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Biological Control of Weeds with Mycoherbicides in the Age of Genomics

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ABSTRACT

Mycoherbicides offer an innovative approach to the management of weeds in disturbed environments using formulated fungal phytopathogens. The efficacy of these mycoherbicides could be improved in the future through the application of genomics (the study of genes and their interactions) to both the target and the biological control agent. In this review, an update is given on approaches to genetic enhancements of mycoherbicides and how a knowledge of, and recent advances in, genomics could be used to improve this process. Specific examples are given of novel approaches that could be used. Genetic modification of mycoherbicidal agents has been shown to be possible, but caution is warranted in terms of public perception and the acceptance of these approaches in the wider community.

Keywords: biological control, bioherbicide, genetics, GMO, weed management

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INTRODUCTION

Bioherbicides are a form of inundative biological control that are used to manage weeds, often in disturbed environments, where they must provide rapid, effective and economic weed control (Weston 1999). The active ingredient in the formulated product is a phytopathogenic microorganism. As the majority of plant pathogens are fungi, it is not surprising that most of the pathogens investigated are fungal, although there are examples of the use of bacteria, oomycetes and viruses (Imaizumi *et al.* 1992; Nishino *et al.* 1997; Anderson and Gardner 1999; DeValerio and Charudattan 1999; Daigle *et al.* 2002; Weissmann *et al.* 2003; Charudattan and Hiebert 2007; Ferrell *et al.* 2008). These organisms may be formulated in various ways but, in most cases, they are applied in a similar fashion as synthetic herbicides using conventional application technology (Gossen *et al.* 2008).

Genomics is a term which was coined in 1987 (McKusick and Ruddle 1987) to describe the quantitative study of genes (both regulatory and non-coding sequences). As compared to genetics in which individual genes and their inheritance is studied, genomics is the study of all of the genes in the genome and their interactions. It encompasses functional, structural and comparative genomics. The study of genomics complements transcriptomics (RNA and gene expression), proteomics (protein expression) and metabolomics (metabolites and metabolic networks). Genomics has been made possible by the large-scale sequencing of genomes of a variety of organisms. The first fungal genome to be fully sequenced was that of the brewer's yeast, *Saccharomyces cerevisiae*, in 1996 (Goffeau *et al.* 1996). This was followed by the sequences of *Schizosaccharomyces pombe* and *Neurospora crassa* (Wood *et al.* 2002; Galagan *et al.* 2003). The sequencing of fungi associated with plant diseases has been initiated more recently. Despite these studies, sequencing of fungal genomes has been comparatively slow (Galagan *et al.* 2005). There are currently over 85 fungal genomes (species and isolates) that have been, or are being, sequenced including plant and animal pathogens. The full list of publicly available fungal genomes is available from the website of the Fungal Genome Initiative of the Broad Institute[1]. Currently available sequence data from non-filamentous fungi such as yeasts or filamentous, non-plant pathogenic fungi may not be overly useful in the search for targets genes involved in plant pathogenesis as they may not contain homologs of pathogenicity or virulence genes (Xu *et al.* 2006). For example, the two closely related species, *Aspergillus fumigates* and *A. fischerianus* have 9226 homologs with 700 genes with no homology between the two species (Nierman *et al.* 2005). Of particular interest to scientists developing mycoherbicides are the genomes of *F. oxysporum* and *Sclerotinia sclerotiorum*, as both species have been considered as candidate mycoherbicides (Riddle *et al.* 1991; Boyette *et al.* 1993; Bourdot *et al.* 1995; Cornwallis *et al.* 1999; Thomas *et al.* 1998; Marley and Shebayan 2005). *F. verticillioides* is also of interest as a direct comparison to *F. oxysporum* notwithstanding the differences in closely related species noted above. The speed of identification of genes involved in plant/pathogen interactions will increase as sequencing information of more plant pathogenic fungal genomes becomes available in the future (Xu *et al.* 2006). This will increase our knowledge of

[1] http://www.broadinstitute.org/science/projects/fungal-genome-initiative/fungal-genome-initiative

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pathogen virulence and may lead to better understanding of regulation of plant pathogen interactions in bioherbicides. The American Phytopathological Society has compiled a list of priority plant pathogens for sequencing, although none of these contain any potential mycoherbicides[2]. So how can genomics assist the research into mycoherbicides? Genomic information on both hosts and pathogens could be used to improve the efficiency of studies of genetic diversity, the production of methods for the tracing and quantification of pathogens and provide insights into methods for selection and improvement of mycoherbicide candidates in the future. This review is concerned with the impact of genomics on genetic diversity and strain improvement in the production of mycoherbicides. Improvement in strains is framed within the context of how new strains of fungal pathogens are produced within agroecosystems, as outlined by Stukenbrock and McDonald (2008).

GENETIC DIVERSITY

Knowledge about the genetic diversity of the target weed is important so that a representative population of the target can be chosen in pathogenicity and host range testing. This will lead to greater field efficiency and reduced variability in management of weeds through the use of mycoherbicides. The assessment of diversity of the target weeds in mycoherbicide programs is becoming common place and is being undertaken using a range of molecular marker approaches (Okoli *et al.* 1997; Ash *et al.* 2003, 2004). The usefulness of DNA markers can be assessed in terms of their genotyping error (a reflection of reproducibility and clarity), their informativeness (a measure of their polymorphic information content) and the multiplex ratio (the number of loci which can be assayed simultaneously). The types of markers that are commonly used include Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA detection (RAPD), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphisms (AFLP), Single Nucleotide Polymorphisms (SNPs) and Diversity Array Technology (DArT). A discussion of the types of markers available and their strengths and weaknesses can be found in Sunnucks (2000) with update on DArT in Xie *et al.* (2006). These of markers vary in their reproducibility, level of polymorphism and cost. Genomic studies are starting to be used to improve the search for appropriate markers for identification, genetic variation and phylogenetics (Fredslund *et al.* 2006; Abdelkrin *et al.* 2009; Duran *et al.* 2009). These approaches can use whole genome sequences or random sequences generated by shotgun sequencing (Abdelkrin *et al.* 2009). Sequencing technologies are also relevant to pathogen studies. Fungi often have ambiguous morphological characters and so the use of genetic fingerprinting and sequencing can assist in their identification (Berthier *et al.* 1996; Tessmann *et al.* 2001; Yourman and Luster 2004; Ash 2010; Ash *et al.* 2010). Charudattan (2001) has suggested that when studies of the genetic diversity of the host and pathogen are combined they may help in predicting suitability of weed/pathogen systems for biological control by either inundative or classical methods. Comparative genomics could also be used to improve the efficiency of production for systems of tracking and persistence of mycoherbicide candidates. Currently these systems are created using exhaustive searches for suitable markers and the creation of quantitative PCR (Zhou *et al.* 2004; Dauch *et al.* 2006; Pitt *et al.* 2006).

