Opportunities and constraints for the biological control of weeds in Europe

M. Vurro¹ and H.C. Evans²

Summary

Although there has been increasing interest in Europe over the last few decades in trying to harness the potential of biological control as a management tool for weeds, securing funding for projects continues to be problematic whilst the successes have been limited. The incentives to use alternative technology are based on a number of interrelated factors, not least the dramatic rise in popularity of organic products (linked to increasing environmental awareness) and a powerful anti-GMO lobby. Combine this with new legislation to remove some chemical herbicides from the market or to restrict their usage, as well as soaring development costs for new products, and the opportunities for biological control have never been better. Here, we analyse the reasons for the limited uptake, and the challenges or constraints facing weed biocontrol from two different approaches: classical and inundative.

Keynote Presenter

Introduction

In a review of the biological control of weeds and its prospect in Europe, Schroeder and Müller-Schärer (1995) stated optimistically that: ‘Although biological weed control so far [has] received little attention in Europe, more recent developments indicate that this may change in the near future’. These developments included the commercialization of, and increasing potential for, mycoherbicide use, particularly in agricultural systems in North America (Charudattan, 1991; Smith, 1991). In the intervening decade or so, has this optimism been realized? Certainly, the odds should have moved favourably in this direction, not least because of the current awareness of environmental issues in Europe by the public and politicians alike, leading to: a dramatic rise in the popularity of organic products; a powerful anti-GM lobby, negating the use of herbicide-resistant crops; and new legislation to remove chemical herbicides from the market, or to restrict their usage.

Here, this question is addressed, together with an assessment of the opportunities and constraints for biological control of weeds in Europe.

Classical

Crispy concepts, soggy concerns: Classical (or inoculative) biological control of weeds has had a long history and has been one of the main strategies for the management of invasive alien plants worldwide, apart from Europe, especially using coevolved arthropod natural enemies (McFadyen, 1998; Julien and Griffiths, 1998). In contrast, the use of coevolved pathogens is still relatively new although there have been notable successes (Evans, 2002). The high success rate with arthropod agents has also been extremely cost effective, with an impres-

1 Institute of Sciences of Food Production, CNR, via Amendola 122/O, 70125 Bari, Italy.
2 CABI E-UK Centre, Silwood Park, Ascot, Berkshire, SL5 7TA, UK.
Corresponding author: M. Vurro <maurizio.vurro@ispa.cnr.it>.
© CAB International 2008

Keywords: bioherbicides, classical biological control, environmental weeds.
Mycosphaerella. This project, funded by a... of yet another alien species: ‘... an issue concerning biodiversity and conservation, Schroeder (1995) has also been stressed recently, classical biological control could be viewed as the introduction of yet another alien species: ‘... an issue of growing public concern’ (Miller and Aplet, 2005). Although these misgivings were aimed specifically at the situation in the United States, where legal issues concerning hidden regulations have been identified, decision-makers in Europe would have taken note. In a ‘blame culture’, perhaps this will color decisions about future project proposals, not to say gaining permission to release classical agents in Europe.

As highlighted by Schroeder and Müller-Schärer (1995), although Europe has been a source of classical biological control agents for use in other continents for over 30 years, only two biological control programmes had been implemented within Europe (Sheppard et al., 2006). One of these programmes was targeted at invasive bracken (Pteridium aquilinum (L.) Kuhn) in the UK but it foundered on legal, political and environmental issues (Lawton, 1988). A potential biological control agent, a leaf-feeding lepidopteran from South Africa, expired in quarantine after lengthy wranglings over additional testing and release protocols. One somewhat bizarre recommendation involved caged releases on bracken infestations in the Isle of Man: The suggested cage size was such that it was deemed both physically and economically impractical (CIBC, 1988, unpublished). Based on weed biological control success stories elsewhere, principally in Australia and South Africa, as well as the changing public opinion concerning biodiversity and conservation, Schroeder and Müller-Schärer (1995) concluded that Europe was ready for classical biological control. Amongst the weeds they identified as potential targets were giant hogweed, Japanese knotweed, and Himalayan balsam. Earlier, Evans and Ellison (1990) also considered these same invasive neophytes to be ideal candidates for this management strategy in the UK because of their increasing environmental or amenity importance. Since this time, biological control programmes have been initiated against these three weeds and, therefore, they make ideal examples to illustrate the opportunities and the constraints for the classical biological control of weeds in Europe.

