Biological control of weeds in European crops: recent achievements and future work

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Summary

Approaches to the biological control of weeds in arable crops and integration of biological weed control with other methods of weed management are broadly discussed. Various types of integrative approaches to biological control of weeds in crops have been studied within the framework of a concerted European Research Programme (COST-816). During the period 1994–99, some 25 institutions from 16 countries have concentrated on five target weed complexes. Some major scientific achievements of COST-816 are: (i) combination of the pathogen *Ascochyta caulina* with an isolated phytotoxin produced by this fungus to control *Chenopodium album* in maize and sugar beet; (ii) the elaboration and preliminary field application of a system management approach using the weed:pathogen system *Senecio vulgaris*:*Puccinia lagenophorae* to reduce the competitiveness of the weed by inducing and stimulating a disease epidemic; (iii) combination of underseeded green cover with the application of spores of *Stagonospora convolvuli* to control *Convolvulus* species in maize; (iv) assessment of the response of different provenances of *Amaranthus* spp. to infection by *Alternaria alternata* and *Trematophoma lignicola*, the development of formulation and delivery techniques and a field survey of native insect species to control *Amaranthus* spp. in sugar beet and maize; (v) isolation of strains of different *Fusarium* spp. that infect all the economically important *Orobanche* spp. and development of novel, storable formulations using mycelia from liquid culture. Although no practical control has yet been reached for any of the five target weeds, potential solutions have been clearly identified. Two major routes may be followed in future work. The first is a technological approach focusing on a single, highly destructive disease cycle of the control agent and optimizing the efficacy and specificity of the agent. The second is an ecological approach based on a better understanding of the interactions among the crop, the weed, the natural antagonist and the environment, which must be managed in order to maximize the spread and impact of an indigenous antagonist on the weed.

Keywords: crop weeds, integrated weed management, biological weed control.

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Crop weeds and their control in Europe

Crop management in Europe is still dominated by mouldboard ploughing, seeding into a completely weed-free seedbed and keeping the crop free of weeds throughout the season (Sommer, 1996). During the last few decades, the importance of mechanical weed control has declined, while there has been increased use of herbicides, often of persistent types. This has allowed long-term, weed-free, bare soil for the first time in the history of farming (Ammon & Müller-Schärer, 1999). More recently, however, farmers and scientists have realized that uncovered ground leads to important disadvantages, such as erosion, water run-off and nitrogen losses (Zimdahl, 1993). A new concept in rational weed control is the tolerance of weeds at a certain threshold level. The availability of chemical herbicides to control weeds effectively and economically greatly favoured the development and establishment of this economic threshold concept. Unfortunately, this concept is presently followed by only a few farmers, as most still want 'clean' fields (Hurle, 1997). Thus, weeds remain the single most important factor causing yield reductions, as measured by the effort spent in their control and by global agrochemical sales (Powell & Jutsum, 1993). Genetic engineering, which has permitted the introduction of herbicide-resistant crops, reflects this fact and indicates the extent to which the industry is prepared to invest to secure weed control markets. However, these developments do not lead to durable agro-ecosystems. The repeated and large-scale application of broad-spectrum herbicides raises further concerns, such as possible selection for weed resistance to new herbicides, spread of resistant volunteer crops and transfer of resistance genes to wild and weedy relatives (Darmency, 1996). In this context, Hurle (1997) proposed the development of ecological thresholds, which consider how many weeds are needed and can be tolerated at an economic threshold. Recent studies (Müller-Schärer & Potter, 1991) clearly show the many positive effects of non-crop (companion) plants for the crop and the agro-ecosystem in general. Further studies are needed to quantify such beneficial effects of weed species, to assess their desirable:undesirable points and, eventually, to build such knowledge into threshold models (Hurle, 1997).

Parallel to these scientific developments and achievements, governments have elaborated important international documents that directly affect future weed control measures. The United Nations Conference on Environment and Development (UNCED), in its Agenda 21, recognized integrated pest management (IPM) as the preferred strategy to achieve sustainable agricultural production (UNCED, 1992). IPM typically involves a reduction in reliance on chemical pesticides, including herbicides. Furthermore, in the Convention on Biological Diversity, the point is clearly made that priority should be given to biological control as a component of future pest management (Glowka *et al.*, 1994).

Methods used to control weeds in arable crops biologically

Biological weed control (for definitions and recent reviews, see Greaves, 1996; Crutwell McFadyen, 1998; Müller-Schärer, 2000) has been used most successfully against invading plant species threatening endangered ecosystems, habitats and species (Crawley, 1989; Hoffmann & Moran, 1991; Holden *et al.*, 1992; Schroeder & Müller-Schärer, 1995) and, most probably, will continue to do so (Cronk & Fuller, 1995). Its application in intensively managed agro-ecosystems, however, is difficult because of the ephemeral nature of these habitats with high disturbance levels and the fast control process needed relative to the short duration of the cropping season. Three methods of biological weed control in crops can be distinguished: the inoculative or classical approach; the inundative or microbial herbicide approach; and the system management or augmentative approach.