STRAIN SELECTION AND IMPROVEMENT

The earliest successes of bioherbicides were with the use of *Phytophthora palmivora* and *Colletotricum gloeosporioides* f.sp. *aeschynomene* (Tebeest *et al.* 1992). Since then there have been a number of products commercialised (Charudattan 2001; Bailey *et al.* 2010) as well as numerous examples of pathogen-weed combinations which had been reported as having potential as bioherbicides (Charudattan 2001; Ash 2010). For a biological control agent to have potential as a mycoherbicide there are a number of desirable characteristics which have been identified including the ability to grow and sporulate on artificial media, genetic stability and host specificity. Host specificity is a consequence of pathogenicity (as defined by Shaner *et al.* (1992)). A number of workers have identified the lack of pathogenicity and narrow host range of many mycoherbicides as reasons for low success rates of mycoherbicides in terms of commercialisation (Auld and Morin 1995; Hallett 2005; Sands and Pilgeram 2009). Virulence, on the other hand, is a quantitative character which refers to the ability of the pathogen to cause disease under certain environmental conditions. Virulence is a trade-off between transmission between hosts and replication within the host (Anderson and May 1982). A highly virulent pathogen will replicate quickly within a host leading to its death, thus leaving less time for spread between hosts, that is, it has a reduced parasitic fitness or aggressiveness (Shaner *et al.* 1992). This may then reduce the likelihood of an epidemic. In most cases this is overcome in mycoherbicides by the application of overwhelming numbers of propagules (Templeton *et al.* 1979). Therefore, in the case of mycoherbicides, the selection for virulence of the fungal pathogen is paramount to the efficacy of the bioherbicide (Charudattan 1988; Ash 2010) as parasitic fitness or aggressiveness is of secondary importance due to the inundative application of mycoherbicides.

In many cases the organisms used in bioherbicide research are endemic pathogens that have co-evolved with their host (Hallett 2005). This co-evolution between pathogen and host could limit their potential as mycoherbicides, as a highly virulent pathogen with high specificity would be committing suicide in an evolutionary sense (Gressel 2001; Rector 2008). This would be the case in host populations with low levels of resistance as these populations are generally thought to harbour less-virulent pathogens (Thrall and Burdon 2002) whereas more virulent pathogens would predominate on resistant hosts (Barrett *et al.* 2007). Although hypervirulent individuals may be produced within sexually reproducing pathogen populations, the chance of isolating a hypervirulent pathogen is probably low as they would have the propensity to kill seedlings and so would be often missed in surveys. Even when pathogens and the host have co-evolved, introduction of pathogens into new environments may lead to significant disease and yield losses in naïve host populations (Stukenbrock and McDonald 2008). Historical examples include the introduction of *Phytophthora infestans* into Ireland (Goodwin *et al.* 1994) and stripe rust into Australia (Wellings 2007). This has also shown to be true in the case of classical biological of weeds, where the release of pathogens of exotic weeds has been highly successful in some cases (TeBeest 1996). For example, the biological control of skeleton weed in Australia by the rust fungus *Puccinia chrondrillina* has been a spectacular success in Australia (Cullen 1985). In many cases it is assumed that host/pathogen relationships have arisen through co-evolution, in the absence of data to the contrary. However, genomic approaches have recently been used to study these relationships and have shown that a number of systems may have evolved differently. Stukenbrock and McDonald (2008) proposed co-evolution (host-tracking) as one of the four most likely scenarios for the emergence of new plant pathogens. The other mechanisms are host shift (when a pathogen infects a new, previously unaffected host), horizontal gene transfer[3] (when there is genomic transfer

[2]http://www.apsnet.org/members/ppb/PDFs/MicrobialGenomicsSeq08revisionfinal.pdf

[3] **Kado CI** (2009) has suggested that intergenetic transfer between organisms within a domain should be referred to as lateral gene transfer and that horizontal gene transfer be restricted to gene transfer between domains. To prevent confusion, in this review lateral and horizontal gene transfer will be considered synonymous.

which affects host range) and hybridization (the fusion of whole genomes) (Stukenbrock and McDonald 2008). Any of these changes can lead to new host ranges and heightened virulence.

Stukenbrock and McDonald (2008) provided a number of examples where there is evidence for host shift or host jump has occurred including in *Rhyncosporium secalis* on barley and *Magnaporthe oryzae* on weeds of rice. In the case of *R. secalis* on barley, evidence for the host jump has been provided through phylogenetic analysis of a toxin-encoding/elicitor gene and several house-keeping genes (Brunner *et al.* 2007; Zaffarano *et al.* 2008). This host jump was facilitated by domestication of host crop plants, anthropogenic modification of the environment and the worldwide distribution of the pathogen. Although not specified by Stukenbrock and McDonald (2008) this host jump may have arisen by a number of means including horizontal gene transfer.

Horizontal gene transfer is common in prokaryotes (Amabilecuevas and Chicurel 1992) to the point that it has been suggested that the prokaryotic community could be viewed as a single multicellular organism with continuous movement of genetic material (Sonea 1991). Naturally occurring horizontal gene transfer has also been documented between prokaryotes and eukaryotes (Binns and Thomashow 1988) and between eukaryotes (Roulin *et al.* 2009). Horizontal gene transfer can occur naturally or can be performed in the laboratory.

There are a number of techniques which have been successfully used in the introduction and genomic integration of foreign DNA into fungi including electroporation, the use of polyethylene glycol and calcium chloride, lithium acetate, restriction enzyme mediated integration, biolistics and the *Agrobacterium*-mediated transformation (Marek *et al.* 1987; Penttilä *et al.* 1987; Dickman 1988; Lorito *et al.* 1993; Redman and Rodriguez 1994; de Groot *et al.* 1998; Zeilinger 2004). *Agrobacterium*–mediated transformation often gives the highest transformation efficiencies of the aforemenioned protocols (de Groot *et al.* 1998; Michielse *et al.* 2008) with frequencies of 200–250 transformants per 1 × 10^6 conidiospores of *Aspergillus awamori* reported by Michielse *et al*. (2008). These protocols can be used to introduce foreign DNA or to produce mutants from insertions which disrupt genes. Dickman *et al*. (1989) demonstrated that the introduction a single gene for cutinase production from *Fusarium solani* f.sp. *pisi* into a *Mycospharella* species could transform this fungus from a wound infecting pathogen into a pathogen that could infect papaya through an intact cuticle. Since this publication there have been numerous reports of the transformation of fungi to increase host range and virulence (Yakoby *et al.* 2000; Screen *et al.* 2001; Cohen *et al.* 2002; Wang and St. Leger 2007). Even though we have made these advances, many of the fundamental questions pertaining to host range, pathotype evolution and the drivers for fungal symbiotic lifestyles remain (Yarden *et al.* 2003).