Opportunities and constraints: examples from projects: Giant hogweed. Heracleum mantegazzianum Sommier and Levier is both an environmental and a health-threat in western Europe and an EU-funded, multidisciplinary project on its ecology and management was initiated in 2002. The outputs of this three-year programme have recently been published (Pysek et al., 2007). Unfortunately the biological control component failed to positively identify any potential biological control agents following surveys for natural enemies in the plant’s centre of diversity, the Caucasus Mountains. A damaging leaf-spot pathogen, Phloeospora heraclei (Lib.) Petr. showed exceptional promise but further evaluation was suspended when it was found to attack several related crop species, including parsnip, in lab-based screening trials. Nevertheless, questions remain unanswered concerning its true (field) host range because this pathogen has never been reported as a disease problem in cultivated parsnip, either in the UK or mainland Europe. Neither has it been found on H. mantegazzianum in its invasive range, despite records of its occurrence on indigenous Heracleum spp. (Seier and Evans, 2007). It would appear, therefore, that pathotypes or formae speciales specific to H. mantegazzianum occur. However, because of scientific and legislative uncertainties, as well as negative public perception or wariness about pathogens (pathophobia), it was decided that this would not be a good model system to launch the classical biological control concept in Europe although it was also concluded that: ‘This does not negate the potential for weed biological control in Europe’ (Cock and Seier, 2007). Nonetheless, how the decision makers and stakeholders in the EU have viewed this ‘failure to deliver’ remains unclear but it is tempting to speculate that it will only provide additional ammunition for critics of classical biological control.

Japanese knotweed. This project, funded by a consortium of UK environmental agencies and local stakeholders, is still ongoing and has now reached the critical phase of submitting the research data on two Japanese natural enemies of Fallopia japonica (Houtt.) R. Decr. for permission to release in the UK, following a four-year assessment. One of these is a hemibiotrophic fungus (Mycosphaerella sp.), ubiquitous and damaging in Japan and specific to the target weed (Kurose et al., 2006). The legislative hurdles still to clear are discussed comprehensively elsewhere in this Proceedings (see Ehlers, R.), as well as in the literature (Sheppard et al., 2006), and at first sight appear to be daunting. However, as well as a test case, this project could serve as the flagship to launch classical biological control into European waters. It is now very much a high-profile weed in the UK, receiving considerable publicity because of its diverse impacts—in natural ecosystems, as a riparian invader; in business/industrial situations, where it dramatically increases the costs of...
construction work; and last but not least, at the urban or ‘grassroots’ level, contaminating gardens and de-valuing property. It has also been in the public domain since London was chosen as the venue for the 2012 Olympics, as the Lea Valley olympic site is infested with both Japanese knotweed and giant hogweed. Initial estimates have put the costs of clearing knotweed from an area smaller than an Olympic swimming pool at around £50,000 or US$100,000 (The Guardian, 13 Sept. 2005). More recent reports on treating the whole site to remove *F. japonica* declared that it would add nearly £70 million to the already burgeoning and highly controversial budget (Sunday Times, March 2007). Such is its infamy that *F. japonica* has now entered the national psyche and vernacular, with one newspaper choosing to describe the ‘success’ of a certain supermarket chain as ‘…spreading through the UK like knotweed’ (The Guardian, 19 April 2007). In fact, an official study on the costs involved in the conventional control of this weed in the UK has arrived at the ‘worryingly precise’ figure of £1.56 billion (DEFFRA, 2003).

*Himalayan balsam.* This is the new project on the block, receiving seed money from several UK agencies to undertake a preliminary survey in parts of its native range in the lower Himalayan region of Pakistan. The results have proven to be extremely encouraging with several fungal and insect natural enemies showing good potential. However, there is still considerable onus on the scientists to raise the stakes with the donors in order to achieve the funding necessary to implement a viable biological control programme against this increasingly problematic and invasive riparian weed in the UK.