Inoculative biological control using exotic control agents

Inoculative (classical) biological weed control has generally been restricted to environmental weeds and extensive agriculture (e.g. rangeland) and has limited application to intensive crop production systems (Watson & Wymore, 1989). Annual weeds of arable crops have long been considered poor targets for inoculative biological control, although theoretically, organisms with excellent search and dispersal abilities (some insects) or those showing persistence (some fungi) can be used for inoculative biocontrol in unstable, disturbed habitats (Reznik, 1996). This phenomenon is illustrated by some well-known pests and diseases, such as *Leptinotarsa decemlineata* Say. (Colorado beetle) on potato (*Solanum tuberosum* L.) or the rust *Puccinia hieracii* Röhl f. sp. *cichorii* on chicory (*Cichorium intybus* L. var. *foliosum* Hegi), which survive and suppress their annual host plant, despite high levels of habitat disturbance and crop rotation. Two examples from weed biocontrol include the introduction of the rust *Puccinia chondrillina* Bubak & Sydenham to control *Chondrilla juncea* L. (skeleton weed) in wheat (*Triticum aestivum* L.)–fallow systems in Australia (Cullen *et al*., 1973; Cullen, 1985; Espiau *et al*., 1998) and the introduction of *Zygogramma suturalis* F. (ragweed beetle) to control *Ambrosia artemisiifolia* L. (common ragweed) in Russia and, more recently, in Croatia, China and Australia (Reznik, 1996).

Inundative biological control using microbial herbicides

The inundative method, primarily using microbial herbicides, has greater opportunity for application in intensive agriculture (Charudattan, 1991). Between 1980 and 1998, three bioherbicides (DeVine, Abbot Laboratories, Chicago, IL; Collego, Encore Technologies, Minnesota, MN; and Dr BioSedge) were registered in the United States, and one was registered in each of Canada (BioMal, PhilomBios, and Agriculture and Agri-food, Saskatoon, Canada), Japan (CAMPERICO, Japan Tobacco, Yokohama) and South Africa (Stumpout, Plant Protection Research Institute, Stellenbosch, S. Africa). Another fungal pathogen, not registered as a microbial herbicide, has been developed for use in the Netherlands (BioChon, Koppert, Biological Systems, Berliel en Rodenrijs, the Netherlands) as a stump-rotter to control regrowth of *Prunus serotina*. Five of these seven bioherbicides are still commercially available for use, while two (BioMal and Dr BioSedge) are unavailable as a result of technical difficulties in production or market considerations (Charudattan, 1999). With regard to type and use of control agents, various approaches can be distinguished: (i) the use of single, highly host-specific agents; (ii) the combination of agents to control a single weed species; (iii) multiple pathogens to control several weeds; and (iv) the use of broad-spectrum bioherbicides. Today, the use of single and host-specific agents is the approach most widely used in crops. Lists with short descriptions of the presently registered bioherbicides and additional, mainly locally produced, fungal biocontrol agents are given by Greaves (1996), Charudattan (1999) and Müller-Schärer (2000).

The exploitation of synergy between pathogens and insects has been used repeatedly and successfully to control environmental weeds (Julien & Griffiths, 1998) and has been proposed for use against crop weeds such as *Orobanche* spp. (Kroschel *et al*., 1999), *Rumex* spp. (Hatcher *et al*., 1994, 1995) and *Senecio vulgaris* L. (Frantzen & Hatcher, 1997). Furthermore, combinations of pathogens that unite the specificity of biotrophs with the virulence of necrotrophs have been suggested (for example, see Morin *et al.*, 1993a,b), but whether or not such interactions can be exploited successfully to control crop weeds remains unknown (Paul *et al.*, 1993).

Preliminary results have shown that control of *Amaranthus hybridus* L. (pigweed), *Senna obtusifolia* (L.) Irwin & Barneby (sicklepod) and *Crotalaria spectabilis* Roth. (showy crotalaria) using a multiple-pathogen strategy consisting of four pathogens applied in a single, post-emergence spray was feasible without loss of efficacy or host-specificity (S. Chandromohan & R. Charudattan, pers. comm.). Further research into this approach is clearly promising.