Genetic modification of fungi to increase virulence and to reduce the reliance of the organisms on the environment during the infection process has been suggested by a number of authors as the future of bioherbicides (Amsellem *et al.* 2002; Gressel *et al.* 2005; Rector 2008); however, the science underpinning these approaches is still in its infancy. The modification or insertion of enzymes of fungal pathogens involved in plant penetration, such as cell wall degrading enzymes and cutinases, does not always lead to increased virulence due to the considerable genetic redundancy in these genes (Xu *et al.* 2006). There are few examples in the literature of fungal transformation to improve the efficacy of mycoherbicides. Cohen *et al*. (2002) reported the increased virulence of *Fusarium oxysporum* using upregulation of indole acetic acid production by transforming with both *iaa*H and *iaa*M. The reported doubling of virulence was not considered sufficient to warrant the use of this transformation in the field (Meir *et al.* 2009). However, Amsellem *et al*. (2002) reported a nine-fold increase in virulence and a reduced requirement for moisture in *Colletotrichum coccoides*, a bioherbicidal candidate against *Abuliton theophrasti*, when using the *NEP1* gene from *F. oxysporum.* The *Nep 1* gene encodes the Nep 1 protein, a 24 kDa necrosis-eliciting protein (Bailey *et al.* 1997). Amsellem *et al*. (2002) reported that although the gene improved the virulence of the organism it also increased the host range to tomato and tobacco, an undesirable characteristic in this case. Additionally, the protein did not increase the bioherbicidal effect of *Pseudomonas syringae* on asteraceae weeds (Gronwald *et al.* 2004). Dauch *et al*. (2006) could not reproduce the increased virulence to *A. theophrasti* when using the *C. coccoides* strain T2O-a when compared to the wild type strain. Furthermore, they could not detect the gene expression in culture or *in planta.* They suggested the gene was being silenced. Amsellem *et al*. (2002) noted that over expression was inhibited within *F. oxysporum* and hypothesised that this would not be the case in unrelated fungi. Bailey *et al*. (2002) demonstrated over expression of the Nep 1 protein in *F. oxysporum* f.sp. *erythroxyli* in high nutrient, liquid formulation. The isolate had the native gene disrupted by insertional mutagenesis and a new construct inserted. This then supports the hypothesis of Amsellem *et al*. (2002) and Meir *et al*. (2009) that the presence of a native gene may lead to silencing when additional copies of the gene are inserted. Meir *et al*. (2009) went further to hypothesise that *Nep 1* gene could be used in fungi that either do not possess the gene for Nep 1 protein production or it could be used in fungi where the gene had been silenced (*sensu* Bailey *et al*. (2002). Care should be taken with this extrapolation, however. In the case of transformation of *C. coccoides*, the pathogen undergoes a biotrophic phase for a period of up to five days post-inoculation, therefore the constitutive expression of a phytotoxin may reduce the biomass of the fungus and could interfere with the plant-pathogen interaction at this stage. This would likely lead to a reduced level of symptom expression. Furthermore, although Bailey *et al*. (2002) demonstrated over expression of Nep 1 in fermentation, this did not lead to increased virulence in the host plant coca. As the authors noted, the regulation of the production could be dependent on a range of factors including transcription, translation and posttranslational effects which may affect protein configuration, stability and excretion. Other genes for a range of polyketide synthetases have been identified in a number of plant pathogenic fungi (Xu *et al.* 2006) which could also be useful in selectively increasing virulence. Use of these types of virulence factors would require the use of inducible promoters which are aligned to the fungal/plant interaction being targeted. As noted by Ash (2010), the host range of some mycoherbicidal agents could be modified by manipulation of genes such as NADPH oxidase genes which are implicated in the regulation of symbiosis in some plant/pathogen interactions (Tanaka *et al.* 2006). The use of comparative genomics will also allow the identification of new families of genes that could be used in the enhancement of mycoherbicides. Recently, de Jonge and Thomma (2009) used publicly available sequence data of fungi to identify putatively secreted LysM-containing proteins. They suggested that these may have a role in nullifying host resistance. All of these strategies rely on the transformation with relatively small segments of DNA, which may lead to erratic expression due to positional effects and copy number-dependent transcription (Peterson 2007). These shortcomings could be overcome by the use of yeast artificial chromosomes (YACs) (Burke *et al.* 1987) which can be used to introduce DNA fragments up to 2 Mb in size (Peterson 2007). These chromosomes can be modified by site-specific recombination (Rothstein 1995). The introduction of these YACs into cells can be problematic, requiring microinjection (Peterson 2007). However, microinjection has been shown to be feasible in fungal systems (Correa and Hoch 1993; Jackson 1995).

Changes in virulence may also be a result of horizontal transfer of whole chromosomes in fungi. It is common in

many fungi for there to be polymorphism in chromosome number between and within species (Covert 1998). Supernumerary chromosomes (also known as B chromosomes, dispensable chromosomes or minichromosomes) are common in many fungi and vary in size from 0.7 Mb up to 4.9 Mb (Covert 1998). These supernumerary chromosomes may carry clusters of pathogenicity factors like toxin genes as well as genes for characters such as rhizosphere competitiveness (Hatta *et al.* 2002; Rodriguez-Carres *et al.* 2008; Aboukhaddour *et al.* 2009). Recently, Ma *et al.* (2010) reported the horizontal transfer of four entire chromosomes between species of *Fusarium* which contained transposon-rich regions and regions associated with pathogenicity. They went onto demonstrate that the transfer of two lineage-specific chromosomes could be correlated with the emergence of changed pathogenicity of the recipient *formae specialis* of *Fusarium oxysporum.*

The fourth scenario for increases in virulence or changes in host range listed by Stukenbrock and McDonald (2008) was hybridisation. There are a number of examples of naturally occurring interspecific hybridisation in fungi and oomycetes which have lead to new host ranges or increases in virulence (Schardl and Craven 2003). In plant pathogenic fungi and oomycetes, there are a range of reports of hybridization including those in *Pythium* species (Nechwatal and Mendgen 2009), *Puccinia lagenophorae* (Morin *et al.* 2009), *Cronartium ribicola* (Joly *et al.* 2006), *Melampsora* species (Newcombe *et al.* 2000) and *Phytophthora* species (Brasier *et al.* 1999; Bonants *et al.* 2000). Barrett *et al*. (2007) demonstrated the likely origin of a lineage of *Melampsora lini* from a hybridisation event which resulted in an increase in virulence in 18% of host lines compared with the presumed parental lineage (Barrett *et al.* 2007). They suggested that the genome arising from the lineage from a different host may not carry potential avirulence elicitors. Therefore, hybridisation of closely related fungi with different host ranges may provide an avenue for the production of new virulent mycoherbicides.

