related to known hosts (Futuyma 2000), and this has been validated by recent reviews of non-target attack by insect (Pemberton 2000; Briese & Walker 2002; Louda et al. 2003; Paynter et al. 2004) and fungal (Barton (née Fröhlich) 2004) weed biological control agents.

We, therefore, consulted the scientific literature to determine the taxonomic position of field horsetail to identify the most closely related plant species present in New Zealand that should be used to assess the risk of non-target attack when conducting host-range tests. To do this we searched the Web of Science® database for recent publications regarding the phylogeny of the genus *Equisetum*. We then referred to the most recent checklist of native New Zealand plants (de Lange et al. 2006) to identify the New Zealand plant species that are most closely-related to field horsetail.

Identifying fungal pathogens of field horsetail

A table was compiled of the fungi that have been reported associated with field horsetail. Information for this was obtained by searching computer databases and Internet sites. The most useful sources for this table were the USDA Fungus-host database or FDSM (which records) includes Zealand disease most New plant at http://nt.arsgrin.gov/fungaldatabases/fungushost/FungusHost.cfm and the Fungal Records Database of Britain and Ireland (FRDBI) at http://194.203.77.76/fieldmycology/FRDBI/FRDBI.asp. The IMI fungal herbarium was also searched online (at http://194.203.77.76/herbIMI/index.htm), but it listed no organisms associated with field horsetail. We also undertook Google searches using the key words 'Equisetum arvense' and 'pathogen' or 'fungi' or 'equiseti' (the species name for many fungi found associated with Equisetum hosts). Once a list had been created from internet sources, further information about each fungus was sought in the published literature. The NZFUNGI database (at http://nzfungi.landcareresearch.co.nz) was used to determine which species were already present in New Zealand. Each fungus was then assessed to determine whether it is likely to be damaging to field horsetail and whether it is likely to be sufficiently specific for use as a classical biological control agent. Note that

viruses and bacteria are not included in this survey because such organisms would be more challenging, and more expensive, to use for biocontrol than fungi.

Identifying arthropod biocontrol agents of field horsetail

Identifying candidate arthropod biocontrol agents of field horsetail was more difficult than identifying pathogens because there is no equivalent of the FDSM or FRDBI databases for all arthropod herbivores. However, a list of lepidoptera recorded from field horsetail was obtained from the Natural History Museum's world listing (http://www.nhm.ac.uk/research-curation/projects/hostplants/). We also searched CAB Abstracts and Google Scholar for field horsetail and sub-searching, first with the keyword "invertebrate*", and then with the keyword "herbivore". The abstracts were then examined and relevant pests added to the list. Useful information was found in the *Handbooks for the Identification of British Insects* series (many of which contain appendices with host-records), which listed species associated with field horsetail in Britain and Ireland.

We referred to checklists of New Zealand fauna to determine whether any of the pest species recorded feeding on/infecting field horsetail already occur in New Zealand.

5. Results

The taxonomy of field horsetail

Equisetum is the sole surviving genus (with two subgenera *Equisetum* and *Hippochaete*) in the 'horsetails and scouring rushes'. The horsetails were traditionally regarded as class Equisetopsida, which were considered to be 'fern allies', rather than true ferns (as below):

- Kingdom: Plantae
 - Division Tracheophyta (vascular plants)
 - Class Lycopsida, (fern-allies) the clubmosses and related plants
 - Class Equisetopsida, (fern-allies) the horsetails and scouring-rushes
 - Class Psilopsida, (fern-allies) the whisk ferns
 - Class Filices, the true ferns
 - Class Spermatopsida (sometimes as several different classes of seed-plants)

Recent molecular analyses, however, place the horsetails within the ferns (Pteridophyta), with the most closely related New Zealand native plants to *Equisetum* currently believed to be the Marattoid ferns (Pryer et al. 2004; Wikström & Pryer 2005; Schuettpelz et al. 2006). Uncertainty remains regarding the exact relationships among horsetail, marattioid, and leptosporangiate ferns (Fig. 2). However, the horsetails are undoubtedly only distantly related to other ferns: using molecular techniques, Pryer et al. (2004) estimated that they diverged by the end of the Devonian (~354 million years ago); an estimate supported by the presence of fossil relatives of horsetails dating back to the late Devonian.

No *Equisetum* species are native to New Zealand and none are commercially important ornamental plants or crops in New Zealand. Due to this taxonomic isolation from native or valued exotics in New Zealand a relatively short test plant list should be sufficient to demonstrate the host-range and, therefore, environmental safety of candidate biocontrol agents. We propose that a test plant list should include a native New Zealand representative from each genus of the following major fern groups:

• Marattoid ferns: There is one native species of Marattoid fern in New Zealand (de Lange et al. 2006): *Marattia salicina* (Para, King Fern). An historical record of another

Marattoid fern *Angiopteris aurata* in New Zealand is considered to be the result of a mislabelled specimen (Brownlie 1959).

- Ophioglossoid ferns: Two genera of Ophioglossoid ferns are present in New Zealand (Allen 1982): *Botrychium*, represented by *Botrychium lunaria* (Moonwort), *B. biforme* and *B. australe* (Parsley fern) and *Ophioglossum*, represented by *Ophioglossum coriaceum* and *O. petiolatum* (de Lange et al. 2006).
- Whisk ferns: Two genera of whisk ferns are present in New Zealand (de Lange et al. 2006); *Tmesipteris*, represented by *Tmesipteris elongata*, *T. lanceolata*, *T. sigmatifolia*, and *T. tannensis* and *Psilotum*, represented by *Psilotum nudum*.
- **Osmundaceous ferns:** One species of *Todea* (*T. barbara*) and two species of *Leptopteris* (*L. hymenophylloides* and *L. superba*) are native to New Zealand (de Lange et al. 2006).

