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Pre-emergence Efficacy of *Phomopsis convolvulus* Ormeno to Control Field Bindweed (*Convolvulus arvensis* L.)

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Short title:

Field bindweed control with *Phomopsis convolvulus*

Susanne Vogelgsang

Abstract

Field and controlled environment experiments were performed to assess the pre-emergence activity of the fungal pathogen, *Phomopsis convolvulus* Ormeno to control *Convolvulus arvensis* L. (field bindweed). A granular barley formulation of the fungal inoculum applied onto the field soil surface resulted in dramatic above-ground biomass reductions of both *C. arvensis* seedlings (94-100%) and regrowth from established plants (53-98%). Under field conditions, surface applications of the granular formulation resulted in greater biomass reductions (93-100%) compared with soil incorporation of the granules (31-97%). Rate of soil applied granules of *P. convolvulus* did not affect the level of weed control and 90-100% *C. arvensis* biomass reductions were obtained for all rates used (30 g, 20 g, and 10 g $0.25m^{-2}$ plot). The use of two different planting substrates in controlled environment studies led to major differences in *C. arvensis* disease development. With an inoculum application on the day of sowing, 81% mortality was obtained for seedlings grown in a sandy loam field soil compared with 50% of seedlings grown in a prepared peat moss medium. The susceptibility of *C. arvensis* biotypes from various geographic locations to *P. convolvulus* was similar following post-emergence foliar inoculum applications. Significant above-ground (65-100%) and new root growth (56-72%) biomass reductions of established plants were obtained for two selected biotypes (Greece, USA-Montana) subjected to a pre-emergence granular inoculum application. In the presence of spring wheat (*Triticum aestivum* L.), field efficacy of *P. convolvulus* was enhanced and above-ground biomass of inoculated *C. arvensis* plants was reduced by 98% compared with inoculated *C. arvensis* plants grown in pure stand.

Résumé

Des expériences au champs et en laboratoire ont permis d'évaluer l'activité en pré-émergence du champignon pathogène *Phomopsis convolvulus* Ormeno pour le contrôle de *Convolvulus arvensis* L. (liseron des champs). Au champs, l'application de l'inoculum du champignon sous forme d'une formulation granulaire d'orge sur la surface du sol a permis d'obtenir des réductions significatives de la biomasse aérienne des plantules (94-100%) et de la repousse des plants établis (53-98%) de *C. arvensis*. De plus, des applications de la formulation granulaire en surface ont causé de plus grandes réductions de la biomasse (93-100%) que l'incorporation de granules (31-97%). Le niveau de contrôle n'a pas été influencé par la dose de granules appliquée sur le sol, ainsi, des réductions de la biomasse de *C. arvensis* de 90-100% ont été obtenues pour toutes les doses appliquées (30 g, 20 g et 10 g 0,25m^{-2} parcelle). En conditions de laboratoire, des différences majeures dans le développement de la maladie sur *C. arvensis* ont été observées lors de l'utilisation de différents substrats de croissance. Dans le cas où l'inoculum a été appliqué au jour du semis, un taux de mortalité de 81% a été obtenu pour des plantules dans un sol de champs loam-sableux comparativement à 50% pour des plantules dans un substrat de croissance préparé de mousse de tourbe. La susceptibilité des biotypes de *C. arvensis* des différentes régions géographiques soumis à *P. convolvulus* était similaire suivant l'application d'inoculum en post-émergence sur le feuillage. Des réductions significatives de la biomasse aérienne (65-100%) et de la croissance de nouvelles racines (56-72%) des plantes établies ont été obtenues pour deux biotypes sélectionnés (Grèce, E.U.-Montana) exposés à une application de l'inoculum granulaire. En présence de blé de printemps (*Triticum aestivum* L.), l'efficacité de *P. convolvulus* a été augmentée et la biomasse aérienne des plants de *C. arvensis* inoculés a été réduite de 98% comparé à celle des plants de *C. arvensis* inoculés en monoculture.

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Description of Thesis Format

This thesis is comprised of original papers that have been submitted to appropriate scientific journals for publication. In accordance with Part B, Section 2 of the "Guidelines for Thesis Preparation" from the Faculty of Graduate Studies and Research, McGill University, I quote the entire text that applies to this format:

*"2/ **Manuscripts and authorship:** Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the Thesis Preparation Guidelines with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.) The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts. The thesis must include the following: A table of contents; an abstract in English and French;, an introduction which clearly states the rationale and objectives of the research; a comprehensive review of the literature (in addition to that covered in the introduction to each paper); a final conclusion and summary; and, rather than individual reference lists after each chapter or paper, one comprehensive bibliography or reference list, at the end of the thesis, after the final conclusion and summary. As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis. In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contribution of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make clearly specify the responsibilities of all the authors of the co-authored papers."*

In order for this thesis to be consistent with the above statement, it is structured in the following manner:

The thesis begins with abstracts in English and French, followed by a table of contents. Chapter 1 comprises a general introduction in which background knowledge and the current state of research of the thesis subject is presented. This section concludes with an outline of the specific thesis objectives. The next five

chapters constitute the body of this thesis, with four chapters being complete manuscripts.

The chapter 2 manuscript was submitted for publication to *Weed Research.*

The chapter 3 manuscript was submitted for publication to *Weed Science.*

The chapter 4 manuscript was accepted for publication to *European Journal of Plant Pathology.*

The chapter 5 manuscript was submitted for publication to *Biological Control.*

The chapter 6 manuscript is not to be submitted for publication.

The various manuscript chapters are linked via connecting texts so as to establish logical bridges between the different papers.

A general discussion and synthesis of the major conclusions of the thesis are presented in Chapter 7. The main contributions to knowledge of this research are outlined in Chapter 8. A bibliography section comprises the final chapter of the thesis and includes all references cited, including those within the manuscript chapters. An Appendices section is also included.

Contribution of Authors

The manuscript from chapter 2 and 5 are co-authored by Drs. A.K. Watson, A. DiTommaso, and K. Hurle. The candidate (S. Vogelgsang) performed all the experimental research, statistical analyses, and is the primary author of the manuscript. Drs. A.K. Watson, A. DiTommaso, and K. Hurle provided supervisory guidance and assisted in manuscript preparation.

Manuscripts from chapters 3 and 4 are co-authored by Drs. A.K. Watson and A. DiTommaso. The candidate (S. Vogelgsang) performed all the experimental research, statistical analyses, and is the primary author of all four manuscripts. Drs. A.K. Watson and A. DiTommaso provided supervisory guidance and assisted in manuscript preparation.

Chapter 1. General Introduction

1.1. Abstract

Convolvulus arvensis L. (field bindweed) constitutes a serious weed problem in various crops throughout many regions of the world. *C. arvensis* is difficult to control with conventional means and has been selected as a target weed for biological control. This research project focuses on the control of *C. arvensis* using the selective fungal pathogen, *Phomopsis convolvulus* Ormeno.

1.2. Introduction

Since the beginning of agriculture, man has been interfering in natural ecosystems in order to create space for the production of useful plants. Despite the implementation of a number of plant protection measures, the deleterious impact of phytophagous pests, pathogens, and wild plant populations on crop plants have continued to be problematic. Until the beginning of this century, hand-weeding and hoeing were the only means of weed control.

After the use of various inorganic compounds for weed control in the 1930's and 1940's, the period of organic herbicides began. In the past 50 years, weed science research has largely been concerned with herbicide efficacy, application, and development (Koch & Hurle 1978). High herbicide efficacy, selectivity, and reliability has resulted in greater crop yields as well as the possibility of implementing new crop rotations. Consequently, traditional control methods were for the most part relegated to a minor role. However, a changing weed flora, development of herbicide resistance (Moss & Rubin 1993), residue problems, and increasing consumer pressure against pesticide use have led to a change in the way chemical control is perceived. It is becoming evident that a total dependence of weed control strategies on chemical herbicides is unacceptable and that alternative and/or complementary control measures must be sought. Hence, the overall goal of new control strategies is to suppress problem weed populations in an ecologically and economically feasible manner by integrating a number of control tactics

(Schroeder 1990). Biological weed control represents a possible strategy that could be integrated into such a system.

Biological weed control is the deliberate use of natural enemies to suppress the growth or reduce the population of a weed species (Watson 1989). There are two main strategies to biological weed control: the inoculative strategy (classical approach) and the inundative strategy (bioherbicide approach) (Wapshere 1982).

In the inoculative strategy, the population density of an introduced plant species is substantially reduced and stabilized by the introduction of specific antagonists. It is therefore considered as a "biological regulation" rather than as a "biological fight" (Schroeder 1990). Moreover, this method is environmentally safe as well as permanent and economical (Templeton *et al.* 1979). However, the classical approach is primarily suitable for relatively stable, less disturbed habitats such as pastures, rangelands, forests, aquatic systems, or fallow land since intensive cultivation is often too disruptive for weed feeding/parasitizing organisms to attain damaging population levels. Moreover, such habitats generally contain a complex of weed species and the control of a single target does not necessarily modify overall competitive effects from the remaining weed complex (Schroeder 1983).

As part of the inundative strategy, the augmentative approach aims at the control of native weed species by manipulating the habitat of existing natural enemies (primarily insects) (Huffaker 1976, Schroeder 1990). Similarly, the recently proposed "system management approach" focuses on "maximizing the natural spread and disease severity of a native or naturalized pathogen" (Frantzen & Müller-Schärer 1996). However, both the inoculative and augmentative approaches require good knowledge of the beneficial agent's life cycle and often necessitate a number of modifications to current agricultural practices including altered mowing and grazing regimes in pastures, and/or pesticide applications (Schroeder 1990). In addition, most native pathogens have only a limited effect on the natural vegetation owing largely to poor virulence, unsatisfactory spore dispersal, or lack of sufficient inoculum. The use of the bioherbicides could overcome some of these constraints.

Bioherbicides are specifically formulated preparations of living inoculum from plant pathogens that are used for the control of a target weed. They are usually applied in a manner similar to chemical herbicides by periodic dispersal of massive doses of virulent inoculum (Watson 1989, Watson & Wymore 1990). In general, the bioherbicide strategy does not include the use of insects or phytotoxins (Watson 1989). Although bacteria, mycoplasma, viruses, and nematodes have been examined for their suitability as biological control agents (Watson 1976, Charudattan *et al.* 1978, Kremer *et al.* 1990, Zhou & Neal 1995), fungal pathogens within the Deuteromycetes or Fungi Imperfecti group constitute by far the majority of plant pathogens used in the bioherbicide approach. An important reason for this is that conidia-producing fungi are usually easily cultured and stimulated to sporulate and possess several modes of action (Freeman 1981). The "mycoherbicide approach" is commonly used as a synonym for the bioherbicide approach since this strategy relies heavily on fungal pathogens.

The bioherbicide strategy involves three major phases: discovery, development, and deployment (Templeton 1982a). During the discovery phase, diseased plant material of the target weed is collected, causal agents isolated and, following demonstration of Koch's postulates, identified. After selection of a suitable organism with sufficient virulence, the pathogen is cultivated and maintained in short- and long-term storage. During the development phase, optimal conditions for spore production, infection, and disease development are determined. Furthermore, the process of infection, mode of action, and possible involvement of toxins are examined. Also included are host specificity and efficacy tests carried out under field conditions. Formulation, scale-up processes, as well as marketing and product introduction are key aspects of the deployment phase.

Most of the registered bioherbicides have been developed and marketed in North America: DeVine®, registered in 1981 in the United States, is a liquid formulation of *Phytophthora palmivora* chlamydospores and was developed for the control of strangler vine [*Morrenia odorata* (H. & A.) Lindl.] in citrus groves (Kenney 1986, Ridings 1986). Following a single post-emergence application,

more than 90% control for at least 2 years is typically achieved (Charudattan 1991). COLLEGO®, a dry spore powder formulation of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* was registered in 1982 and has been used for the control of northern jointvetch (*Aeschynomene virginica* L.) in rice (*Oryza sativa* L.) and soybeans [*Glycine max* (L.) Merrill] (Daniel *et al.* 1973, Bowers 1986). BioMal® is a dry formulation of *C. gloeosporioides* f. sp. *malvae* for control of round-leaved mallow (*Malva pulsilla* Sm.) in flax (*Linum usitatissimum* L.), lentils (*Lens culinaris* Medik.), and wheat (*Triticum aestivum* L.) and although registered in Canada (Makowski & Mortensen 1992), it has not been marketed. Luboa 1 S_{22} contains *C. gloeosporioides* f. sp. *cuscutae* and is used for control of parasitic dodder (*Cuscuta* spp.) in soybeans in China (Templeton 1992, Wan *et al.* 1994). General outlines of mycoherbicide projects are provided by Charudattan (1991), Julien (1992), and Watson (1993).

Bioherbicides are mainly used in intensely cultivated crops where their rapid effect and high degree of control make them superior to the classical or augmentative approach, which often work too slowly or may provide inadequate control of a specific weed species. Moreover, the ease and timing of applications within the bioherbicide strategy, makes their integration in existing farming practices much more feasible (Freeman 1981, Schroeder 1990). According to Templeton *et al.* (1979) and Auld (1991), the application of bioherbicides is especially advantageous for parasitic weeds, for weeds which are difficult to control via chemical means, or in small-scale and specialized crops where the development of specific chemical herbicides is too expensive.

The main limitations to the development of bioherbicides are largely related to biological, technological, environmental, governmental and economical factors (Watson & Wymore 1989). With few exceptions [e.g. tank mixture of two *Colletotrichum* spp., (Boyette *et al.* 1979)], bioherbicides only control single weed species. Therefore, despite the comparatively low costs for development, industry interest for investment is relatively poor due to limited market potential and reduced profits (Templeton 1982b, Wapshere 1982). However, the most frequently

encountered difficulties in the bioherbicide strategy are related to biotic and abiotic constraints including the requirement of most pathogens for rather specific environmental conditions (primarily moisture and temperature) during the infection phase. Appropriate timing of application, improved formulation and/or application methods, as well as genetic manipulation of the organisms could possibly overcome these limitations (Greaves *et al.* 1989, Watson 1989, Auld & Morin 1995).

Granular preparations, either pre- or post-emergence, can possibly protect biotic control agents against competition from other micro-organisms, desiccation, or other unpredictable and unfavourable environmental effects (Walker 1981). A number of pathogens have been formulated in vermiculite-carriers, alginate pellets, inverse emulsions, or pasta-like matrices, leading to improved efficacy and stability (Walker & Connick 1983, Boyette & Walker 1985, Quimby *et al.* 1988, Weidemann & Templeton 1988, Connick *et al.* 1991).

1.3. *Convolvulus arvensis*

1.3.1. Biology, distribution, and agricultural importance

Convolvulus arvensis L. (Tubiflorae: Convolvulaceae), commonly known as field bindweed or small-flowered morning glory, is a perennial herbaceous broadleaf weed, which spreads primarily by means of lateral roots and root buds (Holm *et al.* 1977). Its alternate, simple leaves are long-petioled and often have a V-shaped base and a rounded tip. Leaf shape can also vary greatly in response to environmental conditions so that it has been described as being "triangular to ovate-oblong with hastate, cordate or sagittate basal lobes" (Weaver & Riley 1982). The creeping or twining thin-branched stem (*convolvolo* = to roll/entwine) can form a dense, tangled mat or can attain a height of up to 120 cm. The insect-pollinated flowers of *C. arvensis* are regular with a funnel-shaped, white to pink coloured corolla, usually lasting only one day (Holm *et al.* 1977). After pollination, 2-compartimentalized capsules are formed, with 1 to 4 seeds produced per fruit (Brown & Porter 1942). Estimates of the number of *C. arvensis* seeds produced

per unit area range from 50 000 to 20 million seeds ha^{-1} in a pure stand (Brown & Porter 1942, Whitesides 1979). However, *C. arvensis* seldom sets seed during the first year of growth (Weaver & Riley 1982) and fruit and flower abortion in this species can range from 4 to 40% (Brown & Porter 1942). Seeds can remain dormant for more than 30 years (Timmons 1949, Callihan *et al.* 1990) due to the hard, impermeable seed coat, enabling *C. arvensis* to infest crop lands long after control of flowering parent plants (Rolston 1978). Germination is possible throughout the growing season, but occurs mainly in spring (Weaver & Riley 1982). *C. arvensis* is quite similar in appearance to *Calystegia sepium* L. (hedge bindweed), however, *C. arvensis* can be distinguished by its two bracts below the flower on the peduncle rather than being inserted at the base of the flower.

C. arvensis is of Eurasian origin and was accidentally introduced into North America in 1739 in the state of Virginia, USA (Wiese & Phillips 1976). This weed is a serious problem in 32 crops in more than 44 countries, predominantly in the temperate zones of Europe, Western Asia, and North America (Holm *et al.* 1977). *C. arvensis* is particularly problematic in cereals, corn (*Zea mays* L.), and root crops but can also pose problems in horticultural crops, vineyards, ornamental plantings, and waste areas (Holm *et al.* 1977). Based on a recent European survey, *C. arvensis* is one of the 15 most important weed species in 8 out of 10 main crops (Schroeder *et al.* 1993). Holm *et al.* (1977) ranked *C. arvensis* as the 12th worst weed in the world.

