Phoma macrostoma: as a broad spectrum bioherbicide for turfgrass and agricultural applications

Russell K. Hynes*

Address: Agriculture and Agri-Food Canada,Saskatoon Research and Development Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada.

*Correspondence: Russell K. Hynes. Email: hynesruss@gmail.com

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Abstract

Phoma macrostoma Montagne 94–44B is an effective bioherbicide for broadleaved weed reduction. In 2016, Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the Pest Control Products Act and Regulations, granted full registration for the sale and use of the bioherbicide *P. macrostoma* Montagne 94–44B to control a broad spectrum of broadleaf weeds in established turfgrass and new seeding of grasses as well as in field grown nursery plants, trees and container-grown ornamentals. *P. macrostoma* 94–44B colonizes susceptible and non-susceptible plant roots, however, only in susceptible plants, such as *Taraxacum officinale* (dandelion), do mycelium proliferate around the vascular trachea interfering with the function of neighbouring cells while not entering them. Macrocidins, secondary metabolites secreted by *P. macrostoma* 94–44B mycelia, inhibit multiple steps in carotenoid precursor formation including phytoene desaturase and steps associated with β carotene and lutein carotenoid biogenesis, Fe and Mg chelation and OJIP chlorophyll fluorescence, thus uncoupling the light-harvesting complex of photosystem II from the reaction centre. The combined actions of *P. macrostoma* 94–44B and macrocidins elicit photobleaching of the leaves and eventual weed death. Research is underway to exploit Phoma's bioherbicidal actions for weed management in agriculture. *P. macrostoma* 94–44B can be used to reduce broadleaved weeds in crops such as wheat, corn, potato, forage grasses and established alfalfa (2nd growth or older). Soil residue studies reveal that neither *P. macrostoma* 94–44B nor macrocidins are present after 1 year suggesting that farmers could plant *Phoma*-sensitive crops such as legumes, flax or canola in subsequent years.

Keywords: *Phoma macrostoma*, Bioherbicide, Macrocidin, Weeds

Review Methodology: The author has conducted research on plant-microbial interactions and microbial based biological control for 35 and 25 years, respectively. He has engaged the services of Elsevier B.V., weekly Scopus literature searches for the past 15 years with search terms including biocontrol, formulation development and application. He has frequently supplemented his literature searches using Google Scholar. With regard to the subject area of this manuscript, the author has been actively engaged in research and development of the Phoma technology for the past 12 years.

Introduction

Over the last seven decades, application of synthetic pesticides to agricultural crops has reduced the impact of weeds, insects and plant pathogens resulting in increased crop yield and quality [1, 2]. The pesticide industry is mandated to minimize the risk and effect of pesticides and their residues on human health and on non-target organisms. However, public concerns about their well-being, due to the impact of pesticides on air, water and food have evolved to demands for pesticide-free food [3–5]. The earliest criticisms of pesticide use in agriculture were based on conjecture and intangible elements, however, today the demands are supported by the conclusions of numerous publications on the impact of pesticides that indeed show there have been significant consequences to all trophic