The use of highly virulent, broad-spectrum bioherbicides has been suggested repeatedly for biologically controlling multiple species of target weeds as an alternative to the above approach. One option to permit their safe release might be the use of genetic manipulation to restrict their host range and to prevent their spread or long-term survival (Sands & Miller, 1993). Host-specific promoters or toxins, host-dependency by multiple auxotrophy or mutants dependent on specific environmental conditions may be used in this context (Sands & Miller, 1992). No such mutants, however, are presently in use. Similarly, Yang & Schaad (1996) suggested a further challenging proposal, based on the interaction of low-virulence, broad-spectrum pathogens with special carriers. The specificity is given by the targeted application of the carrier, which must be present for the pathogen to infect the host.

Managing established weed:antagonist systems to stimulate epiphytotics

A third, ecologically oriented approach is based on knowledge of the crop environment, especially of the mechanisms underlying the interactions of the weed, the natural enemy and the environment at the individual and population levels. This approach, despite having received much attention in the literature and being described variously as augmentative control, cultural control, optimization or conservation, has remained largely a theoretical concept. Newman *et al.* (1998) briefly reviewed studies reporting the use of both exotic and native insect herbivores to control environmental, rangeland and aquatic weeds and divided these conservation strategies into three general areas: (i) population protection or the appropriate use of pesticides to maintain native or exotic biological control agents; (ii) habitat protection to preserve critical habitats or refugia; and (iii) plant community management to maintain and enhance the effectiveness of existing biological control agents. This approach is presently being elaborated as the 'system management approach' for use in arable crops (Müller-Schärer & Frantzen, 1996; and see below). By focusing on pathogens, it aims to induce and stimulate disease epidemics within weed populations and, thus, reduce weed competitiveness at the population level. This can be achieved by: (i) the introduction of inoculum in a weed population at the 'right' time or by leaving stands of infected plants to overwinter and to allow early infection in spring; (ii) the careful selection and manipulation of the genetic composition of the pathogen population to match the genetic composition of the weed population; and (iii) the specific management of the infection conditions, mainly through adapted fertilizer and irrigation treatments (Paul *et al.*, 1993). Conservation and facilitation methods of biological control are especially well suited to promoting sustainable agro-ecosystems, in which weed control no longer aims at crop production in a weed-free environment but simply at a reduction in weed-induced crop losses (Watson, 1992; Müller-Schärer & Frantzen, 1996).

Integrating biological control with other methods of weed management

Weed problems in agro-ecosystems are rarely caused by single weed species. Clearly, biological control, which is inherently narrow spectrum, has to be considered as an integrated component of a well-designed pest management strategy, not as a cure by itself. In addition, although microbial herbicides can kill their weed target rapidly, many other biological control agents (especially in the classical strategy) do not lead to initial kill soon after application, which is characteristic of many chemical herbicides (Gressel & Segel, 1982). Nevertheless, those biological agents capable of systemic spread or of natural re-infestation in the field may well reach an 'effective kill', that is the elimination or severe reduction in seed production. In the long term, this effect may be substantially greater than an initial knock-down, as compensatory regrowth of weeds that survive initial injury may allow the weeds to re-establish a competitive population quickly. In this context, 'classical' biological control agents have to be seen rather as stress factors, not as weed-killers. Although microbial herbicides are the exception, in most cases, combinations of biological with other weed management tools will be needed to result in acceptable levels of overall weed control. Such integration can be viewed as a vertical integration of various control tactics against a single weed species, or as a horizontal integration across different weed species in one crop (Watson & Wymore, 1989). Horizontal integration mainly involves the combination of microbial herbicides with chemical herbicides or mechanical methods to broaden the spectrum of weed species controlled. For example, the registered bioherbicides, DeVine and Collego, are routinely integrated with chemical pesticides and other practices to control weeds in citrus fruit and rice (*Oryza sativa* L.) production respectively (Charudattan, 1999). Furthermore, in situations in which particularly high doses of herbicides are needed to control a single weed species, while the rest of the weed flora could be controlled by lower amounts, biological control may allow considerable reduction in herbicide inputs and contribute to maintaining species diversity in crops. Cullen (1996) recently distinguished three types of possible vertical integration, both in time and space, of biological control with other methods of weed management: purpose-specific approaches, ecological integration and physiological integration.

Purpose-specific approaches

The type and level of control are chosen according to the requirements. This often involves different methods to be applied at different sites. For instance, for a weed that is still spreading, chemical herbicides may well be the method of choice to remove new infestations, while biological control may be relied on to give long-term control of large, established infestations.

Ecological integration

This term is given to situations in which different approaches are used on the same infestation, often at the same time. Integration with herbicides (Scheepens, 1987; Wymore *et al.*, 1987) and with (crop) plant competition (DiTommaso *et al.*, 1996; Müller-Schärer & Rieger, 1998) are most widely envisaged. This type of integration essentially summarizes holistic approaches that encompass all modifications to the environment that may favour the effectiveness of biological control agents and facilitate the management of a weed population.