### Inundative

**Stability of research:** Besides the ‘scientific’ constraints that limit weed biological control in Europe, the main problems are the low number of stable or established groups working on inundative weed biocontrol strategies in Europe and the low number of projects that are or have been internationally funded on this topic. The European Framework Programmes, which represent the main source of cooperative research funds, have supported only a limited number of projects specifically dealing with biological control of weeds (http://cordis.europa.eu/en/home.html). In particular, within the 5th Framework only one project was funded. This has been mirrored in the recently completed 6th Framework, where only one project devoted to enhancing and exploiting biocontrol agents, including weed pathogens, was funded. Within the COST programme (European Co-operation in the Field of Scientific and Technical Research), one of the longest-running instruments supporting cooperation among scientists and researchers across Europe, few projects received funding, including ‘Biological control of weeds in Europe’ (COST 816, 1994-1999) and ‘Parasitic plant management in sustainable agriculture’ (COST 849, 2001-2006), which included a biological control component (http://www.cost.esf.org/).

**Favorable and unfavourable legislation:** Thanks to the enforcement of the pertinent EU directives (e.g. 91/414, 2002/2076), many dangerous pesticides have been banned in the EU countries in recent years or are scheduled to be banned within 2007. Since 2001, the use of 127 active ingredients has already been prohibited, and this list should lengthen by 14 more by the end of 2007. Among the 191 compounds recognized as herbicidal active ingredients, 71 of them were already excluded by the so-called Annex 1, which is the list of the permitted herbicides, and many others are under evaluation for their prospective exclusion (data gathered from http://fitorev.imagelinenetwork.com). This should give a renewed impetus to research on mycoherbicides and could revitalize public interest in weed biological control.

However, the Commission Directive 2001/36, which amended the Council Directive 91/414/EEC specifically for biopesticides, is very restrictive with regard to the procedures of risk assessment, registration and use of microbial plant-protection products. This is a further and potent reason why no microbial products are currently included as bioherbicides in the register kept by the Directorate of the Consumer Health Protection.

**Choice of suitable targets and efficacious agents:** The choice of an appropriate target weed is of utmost importance for the success of any biocontrol programme. Weeds that cannot be controlled by traditional methods or by chemicals due to resistance and/or environmental factors should be the preferred targets. The lack of alternative control strategies should increase the acceptability of a biological control agent, even if the price is higher due to a more sophisticated method of application. For example, parasitic plants such as *Orobanche* spp. could represent ideal targets. These weeds represent an unsolved and increasing problem in many countries of the Central European and Mediterranean regions due to their complex life cycle and lack of available control methods, especially in light of the ban on methyl bromide and other dangerous soil fumigants. In addition, perennial weeds, such as *Cirsium arvense* (L.) Scop. or *Sonchus arvensis* L., which occur throughout Europe and whose control appears particularly problematic because of their vegetative spread by subterranean organs, could be appropriate targets at the European level. Even plants spreading in anthropic environments or causing health problems (e.g. the allergic plant *Ambrosia artemisiifolia* L.) could be perfect targets for biological control.

The search for suitable agents has not been pursued to the extent that it has been in other parts of the world, due mainly to funding restrictions (see above). The Mediterranean basin or the Caucasian region, for example, has more often represented sources of agents for classical weed biocontrol programmes overseas rather
than has been considered a source of potential mycoherbicides for European programmes.

**Improving production:** Mycoherbicides can only compete successfully with chemical herbicides if the products are as effective as the chemicals they seek to replace and if they are not significantly more expensive. Apart from the efficacy of the strain of the microorganism used, this is mainly dependent on how it is produced and formulated. The production technology used must ensure the highest possible yield of live propagules and the formulation must be such that an application of the propagules to the soil or to the plant is as easy or nearly as easy as the application of a chemical pesticide. For the development of biological control agents it is usually stated that the formulation must improve or at least assist the effectiveness of the microorganism and must ensure a shelf life of the product of at least one year, preferably two or more years. Most probably the main element is cost-effectiveness in order to make the new product competitive with current technologies. With modern inventory control, shelf life is less important, and if a product is truly efficacious but requires special application techniques or equipment, farmers will make the necessary investments just as they have for other specialized machinery now regularly used in modern agriculture.

