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A rationale for the use of a non-specific fungal seed pathogen to control annual grass-weeds of arable lands

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Abstract. Control of weeds in arable environments has concentrated on reducing densities by killing plants in order to alleviate yield losses from competition. There are few instances where biological control agents have been exploited for this purpose. Yet in other habitats, particularly grassland, rangeland and natural environments, many classical biological control initiatives have targeted reproductive output as a means of regulating populations. Annual grass-weeds have proliferated on arable lands around the world, partly stimulated by conservation tillage practices and partly by an over dependence on herbicides for in-crop control. This has led both to herbicide resistance and a desire to reduce herbicide use. Against this background we consider how biological control may contribute more to integrated weed-management systems for arable lands and we present a case for the inundative use of a seed-borne pathogen for controlling annual grass-weed species. Ecologically, control of reproductive output is rational because many annual grass-weeds characteristically have transient seed-banks. To demonstrate the bioherbicidal potential and complexity of using seed-borne pathogens, some findings of our work with *Pyrenophora semeniperda* are discussed in the light of its non-specificity and its effects on wheat, using preliminary results of field studies.

Introduction

Weed management in arable cropping systems is, more than ever, having to embrace integrated systems of control. These combine fallow management, cultural practices to enhance crop vigour and competitiveness, mechanical, chemical and biological control, crop rotation and various methods of capturing weed-seeds at harvest. Despite the many possible permutations for integration, current management systems are largely reliant on the use of pre- and post-emergence herbicides. Over-dependence on herbicides has, however, provoked a social reaction demanding reduced use of herbicides in food-production enterprises, and this has been legislated in some instances. Researchers have responded to this challenge, largely by trying to 'fine-tune' the current systems through more efficient application of herbicides (Göhlich 1992), through spot and patch spraying (Thompson *et al.* 1991; Felton 1995), by rationalizing herbicide dose rates through factor adjustment (Kudsk and Kristensen 1992) and by developing the tools of prescriptive management through improved decision support (Forcella 1995).

A notable deficiency in the integrated approach is the under utilization of biological control. In Australia, the one prominent example of successful (classical) biological control of a weed of arable land involves skeleton weed rust, *Puccinia chondrillina* Bubak and Syd., for control of skeleton weed, *Chondrilla juncea* L.. Even *P. chondrillina* has had a limited impact in some regions because of the differential susceptibility of the three morphotypes of weed to the rust strains (Panetta and Dodd 1995). This paper explores the potential of a non-specific, seed-borne pathogen, *Pyrenophora semeniperda* (Brittlebank & Adam) Shoem. anamorph *Drechslera campanulata* (Lèv.) B. Sutton, as a bioherbicide to regulate the seed production of some annual, grass-weed species of arable lands.

Strategic control options

In crops, weed management has traditionally concentrated on killing plants in order to minimize yield losses due to competition, with scant regard for the permanent reduction of populations of the weed species. Little is known about the relative importance of the mechanisms that regulate the population dynamics of many of the major weeds and few attempts have been made to determine the factors that are critical for the efficient management of weed populations.

Demographic studies, computer simulations and bioeconomic modelling have all indicated that seed production is the critical factor governing the dynamics of wild oats (Medd and Pandey 1993; Pandey *et al.* 1993; Medd *et al.* 1995). The research has shown that annual grasses such as wild oats have transient seed-banks, albeit with a small, persistent component. When seed production is prevented, populations of the grasses rapidly diminish because the half-life of their seeds is mostly less than six months (Medd 1996). Plants that survive conventional control-measures, together with plants that emerge after treatment, have little direct competitive impact on the crops, but they replenish the seed-banks of the weeds. Methods which directly destroy seeds or prevent their deposition in the seed-bank would therefore assist in the management of these weeds. Agents which attack the reproductive output of weeds are frequently used in biological control programmes against weeds in pastures, rangeland and natural habitats, even against weed species with long-lived seed-banks, where their use would appear to be ecologically futile.