We also searched New Zealand Plantfinder (http://www.plantfinder.co.nz/, accessed 21/2/2008), which indicated that no additional exotic Marattoid, Ophioglossoid, Whisk or Osmundaceous ferns are sold commercially in New Zealand.

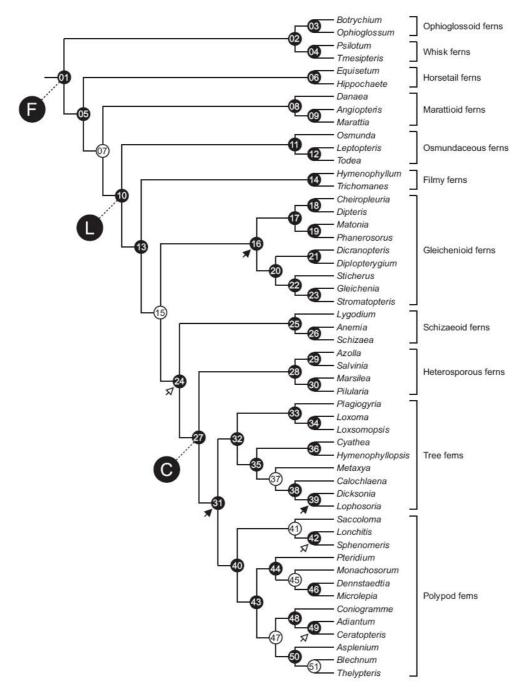


Figure 2. Fern phylogeny resulting from Bayesian analysis of combined 5-gene dataset (reprinted with authors' permission from Schuettpelz *et al.* 2006). F = ferns; L = leptosporangiate ferns; C = core leptosporangiates; major fern groups are also indicated with brackets at right. Filled numbered circles indicate nodes receiving both good posterior probability (≥ 0.95) and good maximum likelihood bootstrap (≥ 70) support; nodes with open circles did not receive good support from both measures.

Potential agents for the biological control of field horsetail in the New Zealand

Fungi

Classical biocontrol

Thirty-seven species of fungi were found associated with field horsetail (listed in Appendix 1). This substantial number probably reflects the fact that field horsetail has been used in traditional medicine for centuries and has, therefore, been relatively well-studied. Of these, 17 have no potential as classical biological control agents either because they are unlikely to be sufficiently damaging to be useful, or because they have a very broad host range, or both (see Appendix 1). There are 14 fungi listed in Appendix 1 for which we know too little to decide whether or not they could be useful for biocontrol. If any of these fungi were found during a pathogen survey of field horsetail, it would be worth testing their virulence and/or host range before rejecting them as possible agents. The remaining six fungi with biocontrol potential that are worthy of further discussion are: Stamnaria persoonii (Moug.) Fuckel; Ascochyta equiseti (Desm.) Grove; Cylindrosporium equiseti W.C. liu et R.L. Bai; Monodidymaria equiseti (Dobrozrakova) U. Braun; Heterosporium equiseti H.C. Greene; and, Mycosphaerella altera (Pass.) House. These fungi are all either Ascomycetes (Stamnaria and Mycosphaerella) or the asexual stages (anamorphs) of Ascomycetes (the other four taxa). The Phylum Ascomycota is the largest group of fungi and includes saprobes, which live on dead organic material; parasites (esp. of plants), which obtain nutrients from living organisms; and, most of the fungi involved in forming lichens (a fungus/algae symbiosis). Most fungi can produce spores both sexually (through meiosis) and asexually (through mitosis). The characteristic that links all Ascomycetes is that when they produce spores sexually, they produce them in a microscopic sack called an ascus. The genus names Ascochyta, Cylindrosporium, Monodidymaria and Heterosporium are all applied to fungi reproducing asexually (anamorphs). However, there is good evidence linking these genera to the Ascomycetes, for example, some *Heterosporium* (Cladosporium) species also produce ascospores characteristic of Ascomycetes in the genus Mycosphaerella (Kirk et al. 2001). The four anamorph species of interest to us either do not produce ascospores on field horsetail, or they do, but no-one has yet linked their anamorph and teleomorph names (a common situation as such pairs usually look completely different from each other).

There have been more than 100 records of *Stamnaria persoonii* and its asexual state *Titaeospora equiseti* (Desm.) Vassiljevsky growing in association with *Equisetum* species (Cooper & Kirk 2006; Farr et al. 2008). While there are also two records of this fungus on unrelated hosts (*Ilex cornuta* and *Lagerstroemia indica*) in Florida (see Appendix 1), these records stand out, and it would be worth confirming their validity. While host range testing would be necessary before any fungus was considered for importation into New Zealand as a biocontrol agent, it would appear that *S. persoonii* is mostly, if not entirely, restricted to hosts in the genus *Equisetum* and would be sufficiently host-specific to be used safely here. Whether or not the fungus is capable of inflicting sufficient damage to field horsetail to be useful for its control is a matter that would require further investigation. Promisingly, it has been reported that *S. persoonii* caused a leaf spot and leaf blight on field horsetail in Korea, where the plant is valued for its medical properties (Shin 1994). Dr Shin reported that

infection was heavy, especially in wet places, and was sometimes associated with insect damage and/or infection with another fungus (a *Gloeosporium* species; Hyeon Dong Shin, Korea, pers. comm.). He said that affected plants "looked like they had been sprayed with herbicide". Given that the damage reported by Dr Shin sounds quite obvious, it is surprising that most other records in the literature do not clearly state that the fungus is associated with disease symptoms. It is possible that the Korean fungus is a different strain to the European/North American one, and this would need to be investigated.