Physiological integration

This type of integration makes use of synergistic interactions between changes in the biochemistry of weeds, often produced by sublethal effects of herbicides and the effectiveness of biological control agents. Herbicides (or other 'synergists') are known to increase the incidence of infection and to enhance the growth of pathogens (Hasan & Ayres, 1990; Sharon *et al.*, 1992; Gressel *et al.*, 1996), but infection by the pathogen may also facilitate the uptake of herbicides, mainly by injuring the cuticle and epidermis of the host. In addition, various studies have shown greatly increased disease severity and agent effects when combined with phytotoxic metabolites produced by the pathogen (Vurro *et al.*, 1997; and see below), or with specific formulation and delivery techniques of microbial herbicides (Greaves, 1996; and see below). Thus, physiological integration is directed towards combined effects with biological control agents on plant individuals.

Ultimately, optimal management with minimal disruptive interventions requires a good understanding of the weed's biology and, especially, population dynamics. Biological weed control requires, and provides, a detailed *ex ante* analysis of the problem situation, especially of the crop environment, revealing interactions between the various components and their underlying interactions. It should, therefore, be the strategy that is basic to integrated production systems. Bridges between different disciplines need to be built to optimize the fit of biological control into existing management systems (Müller-Schärer, 1995; Cullen, 1996; Ammon & Müller-Schärer, 1999). In Europe, there is little tradition of using natural enemies to suppress weeds (Schroeder *et al.*, 1993; Müller-Schärer, 1995; Müller-Schärer & Scheepens, 1997; Julien & Griffiths, 1998). In the early 1990s, interest was expressed by members of the working group on 'Biological Control of Weeds' of the European Weed Research Society (EWRS), to use the expertise gathered in projects for overseas and to co-ordinate activities on biological weed control in Europe (Schroeder *et al.*, 1993). This intention is paralleled by the fact that most European countries must now try to reduce pesticide application levels without satisfactory alternatives being available. In addition, herbicide resistance is increasing in importance in most European countries. Clearly, there is a need for concerted action to develop new control strategies (Müller-Schärer & Scheepens, 1997).

Long-term, interdisciplinary and international research is a prerequisite of biological weed control, as many weed species are widespread, and the biological control strategy often involves the exchange of their natural enemies. Benefits can then easily be shared among many European countries. Moreover, only co-operative and concerted efforts will allow effective completion of weed biocontrol projects within a reasonable period of time. COST actions, as discussed below, provide the ideal framework for such co-operation.

Objectives and results of a concerted European research programme to control problem weeds in arable crops (COST-816)

Objectives

COST (European Co-operation in the Field of Scientific and Technical Research) is principally a framework for R&D co-operation, allowing the co-ordination of nationally funded research projects. COST Action 816 is the result of a Swiss proposal, in co-operation with members of the 'Biological Control Working Group' within the EWRS. The main objectives are (Müller-Schärer & Scheepens, 1997): (i) to gather together European institutions that intend to co-operate in investigating the potential of biological weed control in crops; (ii) to promote a programme for scientific research and exchange; (iii) to draw up a general protocol for biological weed control in Europe; (iv) to integrate biological control into general weed management strategies; (v) to establish a protocol to resolve potential conflicts of interest; (vi) to establish a list of further European weed species for biological control.

To promote co-operation, the research has focused on four target weeds or weed complexes: *Amaranthus* spp., *Chenopodium album* L., *Senecio vulgaris* L. and *Convolvulus* spp. (*Convolvulus arvensis* L. and *Calystegia sepium* (L.) R. Br.). A working group was formed for each of the target weeds. In 1997, *Orobanche* spp. were included as a fifth target complex. These weed complexes were selected because of their abundance, economic importance (Schroeder *et al.*, 1993) and their suitability for biological control (Harley & Forno, 1992; Peschken & McClay, 1992). At the same time, they allow consideration of native and introduced weeds, of various biocontrol methods and of both annual and perennial cropping systems. Hence, initially, key weeds of European agriculture and situations in which the successful control of a single weed species would result in considerable reduction in herbicide input, or of other control measures, were emphasized (Müller-Schärer & Scheepens, 1997). Also, single weeds that may become problems in arable crops as a result of long-term herbicide application or of new, environmentally sound cropping techniques, such as those with living mulch (Müller-Schärer, 1995), were included. A biological control strategy, which may involve several biocontrol methods and general phytosanitary and other crop management measures, was then set up for each of the target weeds that allowed optimal integration into other weed control methods. For reasons discussed above, major emphasis has been given to the use of indigenous fungal pathogens. An estimated 80% of all European research activities on the biocontrol of European weeds are covered by the five working groups. The total cost of national research being co-ordinated is estimated at 10 million euros for the duration of COST-816 (1994–99).