Suitable culture media for the production of fungal propagules should be selected using an appropriate fermentation technology, followed by: evaluation of the most suitable growth conditions; selection of the best technology to separate the propagules from the fermentation product; evaluation of the most suitable methods and conditions for the formulation of the propagules produced; and assessment of the shelf life of the formulated products. The use of biomasses obtained as residues of industrial food processing or agricultural practices could reduce the cost of production. Molasses, exhausted olive cakes, residues from breweries and waste from tinned fruit industries are a few examples of such by-products that still contain nutrients and could be exploited to grow microbes. Often their disposal presents both an economic and environmental problem. Therefore, they could be ideal and inexpensive media for the production of many microbial biocontrol agents.

**Application systems:** One of the main problems in using microbial biocontrol agents to control weeds is to find suitable methods of application that allow the uniform distribution of the agent at the desired site, without waste or excessive consumption of the mycoherbicide that would increase the cost of treatment and the risks of non-target effects. The uniform and precise application of microbial particles close to the target weed and to the crop to be protected can increase the success of a biological control treatment, and the use of systems or technologies which are usually available in agriculture could influence the acceptability of biocontrol agents by farmers and enlarge the market. For example, the use of drip irrigation systems for the application of suspensions containing conidia of potential mycoherbicides has recently been suggested (Boari et al., 2007). An advantage of using propagules of soil-borne pathogens that normally infect at or below the soil surface is that the propagules may be more protected from environmental factors such as wind and UV radiation, which can negatively affect conidial viability and uniformity of distribution. Applying fungal inoculum by drip irrigation does not require growers to invest in new equipment for application since this strategy is already quite widely used in agriculture to supply water, nutrients or chemicals, especially in vegetable crops where perennial and parasitic weeds often represent difficult problems. A further advantage could be the limiting of the applied doses to the crop root zone and not to the whole field, and therefore a reduction in the cost of treatment.

Several potential mycoherbicides could be applied at the soil level (Charudattan, 2001), as could microbial antagonists (Whipp and Lumsden, 2001) and biopesticides (Copping, 1998), during transplanting or through soil-drenching or root-dip, although these techniques of application can be expensive. As the fungal community already in the soil can affect the persistence of microbial treatments, longer watering intervals involving multiple treatments with lower concentrations of spores could be considered. This would result in a better distribution of the microbes in the soil in terms of volume of protected soil and amount of inoculum and reduce the risk of clogging the dripper.

Leaf-applied mycoherbicides could take advantage of sophisticated technologies, such as the use of advanced optics and computer assistance to sense if a weed is present. In this way, a precise amount of mycoherbicide could be applied only to the weeds and not wasted on bare ground. Such systems could be used where weeds occur intermittently, optimizing the consumption of spray suspension, and thus reducing the treatment costs.

**Potential for genetic enhancement of pathogen biological control agents:** Several transformation-based techniques allow reproducible genetic modifications in fungi. It should be possible to knock out genes in a biological control agent, as well as to transfer specific genes into it, and then determine effects on pathogenicity/virulence. Gressel et al. (2007) have recently inserted into some promising biological control agents genes considered ‘soft’, such as those encoding carboxyhydrases, auxin and oxalate, or ‘hard’ such as those encoding phytotoxins.

**Physiological enhancement of biological control activity:** Different approaches are being used to increase the efficacy of biological control agents without using genetic or transgenic manipulation. The transgenically enhanced hypervirulence of a biological control agent has the advantage of constitutiveness; it is already present, and there is no need for additives. Conversely, if the same effect can be achieved physiologically with
an additive, then there is the advantage that the organism is no different from the wild type after the additive has gone. For example, an organism could be engineered to overproduce oxalate (Gressel, 2002) in order to overcome calcium-dependent weed defences, or the biological control organism could be provided with exogenous oxalate to achieve similar hypervirulence (Gressel et al., 2002); yet, the organism lacks hypervirulence when the oxalate is not present.

Another possibility is the use of natural mutants, such as those that are able to overproduce and excrete amino acids that are inhibitory to the target plant, resulting in enhanced virulence and improved efficacy of the bioherbicide (Thompson et al., 2007).