There are at least eight collections of this fungus stored in the Landcare Research Herbarium at Tamaki (PDD), three under the name *S. persoonii*; one under *T. equiseti*; two under *Titaeospora detospora* (Sacc.) Bubák and two under *Cylindrosporium equiseti* (Desm.) Died (Shaun Pennycook, Landcare Research, pers. comm.). All were collected in Europe. Unfortunately, these collections are no longer viable, so this fungus could only be tested for New Zealand if a living culture/specimen could be found. No fungi in this genus have been used previously as classical biocontrol agents (Barton (née Fröhlich) 2004).

Ascochyta equiseti is the next most common fungus with biocontrol potential, with about 40 known records, all from Equisetum species. References consistently report that the organism is associated with disease symptoms (See Appendix 1). While no Ascochyta species have been used as classical biocontrol agents previously (Barton (née Fröhlich) 2004), the genus is biologically and morphologically very similar to the genus Phoma. An exotic strain of Phoma clematidina has been released in New Zealand for the biological control of old man's beard Clematis vitalba but it does not appear to have persisted here and is believed it may have been out-competed by other pathogens already present in old man's beard plant tissues. Ascochyta spores are generally small and moist and so tend to disperse small distances, unlike the dry, wind-dispersed spores of rusts. Therefore, if A. equiseti were to be used as a classical biocontrol agent in New Zealand, it might be necessary to make many releases, or even to apply inoculum as if it were a mycoherbicide, to aid its spread.

Cylindrosporium equiseti was only 'discovered' fairly recently (Lui & Bai 2001). Little information is available, but it is said to be associated with small leaf spots. The limited host range reported in the literature would need to be checked, as it may be an artefact of being little-studied.

There is also little information available about the last three fungi *Monodidymaria equiseti*, *Heterosporium equiseti* and *Mycosphaerella altera* (see Appendix 1). They have all been reported associated with disease symptoms, and only on *Equisetum* species, but with so few records in the literature, further testing would be vital. No fungi in the genera *Cylindrosporium*, *Monodidymaria*, *Heterosporium* or *Mycosphaerella* have been used previously as classical biocontrol agents (Barton (née Fröhlich) 2004). However, that does not mean they could not be useful. While most of the fungi used for classical biocontrol to date have been rusts (Basidiomycota, Uredinales), the remainder have mostly been, like all six of these fungi, Ascomycetes and their anamorphs. Rusts do make very good classical biocontrol agents, so it is unfortunate no rusts have been reported associated with field horsetail. Unfortunate, but not unexpected: rusts are usually associated with seed-plants, and (less frequently) true ferns (Bauer et al. 1999; Kirk et al. 2001). That said, a rust has been discovered associated with a *Selaginella* species (Bauer et al. 1999), and as the Selaginellaceae is an even more primitive plant group than the Equisetaceae

(http://plantphylogeny.landcareresearch.co.nz/), it is possible there is a rust on field horsetail that has yet to be discovered.

To summarise, there are at least six pathogenic fungi that could be useful classical biocontrol agents for field horsetail in New Zealand, either alone or as part of a suite of agents. Four of these occur in the USA, three in Germany and the UK; France and Italy are each home to two

taxa. Thus, surveys for pathogens with biocontrol potential against field horsetail should be conducted in the US, and/or Western Europe.

Inundative biological control: developing a mycoherbicide

The development of a mycoherbicide (as outlined on the final page of this document) requires considerable field testing and other research. Consequently, this technique is usually limited to targets that cause a sufficiently large problem to justify the high developmental costs. Furthermore, mycoherbicides are normally developed using fungi that already occur in the country where they will be used. The exception to this rule is that sometimes a fungus introduced as a classical agent can have its dispersal and efficacy enhanced by being formulated and applied as a mycoherbicide *after* it has been introduced. For example, (Hennecke 2004) proposed that inoculum of the fungus *Phloeospora mimosae-pigrae* should be applied as a spray against the weed *Mimosa pigra*, after it was introduced to Australia, as unassisted it was struggling to keep up with the rapid growth of the weed.

For a mycoherbicide to be developed against field horsetail in New Zealand either a suitable fungus would already need to be here or one of the potential classical agents discussed above would need to be introduced and a decision made to enhance it. Of the 37 fungi listed in Appendix 1, ten already occur in New Zealand. However, none of these have potential as the active ingredient of a mycoherbicide because they are all either saprobes that would cause little damage to the weed (e.g., *Lachnella villosa* and *Lachnum virgineum*), or have very wide host ranges that would make it undesirable to broadcast their inoculum over large areas (e.g., *Verticillium dahliae*). While it has been argued that pathogens with broad host ranges can be used in mycoherbicides as long as the risks to non-target plants are understood (Bourdôt et al. 2003), this is more appropriate for weeds that are problematic in agricultural areas (where the diversity of other species is limited) than for environmental weeds such as field horsetail. A bioherbicide that could damage New Zealand native species would be of little use against field horsetail.

Nematodes

A plant pathogenic nematode, *Pratylenchus penetrans*, has been recorded from field horsetail. It is already present in New Zealand and has a huge host range, including the pasture grasses that compete with field horsetail, and is not, therefore, a suitable control agent for field horsetail.

Arthropods

We found records of 38 arthropod species that feed on field horsetail, of which some are clearly unsuitable for use as biological control agents on the basis of inadequate host-specificity (Appendix 2). However, according to literature host-records, 26 species are apparently sufficiently host-specific to be considered for use against field horsetail in New Zealand. We examine, below, whether this shortlist can be further refined, based on the likelihood that an agent will be sufficiently specific and damaging.