Forced hybridisation by the fusion of protoplasts has been attempted in various fungi and actinobacteria (Agbessi *et al.* 2003; Aiuchi *et al.* 2007; Balasubramanian and Lalithakumari 2008). Fusion of protoplasts of *Helminthosporium graminearum* subspecies *echinochloe* with *Curvularia lunata* has been used in an attempt to complement the pathogenicity and spore production abilities of the two fungi (Zhang *et al.* 2007). This group demonstrated the fusion of the isolates using PCR, but suggested that the majority of the DNA arose from the *Helminthosporium* isolate. Some of the resultant fusants had increased sporulation and production of the phytotoxin ophiobolin A in culture which adequately controlled *Cyperus difformis* in the field. Increases in the biocontrol efficacy of species of *Trichoderma* have been reported by both interspecific hybridisation and self-fusion of protoplasts (Ogawa *et al.* 1989; Prabavathy *et al.* 2006). Self fusion of protoplasts would lead to polyploidy in the resultant fusants. Polyploidy is thought to be common in plants and fungi (Masterson 1994; Deacon 2006). It often gives rise to cascades of novel gene expression patterns which invariably leads to new phenotypic variation arising from over expression of genes, silencing of others or completely unpredicted outcomes. In fungi, polyploids, aneuploids and heterokaryons occur. Smith (1987) first hypothesised that *Saccharomyces cerevisiae* arose from polyploidy. By using genomics, Wolfe and Shields (1997) presented further support for the genome duplication (tetraploidy) of *S. cerevisiae* followed by massive gene deletion. Furthermore, levels of ploidy have been shown to affect the expression and the susceptibility to virally-mediated toxin in yeasts (McBride *et al.* 2008). Barrett *et al*. (2007) postulate that increased heterozygosity in polyploids may lead to a greater chance of recognition by the host and thus reduced virulence. There are currently no records on the improvement of mycoherbicides using increased or altered ploidy levels. Notwithstanding the comments of Barrett *et al*. (2007), it would seem that the investigation of changes in virulence due to changes in ploidy may be an interesting avenue of research.

SUMMARY

Genomics, therefore, offer the potential for a greater understanding of host pathogen interactions and, perhaps through this understanding, a greater opportunity to increase the effectiveness of mycoherbicides. The larger opportunities lie in the genetic manipulation of the pathogen. The interest in developing transgenic biocontrol agents has been limited due to the perceived hazards which include increased host range of the organism, mutation and spread of the transgenes by asexual or sexual recombination (Gressel 2001). In an effort to allay these fears, Gressel (2001) suggested the use of a number of transgenic mitigator genes as failsafe mechanisms in hypervirulent fungi. Amsellem *et al* (2002) suggested that the use of these failsafe mechanisms would be a prerequisite for the release of any hypervirulent outside of containment facilities. Additionally, although the release of genetically modified pathogens with increased virulence and/or altered host range is theoretically possible, there are a number of legislative, social and ethical considerations pertaining to the use of the technology.

Dealings with genetically modified organisms (GMOs) are regulated by different systems in different countries and often involve a number of agencies and legislation. Of particular relevance to researchers contemplating the use of genetically modified mycoherbicides is the legislation surrounding agricultural and veterinary chemicals, biosecurity and plant protection. The regulatory instruments of Australia, New Zealand, the European Union and the United States of America are summarised by Henderson and Murphy (2007). Additionally there are a number of international instruments governing the production and release of GMOs including the Convention on Biological Diversity (CBD)[4], the International Plant Protection Convention (IPPC)[5] and the Sanitary and Phytosanitary Agreement of the World Trade Organisation[6]. Supplementary to the CBD is the Cartagena Protocol on Biosafety, which seeks to protect biological diversity from the potential risks posed by living modified organisms resulting from modern biotechnology. Of the parties to the protocol, Australia and the United States are notable exceptions. Most of these international and domestic instruments use a precautionary approach to dealings with GMOs. The implications of the release of an enhanced biological control are not only of importance to the country of origin but may also have ramifications for other countries in which the target may be native (Henderson and Murphy 2007; Rector 2008). If it is, as Henderson and Murphy (2007), believe that genetic improvement of biological control agents is inevitable, then the negative perceptions of segments of the community need to be overcome. Public perceptions of genetically modified food vary depending on the country in which the surveys are undertaken with more negative perceptions predominating in Europe, ambivalence in Britain and acceptance in the United States (Gaskell 2004; Spence and Townsend 2007). While the research on transgenic mycoherbicides continues in biosecure premises, trust between biocontrol scientists and the community needs to built through dialogue about the risks, safety and the flow of benefits from such research. The maintenance or restoration of trust between scientists (or scientific institutions) and the public is vital to the continued scientific innovation (Barnett *et al.* 2007). It is a common belief that scientists do not put enough effort into informing the public and they should listen to the public's opinion[7]. By building trust between the groups the gap in perspectives between scientists and the lay community will be reduced (Barnett *et al.* 2007).

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[4] http://www.cbd.int/
[5] https://www.ippc.int/IPP/En/default.jsp
[6] http://www.wto.org/english/tratop_e/sps_e/sps_e.htm
[7] http://ec.europa.eu/public_opinion/archives/ebs/ebs_224_report_en.pdf

Rector (2008) proposed that almost any heritable trait can be modified using molecular techniques, if there is sufficient genetic information about the system. Genomics and the other "omics" can be used to supply this information. It is clear that genomic information and its application in mycoherbicides is its infancy. However, by carefully analysing what has occurred in "natural" systems, it is possible to define new genetic directions in research which may be able to improve the utility and success of mycoherbicides in the future. However, simply because we have the technology to do this work is not enough. There must be a clear strategy to engage the lay community in the developing research, a commitment of the scientists to the highest possible ethical standards in performing the research and an early assessment of the feasibility of release of the agent to assure the greatest chance of success of the research.