Preferred habitats for colonization are light, warm, dry or moderately moist, fertile, loamy or clayey soils (Ellenberg 1974, Holm *et al.* 1977). *C. arvensis* has a high light requirement and is not very competitive when shaded. However, due to a deep tap root with numerous lateral roots, *C. arvensis* is very drought resistant and a strong competitor during water shortages. From lateral roots, endogenous root buds are produced, generating new shoots (Kennedy & Crafts 1931), rendering established *C. arvensis* stands very persistent and difficult to control. Close to the soil surface, root pieces of only 1 cm length can produce regrowth while longer sections are able to produce regrowth from greater depths. A *C. arvensis* root

system can cover an area 6 m in diameter and extend to a depth of 9 m (Holm *et al.* 1977).

Crop yield losses are due primarily to competition for nutrients and water, as well as by crop lodging or interference with mechanical harvesting due to the climbing growth habit of the weed. In a soil layer 60 cm deep, one *C. arvensis* plant is capable of reducing available water to below the wilting point of the crop so that in dry years, complete crop failure is a common occurrence (Swan 1980). Heavy *C. arvensis* infestations can reduce winter wheat yields by one-third and summer grown crop yields by three-quarters (Phillips 1976). Similarly, Wiese & Phillips (1976) found severely stunted and dead summer crop plants [e.g. sorghum (*Sorghum* sp.)] as a result of intense *C. arvensis* competition. In Ontario, uncontrolled *C. arvensis* populations reduced corn grain yields by 25 to 70% (O'Toole & Horn 1989). In fruit crops, *C. arvensis* frequently smothers bushes and small trees resulting not only in lower quality fruits because of competition for light, water, and nutrients, but also in longer time expended for pruning and harvesting operations (Davison 1976). In California and Texas alone, the cost of direct yield losses due to *C. arvensis* plus the cost of implementing control measures has been estimated to be in the range of 25 to 50 million US $ (Rosenthal 1983, Bean & Wiese 1989). *C. arvensis* also provides breeding sites for insect pests such as white flies (*Aleyroides* sp.) and cutworms (*Euxoa* sp.), attacking adjacent crops (Landis & Getzendaner 1947, Tamaki *et al.* 1975) and is considered an alternate host for a number of plant pathogenic viruses that cause diseases such as potato X disease, tobacco streak, and tomato spotted wilt (Holm *et al.* 1977).

Possible beneficial aspects of *C. arvensis* include erosion control for roadsides (Rosenthal 1983), cultivation as a fodder or ornamental plant, or use as a purgative (Holm *et al.* 1977, Weaver & Riley 1982).

Changing management and cultivation practices have resulted in an increased prevalence of *C. arvensis*. With the advent of shortened crop rotations, extensive use of mineral fertilizers and chemical herbicides, and the move towards reduced cultivation or zero tillage (Phillips *et al.* 1980), overall weed densities have

decreased but the relative importance of several weed species has shifted (Hurle 1988). A good example is *C. arvensis* which because of its perennial habit has become a dominant weed in a number of important world crops.

1.3.2. Control of Convolvulus arvensis

1.3.2.1. Management, cultivation, and chemical herbicides

Possible control methods include the use of competitive crops that can develop rapidly and shade *C. arvensis* (Koch & Hurle 1978). Such competitive species include winter wheat, alfalfa (*Medicago sativa* L. ssp. *sativa*) and with adequate soil moisture rye (*Secale cereale* L.), soybean, and sorghum (Wiese & Rea 1959, Swan 1980). However, only temporary control is generally achieved and new regrowth often occurs after harvest. Mechanical tillage can be successful at the 3- to 4-leaf stage of the weed after root reserves have been translocated to above-ground plant parts. The frequent removal of all photosynthetic plant parts at 2-to 3-week intervals can effectively reduce stored nutrients (Derscheid *et al.* 1970). In orchards, control of *C. arvensis* can also be achieved by flaming, planting of cover crops or sod, and mowing (Rosenthal 1983). Chemical control can be carried out by using systemically active substances such as 2,4-D (2,4-dichlorophenoxy acetic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), glyphosate [N-(phosphono-methyl)glycine], picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid), or fluroxypyr [([4-amino-3,5-dichloro-6-fluoro-2-pyridinyl]oxy)acetic acid] that are applied either alone or in combination (e.g. Derscheid *et al.* 1970, Westra *et al.* 1992, Macdonald *et al.* 1993). However, variable susceptibility of *C. arvensis* to a number of important herbicides including 2,4-D and glyphosate (Whitworth & Muzik 1967, DeGennaro & Weller 1984, Duncan & Weller 1987, Kosinski & Weller 1989, Yerkes & Weller 1996, Westwood *et al.* 1997) often render chemical control of this weed ineffective. In areas with low precipitation, efficacy is reduced, likely because of the deeper root system, decreased leaf area, as well as greater levels of cuticular wax impeding foliar uptake of the herbicides (Meyer 1978). Moreover, repeated chemical herbicide applications may result in undesirable crop

damage (Gillespie 1990, Schoenhals *et al.* 1990, Heering & Peeper 1991) and groundwater contamination (Anonymous 1990).

1.3.2.2. Biological control

Considerable effort has been placed on the biological control of *C. arvensis* using arthropods (e.g. Rosenthal & Carter 1976, Rosenthal & Buckingham 1982, Maw 1984, Rosenthal 1985, Giannopolitis & Chrysayi 1988, Rosenthal *et al.* 1988, Boldt & Sobhian 1993). In an extensive review by Wang and Kok (1985), various insects and mites have been considered promising biological control agents. Several foliar feeders including the chrysomelids *Galeruca rufa* Germar, *Hypocassida subferruginea* (Schrank), the noctuid *Tyta luctosa* (Denis & Schiffermüller), and the acarid *Aceria convolvuli* (Nalepa), have been evaluated. Among root/stem and seed feeders, the chrysomelid *Longitarsus pellucidus* Foudras and the bruchids *Spermophagus sericeus* (Geoffroy) and *Megacerus discoideus* (Say) also have been evaluated. However, *Galeruca rufa* was removed as a potential biocontrol agent since it fed and reproduced on several sweet potato varieties (*Ipomoea batatas* Monnet Lamarck, Convolvulaceae) (Rosenthal *et al.* 1983). Ciomperlik *et al.* (1992) reported the limited potential of *Tyta luctuosa* as a biocontrol agent for *C. arvensis* due to heavy predation by a fire ant (*Solenopsis invicta* Buren). The gall mite, *Aceria malherbae* Nuzzaci, native to southern Europe and restricted to members of the genera *Convolvulus* and *Calystegia*, has recently been introduced into the United States and South Africa (Rosenthal & Platts 1990, Craemer 1995). Within Canada, attempts to establish new colonies in Alberta with the chrysomelids *Chirida guttata* (Ol.), *Chelymorpha cassidea* (F.), and *Metriona purpurata* Boh. from Saskatchewan have not been successful (Maw 1984). In Pakistan, several insects causing damage to flowers, seeds, roots, aerial and underground stems have been recommended for biological control (Baloch 1977). In a review of biocontrol projects for *C. arvensis*, Julien (1992) concluded that permanent establishment of released organisms and subsequent control was unsatisfactory. Moreover, it has been cautioned that one single organism may likely

not be capable of substantially suppressing *C. arvensis* growth, hence the combined use of multiple stress factors has been suggested (Wang & Kok 1985).

Application of deleterious rhizobacteria to suppress weed seed germination and plant vigor through production of harmful metabolites has also been proposed (Kremer *et al.* 1990, Kennedy *et al.* 1991, Begonia & Kremer 1994). In a recent study, the auxin-producing bacterial isolate *Enterobacter taylorae* inhibited *C. arvensis* root growth by > 77 and 90% when applied alone or in combination with the precursor L-tryptophan, respectively (Sarwar & Kremer 1995). However, such rhizobacteria should selectively colonize weed seedling roots in order to minimize potential damage to crop seedling growth. Selection of sufficiently specific strains might lead to the development of suitable biocontrol agents.

Various fungal pathogens have been investigated as potential bioherbicides for control of *C. arvensis*. Giannopolitis and Chrysayi (1989) isolated three pathogens from the genera *Septoria* and *Phoma* which demonstrated sufficient pathogenicity and host specificity. The powdery mildew, *Erysiphe convolvuli* was found to attack *C. arvensis* leaves, however it was not clearly demonstrated whether the weed damage observed was caused by the pathogen alone or by the combination of environmental stress factors such as hot, dry weather (Rosenthal & Buckingham 1982). The rust fungus, *Puccinia convolvuli*, has been suggested by Hasan (1973) as a potential biocontrol agent for *C. arvensis* but no further investigations have been reported. In a co-operative European research project carried out in France, Switzerland, and England, a fungal isolate from the genus *Stagonospora* has been evaluated for control of *C. arvensis* and the closely related *Calystegia sepium* L. (hedge bindweed) (Hasan *et al.* 1992, Défago *et al.* 1994, Pfirter *et al.* 1996). Conidia mass production, disease mechanism, host specificity, formulation, and integration into existing cropping systems have been investigated. While greater emphasis was laid on control of *C. sepium*, application of spore suspensions in oil emulsion as well as new crop management methods (e.g. corn undersown with a green soil cover) was suggested to enhance the biological control of both "bindweeds" (Défago *et al.* 1994). In the United States, the potential of *Phoma*

proboscis to suppress *C. arvensis* has been examined (Heiny 1990, Heiny & Templeton 1991, Heiny 1994). Culture characteristics, conditions for optimal disease development, field efficacy and survival, and synergism with chemical herbicides were investigated. As with various other potential bioherbicides, the length of dew period during the infection process as well as plant age were two factors found to be critical for disease development.

In the mid 1980's, a fungal pathogen, *Phomopsis convolvulus* Ormeno, was isolated from diseased *C. arvensis* plants in Canada (Ormeno-Nuñez *et al.* 1988b).

1.4. *Convolvulus arvensis-Phomopsis convolvulus* weed-pathogen system

In 1984 and 1985, diseased *C. arvensis* plants growing on the island of Montréal (QC, Canada) were collected. Isolates of the *Phomopsis* genus were recovered and cultivated on artificial media (Ormeno-Nuñez *et al.* 1988b).

By comparing cultures and descriptions of other species, the selected isolate was considered as a new species, *Phomopsis convolvulus* Ormeno (Ormeno-Nuñez *et al.* 1988b). *P. convolvulus* is a member of the Deuteromycetes in the form order Sphaeropsidales. The pathogen lives as a facultative endoparasite with its entire development in the haplophase. After germination of conidia, appressoria are formed. Mycelia then penetrate the plant tissue and form dark pigmented fruiting bodies, the pycnidia, from which new conidia are released.

P. convolvulus has been cultivated on various types of agar media as well as on *C. arvensis* plant tissue (Ormeno-Nuñez *et al.* 1988b). Fungal colonies consist of mycelium with hyaline, branched and septate hyphae, 1 to 4 µm in diameter. The aerial mycelia is white, floccose, dense, and indistinctly zonate. After 2 weeks, at the bottom of usually transparent cultures, small dark brown to black stromata are formed from which dark pycnidia (up to 300 µm in diameter) develop. The pycnidia wall is composed of several layers of light brown cells and the ostiole is often heavily pigmented. The conidiogenous cells arise from the inner layer of the pycnidia and are short to slightly elongate. Both, alpha- and beta-conidia are found. Alpha-conidia are hyaline, oblong to fusiform-ellipsoid, sometimes slightly

constricted in the middle with a length of 11 to 12 µm and a width of 3 to 4 µm. Beta-conidia are only found in artificial culture, are hyaline, filiform, 17 to 33 µm long and 0.5 to 1.5 µm wide.

Inoculation of *C. arvensis* plants with conidia of *P. convolvulus* results in leaf spots and anthracnose symptoms (Ormeno-Nuñez *et al.* 1988b). Lesions are usually light brown in colour, rounded to irregular in outline with a distinct dark margin, often surrounded by a yellowish-green halo, characteristic of phytotoxin production (Morin *et al.* 1989b, Tsantrizos *et al.* 1992). Following prolonged disease development, leaves and eventually the entire foliage, become completely blighted. Dieback symptoms are generally first visible in the youngest leaves. The formation of pycnidia occurs in senescent or dead tissue close to or in direct contact with soil.

P. convolvulus conidia grown on pot barley grains (*Hordeum vulgare* L.), have been tested for viability under different temperature and substrate regimes (Morin *et al.* 1989a). Low temperatures (-70 °C) and the use of sucrose solutions resulted in a shelf-life of several months. Sparace *et al.* (1991) examined the effect of varying matrix concentrations on conidia suspensions produced on potato dextrose agar (PDA). Preparations where stored at room temperature as liquid or dried material. The longest inoculum survival (9 weeks) was achieved with the liquid stage and undiluted matrix, whereas drying of conidia was lethal.

In host specificity tests, various members of the Convolvulaceae, including crops and ornamental plants, have been evaluated (Ormeno-Nuñez *et al.* 1988b). Following inoculation of leaves, shoots, and tubers, plants were exposed to a dew treatment of 18 h and development of symptoms and pycnidia was evaluated at two different dates. Only *C. arvensis* was killed by application of the fungus but all species from the genera *Convolvulus* and *Calystegia* showed leaf lesions and anthracnotic symptoms. However, necrotic areas remained limited to inoculation sites. Species with thick, waxy leaves showed less severe disease symptoms than species with smooth, slender, less waxy leaves. None of the tested crops or ornamental plants was noticeably affected. On *Ipomoea* species such as sweet

potato, pycnidia were observed in senescing tissue, but only after tissues had been placed in humid chambers. Therefore, these host plants were considered "symptomless carriers" (Kulik 1984, Ormeno-Nuñez *et al.* 1988b). It is likely that the long dew period provided resulted in greater survival of either germinated or non-germinated conidia although tissue penetration was absent.

For field inoculum applications, methods need to be developed from which large amounts of infective propagules can be obtained. Inoculum production using solid substrates has consisted of evaluating various agricultural products including barley grains, flax seeds, lentils, oat (*Avena sativa* L.) bran, wheat, and *C. arvensis* foliage; and Richard's (V-8) medium (Tuite 1969) and half-strength potato dextrose broth (PDB) have been examined for liquid fermentation (Morin *et al.* 1990). Overall, *P. convolvulus* grown on pot barley resulted in the production of the highest conidia density, viability, and pathogenicity (Morin *et al.* 1990). The superior sporulation and pathogenicity of pot barley grown *P. convolvulus* was suggested to be due to the large surface area, structure retention of the grains, as well as adequate composition and availability of nutrients.

In order to determine conditions that result in optimal infection and disease development in *C. arvensis*, the effects of inoculum concentration, length and nature of dew period, plant age, and temperature have been evaluated (Ormeno-Nuñez *et al.* 1988a, Morin *et al.* 1989b, 1990). In these studies, *C. arvensis* plants were inoculated with *P. convolvulus* fungal suspensions and disease development and severity were determined from biomass, mortality, and necrotic leaf area data. Both seedlings and clonal material from root pieces were tested. Inoculum dose and moisture provided had a strong effect on biomass reduction. Ormeno-Nuñez *et al.* (1988a) found that for complete necrosis and high mortality (67%) to occur following spraying to run-off, conidia densities of 5×10^6 conidia ml^{-1} and a dew period of more than 6 h were necessary. Using a spray chamber, Morin *et al.* (1989b, 1990) observed a required minimum of 1×10^8 conidia m^{-2} (approximately equivalent to 2×10^6 conidia ml^{-1}) and 18 h dew. Compared with continuous moisture availability, interrupted dew periods resulted in a lower efficacy of the

fungal pathogen (Morin *et al.* 1989a). Younger plants, especially seedlings, were distinctly more susceptible to disease than established plants or shoot regrowth from root sections. Temperatures of 20 or 30 °C resulted in greater infection and disease development of *C. arvensis* than plants subjected to 10 °C (Ormeno-Nuñez *et al.* 1988a).

In an attempt to overcome the requirement for a long dew period during the infection phase and to improve field efficacy, various evaporation-inhibiting additives (e.g. starch, gelatine) have been added to *P. convolvulus* conidia suspensions. Furthermore, conidia suspensions have been combined with the herbicide dicamba and evaluated for synergistic effects (M. Clément pers. comm.). Dicamba and *P. convolvulus* in combination were found to reduce *C. arvensis* biomass to a greater extent at all rates and inoculum densities used than when either was used alone.

More recently, pot barley grains colonized by *P. convolvulus* (Figure 1.1) were milled and air dried. The resulting granular inoculum was applied to the soil surface and tested for pre-emergence activity on *C. arvensis* at different application dates under both controlled environment and the field conditions (Vogelgsang *et al.* 1994). Under controlled environment, time of application and the dew period (18 h continuous moisture versus 4 × 6 h interrupted regime) had a strong effect on mortality and biomass reductions. However, under field conditions, all fungal treatments resulted in biomass reductions ranging from 75 to 83%, regardless of inoculation date. Moreover, dried granular inoculum retained its viability and pathogenicity when stored at 4 °C for a period of at least 6 months.

1.5. Thesis objectives

Granular inoculum of *P. convolvulus* applied onto the soil surface showed high efficacy of control against *C. arvensis* seedlings under both controlled environment and field conditions. However, various aspects of this pathogen-weed system have to be elucidated in order to further develop *P. convolvulus* as a potential bioherbicide. Hence, the main objectives of this research were to:

(1) evaluate the pre-emergence activity of *P. convolvulus* on seedling and established plant growth of *C. arvensis* under both controlled environment and field conditions,

(2) evaluate the effect of pre-emergence soil incorporation of *P. convolvulus* inoculum on *C. arvensis* growth under both controlled environment and field conditions,

(3) determine the effect of reduced rates of granule application on *C. arvensis* growth under both controlled environment and field conditions,

(4) gain additional knowledge about the effects of moisture availability, inoculum production method, and planting substrate on *P. convolvulus* disease development and disease severity under controlled environment conditions,

(5) determine the occurrence of differential disease susceptibility of (a) various *C. arvensis* biotypes to foliar post-emergence applications and of (b) two selected biotypes grown from root stock material to pre-emergence applications of *P. convolvulus*,

(6) determine the pre-emergence efficacy of *P. convolvulus* inoculum to control *C. arvensis* at various application times under field conditions, and

(7) evaluate the impact of *P. convolvulus* on *C. arvensis* growth under competitive cropping situations.