Mites: The eriophyid mite Eriophyes equiseti is likely to be a suitable biological control agent for field horsetail. Eriophyids have been considered to be ideal biological control agents due to the debilitating damage they cause to plants and their largely specialized feeding habits: over 80% of eriophyids found on weeds are monophagous (feed on a single host) (Rosenthal 1996). Two eriophyids have been used successfully as biological control agents against weeds (Aceria chondrillae against skeleton weed Chondrilla juncea in Australia and the USA and A. malherbae against field bindweed Convolvulus arvensis in the USA; Cullen et al. 1982; Rosenthal 1996). Another, Aceria genisteae, was recently (February 2008) released in New Zealand for biological control of Scotch broom Cytisus scoparius. Although Eriophyes equiseti has only been recorded from Hungary (Farkas 1960), this perhaps indicates that few

acarologists have searched for this species (eriophyids are microscopic and unlikely to be identified by casual observation). We have not been able to ascertain the kind of damage caused by this mite but many eriophyids cause plant deformities, such as galls. The remaining candidate agents can be categorised according to the damage they cause (see below).

Sap-suckers: An aphid, two cicadellid leafhoppers and two delphacid planthoppers are apparently specific to Equisetum (Appendix 2). One aphid species, Aphis chloris, has been used as a weed biological control agent in Australia, but did not make an important contribution to St John's Wort Hypericum perforatum control (Briese & Jupp 1995). Aphids are the most important vectors of plant viruses (Burnett & Kawchuk 2002) and could conceivably transmit viral diseases to non-host plants during exploratory feeding and this risk of non-target damage might be expensive to research. Furthermore, aphids (or other sapsuckers that secrete honeydew) may attract ants which may then predate other insects present on the plant, potentially reducing the effectiveness of other agents (Thum et al. 1997). Therefore, careful consideration should be given before sap-suckers are used as weed biological control agents. Nevertheless, few cicadellids and delphacids are attended by ants (Delabie 2001), indicating they may be more suitable than the aphid Macrosiphum equiseti for biological control of field horsetail. A cicadellid Zygina sp. was introduced to control bridal creeper Asparagus asparagoides in Australia. In combination with an introduced rust fungus it caused major reductions of above- and below-ground biomass of the target weed (Morin & Edwards 2006).

Leaf/branch-miners: Several agromyzid flies in the genus Lyriomyza mine the narrow branches (Appendix 2) of field horsetail. Agromyzids are usually easy to rear and establish but they rank among the least successful of weed biocontrol agents: Crawley (1989) found no examples where they resulted in marked control of the target weed. The European agromyzid Phytomyza vitalbae has only a minor impact on old man's beard Clematis vitalba in New Zealand (Paynter et al. 2006), where it is parasitized by at least eight native and introduced parasitoids (Paynter et al. 2008). Agromyzids should therefore be given a low priority for biological control of field horsetail in New Zealand, unless surveys demonstrate they are unusually damaging or if their impact is likely to complement the action of another agent (for example, in combination with a stem-miner).

Stem and root-miners: The flea beetle Hippuriphila modeeri and weevil Grypus equiseti are both apparently specific to Equisetum; their larvae mining the main stems. Hippuriphila modeeri larvae "eat much pith, turning the outer surface of the stem black" (Robbins 1990). The moth Loxoterma tiedemanniana also mines field horsetail stems. However, given its distribution (Appendix 2), it could be a difficult and expensive to collect and it may not be well matched, climatically, to the areas where field horsetail is a weed in New Zealand. A review of successful and unsuccessful weed biocontrol agents indicated that the Chrysomelidae (Crawley 1989; see also Syrett et al. 1996) and weevils (Crawley 1989) have exceptionally good records of success in biocontrol programmes. Moreover, among the Chrysomelidae, various species of stem flea beetles have been extremely successful, for example: Agasicles hygrophila on alligator weed (Stewart et al. 2000); Longitarsus jacobaeae on ragwort Senecio jacobaea (McEvoy et al. 1991); Apthona spp. on leafy spurge Euphorbia esula (e.g., Kirby et al. 2000) – the latter two being stem- and root-miners as larvae. This success implies that both the horsetail flea beetle Hippuriphila modeeri and the horsetail weevil *Grypus equiseti* should be high priority species for consideration as biological control agents of field horsetail in New Zealand.

Defoliators: According to host-records, approximately 15 species of sawfly graze the fronds of, and are apparently specific to, *Equisetum* (Appendix 2). Some, such as *Dolerus bimaculatus* are restricted to relatively cold, northern localities and some, such as *D. pratorum* are rare or local in Britain (Benson 1952). *Dolerus pratensis* and *D. aericeps*,

however, have been described, respectively, as abundant in Great Britain and very common in England (Benson 1952), indicating they must be significant defoliators in the native range. Some adult sawflies are carnivorous (Jervis & Vilhelmsen 2000); although the *Dolerus* species listed by Jervis & Vilhelmsen (2000) were said to feed on pollen, nectar, honeydew and the sugar-containing spermatial fluid of rust fungi, it would be prudent to confirm the adult feeding preferences of any *Dolerus* species being considered for introduction to control field horsetail.

Five exotic sawfly species in the superfamily Tenthredinoidea have been accidentally introduced into New Zealand and become abundant: *Pontania proxima* and *Nematus oligospilus*, which attack willows; *Caliroa cerasi*, which attacks pear, cherry and hawthorn trees; and the raspberry sawfly *Priophorus morio*; and an Australian species *Phylacteophaga froggatti* mines eucalyptus leaves. Therefore, sawfly defoliators should be very promising candidate agents for the biological control of field horsetail in New Zealand. However, one sawfly *Monophadnus spinolae* has been used for biocontrol of an exotic weed (old man's beard *Clematis vitalba*) in New Zealand and its establishment has not been confirmed, even though the first release of this agent was performed in 1996. Mass rearing of the sawfly was difficult due to a 20:1, male: female sex ratio, asynchronous emergence, and poor larval survival (Gourlay et al. 2000).