Environmental impact: One of the main constraints to the release of microbes in the environment for weed biological control is the lack of knowledge about issues such as the fate of the strains after their release into the environment, their stability and the risk of dispersal. The environmental impact of a variety of biological control agents can be assessed by tracking their movement, assayng non-target effects and any changes in host range (especially after genetic or physiological modifications), together with determining long-term environmental persistence. The introduction of biological control agents into soil may pose a risk of unforeseen or detrimental activities on the soil microbial population. The EU Directive 2001/36 states clearly that side effects on non-target soil microorganisms should be addressed, but there are no validated methods available. Until recently, techniques for monitoring direct effects on microorganisms have been restricted to in vitro culture-based methods that ignored 90% or more of the microbial population that could not grow on culture media in the laboratory. The development of DNA-fingerprinting techniques makes it possible to compare the genomes of all strains and to use molecular markers to recognise strains of biological control agents after their release into the environment. The study of the microbial community composition can be based on the direct extraction of DNA from soils or other complex matrices. Practically, techniques such as terminal restriction fragment length polymorphism analysis (T-RFLP), ribosomal intergenic spacer analysis (RISA) and AFLP are relatively rapid, economically feasible, and within the technological capabilities of most microbiological research laboratories. The genetic diversity within species can also be determined by using DNA molecular analyses such as sequencing of nuclear ribosomal DNA, or the beta-tubulin, calmodulin, or elongation factor genes. In addition, real-time PCR techniques can be set up for the qualitative/quantitative detection of DNA from biological control agents (Anderson and Cairney, 2004).

Integration between diverse biological control agents, bioactive fungal metabolites, herbicides, or other chemicals: An important factor that can reduce the efficacy of a potential mycoherbicide is the ability of the target weed to resist invasion and colonization by the biological control agent. Several attempts have been made to combine mycoherbicides with bioactive metabolites, in order to enhance agent efficacy. Such combinations can suppress or weaken herbicide mechanisms by blocking the synthesis of secondary plant metabolites or breaking down physical barriers to pathogen attack, resulting in increased biological control (Duke et al., 2007, and references therein cited).

The effect of herbicides on plant disease is an important but generally overlooked aspect of integrated weed management. Nevertheless, understanding herbicide/plant pathogen interactions can be critical in designing effective and efficient integrated weed management programmes.

Synthetic herbicides have the potential to influence plant disease by several mechanisms. It is not unusual for low rates of herbicides to stimulate in vitro pathogen growth or sporulation (Wyss et al., 2004; Yandoc et al., 2006). On the other hand, herbicides such as glyphosate can also be very effective at lowering plant resistance to pathogens and acting as a synergist for microbial weed biological control products (Duke et al., 2007). In such strategies, dose rates are likely to be highly important to both the direct and indirect effects of herbicides on plant disease.

Enhanced bioherbicidal efficacy of *Eexerohilium monoceras* (Drechs.) K. J. Leonard and E. G. Suggs on *Echinochloa crus-galli* (L.) Beauv., a weed in paddy rice (*Oryza sativa* L.), was obtained when the fungus was applied with δ-aminolevulinic acid, a precursor of tetrapyrroles—compounds which are involved in the bleaching and killing of plant tissue (Hirase et al., 2006). A system to integrate low doses of glyphosate with a foliar desiccant (ammonium sulphate) and the biological control agent *Alternaria destruens* (Smolder) to control *Cucurbita pentagona* Engelm., has also been reported (Cook et al., 2005).

The efficacy of a weak biological control agent, *Colletotrichum coccos* (Wallr.) S. Hughes, on velvetleaf (*Abutilon theophrasti* Medik.) was improved by applying calcium chelators that repressed host-plant defences by reducing callose formation (Gressel et al., 2002). Also phytotoxic metabolites produced by plant pathogens can weaken defence mechanisms of plants, rendering them more susceptible to pathogen attack. Thus, the application of toxins jointly with the pathogens could strongly enhance their bioherbicidal properties (Vurro, 2007).

A mixture of three host-specific pathogens: *Alternaria cassinaria* (specific to sicklepod), *Phomopsis amaranthicola* Rosskopf, Charudattan, Shaban and Benny (specific to pigweeds), and *Colletotrichum dematium* f. sp. crotalariae (specific to showy crotalaria), proved to be very efficacious in the simultaneous control of the three weeds (Chandramohan and Charudattan, 2003). Good control of seven weedy grass species has also been obtained using a
Discussion

Perhaps we can borrow the use of the ‘Anna Karenina Principle’ from McClay and Balciunas (2005), who first applied it to biological control [the list of borrowers, of course, goes back to Tolstoy (1877)] in order to compare the constraints and opportunities for classical vs inundative biological control. For the inundative approach, these are essentially similar for every weed target in every country, region or continent: ‘happy families are all alike’, and the issues involved have been addressed here. However, for the classical approach, especially against invasive environmental weeds, the factors involved are extremely, and often uniquely, complex and therefore must be dealt with on a case-by-case basis: ‘every unhappy family is unhappy in its own way’.