REFERENCES

Abdelkrin J, Robertson BC, Stanton JAL, Gemmell NJ (2009) Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing. *Biotechniques* **46**, 185-191

Aboukhaddour R, Cloutier S, Ballance GM, Lamari L (2009) Genome characterization of *Pyrenophora tritici-repentis* isolates reveals high plasticity and independent chromosomal location of ToxA and ToxB. *Molecular Plant Pathology* **10**, 201-212

Agbessi S, Beausejour J, Dery C, Beaulieu C (2003) Antagonistic properties of two recombinant strains of *Streptomyces melanosporofaciens* obtained by intraspecific protoplast fusion. *Applied Microbiology and Biotechnology* **62**, 233-238

Aiuchi DBaba YInami KShinya RTani MKuramochi KHorie SKoike M (2007) Screening of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains based on evaluation of pathogenicity against cotton aphid and greenhouse whitefly, and viability on the leaf surface. *Japanese Journal of Applied Entomology and Zoology* **51**, 205-212

Amabilecuevas CF, Chicurel ME (1992) Bacterial plasmids and gene flux. *Cell* **70**, 189-199

Amsellem Z, Cohen BA, Gressel J (2002) Engineering hypervirulence in a mycoherbicidal fungus for efficient weed control. *Nature Biotechnology* **20**, 1035-1039

Anderson RC, Gardner DE (1999) An evaluation of the wilt-causing bacterium *Ralstonia solanacearum* as a potential biological control agent for the alien kahili ginger (*Hedychium gardnerianum*) in Hawaiian forests. *Biological Control* **15**, 89-96

Anderson RM, May RM (1982) Coevolution of hosts and parasites. *Parasitology* **85**, 411-412

Ash GJ (2010) The science, art and business of successful bioherbicides. *Biological Control* **52**, 230-240

Ash GJ, Cother EJ, Tarleton J (2004) Variation in lanceleaved waterplantain (*Alisma lanceolatum*) in southeastern Australia. *Weed Science* **52**, 413-417

Ash GJ, Raman R, Crump NS (2003) An investigation of genetic variation in *Carthamus lanatus* in New South Wales, Australia, using intersimple sequence repeats (ISSR) analysis. *Weed Research* **43**, 208-213

Ash GJ, Stodart B, Sakuanrungsirikul S, Anschaw E, Crump N, Hailstones D, Harper JDI (2010) Genetic characterization of a novel *Phomopsis* sp., a putative biocontrol agent for *Carthamus lanatus*. *Mycologia* **102**, 54-61

Auld BA, Morin L (1995) Constraints in the development of bioherbicides. *Weed Technology* **9**, 638-652

Bailey BA, Apel-Birkhold PC, Luster DG (2002) Expression of NEP1 by *Fusarium oxysporum* f. sp *erythroxyli* after gene replacement and overexpression using polyethylene glycol-mediated transformation. *Phytopathology* **92**, 833-841

Bailey BA, Jennings JC, Anderson JD (1997) The 24-kDa protein from *Fusarium oxysporum* f sp. *erythroxyli*: Occurrence in related fungi and the effect of growth medium on its production. *Canadian Journal of Microbiology* **43**, 45-55

Bailey KL, Boyetchko SM, Langle T (2010) Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. *Biological Control* **52**, 221-229

Balasubramanian N, Lalithakumari D (2008) Characteristics of protoplast inter, intra-fusant and regeneration of antagonistic fungi *Trichoderma harzianum* and *Trichoderma viride*. *African Journal of Biotechnology* **7**, 3235-3243

Barnett J, Cooper H, Senior V (2007) Belief in public efficacy, trust, and attitudes toward modern genetic science. *Risk Analysis* **27**, 921-933

Barrett LG, Thrall PH, Burdon JJ (2007) Evolutionary diversification through hybridization in a wild host-pathogen interaction. *Evolution* **61**, 1613-1621

Berthier YT, Bruckart WL, Chaboudez P, Luster DG (1996) Polymorphic restriction patters of ribosomal internal transcribed spacers in the biocontrol fungus *Puccinia carduorum* correlate with weed host origin. *Applied and Environmental Microbiology* **62**, 3037-3041

Binns AN, Thomashow MF (1988) Cell biology of *Agrobacterium* infection and transformation of plants. *Annual Review of Microbiology* **42**, 575-606

Bonants PJM, Hagenaar-de Weerdt M, Man in't Veld WA, Baayen RP (2000) Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology* **90**, 867-874

Bourdot GW, Harvey IC, Hurrell GA, Saville DJ (1995) Demographic and biomass production consequences of inundative treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum. Biocontrol Science and Technology* **5**, 11-25

Boyette CD, Abbas HK, Connick WJ (1993) Evaluation of *Fusarium oxysporum* as a potential bioherbicide for sicklepod (*Cassia obtusifolia*), coffee senna (*C. occidentalis*), and hemp sesbania (*Sesbania exaltata*). *Weed Science* **41**, 678-681

Brasier CM, Cooke DEL, Duncan JM (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences USA* **96**, 5878-5883

Brunner PC, Schurch S, McDonald BA (2007) The origin and colonization history of the barley scald pathogen *Rhynchosporium secalis*. *Journal of Evolutionary Biology* **20**, 1311-1321

Burke DT, Carle GF, Olsen MV (1987) Cloning of large segments of exogenous DNA into yeast by menas of artificial chromosome vectors. *Science* **236**, 806-812

Charudattan R (1988) Assessment of efficacy of mycoherbicide candidates. In: Delfosse ES (Ed) *VIIth International Symposium on Biological Control of Weeds*, 6th-11th March, Rome, Italy, pp 455-464

Charudattan R (2001) Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. *Biocontrol* **46**, 229-260

Charudattan R, Hiebert E (2007) A plant virus as a bioherbicide for tropical soda apple, *Solanum viarum*. *Outlooks on Pest Management* **18**, 167-171

Cohen BA, Amsellem Z, Maor R, Sharon A, Gressel J (2002) Transgenically enhanced expression of indole-3-acetic acid confers hypervirulence to plant pathogens. *Phytopathology* **92**, 590-596

Cornwallis LJ, Stewart A, Bourdot GW, Gaunt RE, Harvey IC, Saville DJ (1999) Pathogenicity of *Sclerotinia sclerotiorum* on *Ranunculus acris* is in dairy pasture. *Biocontrol Science and Technology* **9**, 365-377

Correa A, Hoch HC (1993) Microinjection of urediospore germilings of *Uromyces appendiculatus. Experimental Mycology* **17**, 253-273

Covert SF (1998) Supernumerary chromosomes in filamentous fungi. *Current Genetics* **33**, 311-319

Cullen JM (1985) Bringing the cost benefit analysis of biological control of *Chondrilla juncea* up to date. In: Delfosse ES (Ed) *Proceedings of the VI International Symposium on Biological Control of Weeds*, Agriculture Canada, Ottawa, Canada, pp 145-152