6. Conclusions and recommendations

Prospects for achieving biological control of field horsetail in New Zealand

We conclude that the prospects of finding sufficiently specific classical biological control agents for introduction into New Zealand are extremely good. However, as field horsetail has not been targeted for biological control overseas, a biological control programme would have to start from scratch. Nevertheless, field horsetail has the potential to be a relatively inexpensive target for biological control because:

- As field horsetail is taxonomically distant from New Zealand native plants, an extensive test-plant list is not required and relatively simple laboratory no-choice experiments to determine the fundamental host-range are likely show that the candidate biocontrol agent is sufficiently safe for release in New Zealand. This means costly (and sometimes logistically difficult, or even impossible) field host-range tests are unlikely to be necessary. For arthropods, these tests could be carried out in quarantine in New Zealand. For plant pathogens, host-range testing would have to be conduced overseas as there is currently no pathogen-proof quarantine facility in New Zealand.
- Extensive literature records indicate that a large number of apparently specific arthropods and plant pathogens have already been recorded attacking field horsetail, so extensive surveys in the native range should not be required. Nevertheless, some surveys in the native range (especially in Western Europe and/or the USA) would be prudent to determine which agents appear to be the most damaging and widespread and to investigate whether agents are likely to complement one another by attacking different plant parts and/or by attacking plants at different times of the year.
- Significant opposition to biological control is highly unlikely because, while field horsetail is used in homeopathic preparations (Roy et al. 1998), it is not grown for this purpose in New Zealand (indeed it would be illegal to do so) and it is not valued by other sectors in New Zealand (e.g., as a fodder crop or a nectar source for beekeeping). While horsetails are still grown as ornamentals in some gardens this is unlikely to present a major stumbling block since they can no-longer be sold or propagated.

Two potential complications could arise because field horsetail has a huge native range, over which two subspecies (*ssp. arvense* and *ssp. boreale*) and numerous named forms and varieties occur (http://data.gbif.org/search/taxa/Equisetum%20arvense): (1) ensuring a good climate match between New Zealand and the area in which to search for potential agents and; (2) the potential for host-plant resistance.

To ensure good climate matching, it should be useful to determine the areas within the natural range of field horsetail that, climatically, most resemble New Zealand. This could be achieved using CLIMEX software. However, given that most weed biological control agents have been successfully introduced into New Zealand (directly or indirectly) from Western Europe (Hayes 2005), where field horsetail is native, searching for agents in this region is likely to result in an adequate climate match.

As noted in section 2, above, weed biological control agents, especially rusts and eriophyid mites, can be so specialised that they show host specificity within a given plant species. However, no rusts have been recorded attacking field horsetail (Appendix 1); indeed many of the most promising pathogens and insects attack more than one *Equisetum* species (Appendices 1, 2). We therefore predict that host plant resistant genotypes are unlikely to be an issue except, perhaps, for the eriophyid mite *Eriophyes equiseti*.

Recommendations

- Survey the invertebrate fauna and pathogens of field horsetail in New Zealand. Estimated cost is \$50,000–80,000.
- Contact weed research organisations in other countries where field horsetail is invasive (e.g., Australia, South Africa) to investigate the possibility of collaboration on biological control of field horsetail.
- Survey field horsetail localities in Britain and/or France and Germany and/or the USA and prioritise a shortlist of agents for host-range testing. Approximate cost of three 1-week surveys (Spring, Summer and Autumn) \$40,000 (CABI Europe-UK)—\$80,000 (CSIRO European Laboratory). Both estimates assume samples would be sent to New Zealand for identification, which would be an additional cost.
- Ship most promising arthropod agents into New Zealand quarantine for host-range testing &/or sub-contract an overseas agency (e.g., CSIRO or CABI) to conduct host-range testing on most promising pathogens (or send a pathologist overseas to carry out testing in USA, or Australia, or South Africa).

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Appendices

Appendix 1: Fungi recorded on field horsetail Equisetum arvense and their potential usefulness for biological control

Phylum, Order	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific? (and comments)	Found in NZ/biocontrol
Family					potential?
Ascomycota Dothideales Dothideaceae	Scirrhia castagnei	France; Scotland (Mid Ebudes)	Unknown: Reference is a list of fungi in the Hebrides. No mention of disease symptoms (Dennis 1986)	Yes: 6 records in FRDBI ³ . All on 3 <i>Equisetum</i> species (incl. <i>E. arvense</i>)	%/oN
Ascomycota Helotiales Helotiaceae	Hymenoscyphus ochroleucus	England (Surrey)	Unknown	Unknown: Only 1 record in British fungi: On/with <i>E. arvense</i>	No/?
Ascomycota Helotiales Helotiaceae	Hymenoscyphus rhodoleucus	England (Gloucestershire, Lancaster, Northumberland, Walkers Heath, Worcestershire); Scotland (Mid Ebudes, Wigtownshire); Wales (Pembrokeshire)	No: Saprobe ⁴ found on/with litter, dead material, dead stems and stems	Probably: 39 records in FRDBI, 37 of them on <i>Equisetum</i> species, 1 host not stated, and 1 on dead stems of <i>Calluna</i> vulgaris (Heather)	No/ No
Ascomycota Helotiales Helotiaceae	Stamnaria persoonii (also recorded as S. equiseti {old name}) anamorph = Titaeospora equiseti (anamorph also recorded as Gloeosporium equiseti, and Cylindrosporium	Austria; British Isles; Canada (Ontario); Czech Republic; France; Germany; Italy; Korea; USA (Idaho, Washington, South Dakota). Also found widely in the northern hemisphere on other	Possibly: Recorded: on/with host, on stems and leaves, on living leaves, on withered stems, on dead stems & on stem spots. Reported to cause leaf spot and leaf blight on <i>E. arvense</i> in	Probably: 96 records, 18 host/pathogen combinations in FDSM ⁵ and 16 records in FRDBI. All except 2 collections on <i>Equisetum</i> spp. Exceptions are on <i>Ilex cornuta</i> (Dicotyledon, same genus as Holly) and <i>Lagerstroemia</i>	No, apart from collections in PDD (see main text)/Yes