Figure 1.1. Pot barley grains colonized by *Phomopsis convolvulus* showing pycnidia and fungal matrix.


1 mm

Connecting Text

In previous studies with the *Convolvulus arvensis/Phomopsis convolvulus* weed-pathosystem, granular pre-emergence applications resulted in severe disease development and high mortality of *C. arvensis* seedlings. However, in the field, the vast portion of *C. arvensis* biomass develops from intact or fragmented roots rather than from seedlings. Hence, in this study, the pre-emergence activity of *P. convolvulus* on seedling and established plant growth of *C. arvensis* will be evaluated under both controlled environment and field conditions.

Chapter 2. Effect of the pre-emergence bioherbicide *Phomopsis convolvulus* on seedling and established plant growth of *Convolvulus arvensis*

2.1. Abstract

The effects of the fungal pathogen *Phomopsis convolvulus* on seedling and established plant performance of *Convolvulus arvensis* were compared under both controlled and field conditions. Under a controlled environment, a granular barley formulation of the fungal inoculum that had been applied onto the soil surface of pots containing pregerminated *C. arvensis* seeds resulted in above-ground biomass reductions of up to 87%. However, application of the fungus onto established plants that had been cut to ground level, produced biomass reductions (43%) that were nearly half those obtained for seedlings. In a parallel field experiment conducted over two growing seasons, *P. convolvulus* application resulted in dramatic above-ground biomass reductions for both seedlings and established plants. In one trial, biomass reductions of up to 100 and 98%, respectively, were obtained. *C. arvensis* coverage within field plots was closely correlated with above-ground biomass. Findings in this study indicate that *P. convolvulus* may provide effective control of *C. arvensis* when used pre-emergence.

2.2. Introduction

Convolvulus arvensis L. (field bindweed) is a serious perennial weed in many crops and is prevalent in almost every agricultural area of the world (Holm *et al.* 1977). The move towards reduced cultivation or zero tillage in the past 15 years, has led to an increased prevalence of *C. arvensis* (Phillips *et al.* 1980). Effective control of *C. arvensis* using current methods including cultivation, crop rotation, and chemical herbicides (Derscheid *et al.* 1970) is often not possible due to its extensive root system, high competitiveness, and variable susceptibility to several important herbicides (Whitworth & Muzik 1967, DeGennaro & Weller 1984, Kosinski & Weller 1989, Yerkes & Weller 1996). The first reported incidence of *C. arvensis* being infected by the foliar pathogen, *Phomopsis convolvulus* Ormeno

was in 1988 (Ormeno-Nuñez *et al.* 1988b). Since then, studies on host specificity, conidia mass production, storage, and efficacy of foliar treatments have been carried out (Ormeno-Nuñez *et al.* 1988a,b, Morin *et al.* 1989a,b, Morin *et al.* 1990). One key characteristic of the fungus that has limited its efficacy is the requirement for a long dew period during the germination and infection phases for sufficient disease to develop. In an attempt to overcome this limitation, a granular pre-emergence application of *P. convolvulus* has been tested. Inoculum produced on pot barley grains and applied onto the soil surface showed high efficacy of control against *C. arvensis* seedlings under both controlled environment and field conditions (Vogelgsang *et al.* 1994). However, a major obstacle for effective control of *C. arvensis* is the ability of this vigorous weed to regenerate vegetatively from established plants (Swan & Chancellor 1976). This is especially important given that, within most cropping systems, the largest proportion of *C. arvensis* biomass develops from existing intact or fragmented roots and not seedlings (Weaver & Riley 1982). Hence, the objective of this study was to evaluate the pre-emergence activity of *P. convolvulus* on seedling and established plant growth of *C. arvensis* under both controlled environment and field conditions.

2.3. Materials and methods

2.3.1. Inoculum production of starter cultures

Single conidium isolates of *P. convolvulus* were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 4 °C. From these stock cultures, small pieces of mycelia were placed on 9-cm-diameter Petri dishes with PDA and incubated in the dark at 24 ± 1 °C. After 4 to 5 days, several 1-cm-diameter mycelial plugs were transferred to PDA plates and incubated at room temperature (21 ± 2 °C) and 12 h day^{-1} near-ultraviolet light (F40 BLAB Blacklight, General Electric Lighting, Cleveland, OH, USA). After 3 weeks, conidia were harvested by washing plates with 12 ml of sterile deionized water. Conidia density was adjusted to 1×10^7 conidia ml^{-1} with the aid of a haemocytometer.

2.3.2. Preparation of granular barley inoculum

For controlled environment experiments, 20 ml deionized water were added to 20 g of pot barley grains (*Hordeum vulgare* L.) in 250 ml Erlenmeyer flasks and autoclaved (18 min, 100 kPa, 120 °C). Flasks were cooled to room temperature and inoculated with 1×10^7 conidia (1 ml starter culture). Flasks were incubated at room temperature (21 ± 2 °C) and exposed to 12 h near-ultraviolet light day $^{-1}$ and shaken every second day by hand in order to keep the substrate from clumping. Colonized barley grains were harvested after 3 weeks and milled using an electric coffee grinder (Braun® KSM 2, Lynnfield, MA, USA) by milling each load continously for 10 to 15 sec. The granules produced were dried for 2 days and sieved, resulting in inoculum particles of < 710 µm diameter. In an earlier study, inoculum of this size was found to have a high pre-emergence activity and a shelf-life of at least 6 months (Vogelgsang *et al.* 1994).

For field experiments, 1 L screw cap bottles containing 100 g of autoclaved barley grains and 80 ml deionized water were inoculated with 5×10^7 conidia (5 ml starter culture) and incubated as described above. Following 3 weeks incubation and 2 days before application treatments, a portion of the granules was milled as described above. Since sieving is quite time-consuming and often leads to substantial losses of inoculum, material was ground a second time using an electric meat grinder (Quaker City Mill, Model 4-E, Westinghouse, PA, USA), resulting in a mixture of large and small-sized particles with approximately 70% of the particles being < 710 µm diameter.

Inoculum to be used for both controlled environment and field studies was routinely tested for conidia levels and viability. One gram of granules was suspended in flasks with 100 ml deionized water, and placed on a rotary shaker at 150 rpm for 30 min. Contents were poured through two layers of cheesecloth, and flasks rinsed with 50 ml deionized water in order to wash out any remaining inoculum particles. Conidia quantities were determined and then adjusted to 1×10^6 conidia ml^{-1}. For conidia viability testing, two PDA plugs (10-mm-diameter) were placed on glass slides in a Petri dish, each receiving 30 µl of the conidia

suspension, incubated in the dark at 24 ± 1 °C for 18 h, and conidia killed and stained with 0.1% lactophenol-cotton blue (Tuite 1969). Percent germination was evaluated microscopically (500×) by counting 50 conidia per agar plug. Conidia were considered to have germinated when the germ tube length was greater than the width of the conidium.

2.3.3. Plant production

For controlled environment experiments, *C. arvensis* seeds (Valley Seed Co., Fresno, CA, USA) were washed under warm running tapwater for 2 h and soaked overnight in deionized water. Imbibed seeds were incubated on moist paper towels in a glass Petri dish at 24 ± 1 °C for 24 to 36 h. Four germinated seeds having emerged radicles were sown at a depth of 3 cm into 13-cm-diameter plastic pots containing a mixture of sandy loam (Modugno-Hortibec Inc., St-Laurent, QC, Canada), potting medium (Pro-Mix™ BX, Les Tourbières Premier Ltée, Rivière-du-Loup, QC, Canada), vermiculite (Vil Vermiculite Inc., Montréal, QC, Canada), and peat moss (Les Tourbières Premier Ltée) [3:3:2:1 (V/V/V/V)]. Pot size and soil mixture were selected to allow for substantial root development. Pots were placed in a growth chamber (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 $\mu Em^{-2}s^{-1}$). Plants were grown for 8 weeks and fertilized every second week with 200 ml of 20:20:20 (N:P:K, 3 g L^{-1}). After 8 weeks, above-ground tissue in all pots was cut at the soil line and remaining root matter was considered as the "established" plant material. Two days after cutting "established" plants, "seedlings" were produced by sowing four pre-germinated seeds in 13-cm-diameter pots and placing in a growth chamber under the same conditions as described above.

Field experiments were performed at the Horticulture Research Centre of Macdonald Campus, Ste-Anne-de-Bellevue, QC, Canada. The soil type was a Chicot fine-sandy loam with 70% sand, 20% silt, 10% clay, a pH of 5.3 and 3% organic matter. To produce established plants, seeds were soaked for 20 s in near

boiling water and incubated on moist paper towels for 24 to 36 h at 30 ± 1 °C. Two hundred imbibed seeds were sown in each 0.25 m^2 plot at a depth of approximately 7 cm on 12 and 16 May 1995 and 1996, respectively. Plants were grown for 6 weeks before they were cut at the soil line, dried in paper bags at 60 °C for 4 days, and weighed. Biomass obtained from this harvest was used as a relative value of plant vigour in each plot prior to cutting when compared with biomass data from the final harvest. On the same day established plants were cut, seedling material was produced by sowing 200 imbibed seeds in 0.25 m^2 plots not containing established plant material.

Air temperature and precipitation data were obtained from the McGill Meteorological Observation Centre (1.5 km away from field site). Soil surface temperatures were determined using thermocouple readings from a datapod reader (Model 217 DSM Reader, Omnidata International Inc., Logan, UT, USA) that was placed at ground level within the field. During the 1995 field trial, no precipitation was received for nearly 1 month before and during the application of inoculum. Hence, plots were watered regularly during early plant establishment and once on day 2 of the application treatments (approximately 1 L $plot^{-1}$). For the 1996 field trial, weather conditions were much wetter, and supplemental irrigation was carried out only during the first 2 weeks of initial established plant growth.

2.3.4. Inoculation procedure

All experiments were designed as two-factor experiments involving two *C. arvensis* growth stages and four treatments including three fungal application dates and one uninoculated control.

For controlled environment experiments, the amount of inoculum applied was based on earlier studies that made use of smaller pots (10-cm-diameter) and a dose of 1 g (Vogelgsang *et al.* 1994). Consequently, a dose of 1.7 g of granules per pot was applied in this study, containing 3 to 5 × 10^9 conidia with > 95% germination. The material was manually spread onto the moistened soil surface and pots were

immediately covered with plastic bags until all seedlings emerged (5 to 6 days). Inoculum applications were carried out 0, 1, and 2 days after sowing (DAS).

For field experiments, 30 g of fungal inoculum with 2 to 5 × 10^{10} conidia and > 80% viability was uniformly spread by hand on the soil surface. Uninoculated plots served as controls. During the 1995 field trial, treatments were conducted 3, 4, and 5 DAS. However, in 1996 with cooler weather conditions prevalent, delayed emergence was expected and application dates were changed to 3, 5, and 7 DAS. In both trials, all applications were carried out late in the afternoon.

2.3.5. Assessment of efficacy

For controlled environment experiments, foliar necrosis was evaluated 11 DAS using the following rating system: 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, and 4 = 76-100 % necrosis (Ormeno-Nuñez *et al.* 1988a). Disease rating was performed for each plant in case of seedlings or for each shoot in case of established plants and results were pooled and averaged for each pot. Above-ground and root biomass were determined 14 and 15 DAS, respectively. Plants were cut at the soil line, roots were carefully removed from the potting medium and living tissues dried in paper bags for 4 days at 60 °C, and weighed. Biomass was recorded as total biomass per pot. For the field experiment, above-ground biomass per plot was determined 26 DAS. At harvest, margins of each plot were delineated with white string, and plant coverage was determined with the aid of black and white photographic pictures. Contours of living plant material were traced on the photograph, and percentage coverage of healthy tissues was assessed using an image analyzer (Leco® 2001 Image Analysis System, Instruments Ltée, Longueuil, QC, Canada).

2.3.6. Experimental design and data analysis

All experiments were performed twice. Controlled environment studies were set-up in a completely randomized design with four replicates per treatment. For field trials, a randomized complete block design with five blocks was used. Blocks

were arranged so that their length was perpendicular to a slight slope within the field site. In total, 40 0.25 m^2 plots were established each year. In 1995, the distance between plots and experimental blocks was 0.3 m and 0.8 m, respectively. In 1996, spacing between plots was increased to 0.5 m and to 1 m between blocks in an attempt to reduce rain-splash dispersal of inoculum. Coverage and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test ($\alpha = 0.05$) (Steel & Torrie 1980). Disease ratings were compared using the Kruskal-Wallis one-way analysis of variance by ranks, followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel 1978). Due to significant interactions between the two plant stages, data for seedlings and established plants were analyzed separately. In both controlled environment and field studies, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner *et al.* 1992).

2.4. Results

2.4.1. Controlled environment

Seedlings typically emerged 2 to 3 days after sowing whereas regrowth of established plants occurred during a longer time period, ranging from 3 days to 1 week after cutting. Disease symptoms were visible for all inoculum application dates on both seedlings and established plants. However, control of *C. arvensis* was greatest for delayed application times and seedlings (Table 2.1). All pre-emergence treatments of seedlings resulted in significant reductions of above-ground and root biomass ($\alpha < 0.05$). In Trial 1, above-ground biomass of seedlings was reduced between 41% (0 DAS) and 87% (2 DAS). Disease incidence for established plants was less severe than for seedlings, with a 43% reduction in shoot biomass for the 2 DAS treatment. Root biomass of established plants was variable with no significant differences between control and inoculation treatments being observed. Similar results were obtained in Trial 2 (Appendix 2.1).

2.4.2. Field trials:

2.4.2.1. 1995

Most seedlings and some shoots from established plants emerged 4 to 5 DAS, however shoot emergence of the latter continued until the termination of the experiment. During the inoculum application period, weather conditions were hot and dry, with air temperatures reaching 31 °C. However, based on field thermocouple readings, granules on the soil surface were exposed to temperatures between 27 and 40 °C (Figure 2.1A).

Significant reductions in above-ground biomass were achieved for both inoculated seedlings and established plants (Figure 2.2A). Biomass reductions under field conditions were similar for all inoculum application times. Although observed disease incidence for established plants was lower than for seedlings, above-ground biomass reductions for established plants in the field increased compared with results obtained under a controlled environment. Above-ground biomass reductions for seedlings ranged from 94 to 99%, whereas regrowth from established plants was reduced by 53 to 80% compared with uninoculated controls. A significant difference in biomass of control and inoculated established plants was only observed for the second application day ($\alpha < 0.05$). A possible explanation is that several uninoculated control plots became contaminated with *P. convolvulus*, probably due to conidia dispersal from rain-splash or run-off along a slight slope within the field. Nonetheless, when final harvest biomass data are compared with biomass data from the first harvest after initial establishment (in which plants had been grown for 6 weeks), all inoculum applications resulted in established plants having significantly lower biomass compared with uninoculated controls (data not shown). In general, plot coverage data closely paralleled above-ground biomass results (Figure 2.2A).

2.4.2.2. 1996

The emergence pattern in 1996 was approximately one day behind that in 1995. Throughout the 1996 application period, weather conditions were warm and moist

with heavy precipitation 2 days after the last granular application (Figure 2.1B). Air and soil temperatures reached 28 and 34 °C, respectively. Above-ground regrowth from established plants was drastically reduced by all fungal applications [i.e. 96 to 98 % compared with uninoculated controls (Figure 2.2B)]. In contrast to results for 1995, treatment application for all dates produced significant biomass reductions compared with uninoculated controls (Figure 2.3). This occurred despite substantial cross-infection of control plots even after spacing between experimental plots was increased.

2.5. Discussion

In this study, the pre-emergence potential of the fungal pathogen, *P. convolvulus*, to suppress *C. arvensis* at two growth stages was evaluated. Both seedlings and established plants of *C. arvensis* developed disease symptoms following fungal inoculation. However, under a controlled environment, damage of regrowth tissue from established plants was substantially lower than for seedlings. This confirms results from earlier studies that used aqueous conidial suspensions of *P. convolvulus* as a post-emergence inoculum source (Morin *et al.* 1989b). The possible development of a thicker cuticle by physiologically more mature plants could result in altered defence mechanisms (Martin 1965), and could explain why tissue from established plants was less susceptible to fungal damage. Similarly, a greater amount of constitutive antibiotics and/or phytoalexins in the host tissue from established plants could have led to increased resistance compared to seedlings (Bell 1980). In addition, regrowth from root stocks occurred in a staggered manner and was not completed by the time all seedlings had emerged. Thus, a considerable amount of inoculum could have lost viability lying on the soil surface. Likewise, timing of fungal application was crucial for disease severity. For both growth stages, the level of control declined with increasing time interval between fungal application and actual emergence.

