EVALUATION OF A NON-SPECIFIC FUNGAL SEED PATHOGEN FOR REGULATING SOME ANNUAL GRASS WEEDS

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Summary To alleviate yield losses from competition, control of weeds in arable environments has concentrated on reducing densities by killing plants. In this context, 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.

Ecologically, control of reproductive output is a better strategy for controlling grass populations since many species characteristically have transient seed banks.

In general, biocontrol agents have not been widely exploited in arable systems. Conceptually, seed pathogens have appeal for both reducing the dependence on herbicides by exploiting biocontrol, and gaining better control of populations by attacking seeds. To this end we consider the possible inundative use of a seed borne pathogen for the control of some annual grass weeds. To demonstrate this, some findings of our work with *Pyrenophora semeniperda* are evaluated. Particular reference is made to growth and sporulation requirements with a view to mass production, the infection processes in leaves and seeds, dew requirements and efficacy under field conditions. Implications of its non-specific broad host range are discussed.

INTRODUCTION

Weedy annual species of *Avena, Bromus, Hordeum, Lolium, Phalaris* and *Vulpia* have proliferated on arable lands around the world. This insurgence has been partly stimulated by conservation tillage practices and partly by an over dependence on herbicides for in-crop control. The annual grass weeds are among the most competitive weeds of winter cereal crops and pastures. They are refuges for numerous important diseases of cereal crops, a number have developed resistance to herbicides (e.g. *Lolium* spp. and *Avena* spp.), others are tolerant of registered herbicides so cannot be selectively controlled in cereals (e.g. *Bromus* spp. and *Vulpia* spp.) and some (e.g. *Hordeum* spp.) cause serious physical damage to livestock.

Common to the behaviour of most of the species is high seed production (10 000 to 50 000 seeds m^{-2}) and short lived, transient type seed banks (half life of six months or less), with a small persistent component maintained by enforced or induced dormancy. Newly shed seeds are usually innately dormant and undergo rapid after-ripening. Thus, the critical key to effective management of many annual grass weeds is to break the seed cycle by attacking seed input (seed production and seed rain). Using simulation, Medd and Pandey (1993) predicted that population growth would decline in wild oats, for example, if seed input was reduced by 70% or more.

Conceptually, annual grass weeds could be controlled by pathogens that attack the seed either directly or indirectly to decrease the numbers of seeds available for recruitment. Such pathogens may reduce viability or result in reduced seedling vigour, for example, *Ustilago tritici* (Pers.) Rostrup (the causal agent of loose smut in wheat), *Pyrenophora graminea* Ito & Kuribay (barley leaf stripe) (Agarwal and Sinclair 1987) and *P. teres* Drechsler (net blotch of barley) (Shipton *et al.* 1973). This paper examines the bioherbicidal potential of one such seed borne fungus, *Pyrenophora semeniperda* (Brittlebank & Adam) Shoemaker (anamorph *Drechslera campanulata* (Lèv.) B. Sutton) (ring spot) for regulating seed cycles of annual grass weeds.

ASSESSMENT OF *P. SEMENIPERDA*

Pyrenophora semeniperda has been recorded in North America, New Zealand, South Africa and is common and widespread throughout southern Australia (Medd and Jones 1992). It has a known host range of over 70 grass species in 35 genera, including all of the major winter cereal crops (Medd 1992). Although considered a weak pathogen, its symptoms include small eyespots on seedling leaves which cause little or no damage to the host, and striking stromata on infected seeds which can lead to a reduction in germination and seedling vigour.