Phylum, Order	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific?	Found in NZ/biocontrol
Family					potential?
	equiseti {old names})	Equisetum spp.	Korea (Shin 1994)	indicia (Crêpe myrtle) in Florida	
Ascomycota Helotiales Helotiaceae	Stamnaria sp. anamorph = Titaeospora sp.	USA (California)	Unknown	Unknown: Just 1 record in FDSM	¿ /¿
Ascomycota Helotiales Helotiaceae	Titaeospora detospora teleomorph = Stamnaria sp.	USA (Wisconsin, Wyoming); Poland; West Germany; Czechoslovakia	Unknown. Recorded as on stems or leaves, reference does not state if pathogenic (Greene 1949)	Yes: 9 records in FDSM, all on 5 <i>Equisetum</i> spp. (incl. <i>E. arvense</i>). Lives in temperate climates (Greene 1949)	No/?
Ascomycota Helotiales Hyaloscyphaceae	Lachnella alboviolascens (Note that Lachnella is said to be a synonym of Lachnum in Kirk et al. 2001)	Canada	Probably not: Saprobe?	No: Many distantly related hosts. Widespread on woody and herbaceous stems	Yes, indigenous/No
Ascomycota Helotiales Hyaloscyphaceae	Lachnella (=Lachnum?) villosa	Wales (Brecon)	No: Saprobe, where host given, mostly on dead material	No: 273 records in FRDBI, hosts mostly not given	Yes, exotic/No
Ascomycota Helotiales Hyaloscyphaceae	Lachnum virgineum	Scotland (Wigtownshire)	No: Saprobe, where host given, on dead material	No: 2276 records in FRDBI, hosts mostly not given	Yes/No
Ascomycota Helotiales Hyaloscyphaceae	Psilachnum inquilinum	England (Hertfordshire, Northumberland, Surrey, Yorkshire); Scotland (Wigtownshire); Wales (Cardiganshire, Pembrokeshire)	No: recorded on/with host, stems, dead stems & dead material. Probably saprobe	No: Mostly found on dead tissues of 5 Equisetum species. However, also less commonly found on dead material of unrelated plants such as Populus tremula (aspen) and Abies (fir)	No/ No
Ascomycota Hypocreales	Lasionectria sylvana (also recorded as Nectria	USA (New York)	Unknown	No: 14 records in FDSM, 9 of these from NZ. Hosts include	Yes/No

Phylum, Order Family	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific? (and comments)	Found in NZ/biocontrol potential?
Bionectriaceae	sylvana {old name})			ferns and monocots (e.g., Astelia, Rhopalostylis sapida {nikau} & Phormium tenax {flax})	
Ascomycota Hypocreales Incertae sedis ⁶	Verticillium dahliae	Canada	Yes: Causes wilt disease in many plants (Kirk et al. 2001)	No: 51 records in FRDBI, 343 in FDSM. Found on hosts in many different families, cosmopolitan	Yes, exotic/No
Ascomycota Hypocreales Nectriaceae	Acremonium boreale teleomorph = Nectria tuberculariformis	Canada	Yes. Listed on MAFs unwanted organisms register as 'regulated pest' (HerbariumPDD 2001–2008)	No: Many unrelated hosts	No/ No
Ascomycota Incertae sedis	Ascochyta cf. teretiuscula	England (Surrey)	No: Found on dead stem so may be saprobe.	?: No more information because species ID uncertain	No/?
Ascomycota Incertae sedis	Ascochyta equiseti (also recorded as Stagonosporopsis equiseti {old name})	Belgium; Czechoslovakia; Denmark; England (Lancaster, London, Warwickshire, Yorkshire); France; Germany; Italy; Romania; Switzerland; USA (Wisconsin) & the	Yes: Said to be definitely parasitic (Greene 1950). Associated with dead and dying leaves and stems (Mel'nik 2000) or with indistinct lesions on bleached areas on stems (Punithalingam 1988)	Yes: 21 records in FRDBI, 19 records FDSM, all from various <i>Equisetum</i> species. (Mel'nik 2000; Punithalingam 1988)	No/Yes
Ascomycota Incertae sedis	Cylindrosporium equiseti	China, Poland, Germany	Maybe: Recorded in living foliage, associated with small spots 0.5–8 mm in diameter (Lui and Bai 2001)	Yes: 5 collections, 4 from E. arvense, 1 from E. sp. Probably genus specific. This species (C. equiseti W.C. Liu et R.L. Bai) was described recently and is the only Cylindrosporium	No/Yes