In field trials, biomass reductions were not dependent upon the day of application as was observed for the controlled environment trials. Moreover,

efficacy of control for established *C. arvensis* plants was significantly increased. In these experiments, the fungus was capable of attacking and suppressing both growth stages of the weed under a wide range of environmental conditions. Furthermore, there were indications that the pathogen was more virulent in the field than under controlled growth chamber conditions. This is an unusual observation in bioherbicide research. Frequently, the high levels of disease obtained under laboratory conditions are difficult to reproduce in the field. The narrow environmental range of conditions that is often required to attain high levels of infection has commonly been cited to explain the disparity of results between laboratory and field trials (Watson & Wymore 1990). The precise reason(s) for the observed outcome in this study are not clear. However, several abiotic, biotic as well as methodological factors could have affected the results. Soil type may have had a strong influence on the performance of the fungus. Factors such as particle size, pH, and available nutrients have been shown to favour or inhibit the survival and activity of fungal pathogens (Stotzky 1974, Paulitz & Baker 1987, Höpner & Alabouvette 1996). Furthermore, the presence of numerous micro-organisms under field conditions could lead to intense competition for resources (Waksman 1952), thus preventing *P. convolvulus* conidia from germinating rapidly in the absence of host tissue. The inoculum production method used in this study may also have affected results. Within the larger incubation bottles used for field trials, the greater amounts of conidial matrix observed, could have protected the fungus to a greater extent from desiccation (Sparace *et al.* 1991). In addition, granules prepared for field inoculations were less homogeneous in size. That is, smaller-sized particles that could serve as immediate infection sources were mixed with larger-sized particles possibly being more persistent and having slower inoculum release rates. Finally, the development of a disease epidemic is also dependent upon an adequate distribution of the pathogen near its host. In the field, rain-splash, wind dispersal, as well as run-off are common events that may have contributed to the dissemination of the fungal inoculum (Fitt *et al.* 1989, Madden 1992).

In both years of the field experiment, control of established plants was greater compared with results from growth chamber studies. However, in the first field trial, only one *P. convolvulus* application (2 DAS) resulted in significant reductions of *C. arvensis* above-ground biomass compared with uninoculated controls. This finding was possibly due to the high temperatures and drought conditions present during the 1995 fungal application period. Also, cross-infection of uninoculated control plots, presumably caused by rain-splash dispersal, might have led to less accentuated differences between treatments. In 1996, control of established *C. arvensis* plants was even more apparent, with all fungal treatments resulting in substantial declines in resprouting biomass. During the 1996 application period, weather conditions were more favourable to disease development (i.e. lower temperatures and adequate rainfall). Hence, this increase in the availability of free water could have enhanced *P. convolvulus* germination and infection.

The granular formulation of *P. convolvulus* used in this study improved the capability of the fungus to withstand unfavourable weather conditions. Similar findings were observed by Boyette and Walker (1985) where granular pre-emergence field applications of *Fusarium lateritium* were superiour to post-emergence foliar sprays in suppressing *Abutilon theophrasti*. Likewise, when *Fusarium solani* f. sp. *cucurbitae* was applied pre-emergence, sodium alginate granules provided greater and longer lasting control of *Cucurbita texana* compared with conidial applications (Weidemann & Templeton 1988). Another granular preparation consisting of vermiculite, spores, and mycelia of *Alternaria macrospora* effectively controlled *Anoda cristata* in both greenhouse and field tests (Walker 1981). This method of spore production and formulation was later modified for use with other fungi including a pycnidia-forming fungus, *Phyllosticta* sp. (Walker & Connick 1983).

Plant coverage determined from photographs of field plots closely reflected biomass data. Hence, this method may be more precise than evaluating disease by visual estimates of necrotic leaf area since computer generated surface scans of

plant cover or necrotic area are less subjective and therefore more accurate and reliable.

The results of our study indicate that pre-emergence applications of *P. convolvulus* are effective in controlling different growth stages of *C. arvensis*. Further research on soil-incorporation of granules, dose response, fungal persistence in soil, and effect under competitive cropping situations are in progress and should provide additional information as to the potential of *P. convolvulus* to be an effective biological agent against *C. arvensis*.

Table 2.1. Effect of pre-emergence applied *Phomopsis convolvulus* on disease severity and *Convolvulus arvensis* seedling and established plant growth under controlled environment conditions.- Trial 1 [a]

Plant stage	Treatment [b]	Above-ground biomass (g pot^{-1})	Root biomass (g pot^{-1})	Disease rating [c]
Seedlings	Control	0.095 (0.008) a	0.066 (0.008) a	0.06 (0.06) a
	0 DAS	0.056 (0.009) b	0.031 (0.006) b	1.38 (0.22) ab
	1 DAS	0.030 (0.004) bc	0.023 (0.006) b	2.25 (0.14) bc
	2 DAS	0.012 (0.002) c	0.010 (0.006) b	3.25 (0.31) c
Established plants	Control	1.185 (0.117) a	2.524 (0.313) a	0.05 (0.02) a
	0 DAS	0.991 (0.032) ab	2.328 (0.148) a	0.72 (0.06) ab
	1 DAS	0.845 (0.056) bc	2.156 (0.161) a	0.62 (0.07) ab
	2 DAS	0.674 (0.076) c	2.512 (0.272) a	1.07 (0.24) b

[a] Trials were not combined because variances were not homogeneous.

[b] 0 DAS, 1 DAS, 2 DAS: Application of 1 g of *P. convolvulus* granules plot^{-1} at 0, 1, 2 days after sowing, respectively. Numbers in parentheses are the standard error of the mean. For each plant stage, means in each column with the same letter are not significantly different, according to Tukey's grouping ($\alpha = 0.05$) (biomass) or to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating).

[c] Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.

Figure 2.1. Precipitation, air, and soil temperatures during (A) 1995, (B) 1996 field trials. Temperatures are daily means.

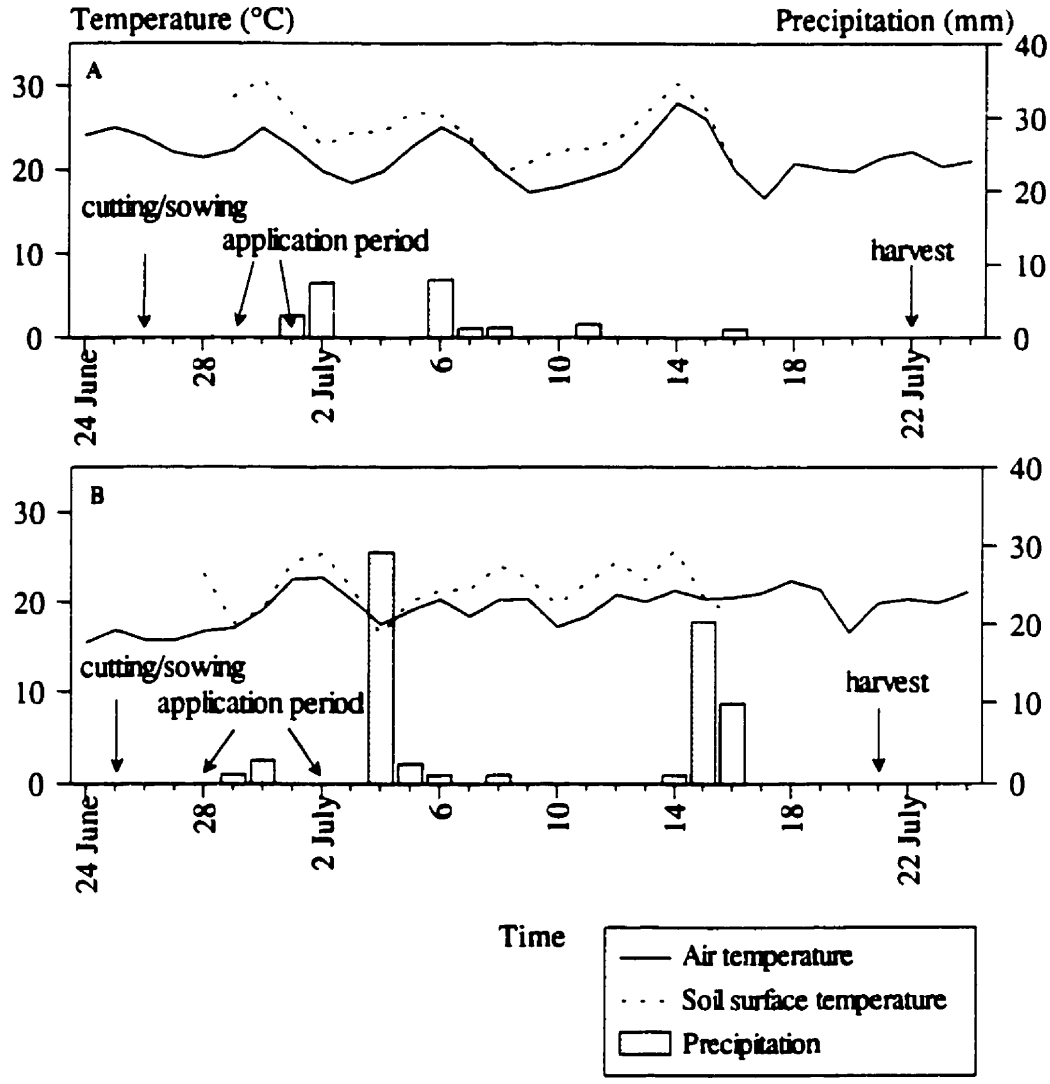

Temperature (°C)
Precipitation (mm)
A
B
cutting/sowing
application period
harvest
24 June
28
2 July
6
10
14
18
22 July
Time
Air temperature
Soil surface temperature
Precipitation

Figure 2.2. Field experiment - Effect of pre-emergence field application of *Phomopsis convolvulus* on *Convolvulus arvensis* above-ground biomass (bars) and plant coverage (circles) for seedlings and established plants in (A) 1995, (B) 1996. Day 1, 2, 3 treatments refer to 30 g *P. convolvulus* granules 0.25 m^2 $plot^{-1}$ applied 3, 4, 5 (1995) or 3, 5, 7 (1996) days after sowing, respectively. Plants were harvested 26 days after sowing. Vertical bars indicate the standard error of the mean.

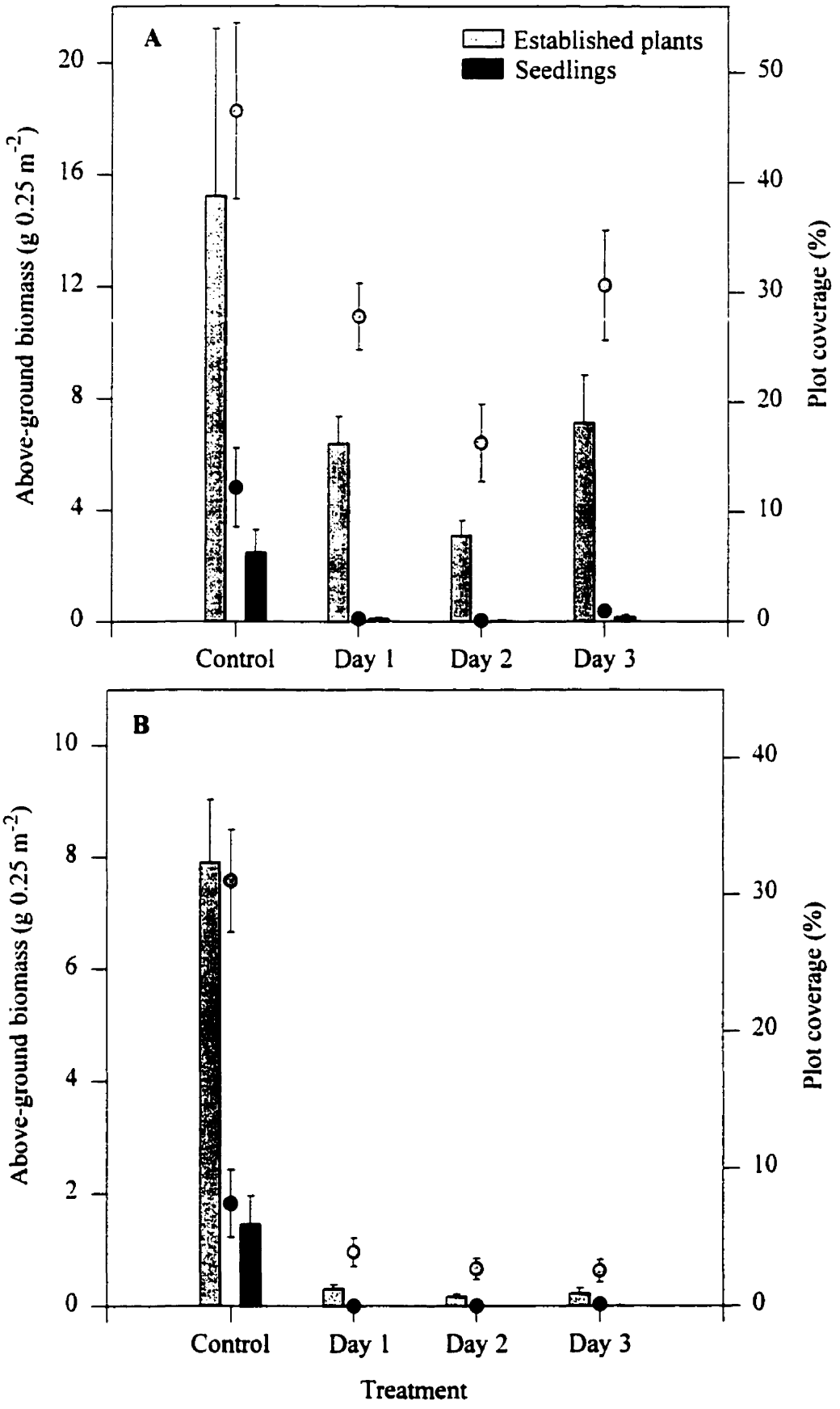
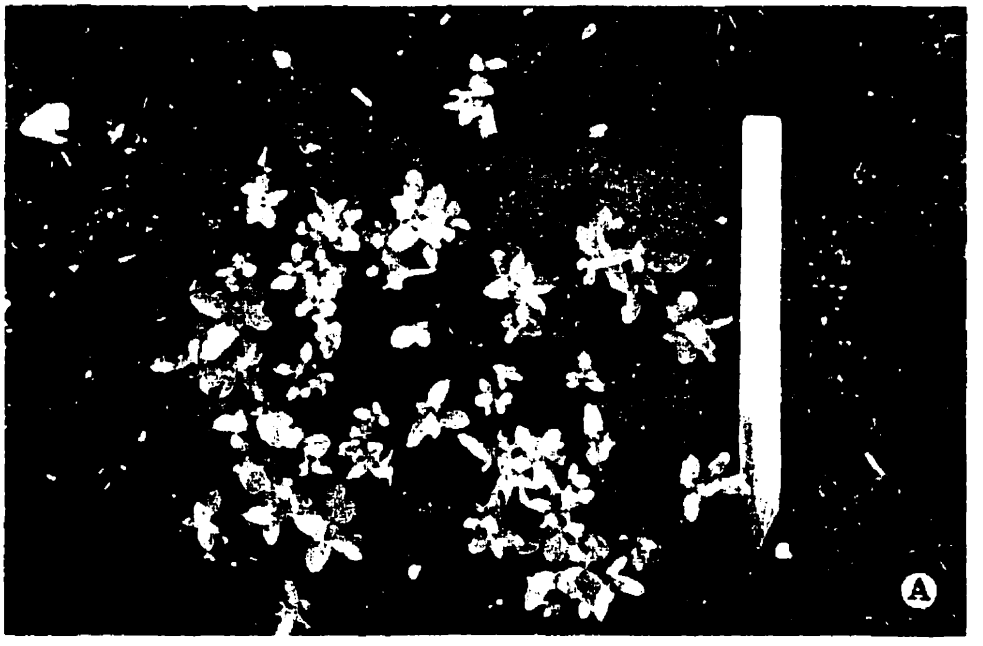
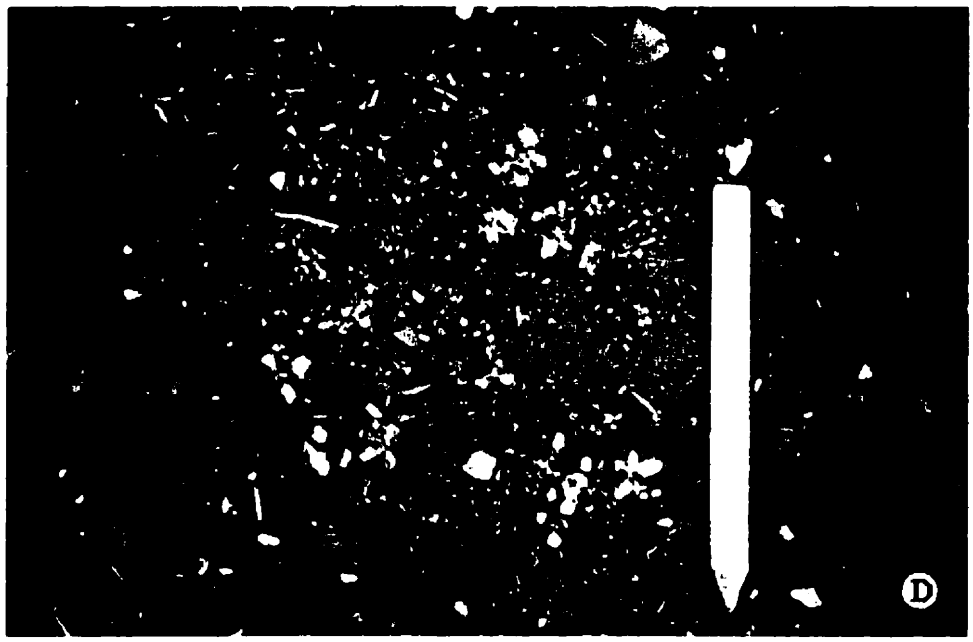

A
Established plants
Seedlings
Above-ground biomass (g 0.25 m-2)
Plot coverage (%)
0
4
8
12
16
20
0
10
20
30
40
50
Control
Day 1
Day 2
Day 3
B
Above-ground biomass (g 0.25 m-2)
Plot coverage (%)
0
2
4
6
8
10
0
10
20
30
40
Control
Day 1
Day 2
Day 3
Treatment

Figure 2.3. Effect of *Phomopsis convolvulus* on *Convolvulus arvensis* seedlings and established plants. Thirty g of *P. convolvulus* granules were applied 5 days after sowing (DAS). At day of harvest (26 DAS): (A) seedlings, control; (B) seedlings, inoculated; (C) established plants, control; and (D) established plants, inoculated.