European participation

The signatory countries to COST-816 are Belgium, Croatia, Denmark, France, Germany, Hungary, Italy, Norway, Switzerland, Spain, Sweden, Slovakia, The Netherlands and the United Kingdom. The Weizmann Institute of Science, Rehovot, Israel, and the National Research Centre, Cairo, Egypt, have been included as institutions from non-COST member states.

Scientific achievements of the working groups

Chenopodium album *(fat hen; common lambsquarters)*

The working group on this target weed now has more than 20 regular participants from eight European countries who have attended several meetings reporting and assessing progress.

One research project from Sweden investigates soil-borne bacteria, several of which showed weed control activity when used as a foliar spray on *C. album* plants. All the other projects focus on the use of *Ascochyta caulina* (P. Karst.) v.d. Aa & vs. Kest. and *A. caulina* in combination with its own toxins or with chemical herbicides as microbial herbicides against *C. album* (Scheepens *et al.*, 1997). This research on *Chenopodium*:*Ascochyta* interactions is based on previous work at Wageningen (The Netherlands) (Kempenaar, 1995). Isolation of toxins from *A. caulina* is based on similar work with other *Ascochyta* species in Italy (Evidente *et al.*, 1998). With the isolate of *A. caulina* used in the initial work at Wageningen, 60–70% kill of plants and a substantial growth reduction in surviving plants can be achieved under field conditions. The activity of the fungus depends on favourable weather conditions (especially humidity) shortly after application and the appropriate growth stage (up to the four-leaf stage) of the weed (Kempenaar & Scheepens, 1996). Most of the work in COST-816 is aimed at ensuring efficacy and reliable field use in a range of situations. An important new initiative was the start of the project 'Optimizing biological control of a major weed in crops' in 1998, funded by the European Commission, in which six of the groups involved in COST-816 and the private company Novartis are collaborating in research to control *C. album* with *A. caulina* and its phytotoxins.

More than 250 new isolates of *A. caulina* have been collected from different European countries (P C Scheepens, unpubl. obs.), some being significantly more virulent than the standard isolate used in earlier work at Wageningen. Also, several of the new isolates require shorter periods of high relative humidity for infection than the standard isolate. The efficacy of *A. caulina* can be enhanced by adding low doses of some chemical herbicides. Similar effects are achieved with culture filtrates containing toxic metabolites of *A. caulina*. The chemical structure of two toxins from culture filtrates of *A. caulina* has been identified (Evidente *et al.*, 1998). By adding a suitable wetting agent, the culture filtrate is toxic to *C. album* without the presence of the living fungus. Progress has also been made with formulations of *A. caulina* spores. Formulations of *A. caulina* based on invert emulsions can result in infections in the absence of dew. However, this type of formulation is regarded as unsuitable for use in practice, because it is difficult to prepare and difficult to spray using conventional equipment. Several adjuvants that increased the reliability of other microbial pesticides under a range of environmental conditions (Greaves, 1998) were not effective with *A. caulina*. Recently, some adjuvants have been identified that have good prospects as formulants of *A. caulina* spores.

Senecio vulgaris *(common groundsel)*

The activities of this working group started in 1993 with participants from five countries who decided to adopt a biological control strategy that we now call the 'system management approach' (Müller-Schärer & Frantzen, 1996). This is a new approach to biological weed control characterized by the use of native pathogens as a relatively small inoculum. Relating disease epidemics to crop:weed interactions at the population level became the major research issue of this working group (Müller-Schärer & Frantzen, 1996; Frantzen & Müller-Schärer, 1998). As a contribution to the development of the system management approach, this research focuses less on the application of biological weed control in practice and more on scientific questions generated by developing this approach. The philosophy behind the research activities has been described by Frantzen & Hatcher (1997).

The rust fungus *Puccinia lagenophorae* Cooke, the selected biological control agent, has been intensively studied in the past with regard to its impact on *S. vulgaris* (Paul *et al.*, 1993). Further studies investigated the overwintering survival of the rust (Frantzen & Müller-Schärer, 1999), mechanisms of avoidance (J Frantzen & H Müller-Schärer, unpubl. obs.) and the mechanisms of resistance (Wyss & Müller-Schärer, 1999). Ongoing research by the group at Fribourg (Switzerland) concerns the effects of temperature on the infection process and seed germination, weed population dynamics, genetic variation and phenotypic plasticity, as well as intraspecific and interspecific competition.