Phylum, Order Family	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific? (and comments)	Found in NZ/biocontrol potential?
				known from the Equisetaceae (Lui & Bai 2001). An earlier fungus initially called <i>Cylindrosporium equiseti</i> (Desm.) Died is a synonym of <i>Titaeospora equiseti</i> (Desm.) Vassiljevsky (teleomorph = <i>Stamnaria persoonii</i>)	
Ascomycota Incertae sedis	Gyrothrix verticillata	England (Surrey)	Unknown	No: Other hosts unrelated (e.g., Fagus sylvatica (Beech), Phormium tenax (NZ Flax))	No/No
Ascomycota Incertae sedis	Monodidymaria equiseti (also recorded as Cercospora equiseti {old name})	Russia; USSR	Probably: "Segments or entire branchlets of the stems discoloured, pale to brown" (Braun 1994)	Possibly: only 2 records in FDSM, both on <i>E. arvense</i> . Reference describes fungus as 'insufficiently known' (Braun 1994)	No/Yes
Ascomycota Incertae sedis	Septogloeum equiseti	England (Norfolk)	Probably not: listed on dead stems so probably saprobe	Probably: 2 records in FRDBI, one on <i>E. arvense</i> and one on <i>E. palustre</i>	No/?
Ascomycota Incertae sedis	Stagonospora sp.	USA (Wisconsin)	Unknown	Unknown: Just 1 record in FDSM. No more information as because species ID uncertain	<i>3/6</i>
Ascomycota Microascales Ceratocystidaceae	Thielaviopsis basicola	Canada	Yes: Causes black root rot (Kirk et al. 2001)	No: Widespread, cosmopolitan, hosts in many plant families	Yes, exotic/No
Ascomycota Mycosphaerellales Mycosphaerellaceae	Cladosporium cladosporioides	England (Surrey)	No: Saprobe	No: Many unrelated plant hosts & organic materials such as dung, mattresses, walls	Yes, origin/No uncertain
Ascomycota	Cladosporium herbarum	USA (Iowa)	No: Saprobe	No: Very common	Yes, exotic/No

fic? Found in NZ/biocontrol potential?	eds of dead isolated etc.	I: 2 from No/ Yes E. nus	DSM: 1 No/ Yes yemale;	FRDBI: on true teridium	f, all on No/? fferent ense	vn hosts No/No	יין זערתיי
Likely to be host-specific? (and comments)	cosmopolitan sp., hundreds of known hosts (mostly on dead tissues), and frequently isolated from air, soil, foodstuffs etc. (Ellis 1971)	Yes: 4 records in FDSM: 2 from E. arvense, 2 from two difference subspecies of E. sylvaticum. Probably genus specific	Probably: 4 records in FDSM: 1 on <i>E. arvense</i> ; 2 on <i>E. hyemale</i> ; 1 on <i>E. telmateia</i>	Probably specific to ferns and fern allies: 8 records in FRDBI: 4 on Equisetum species including E. arvense: 3 on true ferns (Dryopteris sp.; Pteridium spp. & Nephrodium filix-mas)	Yes: 8 records in FDSM, all on Equisetum species (7 different E. spp. including E. arvense	No: more than 100 known hosts	Drobobly 5 room do in CDDDI
Likely to be damaging?		Probably: Described on brown distal portions of branchlets "fungus develops progressively inward from branchlet tips until the entire plant may be involved" Greene 1950)	Probably: Recorded on dead tips of living branches (Greene 1953)	Unknown (References = taxonomic or geographic lists: not helpful)	Unknown (References = geographic lists: not helpful)	Unknown	Dishobly not listed on
Range on field horsetail ²		USA (Wisconsin)	USA (Wisconsin)	Sweden	Sweden	Poland	Unalond (Voulabine)
Species (and other names) ¹	teleomorph = Davidiella tassiana	Heterosporium equiseti (Note that Heterosporium is said to be a synonym of Cladosporium in Kirk et al. 2001)	Mycosphaerella altera	Mycosphaerella aspidii	Mycosphaerella equiseticola	Clathrospora diplospora	C+2000000000000000000000000000000000000
Phylum, Order Family	Mycosphaerellales Mycosphaerellaceae	Ascomycota Mycosphaerellales Mycosphaerellaceae	Ascomycota Mycosphaerellales Mycosphaerellaceae	Ascomycota Mycosphaerellales Mycosphaerellaceae	Ascomycota Mycosphaerellales Mycosphaerellaceae	Ascomycota Pleosporales Diademaceae	A 500mm 100to

Phylum, Order Family	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific? (and comments)	Found in NZ/biocontrol potential?
Pleosporales Incertae sedis			dead stems in 2 of 5 records so probably saprobe	all on <i>Equisetum</i> spp.	
Ascomycota Pleosporales Leptosphaeriaceae	Leptosphaeria arvensis (also listed under name of anamorph Heptameria arvensis)	USA (California); Italy	Unknown: recorded on stems and/or dead stems, no more info. available in literature.	Yes: 5 records FDSM: 4 from E. arvense, 1 from E. hyemale	No/?
Ascomycota Pleosporales Phaeosphaeriaceae	Phaeosphaeria equiseti	Norway	Unknown. Reference does not say if pathogenic (Shoemaker and Babcock 1989)	12 records in FDSM, all on Equisetum sp. (4 difference E. spp. including E. arvense)	No/?
Ascomycota Pleosporales Phaeosphaeriaceae	Phaeosphaeria fuckelii	Sweden	Unknown. Reference does not say if pathogenic (Shoemaker and Babcock 1989)	No: more than 40 hosts. Mostly grasses, but also other monocots, ferns and fern allies. ITS sequence puts this fungus with other <i>Phaeosphaeria</i> found on these types of hosts (Camara et al. 2001)	Yes, exotic. Specimens on two grasses in PDD/No
Ascomycota Pleosporales Phaeosphaeriaceae	Phaeosphaeria lindii	Norway	Unknown. Reference does not say if pathogenic (Shoemaker and Babcock 1989)	Yes: 6 records in FDSM, all on Equisetum sp. (4 difference E. spp. including E. arvense)	No/?
Basidiomycota Agaricales Flagelloscyphaceae	Flagelloscypha minutissima	England (Surrey)	No: Saprobe	No: more than 10 distantly related hosts & wood, bark etc.	No/ No
Basidiomycota Agaricales Pluteaceae	Volvariella gloiocephala	England (Gloucestershire)	No: Mushroom, saprobe	No: 961 records in British fungi, substrate mostly not given, but where given = soil, sand, on/with grasses	No/ No