Inoculum production The ability to produce abundant and durable inoculum in artificial culture is a prerequisite of bioherbicidal candidates. The optimal laboratory conditions for production of conidial inoculum on solid media have been reported by Campbell *et al.* (1996a). Radial growth and sporulation were optimal on modified alphacel medium at 23.2 ± 0.5°C and 18.3 ± 0.3°C respectively. The pH for maximal growth was 4.7 ± 0.5, while that for maximal sporulation was 5.7 ± 0.1 and alternating light/dark and temperature sequences. Growth was enhanced by light of wavelengths longer than 500 nm, while sporulation was enhanced by light of wavelengths shorter than 500 nm (near-ultra violet) and wounding of mycelia. Sealing of Petri plates with Parafilm® had an inhibitory effect on sporulation. Whilst these techniques enabled the copious production (1.6–2.0 × 10^4 conidia cm^{-2}) of inoculum for experimental treatment of plants in the field and glasshouse, they are not necessarily adaptable for commercial scale-up.

Pyrenophora semeniperda grew prolifically in liquid shake cultures of V8 broth, malt extract broth and modified alphacel broth, but could not be induced to sporulate under these conditions. This indicates that fermentation of mycelial fragments and transference of the product to dry substrates is a possibility for mass production of conidial inoculum, as a two step production system (Walker and Riley 1982).

Infection processes Germination of conidia generally exceeded 99% on seedling leaves of both wheat and *Bromus diandrus* Roth. Conidial germination was generally much lower on adult leaves (20–30%), and penetration was rare, which could explain why leaf lesions mostly occur on seedling leaves.

Infection of leaves always involved the formation of appressoria, mostly over the anticlinal epidermal cell walls of wheat and stomata of *B. diandrus* (Campbell *et al.* 1996b). Infection sites develop haloes and papillae form within the leaves as a resistance mechanism. The first post-penetration structures to form are intracellular vesicles with infection hyphae which ramify through the intercellular spaces of the mesophyll, along with cellular disruption in advance of infection hyphae.

For ease of experimentation, infection studies of reproductive tissues were undertaken on wheat. These revealed that ovarial tissues were the most common to be infected. In contrast to leaves, appressoria formation was not essential, as direct hyphal penetration through cracks and wounds also led to infection of ovaries. Infection hyphae subsequently grew intercellularly within the confines of the epidermis and the integuments of the developing caryopsis. Infection of the developing embryo was never observed.

Infection of wheat seeds occurred at all stages of inflorescence development tested. However, the maximal proportion of seed infection occurred at around mid to late anthesis (Campbell *et al.* 1996b).

It is of considerable interest that hyphal fragments of *P. semeniperda* can also produce infection structures within leaf pieces. Low levels of seed infection were also recorded in field trials with mycelial inoculum.

Dew requirement The optimum dew temperature for lesion development of 20.6 ± 0.3°C was lower than that for conidial germination (24 ± 0.5°C), but similar to the optimum for the production of infection structures and lesion development (21.3 ± 0.2°C). The optimum dew temperature for floret infection of 23.6 ± 0.6°C closely matched that for conidial germination (Campbell *et al.* 1996b).

Dew period studies of leaf infection processes found that the maximum number of lesions developed after 21 hours, and did not increase with further provision of dew. An initial dark phase during the dew period was required for leaf infection. Seed infection was more demanding for dew with only 50% of seeds being infected after 48 hours (Campbell *et al.* 1996b).

Market size The broad host and geographic ranges of *P. semeniperda* are seen as advantageous by broadening its potential market. Collectively the annual grasses represent a substantial national and global market. To date, market size has been a severe handicap to most bioherbicidal initiatives (Auld and Morin 1995). On the other hand, the lack of target 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. We consider this aspect in greater detail below.

Efficacy Initial field studies using raw aqueous conidial suspensions have resulted in seed infection levels of up to 62%, a reduction in seedling emergence of up to 70%, and reduced seedling vigour by up to 54%, depending on species (Table 1). In field experiments over 70% infection of seed was recorded in *B. diandrus.* These results confirm the pathogenicity of *P. semeniperda* on a range of hosts.