A


B


C


D

Connecting Text

In the previous chapter, research showed that pre-emergence granular applications of *P. convolvulus* can produce severe disease and important biomass reductions of both *C. arvensis* seedlings and established plants. However, the level of weed control following field inoculum applications can often vary depending on the specific environmental conditions present during the application and infection periods. Soil incorporation of granules is one possible method of protecting fungal inoculum from adverse conditions. Moreover, in previous research with this weed-pathogen system, relatively high inoculum rates have been employed, thus representing a major constraint for practical field applications. In the following chapter, the effect of pre-emergence soil incorporation of *P. convolvulus* inoculum on *C. arvensis* growth under both controlled environment and field conditions will be investigated. In addition, the effect of different doses of soil applied inoculum granules will also be determined.

Chapter 3. Effect of soil incorporation and dose on the efficacy of the pre-emergence bioherbicide *Phomopsis convolvulus* to control *Convolvulus arvensis*

3.1. Abstract

The pre-emergence efficacy of soil surface applications of a *Phomopsis convolvulus* granular formulation to control *Convolvulus arvensis* was compared with its efficacy when inoculum granules were incorporated in soil. In addition, the effect of different doses of soil applied granules was also determined. Under controlled environment conditions, incorporation of the fungal granules resulted in above-ground biomass reductions of between 88 and 96% with no significant differences observed between incorporation depths of 1.5 cm and 3 cm. Granule applications on the soil surface were less effective, reducing above-ground biomass by 40 to 83%. In a parallel field experiment conducted over two growing seasons however, surface applications of inoculum granules resulted in greater weed control compared with soil incorporation of the granules. In 1996 spring and summer trials, surface applications resulted in a 93 and 100% above-ground biomass reduction, respectively, whereas incorporated granules reduced biomass by 62 and 97%. Similar trends were observed in 1997. Different soil applied doses of *P. convolvulus* did not affect the level of weed control under both controlled environment and field conditions. In 1995 and 1996 field trials, all dose rates used (30g, 20g, and 10 g 0.25m^{-2} plot) resulted in substantial (90-100%) *C. arvensis* above-ground biomass reductions. Findings in this study indicate that under field conditions, pre-emergence applications of reduced doses of the bioherbicide *Phomopsis convolvulus* onto the soil surface can provide effective control of *C. arvensis*.

3.2. Introduction

Convolvulus arvensis L. (field bindweed) is a serious perennial weed in many crops and is prevalent in almost every agricultural area of the world (Holm *et al.*

1977). The move towards reduced cultivation or zero tillage in the past 15 years, has led to an increased prevalence of *C. arvensis* (Phillips *et al.* 1980). Effective control of *C. arvensis* using current methods including as cultivation, crop rotation, and chemical herbicides (Derscheid *et al.* 1970) is often not possible due to its extensive root system, high competitiveness, and variable susceptibility to several important herbicides (Whitworth & Muzik 1967, DeGennaro & Weller 1984, Kosinski & Weller 1989, Yerkes & Weller 1996). The first reported incidence of *C. arvensis* being infected by the foliar pathogen, *Phomopsis convolvulus* Ormeno was in 1988 (Ormeno-Nuñez *et al.* 1988b). Since then, studies on host specificity, conidia mass production, storage, and efficacy of foliar treatments have been carried out (Ormeno-Nuñez *et al.* 1988a,b, Morin *et al.* 1989a,b, Morin *et al.* 1990). One key characteristic of the fungus that has limited its efficacy is the requirement for a relatively long dew period during the germination and infection phases for sufficient disease to develop. In an attempt to overcome this limitation, a granular pre-emergence formulation of *P. convolvulus* has been developed and tested. Inoculum produced on pot barley grains and applied onto the soil surface showed high efficacy of control against *C. arvensis* seedlings and established plants under both controlled environment and field conditions (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b). However, levels of weed control after field applications often vary, and are largely dependent on environmental conditions at the time of application (Watson & Wymore 1990) As demonstrated in other studies (Jones *et al.* 1988, Weidemann & Templeton 1988, Jackson *et al.* 1996, Kempenaar *et al.* 1996), infective propagules of soil applied bioherbicides could be protected from adverse environmental conditions through inoculum incorporation into the soil, thus possibly leading to consistently higher levels of weed control. Furthermore, the dose of soil applied *P. convolvulus* in earlier studies was rather high at 30 g $0.25m^{-2}$ (Vogelgsang *et al.* 1994), representing a constraint for practical field applications. The objectives of this study were to: (1) evaluate the effect of pre-emergence soil incorporation of *P. convolvulus* inoculum and (2) determine the effect of reduced rates of granule

application on *C. arvensis* growth under both controlled environment and field conditions.

3.3. Materials and methods

3.3.1. Inoculum production of starter cultures

Single conidia isolates of *P. convolvulus* were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 4 °C. From these stock cultures, small pieces of mycelia were placed on 9-cm-diameter Petri dishes with PDA and incubated in the dark at 24 ± 1 °C. After 4 to 5 days, several mycelial plugs of 1-cm-diameter were transferred to PDA plates and incubated at room temperature (21 ± 2 °C) and 12 h day $^{-1}$ near-ultraviolet light (F40 BLAB Blacklight, General Electric Lighting, Cleveland, OH, USA). After 3 weeks, conidia were harvested by washing plates with 12 ml of sterile deionized water. Conidia density was then adjusted to 1×10^7 conidia ml^{-1} with the aid of a haemocytometer.

3.3.2. Preparation of granular barley inoculum

For controlled environment experiments, 20 ml deionized water were added to 20 g of pot barley grains (*Hordeum vulgare* L.) in 250 ml Erlenmeyer flasks and autoclaved (18 min, 100 kPa, 120 °C). Flasks were cooled to room temperature and inoculated with 1 ml of the previously prepared conidia suspension starter culture. Flasks were incubated at room temperature (21 ± 2 °C) and exposed to 12 h near-ultraviolet light day^{-1} and shaken every second day by hand in order to keep the substrate from clumping. Colonized barley grains were harvested after 3 weeks and milled using an electric coffee grinder (Braun® KSM 2, Lynnfield, MA, USA). The granules produced were dried for 2 days and sieved, resulting in inoculum particles of < 710 µm diameter. In an earlier study, inoculum of this size was found to have a high pre-emergence activity and a shelf-life of at least 6 months (Vogelgsang *et al.* 1994).

For field experiments, 1 L screw cap bottles containing 100 g of autoclaved barley grains and 80 ml deionized water were inoculated with 5 ml of the conidia

suspension starter culture and incubated as described above. Following 3 weeks incubation and 2 days before application treatments, a portion of the granules was milled as described above. Since sieving is quite time-consuming and often leads to substantial losses of inoculum, material was ground a second time using an electric meat grinder (Quaker City Mill, Model 4-E, Westinghouse, PA, USA), resulting in a mixture of large and small-sized particles with approximately 70% of the particles being < 710 µm diameter.

To determine conidia production and viability of inoculum produced on the barley grains, one gram granule samples were routinely tested as described in Chapter 2.

3.3.3. Plant production

For controlled environment experiments, *C. arvensis* seeds (Valley Seed Co., Fresno, CA, USA) were washed under warm running tapwater for 2 h and soaked overnight in deionized water. Imbibed seeds were then incubated in the dark on moist paper towels in a glass Petri dish at 24 ± 1 °C for 24 to 36 h. Four germinated seeds having emerged radicles were sown at a depth of 3 cm into 10-cm-diameter plastic pots containing a commercial potting medium (Pro-Mix BX™, Les Tourbières Premier Ltée, Rivière-du-Loup, QC, Canada). Pots were placed in a growth chamber (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 $\mu Em^{-2}s^{-1}$).

Field experiments were performed at the Horticulture Research Centre of Macdonald Campus (McGill University), Ste-Anne-de-Bellevue, QC, Canada. The soil type was a Chicot fine-sandy loam with 70% sand, 20% silt, 10% clay, a pH of 5.3 and 3% organic matter. For field trials, seeds were soaked for 20 s in near boiling water and incubated in the dark on moist paper towels in a plastic tray for 24 to 36 h at 30 ± 1 °C. Two hundred imbibed seeds were sown in 0.25 m^2 field plots at a depth of approximately 6 cm. For incorporation experiments, both spring and summer trials were carried out and sowing took place on 24/22 May, and

18/31 July 1996/97, respectively. The delay in the 1997 summer trial seeding was due to poor emergence of *C. arvensis* seedlings after sowing on 14 July. Therefore, the trial was repeated and seeds were sown 2 weeks later. For dose response experiments, seeds were sown on 21 July 1995 and 19 July 1996, respectively.

Air temperature, soil temperature, and precipitation data were obtained from the McGill Meteorological Observation Centre (1.5 km from field site). In order to facilitate germination of *C. arvensis* seeds, additional irrigation was carried out for some incorporation trials: During the 1996 and 1997 spring trials, no precipitation was received for nearly 2 weeks after sowing, hence plots were watered as needed (approximately 1 L $plot^{-1}$ per watering). During the 1996 summer trial, weather conditions were much wetter and no supplemental irrigation was required. For the 1997 summer trial, temperature and precipitation were variable and irrigation was carried out once after fungal application.

3.3.4. Inoculation procedures

3.3.4.1. Incorporation experiment

Under controlled environment, applications were performed on the day of sowing. One g of granular inoculum, containing 1 to 2 $\times$ 10^9 conidia with > 80% germination was manually spread either on the soil surface or incorporated at a depth of 1.5 or 3 cm, respectively. Incorporation was performed by filling pots with potting medium up to the final level minus 1.5 or 3 cm, placing inoculum on the surface, and adding the remaining volume of potting medium on top of the granules. Immediately after inoculation, pots were covered with plastic bags until all seedlings had fully emerged (5 to 6 days).

Under field conditions, 30 g inoculum with 8 $\times$ 10^9 to 3 $\times$ 10^{10} conidia and > 90% viability was applied 3 days after sowing (DAS). Inoculum was either spread uniformly by hand onto the soil surface or incorporated by mixing granules in a soil layer of approximately 5 cm with the aid of a hand cultivator. In all trials, inoculations were carried out late in the afternoon.

3.3.4.2. Dose response experiment

Under controlled environment, granule rates of 1, 0.75, 0.5, and 0.25 g were tested, with the highest rate based on the dose used in preliminary studies (Vogelgsang *et al.* 1994). Conidia quantity and viability were equivalent to that of the incorporation experiment under controlled environment described above. Inoculum was manually spread onto the moistened soil surface and pots were immediately covered with plastic bags until all seedlings emerged (5 to 6 days). Inoculum applications were carried out 1 and 2 days after sowing (DAS).

For field experiments, 30, 20, or 10 g of granular inoculum was uniformly spread by hand on the soil surface 3 and 4 DAS. In 1995, 30 g granules contained 4 to 6 $\times$ 10^9 conidia with 50 to 70% viability whereas in 1996, 2 $\times$ 10^{10} conidia with > 85% viability were produced. The low germination rates in 1995 were likely due to an unexpected temperature decline in the incubator used for the germination tests. Controls were either uninoculated (considered 3 DAS) or inoculated with 30 g autoclaved (twice for 30 min) granular inoculum (4 DAS). In both trials, all applications were carried out late in the afternoon.

3.3.5. Assessment of efficacy

For all controlled environment experiments, foliar necrosis was evaluated 8 DAS using the following rating system: 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, and 4 = 76-100 % necrosis (Ormeno-Nuñez *et al.* 1988a). Mortality was assessed 13 DAS by counting the number of seedlings with completely necrotic hypocotyls. Disease rating and mortality determination were performed for each plant and results pooled and averaged for each pot. Above-ground and root biomass were determined 13 and 14 DAS, respectively. Plants were cut at the soil line, roots were carefully removed from the potting medium and living tissues dried in paper bags for 4 days at 60 °C, and weighed. Biomass was recorded as total biomass per pot. For all field experiments, foliar necrosis was evaluated 18 DAS. For incorporation and dose

response experiments, mortality was determined 24 and 25 DAS, respectively and was obtained by subtracting the number of live plants at harvest from the number of initially emerged seedlings. Above-ground biomass per plot was harvested 25 and 26 DAS, respectively.

3.3.6. Experimental design and data analysis

All experiments were performed twice. Controlled environment studies were set-up in a completely randomized design with four replicates per treatment. For field trials, a randomized complete block design with five blocks was used. Blocks were arranged so that their length was perpendicular to a slight slope within the field site. For incorporation and dose response experiments, a total of 15 or 40 0.25 m^2 plots, respectively, were established each year. The distance between plots and experimental blocks was 0.5 m and 1 m, respectively. In an attempt to reduce rain-splash dispersal of inoculum, garden edgings of 15 cm height were placed between experimental plots, leaving approximately a 9 to 10 cm barrier above the ground. Mortality and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test ($\alpha = 0.05$) (Steel & Torrie 1980). For dose response experiments, best fitting regression equations describing the relationship between dose and biomass were determined using regression analysis procedures in Statgraphics® Plus (Statgraphics Plus 1995). Disease ratings for experiments in controlled environment and the field were compared using the Kruskal-Wallis one-way analysis of variance by ranks or by the Friedman test, respectively, followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel 1978). In both controlled environment and field studies, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner *et al.* 1992).

3.4. Results

3.4.1. Incorporation experiment

3.4.1.1. Controlled environment

Seedlings typically emerged 3 to 4 days after sowing. Disease symptoms were visible for all inoculum applications, however incorporation of the granules resulted in greater necroses and higher mortality compared with surface applications (Table 3.1). Different incorporation depths had no significant effect on disease development. Incorporated inoculum produced necrotic spots on both cotyledons and hypocotyls whereas surface applied granules did not lead to disease symptoms on hypocotyls. Similarly, all fungal treatments resulted in significant reductions in *C. arvensis* above-ground and root biomass ($P < 0.05$), but disease incidence for plants subjected to the incorporation treatments was more severe than for soil surface applications (Figure 3.1). In the first and second trial, incorporation of fungal granules reduced above-ground biomass by as much as 96 and 93%, respectively, whereas above-ground biomass reductions following surface applications in the two trials were only 83 and 40%, respectively. A similar trend was observed for root biomass reduction of *C. arvensis* (Figure 3.1A/B).

3.4.1.2. Field trials

Most seedlings emerged 4 to 6 DAS. For spring trials in 1996 and 1997, initial weather conditions were cool and dry (Figure 3.2A/C). Average air temperatures shortly after inoculum application were between 9/10 and 16/17 °C, respectively. In both years, temperatures at night dropped as low as 3 °C. Weather conditions following summer applications in 1996 and 1997 were warm with variable precipitation (Figure 3.2B/D). Average air temperatures were between 20/17 and 22/24 °C, respectively.

In 1997, severe attack by tortoise beetles (Chrysomelidae, Cassidinae, *Chelymorpha* sp.) was observed during both spring and summer trials resulting in generally lower growth rates for plants in all treatments compared with results obtained in 1996.

3.4.1.2.1. 1996 - Spring

Severe disease development and significant reductions in above-ground biomass were achieved for both treatments, however, in contrast to experiments under controlled environment, surface applications resulted in higher disease incidence compared with incorporation of inoculum (Table 3.2A). Necrotic spots on hypocotyls following incorporation were present, however they were not as severe as in experiments under controlled environment. Following application of the granules onto the soil surface, 75% of the seedlings were killed while a mortality rate of only 33% was obtained after soil incorporation of the inoculum. Likewise, above-ground biomass reductions for surface applications reached 93%, whereas biomass for plants subjected to the incorporation treatment was reduced by 62% compared with uninoculated controls (Figure 3.3A).

3.4.1.2.2. 1996 - Summer

Results were similar to the spring trial but with an overall greater level of weed control. Following surface application, a seedling mortality rate of 99% was observed (Table 3.2A) and above-ground biomass was reduced by nearly 100% (Figure 3.3A). In contrast, only 50% of the seedlings were killed after granule incorporation, but biomass reduction reached 97%.

3.4.1.2.3. 1997 - Spring

Following surface application, 44% of *C. arvensis* seedlings were killed whereas a mortality rate of 22% was obtained after granule incorporation (Table 3.2B). Above-ground biomass was reduced by 72% following surface inoculum application while only 31% biomass reduction occurred when plants were subjected to the incorporation treatment (Figure 3.3B).

3.4.1.2.4. 1997 - Summer

As in 1996, overall control efficiency was higher compared with the spring trial. Following surface application, 99% of *C. arvensis* seedlings were killed (Table 3.2B) and above-ground biomass was reduced by nearly 100% (Figure 3.3B, Figure 3.4). Incorporation of fungal inoculum resulted in 80% mortality and a biomass reduction of 96%.