Daigle DJ, Connick WJ, Boyetchko SM (2002) Formulating a weed-suppressive bacterium in "Pesta". *Weed Technology* **16**, 407-413

Dauch AL, Ahn B, Watson AK, Seguin P, Jabaji-Hare SH (2006) Molecular monitoring of wild-type and genetically engineered *Colletotrichum coccodes* biocontrol strains *in planta*. *Plant Disease* **90**, 1504-1510

de Groot MJA, Bundock P, Hooykaas PJJ, Beijersbergen AGM (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. *Nature Biotechnology* **16**, 839-842

de Jonge R, Thomma B (2009) Fungal LysM effectors: Extinguishers of host immunity? *Trends in Microbiology* **17**, 151-157

Deacon J (2006) *Fungal Biology* (4th Edn), Blackwell Publishing, Oxford

DeValerio JT, Charudattan R (1999) Field testing of *Ralstonia solanacearum* [Smith] Yabuuchi et al. as a biocontrol agent for tropical soda apple (*Solanum viarum* Dunal). *Weed Science Society of America Abstracts* **39**, 70

Dickman MB (1988) Whole cell-transformation of the alfalfa fungal pathogen *Colletotrichium trifolii. Current Genetics* **14**, 241-246

Dickman MB, Podila GK, Kolattukudy PE (1989) Insertion of cutinase gene into a wound pathogen enables it to infect intact host. *Nature* **342**, 446-448

Duran C, Appleby N, Edwards D, Batley J (2009) Molecular genetic markers: Discovery, applications, data storage and visualisation. *Current Bioinformatics* **4**, 16-27

Ferrell J, Charudattan R, Elliott M, Hiebert E (2008) Effects of selected herbicides on the efficacy of tobacco mild green mosaic virus to control tropical soda apple (*Solanum warm*). *Weed Science* **56**, 128-132

Fredslund J, Madsen LH, Hougaard BK, Nielsen AM, Bertioli D, Sandal N, Stougaard J, Schauser L (2006) A general pipeline for the development of anchor markers for comparative genomics in plants. *BMC Genomics* **7**, 207

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang SG, Nielsen CB, Butler J, Endrizzi M, Qui DY, Ianakiev P, Pedersen DB, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stabge-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metzenberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li WX, Pratt RJ, Osmani SA, DeSouza CPC, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seller S, Dunlap J, Radford A,

Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* **422**, 859-868

Galagan JE, Henn MR, Ma LJ, Cuomo CA, Birren B (2005) Genomics of the fungal kingdom: Insights into eukaryotic biology. *Genome Research* **15**, 1620-1631

Gaskell G (2004) Science policy and society: The British debate over GM agriculture. *Current Opinion in Biotechnology* **15**, 241-245

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert, F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 genes. *Science* **274**, 546-567

Goodwin SB, Cohen BA, Fry WE (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences USA* **91**, 11591-11595

Gossen BD, Peng G, Wolf TM, McDonald MR (2008) Improving spray retention to enhance the efficacy of foliar-applied disease- and pest-management products in field and row crops. *Canadian Journal of Plant Pathology* **30**, 505-516

Gressel J (2001) Potential failsafe mechanisms against the spread and introgression of transgenic hypervirulent biocontrol fungi. *Trends in Biotechnology* **19**, 149-154

Gressel J, Al-Ahmad H, Amsellem Z, Babalola O, Meir S (2005) Transgenic enhancement of biocontrol agents. *Phytopathology* **95**, S126-S126

Gronwald JW, Plaisance KL, Bailey BA (2004) Effects of the fungal protein Nep1 and *Pseudomonas syringae* on growth of Canada thistle (*Cirsium arvense*), common ragweed (*Ambrosia artemisiifolia*), and common dandelion (*Taraxacum officinale*). *Weed Science* **52**, 98-104

Hallett SG (2005) Where are the bioherbicides? *Weed Science* **53**, 404-415

Hatta R, Ito K, Hosaki Y, Tanaka T, Tanaka A, Yamamoto M, Akimitsu K, Tsuge T (2002) A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen *Alternaria alternata*. *Genetics* **161**, 59-70

Henderson WR, Murphy EC (2007) Pest or prized possession? Genetically modified biocontrol from an international perspective. *Wildlife Research* **34**, 578-585

Imaizumi S, Nishino T, Miyabe K, Fujimori T, Yamada M (1997) Biological control of annual bluegrass (Poa annua L) with a Japanese isolate of *Xanthomonas campestris* pv poae (JT-P482). *Biological Control* **8**, 7-14

Jackson SL (1995) Microinjection of fungal cells - A powerful experimental technique. *Canadian Journal of Botany* **73**, S435-S443

Joly DL, Langor DW, Hamelin RC (2006) Molecular and morphological evidence for interspecific hybridization between *Cronartium ribicola* and *C. comandrae* on *Pinus flexilis* in southwestern Alberta. *Plant Disease* **90**, 1552-1552

Kado CI (2009) Horizontal gene transfer: sustaining pathogenicity and optimizing host-pathogen interactions. *Molecular Plant Pathology* **10**, 143-150

Lorito M, Hayes CK, Dipietro A, Harman GE (1993) Biolistic transformation of *Trichoderma hrazianum* and *Gliocladium virens* using plasmid and genomic DNA. *Current Genetics* **24**, 349-356

Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, Di Pietro A, Dufresne M, Freitag M, Grabherr M, Henrissat B, Houterman PM, Kang S, Shim WB, Woloshuk C, Xie X, Xu JR, Antoniw J, Baker SE, Bluhm BH, Breakspear A, Brown DW, Butchko RA, Chapman S, Coulson R, Coutinho PM, Danchin EG, Diener A, Gale LR, Gardiner DM, Goff S, Hammond-Kosack KE, Hilburn K, Hua-Van A, Jonkers W, Kazan K, Kodira CD, Koehrsen M, Kumar L, Lee YH, Li L, Manners JM, Miranda-Saavedra D, Mukherjee M, Park G, Park J, Park SY, Proctor RH, Regev A, Ruiz-Roldan MC, Sain D, Sakthikumar S, Sykes S, Schwartz DC, Turgeon BG, Wapinski I, Yoder O, Young S, Zeng Q, Zhou S, Galagan J, Cuomo CA, Kistler HC, Rep M (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**, 367-373

Marek ET, Richey MG, Schardl CL, Smith DA (1987) Transformation of fungal protoplasts by electric-pulse treatment. *Phytopathology* **77**, 1740-1740

Marley PS, Shebayan JAY (2005) Field assessment of *Fusarium oxysporum* based mycoherbicide for control of *Striga hermonthica* in Nigeria. *Biocontrol* **50**, 389-399