Several biotic and abiotic factors may influence both the speed of epidemic spread and the subsequent impact of *P. lageophorae* on *S. vulgaris*. Insects are abundant in the field and may be a biotic factor influencing biological weed control. Also, the interactions between insects and the rust have been investigated (Tinney *et al.*, 1998a,b), but the results have not yet been evaluated with respect to the system management approach of biological weed control. The effects of the abiotic factor temperature on *P. lagenophorae* epidemics are currently under investigation. A preliminary result is that the velocity of epidemic spread increases linearly with temperature in the range of 10–22 °C (R Kolnaar, unpubl. obs.). The effects of chemical herbicides on the infection process were also investigated, and the preliminary results suggest that herbicides have a negative effect on *P. lagenophorae* epidemics (F Rossi & G S Wyss, unpubl. obs.).

Estimates of basic parameters of epidemiological models allow the calculation of velocities of epidemic spread that are presently being tested in field experiments. The impact of induced epidemics on celeriac (*Apium graveolens* L. var. *rapaceum* cv. Kojak):*S. vulgaris* interactions (Müller-Schärer & Rieger, 1998) will be quantified further in future field experiments.

Convolvulus arvensis *(field bindweed) and* Calystegia sepium *(hedge bindweed)*

Fourteen scientists from nine institutions in seven countries are participating in this working group on studies that concentrate on *Stagonospora convolvuli* Dearness & House strain LA39, which was isolated at Long Ashton, UK (Pfirter & Defago, 1998).

This pathogen induces severe disease symptoms on both *C. arvensis* and *C. sepium*. All tested growth stages and ecotypes of the weed were susceptible (Pfirter & Defago, 1998). Several phytotoxins of the pathogen have been isolated and identified, including leptosphaerodione (B Nicolet, pers. comm.). The genetic variation among *Stagonospora* spp. isolates collected in different European countries has been studied using random fragment length polymorphism (RFLP) analysis of the ITS region and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) assay (H A Pfirter, unpubl. obs.). The results obtained will allow us to track this control agent once released in the field.

Formulation of the pathogen in a 10% rapeseed oil-in-water emulsion significantly improved its efficacy and reduced its dependence on extended dew periods. Inoculation of the weed with 10^7 spores mL^{-1} in the oil emulsion applied to run-off produced a high level of leaf necrosis even in the absence of 100% relative humidity (Pfirter & Defago, 1998). Solid-state fermentation on couscous (cracked hard wheat) produced up to 4×10^8 spores g^{-1} substrate. These spores were as pathogenic as those grown on V8 juice agar. After air drying on kaolin, followed by storage at 3 °C, the spores remained viable and pathogenic for 140 days (H A Pfirter, unpubl. obs.).

From 1995 to 1997, *S. convolvuli* strain LA39 has been applied in a maize (*Zea mays* L.) field heavily infested by *C. sepium*. In all years, a high disease level was observed with defoliation of the weed. In addition, ground cover of *C. sepium* was significantly reduced (Pfirter *et al.*, 1997; Guntli *et al.*, 1999a). The potential of *S. convolvuli* strain LA39 as a bioherbicide has also been demonstrated in a non-crop situation. Application in a cemetery, where cotoneaster (*Cotoneaster dammeri* C. K. Schneider) was heavily infested by *C. arvensis*, resulted in a decrease in ground cover density of *C. arvensis* from 40% to 17% in the plots inoculated with *S. convolvuli* strain LA39 within 20 days after application (Guntli *et al.*, 1998).

In greenhouse experiments, *Lolium multiflorum* Lam. and *Trifolium pratense* L. reduced the growth of bindweed (*Calystegia sepium*). In the field, undersowing maize with *T. pratense* had no positive effect on weed control (Guntli *et al.*, 1998). Nevertheless, a green cover (in maize), which controls most weeds, together with application of *S. convolvuli* strain LA39 to control bindweed (*C. sepium*) plants that escape the green mulch, would be perfectly suited to an integrated pest management system (Pfirter *et al.*, 1997). Bindweeds (*C. sepium*) produce large amounts of calystegines in their roots. The rhizobacterium *Sinorhizobium meliloti* strain Rm41 [with a plasmid containing genes for calystegine degradation (*cac*)] had a significant advantage over a *cac*$^-$ derivative in root colonization. Such *cac* plasmids could be used to improve microbial biocontrol agents of bindweeds (*C. sepium*) (Guntli *et al.*, 1999b,c). A mixture of two *Stagonospora* spp. (*S. convolvuli* strain LA39 and *Stagonospora* sp. isolate LA30B) increased control of the weed (H A Pfirter, unpubl. obs.), and formulating the pathogen with sublethal doses of different chemical herbicides increased the susceptibility of bindweed (*Convolvulus arvensis*) to *S. convolvuli* strain LA39 (H A Pfirter, pers. comm.).