3.4.2. Dose response experiment

3.4.2.1. Controlled environment

Seedling emergence occurred 3 to 4 days after sowing. All fungal applications resulted in severe disease development but the effect of dose was limited (Table 3.3A/B). As demonstrated in other studies using *P. convolvulus*, the date of inoculation was more important and disease response less pronounced when granules were applied shortly after sowing. Following fungal application, seedling mortality in the first trial ranged between 50% (1 DAS, 0.25 g pot^{-1}) and 100% (2 DAS, 0.5 or 0.75 g) (Table 3.3A). Similarly, *C. arvensis* above-ground biomass was reduced between 74% (1 DAS, 0.25 g) and 100% (2 DAS, 0.75 g) (Figure 3.5A). Surprisingly, the average biomass of plants subjected to the 1 g pot^{-1} treatment 1 DAS, was slightly greater than the biomass for other dose treatments on the same application date. A possible explanation for this unexpected result is the escape from inoculation of two plants in one of the treated pots. In the second trial, trends were similar with 31% (1 DAS, 0.5 g) and up to 94% (2 DAS, 1 g) of seedlings killed (Table 3.3B). Furthermore, *C. arvensis* above-ground biomass was reduced between 48 (1 DAS, 0.5 g) and 98% (2 DAS, 1 g) (Figure 3.5B).

3.4.2.2. Field trials

Most *C. arvensis* seedlings emerged 3 to 5 DAS. Weather conditions during the 1995 trial were generally warm with variable precipitation (Figure 3.6A). Average air temperatures shortly after inoculum application ranged between 21 and 25 °C.

During the 1996 trial, weather conditions were initially cooler (average air temperatures between 17 and 22 °C) and drier than in 1995 (Figure 3.6B).

3.4.2.2.1. 1995

Following fungal application, all *C. arvensis* seedlings rapidly developed disease symptoms, regardless of the dose applied. In contrast to experiments under controlled environment, disease incidence was similar for both application days. Some control plants also showed necrotic spots, indicating that the garden edgings placed around field plots did not completely prevent splash dispersal of the pathogen. Moreover, 'control' plants subjected to an application of autoclaved fungal granules developed a slightly greater degree of disease severity (Table 3.4). This might have been caused by phytotoxins (Tsantrizos *et al.* 1992) and/or by insufficient autoclaving, resulting in some conidia viability. However, a germination test, carried out shortly after field application, showed no viable conidia. The above-ground biomass of *C. arvensis* seedlings exposed to any of the fungal granule rates was reduced by nearly 100% compared with uninoculated controls (Figure 3.7A). Similarly, 99 to 100% of inoculated seedlings were killed (Table 3.4).

3.4.2.2.2. 1996

Initial disease development on inoculated *C. arvensis* seedlings was very low and most plants showed normal growth and the presence of only a few necrotic spots. However, 2 to 3 days after a relatively heavy rainfall (20 mm - 12 DAS), all treated seedlings showed severe necrotic symptoms (Table 3.4). In contrast to 1995, plants were not affected by autoclaved inoculum. Although *C. arvensis* control was slightly lower than in 1995, above-ground biomass was reduced between 94 (20 g, 4 DAS) and 98% (30 g, 4 DAS) when compared with uninoculated controls (Figure 3.7B). Likewise, mortality of inoculated seedlings ranged between 85 and 93% (Table 3.4). Compared to 1995, *C. arvensis* biomass

for all treatments was considerably lower (Figure 3.7A/B), possibly due to poor seedling emergence.

3.5. Discussion

In this study, soil incorporation of *P. convolvulus* granular inoculum was compared with surface applications and the effect of dose of the pathogen on control of *C. arvensis* was also examined. Under a controlled environment, incorporation of fungal granules resulted in higher disease development and enhanced weed control regardless of incorporation depth. Granules covered by a layer of soil were likely protected from desiccation and alternating temperatures, whereas inoculum on the soil surface could have lost viability. This protective effect may have been crucial since inoculations under controlled environment were performed immediately after sowing, resulting in a 3 to 4 day gap between fungal application and emergence of *C. arvensis* seedlings. This finding supports earlier studies using pre-emergence applied *P. convolvulus* inoculum, showing that the level of weed control declines with increasing time interval between inoculation and seedling emergence (Vogelgsang *et al.* 1994). Necroses observed on hypocotyls of plants subjected to the granule incorporation treatments also suggest improved contact between conidia and vulnerable seedling stages. Movement of conidia in soil water channels could have had a similar effect, resulting in a greater infection rate, regardless of depth of the incorporated inoculum. Furthermore, early infection by way of hypocotyls beneath the soil surface could have led to advanced disease development compared with infection of later emerging cotyledons.

Field trial results differed from findings obtained under controlled environment. Inoculum applied on the soil surface in the field produced more severe disease symptoms thus resulting in more effective control compared with the incorporation of granules. It should be noted that granule incorporation in the field was achieved by mixing inoculum with soil throughout a 5 cm soil layer rather than placing the inoculum at a specific soil depth. Hence, the density of infective propagules could have been decreased, resulting in fewer contacts between the fungal pathogen and

its weed host. Another possible reason for these varying results may be linked to the relative humidity present within the two environments. In the growth chamber, the constant air movement over the pot surfaces might have resulted in drier conditions compared with more mesic conditions in the field as a consequence of relatively little air movement. Hence in the growth chamber, incorporated inoculum might have resulted in greater efficacy of control. Moreover, rain-splash, wind dispersal, as well as run-off are common events in the field that may have enhanced the dissemination of the fungal inoculum on the soil surface (Fitt *et al.* 1989, Madden 1992). Apart from climatic conditions, the contrasting results obtained in the field versus in controlled environment could also be explained by several biotic and/or methodological factors. Soil type may have had a strong influence on the performance of the fungus. Factors such as particle size, pH, and available nutrients have been shown to favour or inhibit the survival and activity of fungal pathogens (Stotzky 1974, Paulitz & Baker 1987, Höpner & Alabouvette 1996). The enhanced activity of field soil applied inoculum compared with granules that were applied on sterilized potting medium in controlled environment could be explained by the greater densities of soil micro-organsims typically found under field conditions. The presence of these miccroorganisms may have led to intense competition for resources (Waksman 1952), thus preventing *P. convolvulus* conidia from germinating rapidly in the absence of host tissue. The inoculum production method used in this study may also have affected results. Within the larger incubation bottles (1 L) used for field trials, greater amounts of conidial matrix were observed, thus possibly protecting the fungus to a greater extent from desiccation (Sparace *et al.* 1991). The impact of a number of the above mentioned factors on *P. convolvulus* disease incidence and severity have been recently investigated (Vogelgsang *et al.* 1998a). This research demonstrated that planting substrate has a significant effect on the ability of *P. convolvulus* to cause severe disease on *C. arvensis* plants.

In the present study, field and controlled environment trial results were not consistent, however, the potential of soil incorporating fungal pathogens to

efficiently control weeds has been demonstrated elsewhere. An incorporated *Alternaria macrospora* granular preparation consisting of vermiculite, spores, and mycelia effectively controlled *Anoda cristata* in field tests (Walker 1981). More recently, incorporated microsclerotia of *Colletotrichum truncatum* resulted in almost complete (> 95%) control of the target weed hemp sesbania (Jackson *et al.* 1996). Similarly, conidia of *Ascochyta caulina* mixed into the soil remained infective for at least 2 weeks and effectively suppressed *Chenopodium album* seedlings (Kempenaar *et al.* 1996). *Fusarium solani* f. sp. *cucurbitae*-infested sodium alginate granules applied pre-emergence provided greater and longer lasting control of *Cucurbita texana* compared with conidial applications (Weidemann & Templeton 1988). Some of the above mentioned pathogens are known to be soil borne and to cause systemic infections. Hence, the ability of *P. convolvulus* conidia to attack *C. arvensis* underground plant parts or the possibility that they may cause systemic infection requires further investigation.

Spring and summer trials generally produced similar results, however, treatment differences were most evident during spring trials. For summer fungal applications, the overall level of *C. arvensis* control was generally greater than for spring trials and treatment differences were less pronounced. Higher air and soil temperatures and more frequent thunderstorms during July and August could have favoured both the infection rate and dissemination of fungal inoculum through splash dispersal.

Reduced rates of soil applied granules had no effect on disease development and subsequent control of *C. arvensis* in both controlled environment and field trials. Apparently, at the lowest granule rates used in this study (10 g 0.25 m^{-2}), the threshold minimum density of infective propagules required for optimal *C. arvensis* control had not been attained. This was particularly the case for field trials where disease development was improved and more consistent compared with controlled environment trials.

The findings in this study indicate that under field conditions, surface applications of *P. convolvulus* provide better control of *C. arvensis* compared with

inoculum that is soil incorporated. From a practical perspective, this might be advantageous because of a reduction in the number of steps necessary for application. Moreover, further inoculum dose reductions may be possible thus decreasing input costs and improving the economic feasibility of this control strategy. Finally, the contrasting results obtained under field and controlled environment with regards to soil substrate used, suggests that caution should be taken when assessing the efficacy of biocontrol agents applied to artificial soil substrates. Further research on fungal persistence in field soil, and the impact of fungal infection under competitive cropping situations are in progress and should provide additional information as to the potential of *P. convolvulus* to be an effective bioherbicide against *C. arvensis*.

Table 3.1. Effect of surface applied versus soil incorporated granules of *Phomopsis convolvulus* on disease severity of *Convolvulus arvensis* under controlled environment conditions. [a]

Treatment [b]	Disease Rating [c]		Mortality (%)	
	Trial 1[d]	Trial 2	Trial 1	Trial 2
Control	0.1(0.1) a	0.0(0.0) a	0 (0) a	0 (0) a
Surface applied	2.3(0.6) ab	1.4(0.3) ab	56 (21) b	13 (7) a
Incorporated - 1.5 cm	3.0(0.5) b	3.1(0.4) b	88 (7) b	81 (12) b
Incorporated - 3 cm	2.3(0.5) ab	2.5(0.5) b	75 (0) b	81 (6) b

[a] Trials were not combined because variances were not homogeneous.

[b] Surface applied, Incorporated - 1.5/3 cm: Application of 1 g of *P. convolvulus* granules pot^{-1} at the day of sowing on the soil surface or incorporated at a depth of 1.5 or 3 cm, respectively.

[c] Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.

[d] Numbers in parentheses are the standard error of the mean. Means in each column with the same letter are not significantly different, according to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating) or to Tukey's grouping ($\alpha = 0.05$) (mortality).

Table 3.2A. Effect of surface applied versus soil incorporated granules of *Phomopsis convolvulus* on disease severity of *Convolvulus arvensis* under field conditions in 1996. [a]

Treatment [b]	Disease Rating [c]		Mortality (%)	
	Spring [d]	Summer	Spring	Summer
Control	0.2 (0.0) a	0.6 (0.1) a	9 (2) a	5 (2) a
Surface applied	1.3 (0.1) b	2.7 (0.1) b	75 (3) c	99 (1) c
Incorporated	1.1 (0.1) ab	1.3 (0.1) ab	33 (5) b	50 (3) b

[a, c] As for Table 3.1.

[b] Surface applied, Incorporated: Application of 30 g of *P. convolvulus* granules 0.25 m^{-2} plot 3 days after sowing on the soil surface or incorporated at a depth of approximately 5 cm, respectively.

[d] Numbers in parentheses are the standard error of the mean. Means in each column with the same letter are not significantly different, according to the Friedman test followed by a multiple comparison procedure ($P = 0.15$) (disease rating) or to Tukey's grouping ($\alpha = 0.05$) (mortality).

Table 3.2B. Effect of surface applied versus soil incorporated granules of *Phomopsis convolvulus* on disease severity of *Convolvulus arvensis* under field conditions in 1997.[a]

Treatment[b]	Disease Rating[c]		Mortality (%)	
	Spring[d]	Summer	Spring	Summer
Control	0.5 (0.1) a	0.9 (0.1) a	3 (2) a	5 (3) a
Surface applied	2.2 (0.2) b	3.9 (0.1) b	44 (5) c	99 (1) c
Incorporated	1.6 (0.1) b	3.2 (0.2) ab	22 (6) b	80 (11) b

[a, c] As for Table 3.1.

[b, d] As for Table 3.2A.

Table 3.3A. Effect of various *Phomopsis convolvulus* doses on disease severity of *Convolvulus arvensis* seedlings under controlled environment conditions. - Trial 1 [a]

Date of application	Dose (g pot^{-1}) [b]	Disease rating [c, d]	Mortality (%)	Root biomass (g × 10^{-3} pot^{-1})
1 DAS	0 g	0.3 (0.1)	0 (0)	16.0 (1.3)
	0.25 g	1.9 (0.3)	50 (10)	6.0 (0.7)
	0.5 g	2.9 (0.3)	81 (6)	3.8 (0.6)
	0.75 g	3.3 (0.4)	81 (12)	3.0 (0.4)
	1 g	2.9 (0.2)	69 (6)	3.5 (0.3)
2 DAS	0 g	0.2 (0.1)	6 (6)	15.0 (2.9)
	0.25 g	3.2 (0.3)	88 (7)	2.3 (1.1)
	0.5 g	3.6 (0.2)	100 (0)	1.8 (0.6)
	0.75 g	3.6 (0.2)	100 (0)	2.5 (0.5)
	1 g	3.2 (0.5)	81 (12)	4.0 (1.0)

[a, c,] As for Table 3.1.

[b] 1 DAS, 2 DAS: Application of *P. convolvulus* granules at 1, 2 days after sowing, respectively.

[d] Numbers in parentheses are the standard error of the mean.

Regression equations for disease rating are: (1 DAS) $Y=0.453+2.979x^{0.5}$ $r^2=0.77$; (2 DAS) $Y=0.750+3.254x^{0.5}$ $r^2=0.67$; for mortality: (1 DAS) $Y=22.5+67.5x^{0.5}$ $r^2=0.51$; (2 DAS) $Y=22.879+84.802x^{0.5}$ $r^2=0.64$; for root biomass: (1 DAS) $Y=0.015-0.013x^{0.5}$ $r^2=0.84$; (2 DAS) $Y=0.012-0.011x^{0.5}$ $r^2=0.51$.

Table 3.3B. Effect of various *Phomopsis convolvulus* doses on disease severity of *Convolvulus arvensis* seedlings under controlled environment conditions. - Trial 2[a]

Date of application	Dose (g pot^{-1}) [b]	Disease rating [c, d]	Mortality (%)	Root biomass (g × 10^{-3} pot^{-1})
1 DAS	0 g	0.3 (0.1)	13 (13)	18.0 (3.0)
	0.25 g	2.1 (0.4)	38 (24)	8.8 (1.4)
	0.5 g	1.9 (0.3)	31 (6)	8.3 (1.5)
	0.75 g	2.3 (0.1)	63 (7)	4.8 (0.6)
	1 g	2.7 (0.1)	69 (6)	5.8 (0.8)
2 DAS	0 g	0.1 (0.1)	0 (0)	20.8 (2.5)
	0.25 g	3.5 (0.2)	88 (7)	2.3 (0.9)
	0.5 g	2.5 (0.5)	69 (16)	4.5 (1.0)
	0.75 g	3.3 (0.3)	88 (7)	3.3 (1.1)
	1 g	3.1 (0.5)	94 (6)	3.0 (0.4)

[a, c] As for Table 3.1.

[b, d] As for Table 3.3A.

Regression equations for disease rating are: (1 DAS) $Y=0.480+2.229x^{0.5}$ $r^2=0.76$; (2 DAS) $Y=0.714+2.932x^{0.5}$ $r^2=0.55$; for mortality: (1 DAS) $Y=12.219+6.635x^{0.5}$ $r^2=0.47$; (2 DAS) $Y=12.738+89.098x^{0.5}$ $r^2=0.67$; for root biomass: (1 DAS) $Y=0.017-0.013x^{0.5}$ $r^2=0.66$; (2 DAS) $Y=0.017-0.017x^{0.5}$ $r^2=0.67$.

Table 3.4. Effect of various *Phomopsis convolvulus* doses on disease severity of *Convolvulus arvensis* under field conditions.

Date of application[a]	Dose (g $0.25m^{-2}$)[b]	Disease Rating[c, d]		Mortality (%)	
		1995	1996	1995	1996
3 DAS	Control	1.0 (0.0)	1.5 (0.2)	19 (3)	10 (6)
	10 g	2.9 (0.7)	3.6 (0.1)	99 (1)	87 (6)
	20 g	3.6 (0.4)	3.7 (0.1)	100 (0)	90 (4)
	30 g	3.8 (0.2)	3.5 (0.1)	100 (0)	87 (1)
4 DAS	Control	1.1 (0.0)	1.5 (0.2)	30 (5)	10 (5)
	10 g	3.4 (0.6)	3.6 (0.1)	100 (0)	89 (3)
	20 g	3.9 (0.1)	3.4 (0.2)	100 (0)	85 (6)
	30 g	3.4 (0.6)	3.8 (0.1)	100 (0)	93 (3)

[a] 3, 4 DAS: Application of *P. convolvulus* granules 3, 4 days after sowing, respectively.

[b] Control 3 DAS and 4 DAS refer to uninoculated plots or with 30 g of autoclaved *P. convolvulus* granules inoculated plots, respectively.

[c] As for Table 3.1.

[d] Numbers in parentheses are the standard error of the mean.