Table 1 *Phoma* sp. reported in the literature with weed biocontrol activity

Phoma spp	Target plant	Common name	Reference
P. sorghina	*Etchhornia crassipes*	Water hyacinth	[26]
P. aquilina	*Pteridium aquilinum*	Eastern Bracken fern	[27]
P. proboscis	*Convolvulus arvensis*	Bindweed	[28]
P. sorghina	*Lythrum salicaria*	Purple loosestrife	[29]
P. macrostoma 94–44B	*Taraxacum officinale Cirsium arvense* and others	Several broadleaved weeds	[48]
P. herbarum	*Taraxacum officinale*	Dandelion	[30]
P. exigua	*Acroptilon repens*	Russian knapweed	[31]
P. destructiva, *P. hedericola*	*Cirsium arvense*	Thistle	[32]
P. herbarum, *P. exigua*	*Taraxacum officinale*	Dandelion	[33]
P. exigua	*Centaurea solstitialis*	Yellow starthistle	[34]
P. macrostoma	*Cirsium arvense*, *Taraxacum officinale*	Thistle, dandelion	[16]
P. destructiva	*Cirsium arvense*	Thistle	[35]
P. exigua	*Gaultheria shallon*	Salal	[36]
P. herbarum FGCC 75	*Parthenium hysterophorus*	Santa Maria feverfew	[37]
P. tropica	*Etchhornia crassipes*	Water hyacinth	[38]
Phoma spp	*Lonicera japónica*	Japanese honeysuckle	[39]
P. exigua	*Sonchus arvensis*	Sowthistle	[40]
P. argillacea	*Rubus spectabilis*	Salmonberry	[41]
P. lingam	*Raphanus raphanistrum*	Wild radish	[42]
P. clematidina	*Clematis vitalba*	Clematis	[43]
Phoma sp. FGCC#18	*Parthenium hysterophorus*	Santa Maria feverfew	[51]
P. chenopodiicola	*Chenopodium album*	Lambsquarters	[49]
P. herbarum	*Trianthema portulacastrum*	Horse purslane	[44]
P. macrostoma	*Convolvulus arvensis*	Bindweed	[45]
P. commelinicola	*Commelina diffusa*	Spreading dayflower	[46]
Phoma sp	*Cucumis sativus, Sorghum bicolor*	Cucumber, sorghum	[50]
P. sorghina	*Oxalis debilis*	Pink woodsorrel	[47]
P. crystallifera	*Convolvulus arvensis*	Bindweed	[52]

levels of the food chain following decades of pesticide application. These include a decline in the populations of beneficial organisms such as predators, pollinators and earthworms, changes in soil microbial diversity and development of pest resistance to pesticides [6–11]. Regarding the effect on humans, the persistent nature of some pesticides has impacted our ecosystem to such an extent that pesticides have entered into various food chains and into the higher trophic levels such as those of humans and other mammals [12]. Development of new pest management strategies that have no appreciable effect on the environment and non-target organisms, as well as, ensure no reduction in crop production levels is a challenge facing the global agriculture industry today. New pest management strategies are being sought that can be integrated into current farming practice and that match or improve on crop productivity. A promising pest management strategy is biological control with biopesticides, i.e. the application of microorganisms such as fungi, bacteria or virus [13]. This approach is gaining global attention as a new tool to kill or suppress pest populations such as weeds, plant pathogens and insects while posing a low risk to humans and the environment an having no persistence in soil [14–16]. The synthetic pesticide manufacturing sector has noticed the steady achievements in biopesticide research and development and have aligned with or purchased start-up biopesticide companies to broaden their portfolio of pest management products. Olsen [17] reported that recent financial expenditures by the large multinational companies demonstrate a significant interest in the biopesticide strategy. With regard to weed management using the bioherbicide approach, logical requirements for advancing this technology include identifying a plant pathogen that can be industrially mass-produced and the pathogen–host interaction manipulated to reduce the density of a weed and provide an economic return [18]. Bioherbicides are typically applied to the target pest so that their antagonistic properties align with the most vulnerable time of the weed's life cycle. This review will focus on the inundative applications for weed control using microbial biocontrol. Microorganisms have been considered as potential biocontrol agents of economically important weeds and *Phoma* spp. appear to be the dominant fungal species reported in the literature with bioherbicidal properties [Table 1; 19–21].

Phoma Sacc. – A Genus with Bioherbicide Potential

The ubiquitous fungal genus *Phoma* consists of a large group of species responsible for saprophytic, phytopathogenic and more recently bioherbicidal activity. *Phoma* spp. show great potential for bioherbicidal activity of infestations and weed damage based on the frequency of reports on this species (Table 1). Taxonomic designation of *Phoma* is challenging and species identification is often difficult. Boerema *et al.* [22] divided *Phoma* into nine sections using culturing techniques and morphological data in a

Table 2 Herbicidal phytotoxins produced by *Phoma* sp.