Amaranthus *spp. (pigweeds)*

The working group on *Amaranthus* involves 12 institutions from nine countries. An extensive survey of insects associated with *Amaranthus* spp. has been made in Switzerland, Hungary and Slovakia (Bürki *et al.*, 1997). Some 150 phytophagous insect species were found, many in all three countries. All of these insects were either polyphagous or oligophagous, and none was monophagous. In addition, some are known crop pests. Based on the results of these field surveys and on an extensive literature review (El Aydam & Bürki, 1997), none of the phytophagous insect species associated with target *Amaranthus* spp. in Europe has potential as a biological control agent. Thus, South, North and Central America, the centres of origin of noxious *Amaranthus* weeds in Europe, will need to be surveyed to locate potential insect control agents for use in Europe. In Hungary, several *Amaranthus* spp. are cultivated as crops because of their high content of protein, vitamins and amino acids. The leaves and grains are fed to chickens and pigs, and grains for human nutrition are exported to western Europe. A gene bank has been established with some 100 cultivars and strains of *A. hypochondriacus* L., *A. cruentus* L. and *A. caudatus* L. Some of these biotypes appear to be resistant to phytophagous insects. In stands of heavily infested plants, some individuals remained free from insect attack. Interestingly, few differences were found in the composition of the insect faunas associated with crop and weedy *Amaranthus* spp. However, the species found always occurred in greater abundance on crop species, which have higher biomass, more leaves and bigger flowerheads, than on weedy species (L Szabo-Maraz, pers. comm.).

Several pathogenic fungi (*Alternaria*, *Fusarium*, *Phomopsis* and *Trematophoma* spp.) have been isolated from *Amaranthus* spp. from different European locations (Bürki *et al.*, 1997). Among these, *Alternaria alternata* (Sr.) Keissler (Lawrie *et al.*, 2000) and *Trematophoma lignicola* (Petrak) (Lawrie *et al.*, 1999) appear to be the best potential candidates as microbial herbicides. *Alternaria alternata* is effective against *A. retroflexus* L. when applied as a foliar spray in a rapeseed oil emulsion containing 10^7 conidia mL^{-1} (three or four true-leaf plants) and given a 6–8 h dew (Lawrie *et al.*, 2000). *Trematophoma lignicola* is also effective against this weed when given a 16-h dew (Lawrie *et al.*, 1999). Further work on formulation and application parameters to ensure even distribution of the pathogen on its target and, thus, optimal efficacy in the field are in progress (Lawrie *et al.*, 1997).

In Germany, the intraspecific variability of *A. retroflexus*, including its hybridization with the related species *A. powellii*, has been studied (Jüttersonke, 1996), the emphasis being on the variability of reactions to ecological and crop management-related factors. The 110 provenances investigated showed great genetically based variation in morphological characters and growth patterns. In addition, the spontaneous infection of plants in the field by the fungus *Albugo amaranthi* (Schw.) Kuntze was investigated to determine potential variability between different provenances in their response to the fungus. While the leaves of biotypes originating from eastern Germany showed severe infection, the provenances from other countries were not or only slightly infected. Susceptibility was negatively correlated with growth rate.

Orobanche *spp. (broomrapes)*

This working group brought together 10 participants from various institutions in five countries. Many new ideas and approaches have been discussed and are being developed. Presently, a joint research programme has been set up by the University of Hohenheim, Germany, and the Weizmann Institute Rehovot, Israel, which is funded by the German Research Society (DFG) as a trilateral project including researchers from the Palestinian authorities. This project will usefully supplement the search for *Orobanche*-attacking fungi by including bacteria that are pathogenic to *Orobanche* and that may be used alone or in combination with fungi isolated by the other two groups, for additive or synergistic control. Some promising bacteria have already been isolated (J Gressel, pers. comm.).

The Italian group has done some preliminary work to characterize the toxins produced by organisms pathogenic to *Orobanche*. Typically, in the past, much effort was given to the formulation of spores, which could often only be produced on solid substrates. It was never considered feasible to formulate mycelium, even though it is produced more abundantly and easily in liquid formulation. The Israeli group has established an informal collaboration on formulation with the USDA-ARS European Biocontrol Laboratory at Montpellier led by Dr P C Quimby. The new formulation techniques have resulted in the development of an effective formulation containing chopped mycelia from liquid cultures, which may lead to a totally different approach to mycoherbicides. These formulations of mycelium can be stored in the cold for over a year, without unacceptable loss of viability. This development is important well beyond the confines of this working group (J Gressel, pers. comm.).