Regression equations for disease rating are: (1995-1 DAS) $Y=1.170+0.0526x^{0.5}$ $r^2=0.64$; (1995-2 DAS) $Y=1.380+0.481x^{0.5}$ $r^2=0.51$; (1996-1 DAS) $Y=1.781+0.398x^{0.5}$ $r^2=0.76$; (1996-2 DAS) $Y=1.690+0.423x^{0.5}$ $r^2=0.79$; for mortality: (1995-1 DAS) $Y=27.952+15.643x^{0.5}$ $r^2=0.85$; (1995-2 DAS) $Y=38.339+13.459x^{0.5}$ $r^2=0.83$; (1996-1 DAS) $Y=18.862+15.091x^{0.5}$ $r^2=0.78$; (1996-2 DAS) $Y=18.882+15.323x^{0.5}$ $r^2=0.81$;

Figure 3.1. Incorporation experiment under controlled environment - Effect of surface application or incorporation of *Phomopsis convolvulus* inoculum on above-ground and root biomass of *Convolvulus arvensis* in (A) Trial 1, (B) Trial 2. Surface, 1.5 cm, and 3 cm treatments refer to 1 g of *P. convolvulus* granules pot^{-1} surface applied, incorporated at a depth of 1.5 or 3 cm on the day of sowing, respectively. Above-ground and root biomass were harvested 13 and 14 days after sowing, respectively. Vertical bars indicate the standard error of the mean.

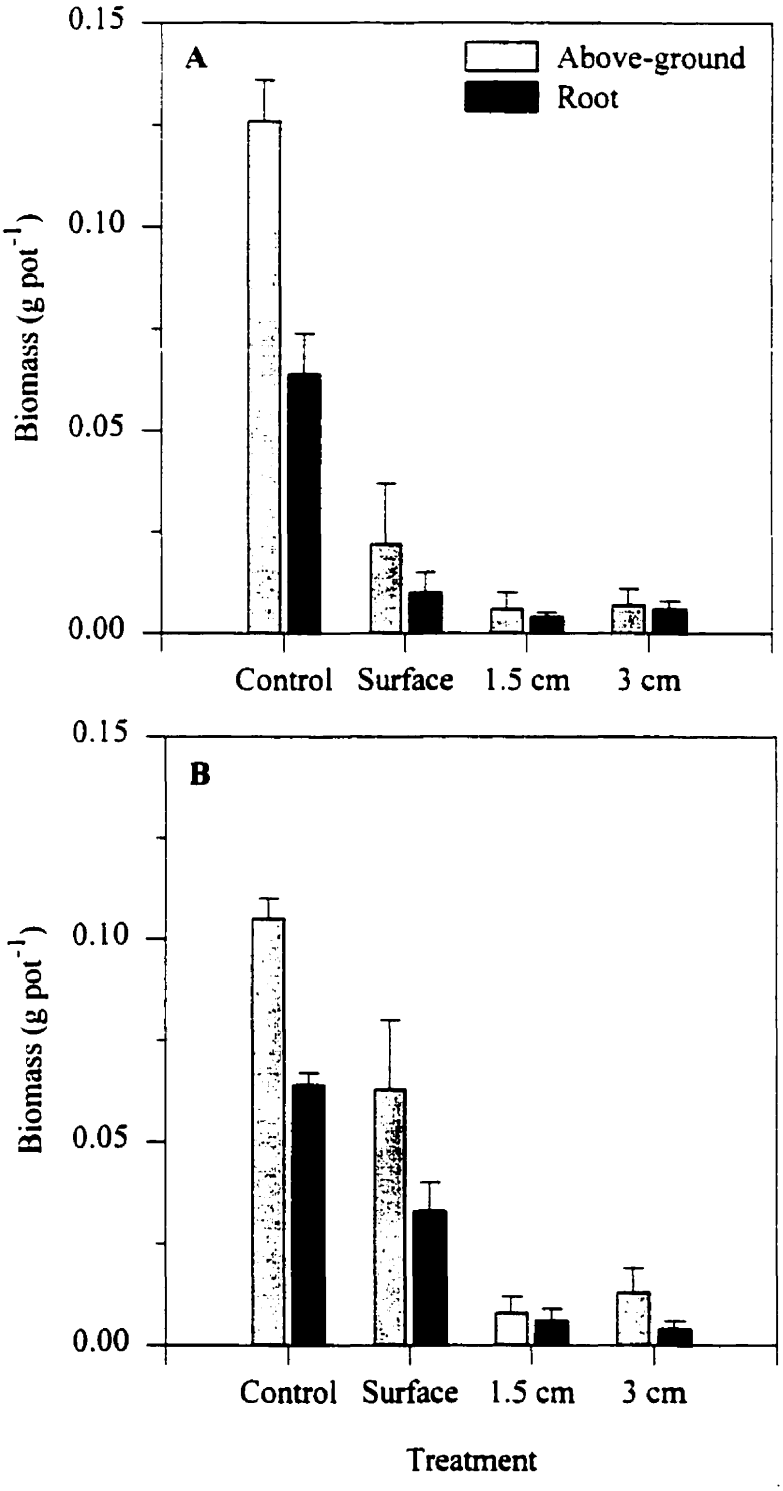
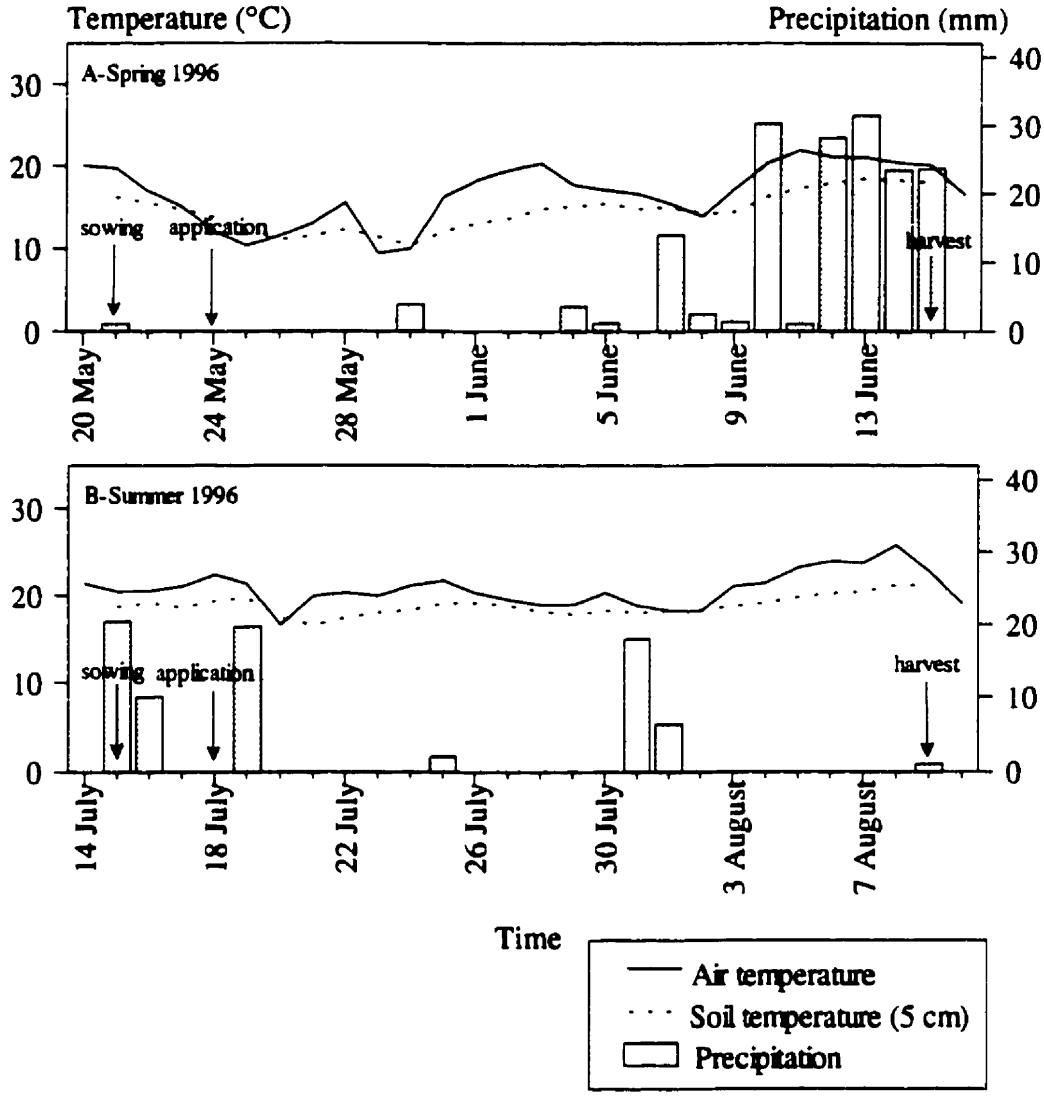

A
Above-ground
Root
0.15
0.10
0.05
0.00
Biomass (g pot-1)
Control
Surface
1.5 cm
3 cm
B
0.15
0.10
0.05
0.00
Biomass (g pot-1)
Control
Surface
1.5 cm
3 cm
Treatment

Figure 3.2. Incorporation experiment - Precipitation, air, and soil surface temperatures during (A) spring, (B) summer 1996, and (C) spring, (D) summer 1997 field trials. Temperatures are daily means.


Temperature (°C)
Precipitation (mm)
A-Spring 1996
sowing
application
harvest
20 May
24 May
28 May
1 June
5 June
9 June
13 June
B-Summer 1996
sowing
application
harvest
14 July
18 July
22 July
26 July
30 July
3 August
7 August
Time
Air temperature
Soil temperature (5 cm)
Precipitation

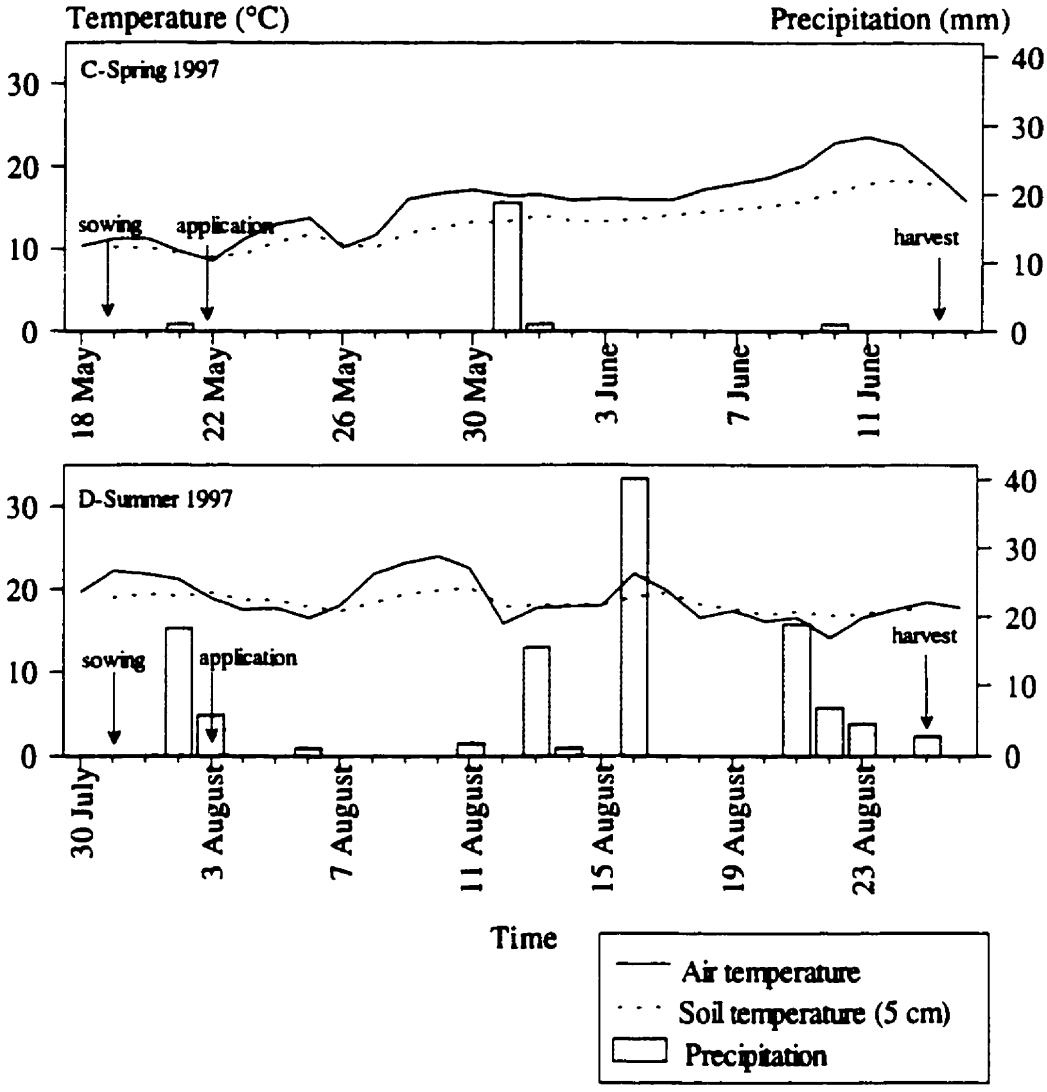
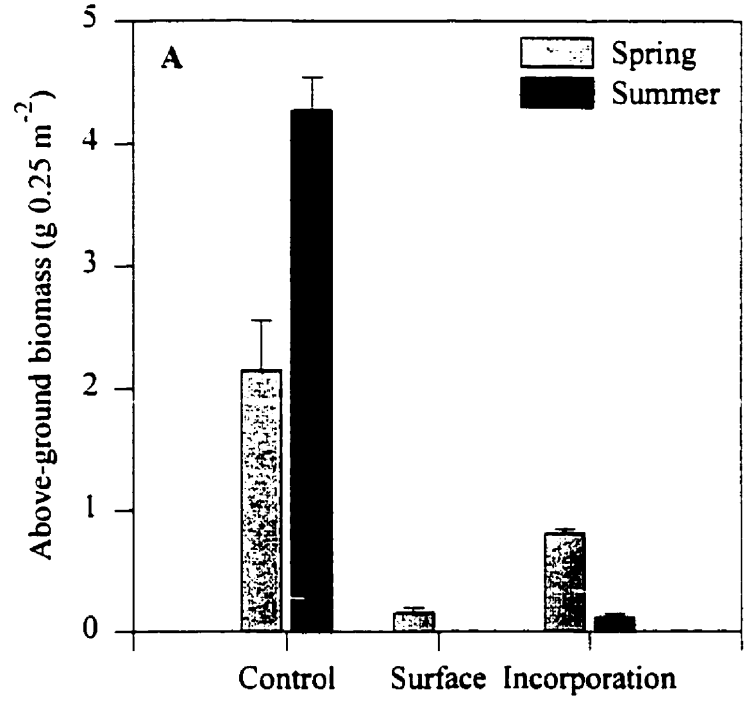

Temperature (°C)
Precipitation (mm)
C-Spring 1997
sowing
application
harvest
18 May
22 May
26 May
30 May
3 June
7 June
11 June
D-Summer 1997
sowing
application
harvest
30 July
3 August
7 August
11 August
15 August
19 August
23 August
Time
Air temperature
Soil temperature (5 cm)
Precipitation

Figure 3.3. Incorporation experiment under field conditions - Effect of surface application or incorporation of *Phomopsis convolvulus* inoculum on above-ground biomass of *Convolvulus arvensis* in the field (A) 1996, (B) 1997. Surface and Incorporation treatments refer to 30 g of *P. convolvulus* granules 0.25 m^{-2} plot surface applied or incorporated at a depth of approximately 5 cm 3 days after sowing, respectively. Plants were harvested 25 days after sowing. Vertical bars indicate the standard error of the mean.


A
Spring
Summer
Above-ground biomass (g 0.25 m-2)
5
4
3
2
1
0
Control
Surface
Incorporation

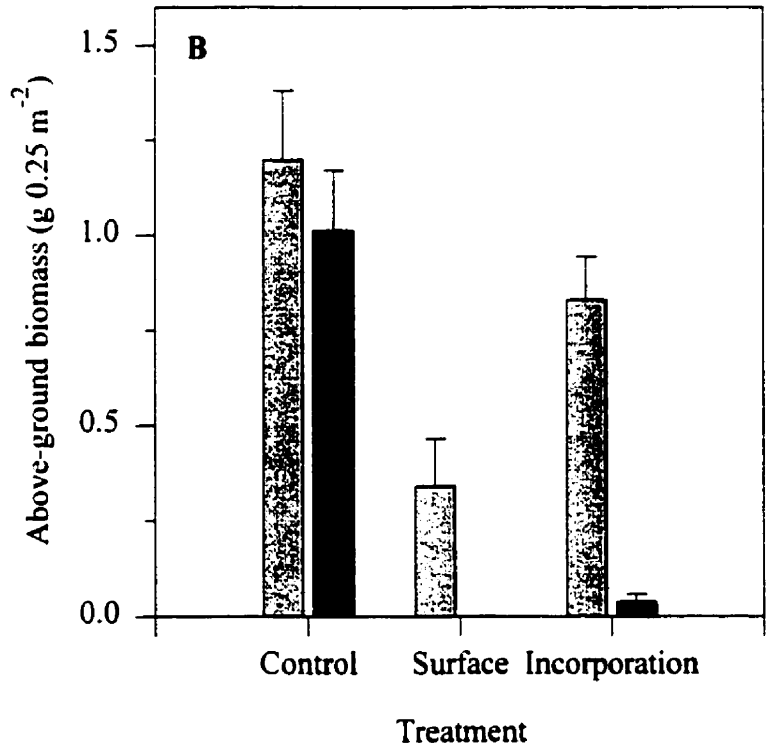

B
Above-ground biomass (g 0.25 m-2)
1.5
1.0
0.5
0.0
Control
Surface
Incorporation
Treatment

Figure 3.4. Effect of surface application or incorporation of *Phomopsis convolvulus* on *Convolvulus arvensis* above-ground and root biomass. From left to right: control, incorporated, and surface applied inoculum.