Phoma spp	Target plant	Phytotoxin	Reference
P. macrostoma 94–44B	*Taraxacum officinale*	Macrocidins A, B	[53]
P. herbarum FGCC 75	*Parthenium hysterophorus*	3-nitro-1,2-benzenedi-carboxylic acid (3-natrophthalic acid)	[37]
P. exigua	*Sonchus arvensis*	deoxaphomin	[40]
Phoma sp. FGCC#18	*Parthenium hysterophorus*	Unidentified	[51]
P. chenopodiicola	*Chenopodium album*	Chenopodolans A-C	[49]
Phoma sp.	*Cucumis sativus*, *Sorghum bicolor*	Pyrrolo[1,2a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	[50]
P. crystallifera	*Convolvulus arvensis*	Unidentified	[52]

comprehensive manual to aid identification. Taxonomic revision of *Phoma* and its teleomorphs, with a focus on its genetic code, have been reviewed; see Aveskamp *et al.* [23] and Chen *et al.* [24]. *Phoma* distribution and secondary metabolite production were reviewed by Rai *et al.* [25]. *Phoma* sp. are widely distributed in nature and are found on many hosts as a phytopathogen and in numerous ecological niches contributing to global nutrient turnover as a saprophyte. Several *Phoma* spp. have been described as having the potential for use as a biocontrol agent of weeds and to the best of my knowledge, the earliest report was by Abdel Rahim and Tawfig [26] studying *Etchhornia crassipes* (water hyacinth) management by *Phoma sorghina*. Since then, numerous other *Phoma* spp. have been reported to elicit disease on economically important weeds (Table 1).

Herbicidal activity associated with *Phoma* sp. was observed following application of conidia and/or mycelium onto attached or detached leaves [26–47] or applied to soil seeded with broadleaved weeds [48, 16] and assessed using classic plant disease symptomology, i.e., necrosis, chlorosis, macerations. However, several researchers have advanced their research and reported the isolation of a partially purified or purified active ingredient from the spent media of *Phoma* sp. that contributes to herbicidal activity (Table 2). Vikrant *et al.* [37] characterized a phytotoxic metabolite from cell-free spent medium of *P. herbarum* FGCC #75. Tests with cell-free spent medium in post-emergent and detached-leaf assays on *Parthenium hysterophorus* (Santa Maria feverfew) revealed that the plant tissue turned yellow by 24 h and seedlings had collapsed by 72 h. The active ingredient was presumed to be 3-nitro-1, 2-benzenedicarboxylic acid (3-nitrophthalic acid) based on GC–MS spectroscopy results [37]. *Phoma exigua* var. *exigua* was reported to produce deoxaphomin in liquid and solid phase fermentation culture that exhibited herbicidal activity on *Sonchus arvensis* and *Cirsium arvense* (perennial thistles) [40]. When applied to leaf disks, deoxaphomin elicited necrotic lesions on these thistles. Cimmino *et al.* [49] reported that *P. chenopodiicola* produced phytoxic furopyrans named chenopodolans A, B and C. Leaf samples from *Sonchus oleraceus* (sow thistle), *P. annua* (grass) and *C. album* (lambsquarters) revealed that chenopodolans A and C caused spreading necrosis while chenopodolan B was inactive when examined by the punctured detached-leaf assay. Brun *et al.* [50] reported that cell-free spent fermentation broth from *Phoma* sp. inhibited *Cucumis sativus* (cucumber) and *Sorghum bicolor* (sorghum) seed germination 100% as compared with fresh media and water treatments. As observed in the detached-leaf assay, cell-free spent broth elicited yellow colouration and necrotic lesions on *C. sativus* and *S. bicolor* leaf tissue, respectively [50]. Methanol, ethanol and ethyl acetate fractionation of the cell-free spent fermentation broth from *Phoma* sp. suggested that pyrrolo[1,2a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) may be responsible for reduced plant height [50]. Quereshi *et al.* [51] and Razaghi and Zafari [52] reported herbicidal activity on *P. hysterophorus* and *Convolvulus arvensis* (bind weed) from cell-free spent medium of *Phoma* sp. FGCC #18 and *P. crystallifera*. The identities of the active ingredients from these two bioherbicidal *Phoma* sp. were not provided. Graupner *et al.* [53] applied bio-assay directed isolation to discover that macrocidins A and B, the first representatives of a new family of cyclic tetramic acids, were the herbicidal metabolites secreted by *P. macrostoma* 94–44B. Macrocidins A and B and putative upstream metabolite macrocidin Z are secondary metabolites and are produced during liquid and solid-state fermentation by *P. macrostoma* 94–44B [54]. Considerable basic and applied research has focused on *P. macrostoma* 94–44B including host range, global distribution, mode of action and identity of its phytotoxic metabolites with the goal of advancing biological control of weeds and bioherbicide product development [55–58]. *Phoma macrostoma* 94–44B was registered in Canada and the USA for broadleaved weed management in turf [59] and is in the advanced stage of product development by an industry partner [60]. The balance of this review will highlight the work that has led to product development of *P. macrostoma* 94–44B, results of which show promise for weed control in agricultural crop production and contribute to the advancement of a new technology for broadleaved weed management.