Non-specific, broad host range Whilst the level of damage achieved for some weed species is considered adequate to significantly deplete seed banks (Medd and Pandey 1993), the cross infection of wheat (or grain of other economic species) may be commercially unacceptable. By primarily infecting ovarian tissue (Campbell *et al.* 1996b), *P. semeniperda* effectively colonizes developing seeds (caropyses) without affecting grain development or yield. Consequently, strategies may be deployed to either minimize undesirable spillover effects or mitigate their impact. The selection of specific isolates, timing of inoculation to avoid the crop but coincide with the target, application of the agent to the non-crop phase, e.g. pasture, could all be possible options for minimizing spillovers.

Leaf spot damage is unlikely to occur in wheat due to natural resistance of older leaves, and even if it did occur it is unlikely to result in economic damage (Medd 1992).

Medd and Campbell (1996) found that should the grain of the crop be infected, as is periodically the case in nature (Medd 1992), there is unlikely to be any adverse economic effect. Milling and baking quality of black pointed grain heavily infected with *P. semeniperda* were not different from undamaged grain.

As there have been no adverse consequences reported with naturally infected grain, there would not appear to be cause for concern regarding grain quality. Clearly, if the agent is inundatively applied *en masse*, thorough tests will be needed to confirm its benign association in grain intended for consumption or processing.

Infected grain retained for grow-on does, however, appear to present some unmanageable damage since infected grain does not respond to seed dressings, resulting in a detrimental loss of vigour (Medd and Campbell 1996). Indeed, as is often the case, seed treated with several of the fungicidal dressings had adverse effects on seedling vigour (Murray and Kuiper 1988).

If seedling vigour were seen to be a problem to utilizing this agent, it would be simple to avoid by retaining seed grain only from clean untreated fields.

DISCUSSION

We have suggested that a strategy of reducing seed banks is required to improve the management of annual grass weeds. One appealing way of regulating seed input is to develop inundative biological control methods that directly attack seeds. To this end the ability of *P. semeniperda* to colonize seeds when applied as conidial suspensions to the inflorescence of several grass weeds has been demonstrated, verifying its potential for inundative use. Infection 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 significant population decline in some species.

A provisional patent has been granted for the novel use of the agent and further work on screening isolates and field delivery systems is planned. NSW Agriculture is currently seeking a commercial partner to assist with this further development and product evaluation.

Opportunities for refinement We see a number of possibilities for enhancing the potential of this fungus as a commercial proposition. These involve the use of mycelial inoculum, formulation to minimize dew requirements, interactions with low doses of herbicides and the use of ‘cocktails’ of isolates.

To minimize the cost of inoculum production, the use of mycelium fragments may be an attractive alternative to conidial inoculum. Mycelium could be more economically produced by commercial fermentation as compared with systems needed to produce conidia of dry sporing fungi like *P. semeniperda*. Although we have not studied the possibilities, the behaviour of the fungus in liquid shake culture indicates it could be readily adaptable to fermentation.

Infection of seedling leaf material with conidiophores and hyphal fragments is clearly possible. However, mycelial inoculum appears to be less infective than conidia, based on our limited studies of this aspect. Further investigations to promote the infectivity of mycelium and to examine methods for preserving mycelial inoculum, to enhance storage longevity, would certainly be worth pursuing.

Overcoming the requirement for prolonged periods of dew or free water is a major constraint to this, and

Table 1. The effect of field applications (8.0×10^6 conidia m^{-2}) of a single isolate of *P. semeniperda* on caryopsis infection, germination and development of wheat and several annual grass weeds. Means within a column followed by the same letter are not significantly different.

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

[A] Seeds with stroma.

[B] Difference between seeds harvested from uninoculated and inoculated plants.

[C] Difference in coleoptile length in seeds harvested from uninoculated and inoculated plants, using a bioassay technique described by Murray and Kuiper (1988).

indeed, most bioherbicidal candidates. However, there are encouraging developments in the use of vegetable oils and surface active agents to form invert emulsions (Auld and Morin 1995) which could overcome this constraint. A further unexplored possibility for improving the efficacy of *P. semeniperda* is its combination with sub lethal doses of herbicides to weaken plant defences.