Phylum, Order Family	Species (and other names) ¹	Range on field horsetail ²	Likely to be damaging?	Likely to be host-specific? (and comments)	Found in NZ/biocontrol potential?
Basidiomycota Ceratobasidiales Ceratobasidiaceae	Rhizoctonia solani teleomorph = Thanatephorus cucumeris	UA (Illinois, Minnesota, Texas)	Yes: Causes root rot. Listed by MAF as a 'regulated pest' (HerbariumPDD 2001- 2008)	No: More than 2400 fungus/host combinations. Cosmopolitan, plurivorous, commonly found on roots but also stems, seedlings, leaves and fruits	Yes, exotic/No
Zygomycota Mucorales Choanephoraceae	Poitrasia circinans	India	Probably not: Described on roots of living plant (Rai 1990), and this seems to be the only record	Unknown: Only the single record on <i>E. arvense</i> in FDSM	No/?

¹ Many fungi have more than one Latin name because they can produce more than one type of spore. The name given when they are producing 'sexual' spores column 2 uses whichever name/names were recorded when the fungus was found on E. arvense. If a fungus was listed under an out-of-date name (synonym) classified according to their 'teleomorph' name, unless the 'anamorph' is the only form known. So, appendix 1 gives the taxonomy of the teleomorph, but is called the teleomorph, whereas the stage producing 'asexual' spores is called the anamorph. The two stages often look completely different. Fungi are this is also stated in column 2.

² Only the places where the organism was found associated with E. arvense are listed here. It may also be found elsewhere on other hosts.

³ FRDBI = the Fungal Records Database of Britain and Ireland (FRDBI) at http://194.203.77.76/fieldmycology/FRDBI/FRDBI.asp

Saprobe: An organism using dead organic material as food and commonly causing its decay (Kirk et al. 2001). Unlikely to cause disease and therefore probably insufficiently damaging to be useful for biocontrol.

FDSM = USDA Fungus-host database at http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm

Insertae sedis = of uncertain taxonomic position within a higher taxonomic order (e.g., Phylum known, but order within that phylum uncertain).

HYMENOPTERA Tenthredinidae

Yes: Host plants <i>E. arvense</i> (Medvedev & Roginskaja 1988); <i>Equisetum</i> spp. <i>E. palustre</i> (Bullock 1992).	No: Host plants <i>E. arvense</i> , <i>Hosta</i> sp. (Agavaceae) (Gressitt & Kimoto 1963).	Yes: Host plants <i>E. palustre</i> and <i>E. arvense</i> (Morris 2002)	Yes: Host plant <i>E. arvense</i> (Spencer 1972) Yes: Host plant <i>E. arvense</i> in stems and thicker branches; larva eats	Yes: Main host plant E . fluviatile, also recorded from E . arvense	No: Highly polyphagous (NHM HOSTS database)	No: Highly polyphagous (NHM HOSTS database)	No: Highly polyphagous tunnels branches (Grehan 1989) No: Highly polyphagous root-feeder	Yes: Host plant Equisetum (in stems; NHM HOSTS database)
W Palaearctic to Mongolia	Japan, Korea, N. China	Holarctic; widespread in Britain (Morris 2002)	Europe Holarctic	Holarctic	Native to Europe, introduced North	America Europe	East Asia Europe	Palearctic (Finland, South Siberian mountains)
Flea beetle	Flea beetle	Weevil	Leaf-mining fly Leaf-mining fly	Leaf-mining fly	Moth	Moth	Moth Moth	Moth
Hippuriphila modeeri	Liprus punctatostriatus	Grypus equiseti	Liriomyza occipitalis Liriomyza equiseti	Liriomyza virgo	Hydraecia micacea	Xylena vetusta	Endoclita excrescens Triodia sylvina	Loxoterma tiedemanniana
COLEOPTERA Chrysomelidae		Erirhinidae	DIPTERA Agromyzidae		LEPIDOPTERA Noctuidae		Hepialidae	Tortricidae

Appendix 3: Steps in a Biocontrol Project

A classical biocontrol programme typically works through the following steps; this is usually done in a sequential manner, but some activities may occur concurrently.

- 1. Explore the feasibility of project. If project looks feasible, proceed.
- Survey weed in places where biocontrol is desired. If any potential agents are found explore ways to maximise them. If any likely impediments are found look for ways to mitigate them.
- 3. Undertake molecular studies of the weed to help narrow down the best place in the native range to find natural enemies.
- 4. Unless natural enemies are already well known, survey weed in native range. Identify and study life cycles of natural enemies found.
- 5. Determine host range for potential agents. Abandon any species that do not appear to be safe or effective enough.
- 6. Apply to authorities for permission to release agents.
- 7. If permission is granted import, clear through quarantine, and develop rearing techniques for new agents (if not already known)
- 8. Mass rear and release agents over several years.
- 9. Monitor establishment success and dispersal of agents over several years.
- 10. Harvest and redistribute agents
- 11. Evaluate success of project. Decide if further agents are needed.

An inundative biocontrol programme where mycoherbicides are developed for use, typically works through the following steps in a sequential manner:

- 1. Survey the weed to look for potentially useful pathogens (if not already known)
- 2. Undertake glasshouse trials to explore host range and pathogenicity
- 3. Develop prototype formulation and test under field conditions
- 4. Refine formulation
- 5. Explore techniques for mass production and prospects for commercialisation
- 6. Undertake risk analysis and apply for registration