P. macrostoma 94–44B: Discovery

P. macrostoma 94–44B was isolated from chlorotic lesions on *C. arvense* (Canada thistle, CT), in Melfort, Saskatchewan, Canada, identified by the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre as *P. macrostoma* var. *macrostoma* and deposited within the International Deposit Authority of Canada (IDAC), Health Canada. *P. macrostoma* var. *macrostoma* Montagne 94–4B is in clade 6, *Didymellaceae*, *Didymella macrostoma* Montagne; it is a coelomycetous anamorph, reproducing by conidia released from pycnidia which are then dispersed by wind and water [20]. The sexual genus of *P. macrostoma* is unresolved [20]. *P. macrostoma* 94–4B was one of over a thousand fungal and bacterial isolates collected and screened for bioherbicidal activity to CT for potential advancement as a new class of biopesticide for managing economically important weeds in western Canada. Serendipity and changes in the biocontrol screening protocol that included soil inoculation with the fungus played a significant role in advancing *P. macrostoma* 94–44B as the top isolate for broadleaved weed suppression [56]. It is remarkable that this plurivorous and weakly opportunistic fungal isolate had the right mix of metabolic and growth characteristics to advance through lengthy biopesticide screening experiments, satisfying Canadian and USA government regulatory obligations, to move into the industrial product development stage [61]. *P. macrostoma* 94–44B suppresses root and shoot growth and most notably causes severe chlorosis or photobleaching of the foliar parts of broadleaved weeds while having no effect on monocotyledonous weeds and crop species [48, 62]. Three factors that contributed significantly to the prospects of *P. macrostoma* 94–44B advancing to turf bioherbicide product development were: (i) Broad bioherbicidal activity over several plant families including Asteraceae, Brassicaceae, Linaceae and Fabaceae while having no herbicidal activity on plant families Poaceae, Pinaceae and Lamiaceae [62], (ii) Discovery of macrocidins, metabolites secreted by *P. macrostoma* 94–44B, that cause chlorosis or photobleaching and death of broadleaved weeds, thus providing an unmistakeable visual response to this microbe [53], and (iii) Need for a natural product in Canada for weed control in turf due to municipal and provincial laws banning the use of synthetic herbicides in urban environments. Canada's first municipal pesticide bylaw was passed 6 May 1991 by the small community of Hudson, Quebec, west of Montreal. To date the eight provinces that have banned synthetic pesticides for cosmetic purposes in urban environments include Quebec 2003, Ontario and New Brunswick 2009, Alberta and Prince Edward Island 2010, Nova Scotia 2011, Newfoundland and Labrador 2012, and Manitoba 2014.