For most of our studies, a single, stable and prolific isolate was selected. Given the diverse host and geographic range of the fungus, it is highly probable that strains with differential species specific virulence could be isolated. The desirable traits of each could be combined by mutagenesis or genetic engineering, or more simply by the application of cocktail mixtures of isolates to broaden the virulence spectra.

Conceptual barriers Although this is an unconventional bioherbicidal approach involving complex concepts, and is fraught with many difficulties, the potential pay-offs are noteworthy. Foremost of these is the universal desire to reduce dependence on herbicides, and the development of biocontrol options for integrated weed management systems. Any biocontrol opportunity must therefore be thoroughly and thoughtfully evaluated for this group of weeds which have a substantial global market potential.

Like many seed pathogens, *P. semeniperda* is a non-specific 'generalist' micro-organism. Its subtle destruction of seeds or weakening of seedlings, compared to the obvious necrotic damage expected of phytotoxin or phytopathic control agents, may be seen by some as a deterrent to its development, simply because its impact is not immediately conspicuous. Furthermore, because the strategy aims to reduce populations in the long term it will not necessarily provide an immediate economic benefit. However, this need not be a constraint. Increasingly it is being realised that weed management should be evaluated in a long term framework. Manipulating weed populations into decline will result in savings in the overall cost of weed control and increased profits, as demonstrated for wild oats by Pandey and Medd (1991).

Thus, in an arena where there are limited opportunities for biological control such unconventional, even bizarre, opportunities should not be too lightly dismissed.

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REFERENCES

Agarwal, V.K. and Sinclair, J.B. (1987). 'Principles of Seed Pathology', Volume 1. (CRC Press, Boca Raton).

Auld, B.A. and Morin, L. (1995). Constraints in the development of bioherbicides. *Weed Technology* 9, 638-52.

Campbell, M.A., Medd, R.W. and Brown, J.F. (1996a). Growth and sporulation of *Pyrenophora semeniperda in vitro*: Effects of culture media, temperature and pH. *Mycological Research* 100, 311-17.

Campbell, M.A., Medd, R.W. and Brown, J.F. (1996b). The bioherbicidal potential of the seed-borne pathogen *Pyrenophora semeniperda* for control of annual grass weeds in cereal crops. Proceedings of the IX International Symposium on Biological Control of Weeds. In press.

Medd, R.W. (1992). A review of the world distribution and host range of *Pyrenophora semeniperda* (Brittlebank & Adam) Shoemaker. *Review of Plant Pathology* 71, 891-901.

Medd, R.W. and Campbell, M.A. (1996). Regulation of some annual grass weeds of arable lands using a fungal seed pathogen. Proceedings of the IX International Symposium on Biological Control of Weeds. In press.

Medd, R.W. and Jones, K.H. (1991). Host range, distribution and importance of *Pyrenophora semeniperda* (Brittlebank & Adam) Shoemaker (Ascomycotina: Pyrenomycetes) in Australia. *Proceedings of the Linnean Society of New South Wales* 113, 15-26.

Medd, R.W. and Pandey, S. (1993). Compelling grounds for controlling seed production in *Avena* spp. (wild oats). Proceedings 8th European Weed Research Society Symposium, Quantitative approaches in weed and herbicide research and their practical application, pp. 769-76.

Murray, G.M. and Kuiper, J. (1988). Emergence of wheat may be reduced by seed weather damage and azole fungicides and is related to coleoptile length. *Australian Journal of Experimental Agriculture* 28, 253-61.

Pandey, S. and Medd, R.W. (1991). A stochastic dynamic programming framework for weed control decision making: an application to *Avena fatua* L. *Agricultural Economics* 6, 115-28.

Shipton, W.A., Kahn, T.N. and Boyd, W.J.R. (1973). Net blotch of barley. *Review of Plant Pathology* 52, 269-90.

Walker, H.L. and Riley, J.A. (1982). Evaluation of *Alternaria cassiae* for the biocontrol of sicklepod (*Cassia obtusifolia*). *Weed Science* 30, 651-4.