Masterson J (1994) Stomatal size in fossil plants - evidence for polyploidy in majority of angiosperms. *Science* **264**, 421-424

McBride R, Greig D, Travisano M (2008) Fungal viral mutualism moderated by ploidy. *Evolution* **62**, 2372-2380

McKusick VA, Ruddle FH (1987) A new discipline, a new name, a new journal. *Genomics* **1**, 1-12

Meir S, Arnsellem Z, Al-Ahmad H, Safran E, Gressel J (2009) Transforming a NEP1 toxin gene into two *Fusarium* spp. to enhance mycoherbicide activity on *Orobanche* - failure and success. *Pest Management Science* **65**, 588-595

Michielse CB, Hooykaas PJJ, van den Hondel C, Ram AFJ (2008) *Agrobacterium*-mediated transformation of the filamentous fungus *Aspergillus awamori*. *Nature Protocols* **3**, 1671-1678

Morin L, Van Der Merwe M, Hartley D, Muller P (2009) Putative natural hybrid between *Puccinia lagenophorae* and an unknown rust fungus on *Senecio madagascariensis* in KwaZulu-Natal, South Africa. *Mycological Research* **113**, 725-736

Nechwatal J, Mendgen K (2009) Evidence for the occurrence of natural hybridization in reed-associated *Pythium* species. *Plant Pathology* **58**, 261-270

Newcombe G, Stirling B, McDonald S, Bradshaw HD (2000) *Melampsora* x *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. *Mycological Research* **104**, 261-274

Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J, Berriman M, Abe K, Archer DB, Bermejo C, Bennett J, Bowyer P, Chen D, Collins M, Coulsen R, Davies R, Dyer PS, Farman M, Fedorova N, Fedorova N, Feldblyum TV, Fischer R, Fosker N, Fraser A, García JL, García MJ, Goble A, Goldman GH, Gomi K, Griffith-Jones S, Gwilliam R, Haas B, Haas H, Harris D, Horiuchi H, Huang J, Humphray S, Jiménez J, Keller N, Khouri H, Kitamoto K, Kobayashi T, Konzack S, Kulkarni R, Kumagai T, Lafon A, Latgé JP, Li W, Lord A, Lu C, Majoros WH, May GS, Miller BL, Mohamoud Y, Molina M, Monod M, Mouyna I, Mulligan S, Murphy L, O'Neil S, Paulsen I, Peñalva MA, Pertea M, Price C, Pritchard BL, Quail MA, Rabbinowitsch E, Rawlins N, Rajandream MA, Reichard U, Renauld H, Robson GD, Rodriguez de Córdoba S, Rodríguez-Peña JM, Ronning CM, Rutter S, Salzberg SL, Sanchez M, Sánchez-Ferrero JC, Saunders D, Seeger K, Squares R, Squares S, Takeuchi M, Tekaia F, Turner G, Vazquez de Aldana CR, Weidman J, White O, Woodward J, Yu JH, Fraser C, Galagan JE, Asai K, Machida M, Hall N, Barrell B, Denning DW (2005) Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* **438**, 1151-1156

Ogawa K, Ohara H, Koide T, Toyama N (1989) Intraspecific hybridization of *Trichoderma reesei* by protoplast fusion. *Journal of Fermentation and Bioengineering* **67**, 207-209

Okoli CAN, Shilling DG, Smith RL, Bewick TA (1997) Genetic diversity in purple nutsedge (*Cyperus rotundus* L) and yellow nutsedge (*Cyperus esculentus* L). *Biological Control* **8**, 111-118

Penttilä M, Nevalainen H, Rättö M, Salminen E, Knowles J (1987) A versatile transformation system for the cellulytic filamentous fungus *Trichoderma reesei*. *Gene* **61**, 155-164

Peterson KR (2007) Preparation of intact yeast artificial chromosome DNA for transgenesis of mice. *Nature Protocols* **2**, 3009-3015

Pitt WM, Bailey KL, Derby JA (2006) Movement and activity of *Phoma macrostoma* in soil and water, following simulated flooding - environmental fate of a biocontrol agent for dandelion. *Canadian Journal of Plant Pathology* **28**, 362-362

Prabavathy VR, Mathivanan N, Sagadevan E, Murugesan K, Lalithakumari D (2006) Self-fusion of protoplasts enhances chitinase production and biocontrol activity in *Trichoderma harzianum*. *Bioresource Technology* **97**, 2330-2334

Rector BG (2008) Molecular biology approaches to control of intractable weeds: New strategies and complements to existing biological practices. *Plant Science* **175**, 437-448

Redman RS, Rodriguez RJ (1994) Factors affecting the efficient transformation of *Colletotrichum* species *Experimental Mycology* **18**, 230-246

Riddle GE, Burpee LL, Boland GJ (1991) Virulence of *Sclerotinia sclerotiorum* and *S. minor* on dandelion (*Taraxacum officinale*). *Weed Science* **39**, 109-118

Rodriguez-Carres A, White G, Tsuchiya D, Taga M, Van Etten HD (2008) The supernumerary chromosome of *Nectria haematococca* that carries pea-pathogenicity-related genes also carries a trait for pea rhizosphere competetiveness. *Applied and Environmental Microbiology* **74**, 3849-3856

Rothstein R (1995) Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. In: Guthrie C, Fink GR (Eds) *Guide to Yeast Genetics and Molecular Biology*, Academic Press, New York, pp 281-301

Roulin A, Piegu B, Fortune PM, Sabot FD, Hont A, Manicacci D, Panaud O (2009) Whole genome surveys of rice, maize and sorghum reveal multiple horizontal transfers of the LTR-retrotransposon Route66 in Poaceae. *BMC Evolutionary Biology* **9**, 58-68

Sands DC, Pilgeram AL (2009) Methods for selecting hypervirulent biocontrol agents of weeds: why and how. *Pest Management Science* **65**, 581-587

Schardl CL, Craven KD (2003) Interspecific hybridization in plant-associated fungi and oomycetes: a review. *Molecular Ecology* **12**, 2861-2873

Screen SE, Hu G, St. Leger RJ (2001) Transformants of *Metarhizium anisopliae* sf. *anisopliae* overexpressing chitinase from Metarhizium anisopliae sf. *acridum* show early induction of native chitinase but are not altered in pathogenicity to *Manduca sexta*. *Journal of Invertebrate Pathology* **78**, 260-266

Shaner G, Stromberg EL, Lacy GH, Barker KR, Pirone TP (1992) Nomenclature and concepts of pathogenicity and virulence. *Annual Review of Phytopathology* **30**, 47-66