Phoma Host Range

Phoma displays a broad spectrum of weed species control that include *Bellis perennis* (English daisy), *C. arvense* (Canada thistle), *Sinapis arvensis* (wild mustard), *Stellaria media* (chickweed), *Linum usitatissimum* (flax), *Medicago lupulina* (black medic) from the plant families Asteraceae, Brassicaceae, Linaceae and Fabaceae. However, caution is warranted with flowering ornamentals species such as sweet pea (*Lathyrus odorata* L. Fabaceae), petunia (*Petunia* × *hybrida*, Solanaceae) and pot marigold (*Calendula officinalis*, Asteraceae) showing dose-related susceptibility to *Phoma*. No herbicidal activity is observed on species from the plant families Poaceae, Pinaceae and Lamiaceae, such as *Agrostis palustris* (bentgrass), *Poa pratensis* (Kentucky bluegrass), *Picea mariana* (black spruce), *Pinus* spp. (pine), *Salvia coccinea* (Crimson sage). A comprehensive list of sensitive and resistant plants to the bioherbicidal action of *Phoma* is reported in Bailey *et al.* [62]. The spring season is the optimum time for application of *Phoma* for weed control. With an air temperature of approximately 10° C weed seedlings are activity growing; *Phoma* is colonizing the seedling roots and secreting herbicidal macrocidins (Hynes and Bailey, unpublished). However, care with the placement of the *Phoma* product must be practiced so that desired sensitive ornamental plants are not affected.

P. macrostoma 94–44B is highly efficacious on *Taraxacum officinale* (dandelion) and *Trifolium* sp. (clover) [56] which attracted the attention of companies seeking new products to control broadleaved weeds in turf. Smith *et al.* [63] and Wolfe *et al.* [64] have confirmed the bioherherbicidal activity of *P. macrostoma* 94–44B on dandelion in turf.

P. macrostoma 94–44B: Mode of Action

Proof of concept experiments with *P. macrostoma* 94–44B on CT and dandelion in greenhouse experiments revealed that inundating soil seeded with dandelion or CT root pieces with 3 $\log_{10}$ colony forming units/g or more from an agar mat, liquid cultures or formulated granules of *P. macrostoma* caused foliar photobleaching, significant weed biomass reduction and weed death. This is in stark contrast to infections of CT by *P. macrostoma* in nature where one observes photobleaching limited to the youngest leaves and survival of the plant. *P. macrostoma* 94–44B mycelium rather than its spores proved to be the infective fungal propagule, from which a strategy for mass production could be planned. Bailey *et al.* [65] reported that soil application of *P. macrostoma* 94–44B mycelium to dandelion under pre- and post-emergence conditions elicited weed death while under these conditions application of its spores did not. Only *P. macrostoma* 94–44B mycelium has the capacity to produce and secrete the herbicidal molecules macrocidins [53]. *P. macrostoma* 94–44B is capable of colonizing a wide range of plant families, but only broadleaved plants were sensitive to *Phoma*, showing severe chlorosis particularly on new emerging leaves. There were dramatic differences observed microscopically between dandelion and barley roots colonized by *P. macrostoma* 94–44B [65]. While colonization of barley by *Phoma* was limited to root hairs and outer root surfaces, colonization of dandelion was extensive, infecting the inside of root hairs and the centre of the root and surrounding but not entering the root cells or vascular trachea. Appressoria or infection pegs were never observed [65].