On this premise, agents which directly attack the reproductive capacity of species with short-lived seed-banks, either during formation or whilst in the soil, should be explored for biological control. Given the unacceptable risk of using exotic agents for biological control of some annual grasses, because they are too closely related to cereal crops, a logical starting point is to evaluate endemic microorganisms for possible inundative use.

Seed pathogens as potential biological control agents

Pathogens that infect seeds on the developing inflorescence may reduce seed-viability or result in reduced seedling-vigour, e.g. *Ustilago tritici* (Pers.) Rostrup (the causal agent of loose smut in wheat), *Pyrenophora graminea* Ito and Kuribay (barley leaf stripe) (Agarwal and Sinclair 1987), *P. semeniperda* Medd (1992) and *P. teres* Drechsler (net blotch of barley) (Shipton *et al.* 1973). Other pathogens adhere to seeds, either in the inflorescence or in the soil, and, mostly, cause seedling blight or damping-off symptoms, e.g. *Tilletia tritici* (Bjerk.) R. Wolff (common bunt), *Cochliobolus sativus* (Ito and Kurib) Drechsler ex Dastur (Agarwal and Sinclair 1987), and *Pyrenophora avenae* Ito and Kuribay (oat leaf stripe) (Wilson and Hall 1987). Pathogens that replace the seed in the inflorescence or prevent flowering also have potential for biological control. Notable examples of such pathogens are some fungal endophytes and species of *Claviceps*, *Tilletia* and *Ustilago* (Siegel *et al.* 1987; Clay 1989).

Many of the potentially most devastating agents, such as the smuts, are obligate parasites and cannot be cultured on artificial media, but this need not be a limiting factor if an augmentative rather than inundative approach is pursued. The possibility of using smut fungi may be enhanced by developing *in vitro* culturing techniques, as has been the case for *Entyloma compositarum* Farlow (Trujillo *et al.* 1988).

Pathogens that attack seeds in the soil may also be used for biological control, but the prospects are not encouraging (Kremer 1993). Damping-off fungi such as species of *Pythium*, *Rhizoctonia* and *Phytophthora* and some species of *Fusarium* (Kiewnick 1963) may affect seed dormancy or viability, or cause seedling mortality. The soil phase, as opposed to the aerial, of the seeds is perceived to be a more hospitable and protected environment (Auld and Morin 1995). However, a major limiting factor with soil-borne pathogens is the requirement for high inoculum dosages. For example, studies of soil-borne diseases of fungi have shown that approximately 1.8 t ha^{-1} of inoculum was required to provide adequate control (Huang 1980). New application technology may be required to deliver such amounts of inoculum effectively. Furthermore, such a bioherbicide would have to come into contact with the weed seeds in the soil and it would require a well developed saprophytic ability, or would need to possess other survival mechanisms, such as the production of chlamydospores or sclerotia.

Given these constraints, it is probably more appropriate to concentrate on agents that can be applied onto the aerial phase of the seeds, using conventional spray equipment. However, pathogens that infect the seeds in the inflorescences, or that reduce the numbers of seeds that are set, may require special considerations. Seed production in most annual grass-weeds is asynchronous so, to be effective, a pathogen would need to be active over a long period. Pathogens such as *Pyrenophora teres* (Shipton *et al.* 1973), which sporulate on soil- or leaf-surfaces, may produce airborne inoculum over a prolonged period to overcome problems of asynchrony. A pathogen that has a narrow infection window, e.g. restrictions to anthesis, would require repeated applications. Pathogens encapsulated in sodium alginate pellets, or other solid substrates, and applied to the surface of the soil may also deliver inoculum over a protracted period (Walker and Connick 1983; Boyette *et al.* 1991; Auld and Morin 1995).