The *Fusarium* spp. that have been isolated from diseased *Orobanche* have been transformed with three different genes: the *gus* gene allowing specific visualization of these organisms within *Orobanche* tissue; the *gfp* gene for visualization of the organisms on the surface of the parasitic weed and in the soil; and a gene leading to overproduction of IAA that will hopefully enhance pathogenicity (J Gressel *et al.*, unpubl. obs.).

Co-operative activities

Working group meetings have been held regularly (one or two per group per year) to discuss items specific to the individual target weeds, and five workshops with a thematic character, of equal importance to all working groups, have been organized. The latter, which provided good platforms for exchanging knowledge and ideas between working groups, were: Genetic variation in weed and pathogen/insect populations: implications for weed biocontrol (1995); Application and formulation of biological herbicides (1996); Integrating biological weed control into pest management strategies (1997); Risk assessment and registration (1998); Biological weed control applied in the field as part of IPM (1999).

A challenge to any basic research programme is to translate the results into the development of practical systems. This challenge has been met, at least in part, by setting up joint field experiments. This also encouraged the members of working groups to collaborate intensively and to integrate their different methods and approaches into one general biological weed control strategy. Each of the initial four working groups was allocated a field site at Zürich-Reckenholz, Switzerland, for trials in 1998 and 1999. Several of these trials have been replicated at sites in other countries. Joint field trials have also been established in Israel by the *Orobanche* working group using a *Fusarium oxysporum* strain, isolated by the German research group, that specifically attacks *O. cumana* on sunflower (*Helianthus annuus* L.). These intensive collaborations have greatly strengthened European research in the field of biological weed control.

Education and training

Short-term scientific missions proved to be a powerful tool for transferring expertise between institutes or bringing in experts from outside biological weed control to a COST-816 working group. A total of 50 exchanges of scientists and students, each lasting from 3 to 30 days, has been financed through COST-816. It is particularly important that a substantial base of human resource has been established through the training of some 40 PhD and MSc students, probably the most significant 'product' of this research programme, besides the basic knowledge derived.

To conclude, both the concentration on a few weed species and the excellent collaboration in joint field experiments have proved to be most efficient and productive. Although no practical control measure has yet been developed for any of the five target weeds, our research has resulted in substantial progress in the development of methods and strategies for biological control of weeds in crops and has clearly identified potential control solutions for all five target weed complexes. Furthermore, through specific training and education schemes, a substantial base of 'new experts' has been established. The initial hypothesis, however, that successful implementation of biological control will reduce pesticide input and increase biodiversity in agro-ecosystems remains to be demonstrated.

Future research directions

For biological control of weeds in crops, two major routes may be followed in future work. The first is a technological approach focusing on a single, highly destructive disease cycle of the control agent and optimizing the efficacy and specificity of the agent through gene, formulation and delivery techniques. In this way, the agent is to be developed as a product that can be marketed as a biopesticide or a microbial herbicide. The second is an ecological approach based on a better understanding of the interactions among the crop, the weed, the natural antagonist and the environment, which must be managed in order to maximize the spread and impact of an indigenous antagonist on the weed (to be developed further). Future emphasis should to be given to: (i) studying the interactions between the weed and the control agent to improve efficacy and safety, and to overcome (partial) resistance by the host weed; (ii) improving formulation and delivery technologies to guarantee reliable control under a broad range of environmental conditions, and (iii) combining biocontrol with other weed control and general phytosanitary measures, as well as with specific cropping techniques, to optimize pest control.

Although biological control of weeds in crops is, as yet, only a minor component of practical weed control measures on a global scale, the value of many biological weed control programmes on a local and regional scale has been considerable in terms of the resulting benefits to the economy and to human welfare (Charudattan, 1999). In spite of the dearth of commercial interest in weed biocontrol studies, research in this field has been sustained by the public's demand for non-chemical weed control alternatives. This demand has increased recently, as governments have imposed severe use limitations on pesticides and implemented various agro-environment schemes to support the restoration of biological diversity on agricultural land. Over 20% of European farmland (equivalent to 27 million ha) is now subject to management in such schemes. In many areas, this involves the conversion of intensively managed arable land to extensive pastures. Colonization of such land by weeds may impose severe limitations to the successful implementation of restoration schemes, and biological control may well be the appropriate means of control because of its high degree of selectivity and environmental safety.

A proposed continuation of COST-816 is presently being developed with emphasis on 'biological management of key weeds in agro-ecosystems to promote biodiversity'. This will include weed management of extensive pastures, and both horizontal and vertical integration of biological control with other methods of weed control will be a central component. As scientists concerned with reducing disruptive chemical input by establishing an ecologically based approach to weed control, we have a responsibility to extend our expertise in order to help provide the basis for programmes of integrated weed management.

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