Prior to the discovery of macrocidins, it was noted that the spent liquid fermentation medium of *P. macrostoma* 94–44B also elicited photobleaching and biomass reduction of dandelion but at a reduced level compared with that of *P. macrostoma* mycelium plus the spent medium. The photobleaching symptoms that develop in susceptible plants following exposure to *P. macrostoma* 94–44B was hypothesized to be a result of significant interference by macrocidins with the plant's carotenoid pathway [57]. The commercial synthetic herbicide diflufenican (Bayer Crop Science) similarly elicits photobleaching in broadleaved weeds and is known to inhibit biosynthesis of carotenoid precursors. This hypothesis was tested by comparing the carotenoid profiles and other physiological parameters of macrocidin- and diflufenican-treated Canada thistle, dandelion, *Cucurbita pepo* (pumpkin) and *Triticum aestivum* (wheat) [57]. In susceptible plants (CT, dandelion and pumpkin), diflufenican and macrocidins induced photobleaching symptoms, lowered total chlorophyll content, reduced photosynthetic exchange and increased the amount of carotenoid precursor phytoene. In the non-susceptible plant, wheat, neither diflufenican nor macrocidins induced any changes in the parameters measured as compared with the control treatments. While diflufenican provided complete inhibition of the carotenoid biosynthetic enzyme phytoene desaturase, based on near 100% accumulation of phytoene, macrocidin inhibition of phytoene desaturase was not as complete and appeared to inhibit one or more additional steps, notably those associated with β carotene and lutein carotenoid biogenesis [57]. It had been observed in the earlier field and greenhouse studies with individual plants that some broadleaved weeds responded differently to *P. macrostoma* 94–44B treatment, for example, photobleaching and death were observed with dandelion, whereas, only death was noted with *Senecio vulgaris* (groundsel) [62]. In the laboratory, inhibition of phytoene desaturase was more complete in dandelion as compared with groundsel [58]. This suggests that the impact of macrocidins on the carotenoid pathways varied by plant species. The effect of macrocidins on chelation of Fe and Mg by macrocidins plus macrocidin-induced changes in the OJIP chlorophyll fluorescence parameters is consistent with inhibition of electron transport from Q_A to Q_B and uncoupling of the light-harvesting complex of photosystem II from the reaction centre [58]. This work concluded that the impact of *P. macrocidin* 94–44B on broadleaved weed growth suppression was multi-faceted.

P. macrostoma 94–44B: Bioherbicide for Agricultural Broadleaved Weed Management

With no weed control alternatives for dandelion in turf, the action was directed to forming an industrial collaboration to advance *P. macrostoma* 94–44B from the research phase to the commercialization stage. Two objectives were then established: (i) a short-term objective of developing *Phoma* as a pre and post-emergent bioherbicide for control of dandelion in turf and (ii) a long-term objective of label expansion of *Phoma* to include broadleaved weed reduction in agricultural crops. The research phase of the turf *Phoma* bioherbicide included plant tolerance assessment of many genus and species of Poaceae, trees, shrubs, ornamentals and others in greenhouse and field studies to *Phoma*, thus providing a comprehensive list of tolerant and intolerant plants for weed control in turf [62]. Research on *Phoma* for use in agriculture followed a similar path; however, adjustments in this research plan were made which included (i) examination of crop tolerance to *P. macrostoma* 94–44B at multiple field sites and, (ii) efficacy evaluation following application of *Phoma* for pre and post-emergent control of broadleaved weed in wheat crops, (iii) residue studies on *Phoma* activity in crop stubble soil 1 year after use. Data were also collected on environmental and agronomic factors affecting bioherbicide application and weed management to satisfy some of the regulatory requirements for product label expansion and to provide guidelines for use. At this time, 6 years of field studies have examined crop production systems with application of *Phoma*. Field experiments were conducted at two field sites in Saskatchewan in soils characterized as dark brown chernozemic, loam to clay loam and a brown chernozemic, sandy loam. *Hordeum vulgare* (barley), wheat, *Zea mays* (corn) and *Solanum tuberosum* (potato) emergence, growth and dry matter yield or tuber yield and market grade were unaffected following application of *P. macrostoma* 94–44B. Crop tolerance studies revealed that *Phoma* could be incorporated into broadleaved weed management strategies for these crops and established *Medicago sativa* (alfalfa) following first cut or older. Crops sensitive to *Phoma* application at the time of crop seeding included alfalfa, canola, flax, lentil and peas, all of which displayed photobleaching following germination or reduced germination and yield loss. Applying conventional western Canada crop rotation practice, crops sensitive to *P. macrostoma* 94–44B were grown on the previous year's cereal plots to determine if there were any soil residues of *Phoma* present to impact plant growth. Neither photobleaching nor differences in plant stand or yield as compared with plots not-treated with *Phoma* were observed concluding that *Phoma* residue was not present 1 year after application. This result is in agreement with earlier work in which a *P. macrostoma* 94–44B-specific molecular probe was used to detect and monitor its survival characteristics in the soil. The frequency of positive detection of *P. macrostoma* 94–44B did not decrease significantly in the 2 and 4-month soil samples; however, the fungus was not detected in soil and root samples 1 year after treatment [16]. In conclusion, farmers can follow their traditional crop rotations, using *Phoma* on cereals for control of weeds, without concerns of any impacts or any limitations on the following year's crops.