Since some annual grasses may occur in pastures or crops used as forage, any potential bioherbicidal agent should be devoid of toxic effects on livestock. Equally, there should be no risk of introducing toxins to grain that is harvested for human consumption. These considerations negate the use of fungi such as *Claviceps* species which produce ergot alkaloids. Also, some species of *Pyrenophora* and *Fusarium* are known to produce mammalian toxins (Agrios 1988; Scott 1988).

Conceptually, annual grass-weeds may be controlled effectively by bioherbicides that attack the seeds directly or that decrease the numbers of seeds available for recruitment. Pathogens that attack both seed and foliage, giving a 'two-pronged' effect must also rank highly as potential bioherbicidal agents. Infected seed would have reduced viability, whilst damage to foliage would weaken the seedling as a competitor or, ideally, kill it. It is possible that seed-and-foliage pathogens may have a broader infection window compared with those that only infect the seed.

Assessment of *Pyrenophora semeniperda*

In accordance with the above rationale, *P. semeniperda* was selected for study as a possible biological control agent. The fungus is known as a weak, seed-borne pathogen which causes seedling blight and leaf lesions and is highly sporadic in occurrence. Campbell *et al.* (this Volume) have considered some aspects of the utility of *P. semeniperda* for bioherbicidal seed-control. Here, we consider its seed-kill efficacy under field conditions and some consequences of its broad host-range.

Pyrenophora semeniperda occurs in Australia, New Zealand, North America and South Africa, but its origin is unknown (Medd 1992). One advantage of its broad geographic- and host-ranges is that the agent could have utility across a suite of weedy grasses in a number of countries, broadening its market potential (Auld and Morin 1995). On the other hand, the lack of host-specificity and the organism's capacity to attack all of the major winter cereal crops, may be seen as grounds to condemn this potential agent, since it is generally held that biological control agents must be host-specific.

Initial field-studies, using raw aqueous conidial suspensions, have measured seed infection-levels of up to 62%, a reduction in seedling emergence of up to 70%, and reduced seedling vigour of up to 54% in a range of host-plant species (Table 1). These results confirm the pathogenicity of *P. semeniperda* and its potential as a bioherbicide.

While the levels of damage on some weed species is considered adequate to deplete their seed-banks significantly (Medd and Pandey 1993), the cross infection of wheat may be commercially unacceptable. By primarily infecting ovarian tissue (Campbell *et al.* this Volume), *P. semeniperda* colonizes developing seeds (caropyses) without inhibiting grain development or yield. Consequently, strategies may be developed to minimize undesirable spillover-effects or

Table 1. The effect of field applications of a single isolate of *P. semeniperda* on caryopsis infection (measured as the percentage of seeds with stroma) and on the reduction in germination, emergence and vigour of seeds harvested from inoculated plants, compared with seeds harvested from uninoculated plants of wheat and several annual grass weeds.. The reduction in vigour of the seeds was determined from differences in coleoptile lengths using a bioassay technique described by Murray and Kuiper (1988). Means within a column followed by the same letter are not significantly different.

	Seed infected(%)	Reduction in germination (%)	Reduction in emergence (%)	Reduction in vigour (%)
Triticum aestivum L. cv. Cook	21c	2c	55b	34b
Bromus diandrus Roth	62a	35a	51b	26b
Avena fatua L.	8d	2c	11d	10c
Lolium rigidum Gaudin	18c	12b	12d	22b
Hordeum leporinum Link	38b	15b	38c	30b
Vulpia bromoides (L.) Gray	59a	16b	70a	54a

Table 2. Physical, milling and baking-qualities of wheat harvested from a field-crop heavily infected with symptoms attributed to *P. semeniperda*. Grain was separated into lots according to visual symptoms of black point. * - qualitative measures. nsd - no significant difference.