Smith MM (1987) Molecular evolution of the *Saccharomyces-cerevisiae* histone gene loci. *Journal of Molecular Evolution* **24**, 252-259

Sonea S (1991) Bacterial evolution without speciation. In: Margulis L, Fester R (Eds) *Symbiosis as a Source of Evolutionary Innovation*, MIT, Cambridege Massachuesetts, pp 95-105

Spence A, Townsend E (2007) Predicting behaviour towards genetically modified food using implicit and explicit attitudes. *British Journal of Social Psy-*

chology **46**, 437-457

Stukenbrock EH, McDonald BA (2008) The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology* **46**, 75-100

Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology and Evolution* **15**, 199-203

Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B (2006) Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. *Plant Cell* **18**, 1052-1066

TeBeest DO (1996) Biological control of weeds with plant pathogens and microbial pesticides. In: *Advances in Agronomy* (Vol 56), Academic Press Inc., San Diego, pp 115-137

Tebeest DO, Yang XB, Cisar CR (1992) The status of biological-control of weeds with fungal pathogens. *Annual Review of Phytopathology* **30**, 637-657

Templeton GE, TeBeest DO, Smith RJ (1979) Biological weed control with mycoherbicides. *Annual Review of Phytopathology* **17**, 301-310

Tessmann DJ, Charudattan R, Kistler HC, Rosskopf EN (2001) A molecular characterization of *Cercospora* species pathogenic to water hyacinth and emendation of *C. piaropi. Mycologia* **93**, 323-334

Thomas HSauerborn JMuller-Stover DZiegler ABedi JSKroschel J (1998) The potential of *Fusarium oxysporum* f.sp. *orthoceras* as a biological control agent for *Orobanche cumana* in sunflower. *Biological Control* **13**, 41-48

Thrall PH, Burdon JJ (2002) Evolution of gene-for-gene systems in metapopulations: The effect of spatial scale of host and pathogen dispersal. *Plant Pathology* **51**, 169-184

Wang CS, St. Leger RJ (2007) A scorpion neurotoxin increases the potency of a fungal insecticide. *Nature Biotechnology* **25**, 1455-1456

Weissmann R, Uggla C, Gerhardson B (2003) Field performance of a weed-suppressing *Serratia plymuthica* strain applied with conventional spraying equipment. *Biocontrol* **48**, 725-742

Wellings CR (2007) *Puccinia striiformis* in Australia: A review of the incursion, evolution, and adaptation of stripe rust in the period 1979-2006. *Australian Journal of Agricultural Research* **58**, 567-575

Weston VCM (1999) The commercial realization of biological herbicides. In: *Brighton Crop Protection Conference – Weeds*, Farnham, UK, pp 281-289

Wolfe KH, Shields DC (1997) Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387**, 708-713

Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, Sgouros J, Peat N, Hayles J, Baker S, Basham D, Bowman S, Brooks K, Brown D, Brown S, Chillingworth T, Churcher C, Collins M, Connor R, Cronin A, Davis P, Feltwell T, Fraser A, Gentles S, Goble A, Hamlin N, Harris D, Hidalgo J, Hodgson G, Holroyd S, Hornsby T, Howarth S, Huckle EJ, Hunt S, Jagels K, James K, Jones L, Jones M, Leather S, McDonald S, McLean J, Mooney P, Moule S, Mungall K, Murphy L, Niblett D, Odell C, Oliver K, O'Neil S, Pearson D, Quail MA, Rabbinowitsch E, Rutherford K, Rutter S, Saunders D, Seeger K, Sharp S, Skelton J, Simmonds M, Squares R, Squares S, Stevens K, Taylor K, Taylor RG, Tivey A, Walsh S, Warren T, Whitehead S, Woodward J, Volckaert G, Aert R, Robben J, Grymonprez B, Weltjens I, Vanstreels E, Rieger M, Schäfer M, Müller-Auer S, Gabel C, Fuchs M, Düsterhöft A, Fritzc C, Holzer E, Moestl D, Hilbert H, Borzym K, Langer I, Beck A, Lehrach H, Reinhardt R, Pohl TM, Eger P, Zimmermann W, Wedler H, Wambutt R, Purnelle B, Goffeau A, Cadieu E, Dréano S, Gloux S, Lelaure V, Mottier S, Galibert F, Aves SJ, Xiang Z, Hunt C, Moore K, Hurst SM, Lucas M, Rochet M, Gaillardin C, Tallada VA, Garzon A, Thode G, Daga RR, Cruzado L, Jimenez J, Sánchez M, del Rey F, Benito J, Domínguez A, Revuelta JL, Moreno S, Armstrong J, Forsburg SL, Cerutti L, Lowe T, McCombie WR, Paulsen I, Potashkin J, Shpakovski GV, Ussery D, Barrell BG, Nurse P (2002) The genome sequence of *Schizosaccharomyces pombe. Nature* **415**, 871-880

Xie Y, McNally K, Li CY, Leung H, Zhu YY (2006) A high-throughput genomic tool: Diversity array technology complementary for rice genotyping. *Journal of Integrative Plant Biology* **48**, 1069-1076

Xu JR, Peng YL, Dickman MB, Sharon A (2006) The dawn of fungal pathogen genomics. *Annual Review of Phytopathology* **44**, 337-366

Yakoby N, Freeman S, Dinoor A, Keen NT, Prusky D (2000) Expression of pectate lyase from *Colletotrichum gloeosporioides* in *C. magna* promotes pathogenicity. *Molecular Plant-Microbe Interactions* **13**, 887-891

Yarden O, Ebbole DJ, Freeman S, Rodriguez RJ, Dickman MB (2003) Fungal biology and agriculture: Revisiting the field. *Molecular Plant-Microbe Interactions* **16**, 859-866

Yourman LF, Luster DG (2004) Generation of molecular markers for the identification of isolates of *Puccinia jaceae*, a potential biological control agent of yellow starthistle. *Biological Control* **29**, 73-80

Zaffarano PL, McDonald BA, Linde CC (2008) Rapid speciation following recent host shifts in the plant pathogenic fungus *Rhynchosporium*. *Evolution* **62**, 1418-1436

Zeilinger S (2004) Gene disruption in *Trichoderma atroviride* via *Agrobacterium*-mediated transformation. *Current Genetics* **45**, 54-60

Zhang ZB, Burgos NR, Zhang JP, Yu LQ (2007) Biological control agent for rice weeds from protoplast fusion between *Curvularia lunata* and *Helminthosporium gramineum*. *Weed Science* **55**, 599-605

Zhou LC, Bailey KL, Derby J (2004) Plant colonization and environmental fate of the biocontrol fungus *Phoma macrostoma*. *Biological Control* **30**, 634-644