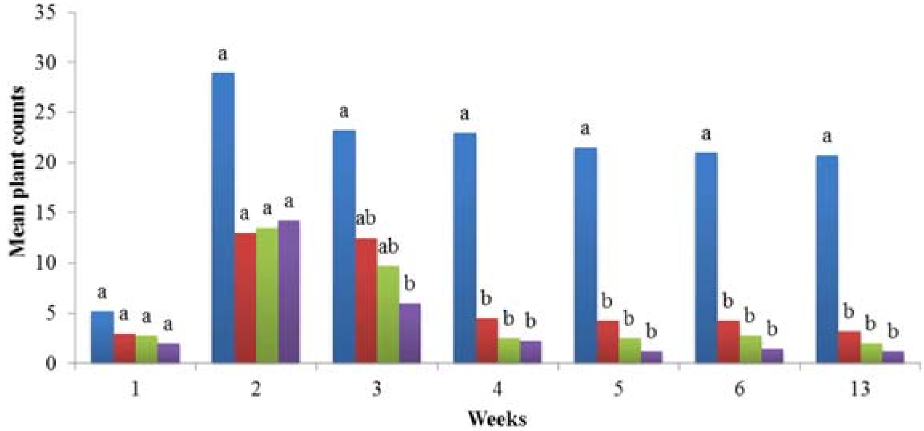

Figure 1 Effect of pre-emergent application of *P. macrostoma* 94–44B on *Sinapis arvensis* (wild mustard) seeded with *Triticum aestivum* (wheat). Blue, red, green and purple bars represent mean weed counts treated with 0, 0.25X, 0.5X and 1X *Phoma* application. The 1X *Phoma* application was 85 g/m^2. Analysis of variance conducted using SAS; data with a different letter are significantly different at $P = 0.05$.