	Black point		
	Without	With	Significance
1000 Kernel weight (mg oven dry)	40.9	48.3	P<0.05
Protein at 11% water (%)	10.2	10.6	nsd
Total flour (%)	74.8	75.4	nsd
Flour protein at 11% water (%)	8.4	9.0	nsd
Flour colour (Minolta 200 L-b)*	83.3	83.5	nsd
Loaf volume (relative)*	576	613	nsd
Baking score (relative)*	35.7	38.4	nsd
Loaf colour (Minolta 200 L-b)*	57.8	58.4	nsd

Table 3. Mean coleoptile length (mm) of wheat seedlings arising from normal or black-pointed grain treated with a range of seed-dressing fungicides at the recommended rates, using a bioassay technique described by Murray and Kuiper (1988). Means within a column followed by the same letter are not significantly different.

	Black point	
	Without	With
Untreated	90.0^{ab}	12.7^{abc}
Surface sterilized	92.5^{a}	12.7^{abc}
Bitertanol (Sibutol®)	91.3^{ab}	20.8^{ab}
Carboxin (Vitavax®)	89.6^{ab}	21.1^{ab}
Thiram (Thiram®)	88.7^{ab}	21.5^{a}
Hexachlorobenzene (Hexachlor®)	88.1^{b}	19.9^{abc}
Fenaminosulf (Le-san®)	87.6^{b}	13.5^{abc}
Tebuconazole (Raxil®)	77.8^{c}	11.7^{abc}
Flutriafol (Vincit®)	75.0^{c}	8.9^{c}
Triadimenol (Baytan®)	61.3^{d}	14.1^{abc}
Triadimefon (Erex®)	60.5^{d}	8.1^{c}

to mitigate their impact. The selection of specific isolates, timing of inoculation to avoid the crop but coincide with the target weed, application of the agent to the non-crop phase, e.g. pasture, are all options for minimizing spillovers. Leaf-spot damage is unlikely to occur in wheat due to the natural resistance of older leaves (Campbell 1996), and even if it did occur it is unlikely to result in economic damage (Medd 1992). Should the grain of the crop be infected, as happens periodically under natural conditions (Medd 1992), there would be no economic losses. The findings presented in Table 2 show that the milling and baking qualities of black-pointed grain that is heavily infected with *P. semeniperda* were not different from undamaged grain.

As there have been no adverse reports of grain that is infected during natural epidemics, there should be no cause for concern about grain quality. However, if the potential of the agent is pursued, this aspect will need exhaustive tests. Infected grain retained for grow-on does suffer some damage because infected grain does not respond to commercial seed-dressings, resulting in a loss of vigour (Table 3). Indeed, as is often the case, several of the fungicidal dressings had adverse effects on seedling vigour (Murray and Kuiper 1988). If loss of seedling vigour was perceived to be a problem, it could be simply avoided by retaining seed grain from clean, untreated fields only.

In conclusion, we have argued that a strategy to reduce seed-banks is required to improve the management of annual grass-weeds in arable lands and that research should emphasize the development of inundative biological control methods that directly destroy seeds. The ability of *P. semeniperda* to infect seeds when applied as conidial suspensions to the inflorescence of several, grassy weed-species has been demonstrated. This has resulted in partial control through dual impacts on seed viability and seedling vigour. Based on the simulated predictions made by Medd and Pandey (1993), this level of damage should promote a significant decline in populations of the weeds. Although this is an unconventional bioherbicidal approach and the concept is fraught with difficulties, a provisional patent has been granted for the use of *P. semeniperda* and further work on screening isolates and field delivery-systems is planned. Like many seed pathogens, *P. semeniperda* is a non-specific 'generalist' microorganism. Its subtle destruction of seeds or weakening of seedlings, compared to the obvious necrotic damage expected of phytotoxin or phytopathic control agents, is another obstacle to its development as a biological control agent because its impact is not immediately obvious. Furthermore, because the strategy aims to reduce populations in the long-term it will not necessarily provide an immediate economic benefit. In a field where there are limited opportunities for biological control such unconventional approaches should not be dismissed lightly.

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