In the European context, many of these issues have already been highlighted and reviewed comprehensively by Sheppard et al. (2005). Suffice it to say that no biological control agents have been released thus far, and the few that are in the pipeline face an uphill struggle and uncertain future for acceptance, despite the fact that: ‘Classical biological control remains the only tool available for permanent ecological and economic management of invasive alien species …’ (Sheppard et al., 2005). This approach has even received the seal of approval from the Convention on Biological Diversity (CBD), the European and Mediterranean Plant Protection Organisation (EPPO) and the European Strategy on Invasive Alien Species (ESIAS). However, is this the kiss of death, as the bureaucratic red tape kicks in? As previously mentioned, there are now so many more environmental concerns to address, compared to earlier times, that the costs of implementing all of them would put any biological control project out of the financial reach of traditional donors. Certainly, we have moved on from the ‘hunter-gatherer, quick-release, let’s-try-this-one’ approach to the position where it is essential to abide by the CBD and to undertake scientifically driven risk assessments. These are in place but still subject to the whims and interpretations of individual countries and international organisations, as well as the critics of biological control. In the present climate, it would still take only one mistake, or unexpected non-target issue, to seriously undermine the solid scientific foundations on which classical biological control is based.

In the case of the CBD, this has recently created additional barriers hampering free exchange of germplasm between countries. For example, permission to release an Argentinian strain of the rust, Puccinia spegazzinii DeToni for use against the highly invasive mile-a-minute weed (Mikania micrantha Kunth) in China has been blocked, seemingly permanently, by Misiones Department, which has a separate CBD policy from that of Argentina. Thus, biological control scientists are now expected to negotiate delicate political issues in order to implement classical projects. In addition, there are constant attacks on or criticisms of the safety-testing procedures employed to screen classical weed biological control agents, despite an impeccable track record (McFadyen, 1998), as well as new concerns about the indirect impacts of even host-specific agents on non-target species (Pearson and Callaway, 2003; Louda et al., 2003). These concerns provide further ammunition for biological control sceptics to shoot down any proposals, which are based solely on the classical biological control approach, before they have gotten off the ground. Moreover, this relates only to relatively ‘inoffensive’ insect agents! What hope is there for pathogens? It could be argued that if all the environmental concerns and risks involved in undertaking a motorized shopping trip were analysed as critically, supermarkets would go out of business (even the ones spreading like knotweed)!

As flagged by Thomas et al. (2004), such concerns serve to highlight ‘... the need for proper ecological and socioeconomic evaluation of pests before control to determine probable costs and benefits’. The financial stakes are raised yet again, as well as time frames, before biological control can even be considered as a management option, putting such proposals on a different donor level. The giant hogweed project, for example, was large by EU standards because of its multidisciplinary approach and composition. Even so, fundamental questions relating to the safety of several potential biological control agents still remained unanswered because of insufficient time and funding. Thus, we are left with the possibility that future classical weed biological control projects for Europe need to be multidisciplinary and last a minimum of 5–10 years to achieve all the goals now expected, once the target posts have been moved. Such multi-million euro proposals must thus be the norm if proposals are to be approved and project aims realized. It is no coincidence, perhaps, that the multinational, multi-organisational, multidisciplinary biological control project against migratory locusts did achieve its objectives, but only after a massive injection of funds from a consortium of international donors for over more than a decade.

This leads finally to the perennial question: biological control—risky or necessary? (Thomas and Willis, 1998). Critical decisions need to be taken in Europe regarding the long-term management of invasive weeds, especially those with serious environmental impacts, but perhaps it is better not to fiddle around too much while the aliens continue their destruction of fragile ecosystems.
Opportunities and constraints for the biological control of weeds in Europe

References


Tolstoy, L. (1877) Anna Karenina. Ruskii Vestnik, Moscow, Russia.