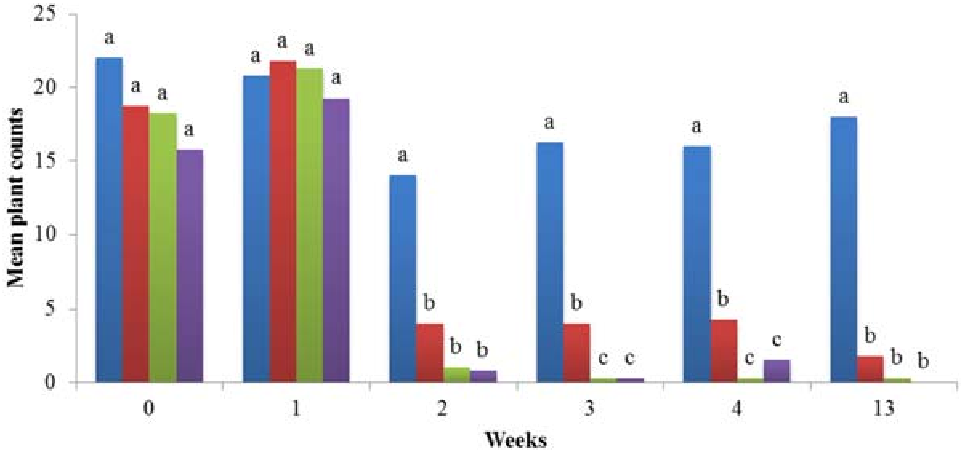

Figure 2 Effect of post-emergent application of *P. macrostoma* 94–44B on *Sinapis arvensis* (wild mustard) seeded with *Triticum aestivum* (wheat). Blue, red, green and purple bars represent mean weed counts treated with 0, 1X, 2X and 3X *Phoma* application. The 1X *Phoma* application was 85 g/m^2. Analysis of variance conducted using SAS; data with a different letter are significantly different at $P = 0.05$.

Field research examined the efficacy of several rates of application of a granular formulation of *P. macrostoma* 94–44B on control of *S. arvensis* (wild mustard) in wheat crops. Pre and post-weed emergence application of *Phoma* reduced weed count and elicited weed photobleaching, death and growth stunting. Wild mustard was effectively controlled by pre and post-emergence applications of *P. macrostoma* to plots seeded with wheat (Figures 1 and 2). Photobleaching of the wild mustard cotyledons was evident following plant emergence, then plant death (Figure 1). With the advancements that have been made in farm machinery equipment, post-emergence weed control by *Phoma* is most logical approach thus allowing a farmer to apply the bioherbicide where required. This work revealed that the bioherbicide approach to broadleaved weed management in a wheat crop could be put into practice.

Field studies identified environmental factors that could affect weed biocontrol efficacy under agricultural field conditions. The role of environment in biocontrol effectiveness was studied by recording weed reductions with *Phoma* applied at different dates representing early, mid and late season applications. Per cent weed reduction was greatest with an early pre-emergent and mid-season application of a granular formulation of *P. macrostoma*. Precipitation and air temperature data indicated that the greatest reduction in weeds occurred when the *Phoma* was applied at air temperatures consistently above 10° C with precipitation occurring within a few days prior and after application.

Future Research

Studies were recently initiated to isolate large quantities of macrocidins from *P. macrostoma* 94–44B to gain greater insight into its movement in soil and uptake by plants. Research on the genomic and metabolic processes governing macrocidin production would improve our understanding of how and when macrocidins are formed in the fungus which could lead to improvements in the production of the herbicidal molecules and increased the efficacy of *P. macrostoma* 94–44B at lower doses of application.

Conclusions

A growing plant has an exceptionally rich and diverse population of microbial life that can be found colonizing its outer surfaces, internal cells and structures (endophytes) and its rhizosphere. From this resource, a microbial collection is created and by applying screening processes that include the key traits required for pest management potential candidates are selected. Following the initial discovery and basic research phases with *P. macrostoma* 94–44B, collaborative research and development with an industrial partner provides the much-needed support to advance the technology through a Stage/Gate process and pass key hurdles at the regulatory approval, commercial scale-up, application technology and technology adoption phases [56]. Commercial interest in *P. macrostoma* 94–44B was strengthened by its broad spectrum weed reduction [62], long shelf-life, high efficacy, short persistence and low risk to the environment. Marketing interests in *P. macrostoma* 94–44B were satisfied by the strong visual symptomology (photobleaching effect) that *Phoma* elicits on broadleaved weeds which signal to consumers that the product is working.

Biopesticides and biofertilizers (i.e. pro-biotics, plant growth promoters) are being developed around the world for agricultural use and in some cases, biopesticide and biofertilizer, stacking technology, are being combined to meet the needs of organic production and conventional farming practices wishing to diversify their pest management strategy. Research and development on *P. macrostoma* 94–44B has contributed significantly to understanding the interaction between a fungus and a plant [62, 65, 57, 58] and provides a roadmap for bioherbicide product development [56].

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