Figure 3.5. Dose response experiment under controlled environment - Effect of *Phomopsis convolvulus* dose when applied 1 day after sowing (DAS) (O--O) and 2 DAS (●—●) on *Convolvulus arvensis* above-ground biomass in (A) Trial 1, (B) Trial 2. Dose treatments of 0, 0.25, 0.5, 0.75, and 1 refer to soil applications of granular inoculum in the range of 0 to 1 g pot^{-1}. Regression equations are: (Trial 1-1 DAS) $Y=0.030-0.030x^{0.5}$ $r^2=0.74$; (Trial 1-2 DAS) $Y=0.030-0.035x^{0.5}$ $r^2=0.65$; (Trial 2-1 DAS) $Y=0.032-0.026x^{0.5}$ $r^2=0.63$; (Trial 2-2 DAS) $Y=0.033-0.037x^{0.5}$ $r^2=0.70$.

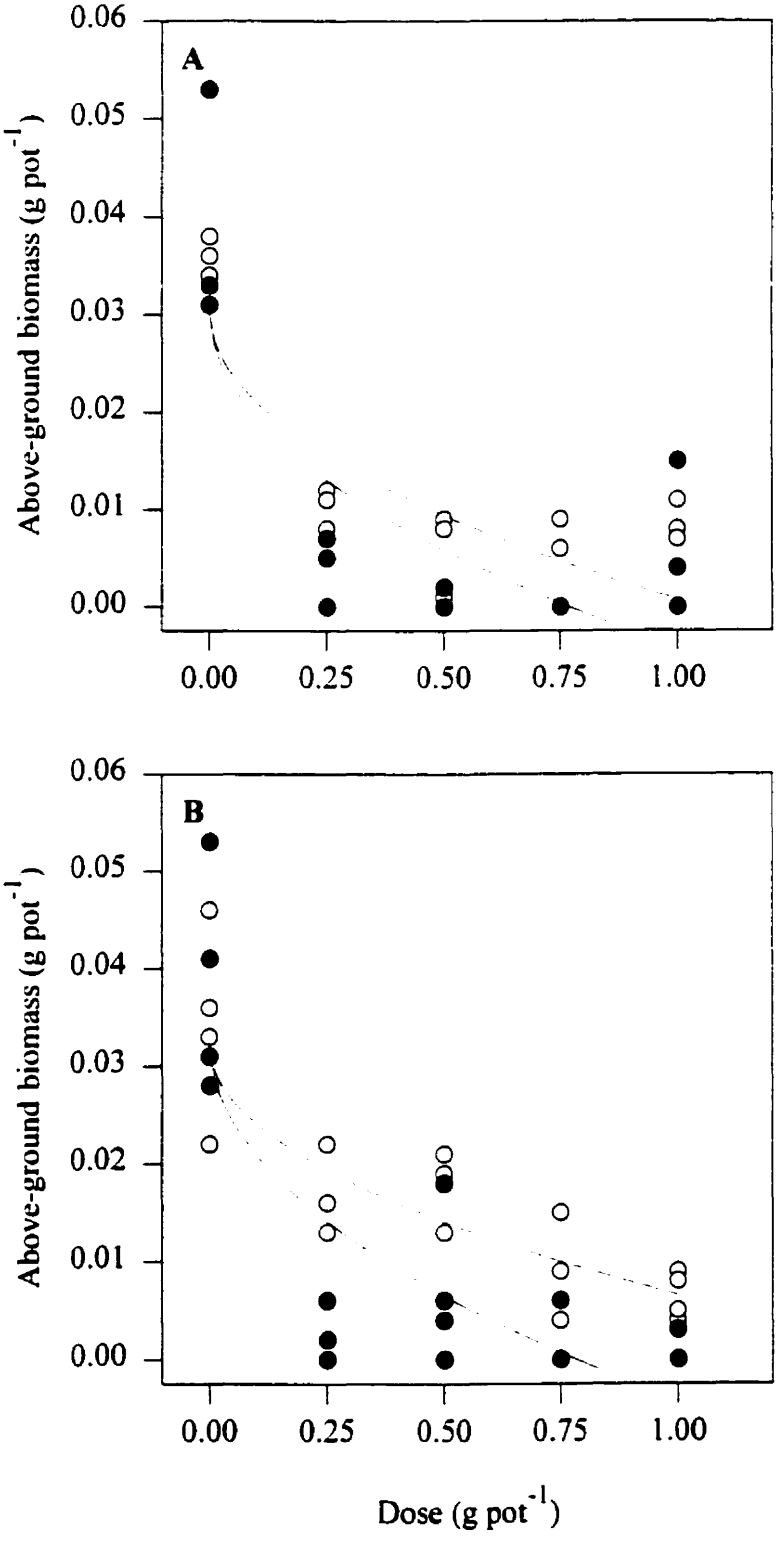

A
B
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Above-ground biomass (g pot-1)
0.00
0.25
0.50
0.75
1.00
Dose (g pot-1)

Figure 3.6. Dose response experiment - Precipitation, air, and soil surface temperatures during (A) 1995 and (B) 1996 field trials. Temperatures are daily means.

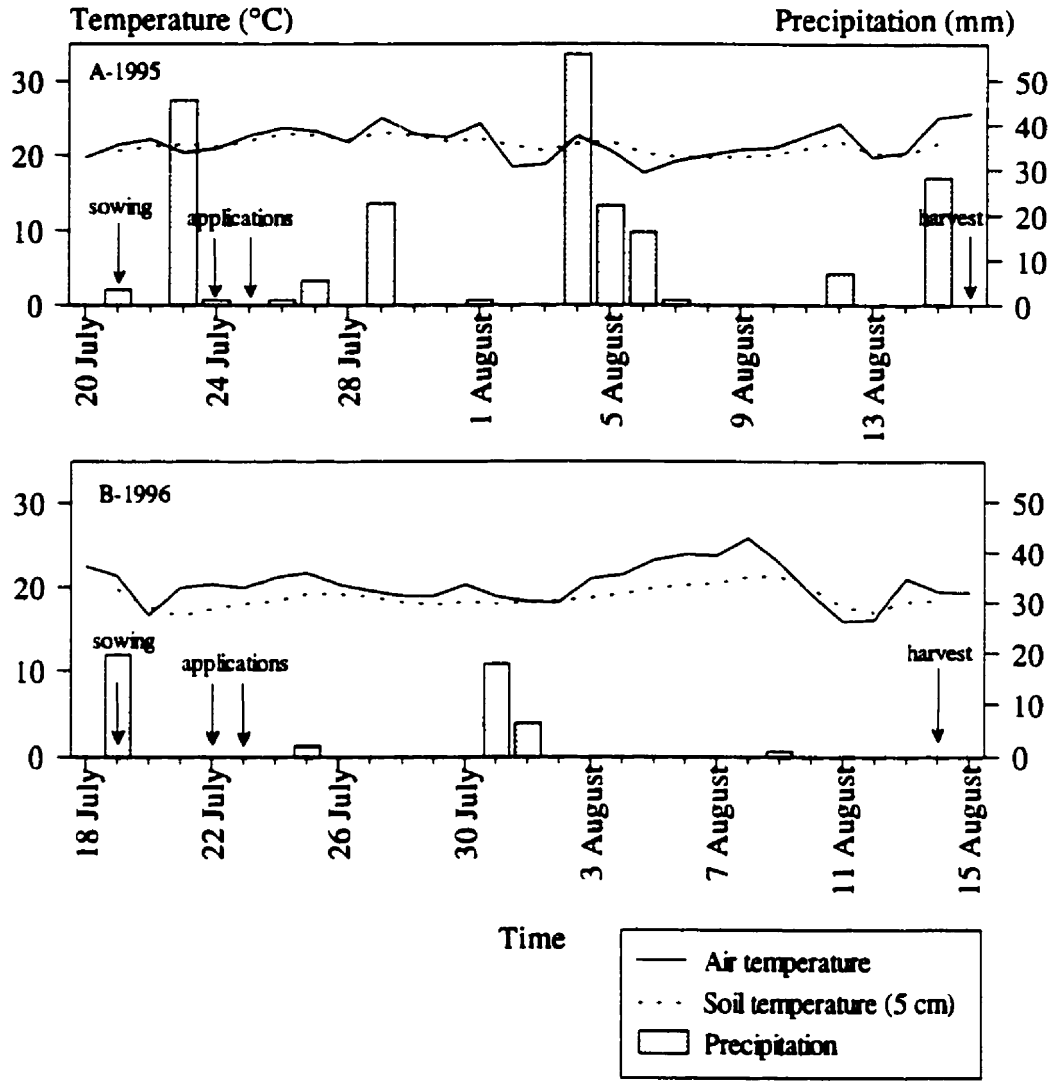
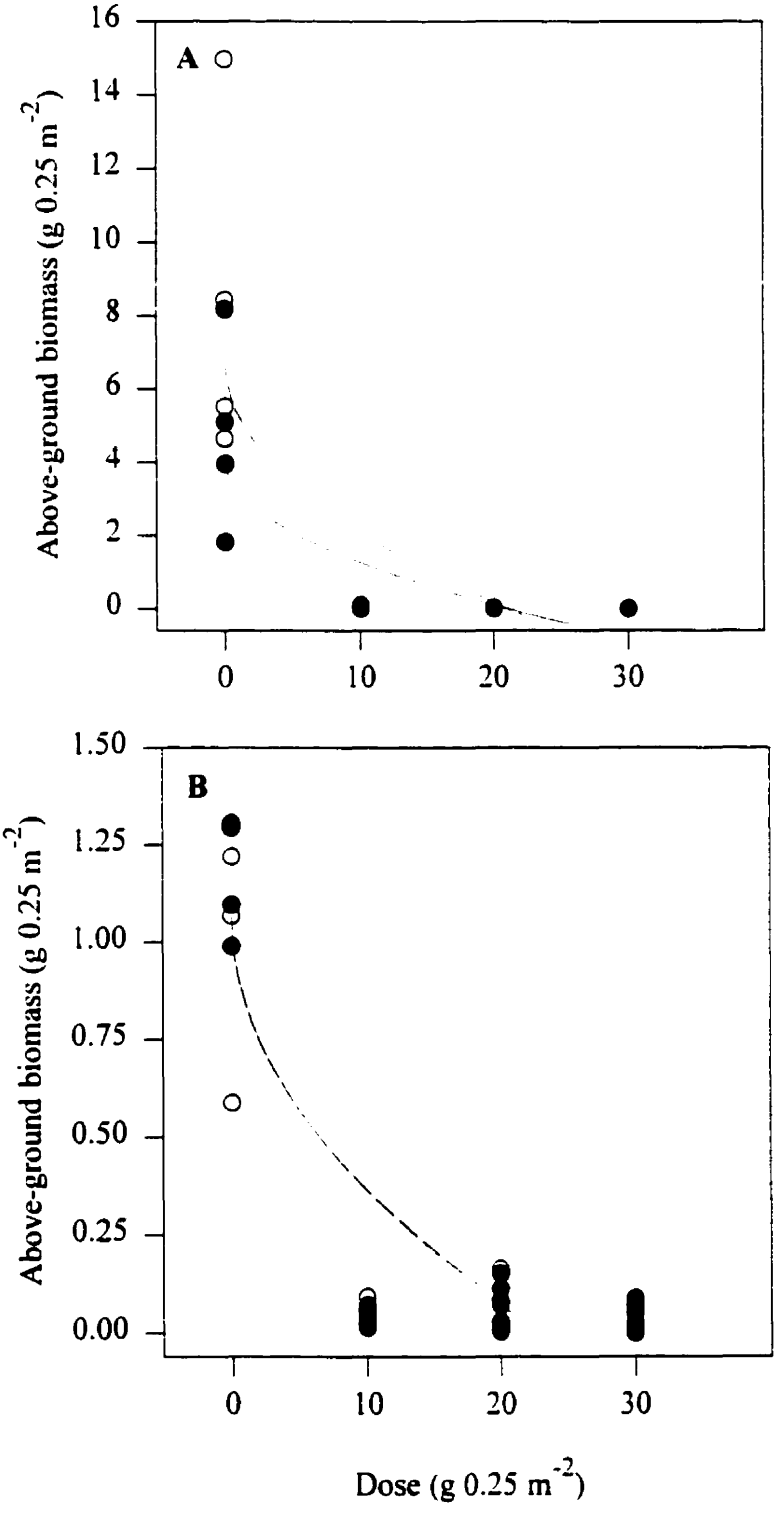

Temperature (°C)
Precipitation (mm)
A-1995
sowing
applications
harvest
20 July
24 July
28 July
1 August
5 August
9 August
13 August
B-1996
sowing
applications
harvest
18 July
22 July
26 July
30 July
3 August
7 August
11 August
15 August
Time
Air temperature
Soil temperature (5 cm)
Precipitation

Figure 3.7. Dose response experiment under field conditions - Effect of *Phomopsis convolvulus* dose when applied 3 days after sowing (3 DAS) (O--O) and 4 DAS (●—●) on *Convolvulus arvensis* above-ground biomass in (A) 1995, (B) 1996. Dose treatments of 0, 10, 20, and 30 refer to soil application of granular inoculum in the range of 0 to 30 g 0.25m^{-2} plot. Regression equations are: (1995-3 DAS) $Y=6.739-1.471x^{0.5}$ $r^2=0.62$; (1995-4 DAS) $Y=4.063-0.887x^{0.5}$ $r^2=0.66$; (1996-3 DAS) $Y=1.056-0.218x^{0.5}$ $r^2=0.72$; (1996-4 DAS) $Y=1.066-0.222x^{0.5}$ $r^2=0.83$.


A
B
Above-ground biomass (g 0.25 m-2)
Above-ground biomass (g 0.25 m-2)
Dose (g 0.25 m-2)
16
14
12
10
8
6
4
2
0
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
10
20
30

Connecting Text

In contrast to most other weed-pathosystems, control of *C. arvensis* through pre-emergence *P. convolvulus* inoculum applications has been shown to be typically greater under field conditions than in a controlled environment. In order to explore some possible causes for this research anomaly, the effect of moisture availability, inoculum production method, and planting substrate on *P. convolvulus* disease development in *C. arvensis* plants will be examined under controlled environment conditions.

Chapter 4. Effect of moisture, inoculum production, and planting substrate on disease reaction of *Convolvulus arvensis* to the fungal pathogen, *Phomopsis convolvulus*

4.1. Abstract

Phomopsis convolvulus is being evaluated as a possible bioherbicide for *Convolvulus arvensis* control. A granular barley formulation was applied pre-emergence onto the soil surface of pots containing pregerminated *C. arvensis* seeds. Covering the pots with transparent plastic bags immediately after application increased disease incidence and resulted in up to 81% reduction in above-ground dry biomass, whereas a treatment of interrupted dew periods (8 h day^{-1}) for 6 days, resulted in only 56% biomass reduction. The size of container used for producing and for incubating the fungus granules had no significant effect on disease incidence and subsequent weed control of *C. arvensis*. Likewise, no significant differences in efficacy were observed using inoculum that was milled once and then sieved or repeatedly milled and non-sieved. For early application dates, the use of two different planting substrates led to major differences in disease development. Pre-emergence application of inoculum on the surface of field collected soil on the same day that *C. arvensis* seeds were planted resulted in an 81% mortality of seedlings emerging. In contrast, only 50% of emerging seedlings were killed when inoculum was applied on the surface of peat moss. Findings in this study indicate that moisture conditions and planting substrate may affect disease incidence and subsequent control of *C. arvensis* by pre-emergence application of the selective fungal pathogen, *P. convolvulus*.

4.2. Introduction

The foliar pathogen, *Phomopsis convolvulus* Ormeno, selectively infects *Convolvulus arvensis* L. (field bindweed) and is currently being investigated as a possible bioherbicide against this serious perennial agricultural weed. Traditional control methods including crop rotation, cultivation, and chemical herbicides have met with only limited success (Swan 1980). In addition, reduced cultivation

practices (Phillips *et al.* 1980) and variable susceptibility of *C. arvensis* to several important herbicides (Whitworth & Muzik 1967, DeGennaro & Weller 1984, Kosinski & Weller 1989, Yerkes & Weller 1996) have led to an increased prevalence of *C. arvensis*. *P. convolvulus*, isolated in Québec, Canada, was first reported on *C. arvensis* in 1988 (Ormeno-Nuñez *et al.* 1988b). Subsequently, studies on host specificity, conidia mass production, storage, and efficacy of foliar treatments have been carried out (Ormeno-Nuñez *et al.* 1988a,b, Morin *et al.* 1989a,b, Morin *et al.* 1990). Weed control efficacy has been limited by the long dew period required for the germination and infection phases of this fungus.

In an attempt to overcome this limitation, a granular pre-emergence application of *P. convolvulus* has been evaluated (Vogelgsang *et al.* 1994). Inoculum produced on pot barley grains and applied onto the soil surface has effectively controlled seedlings and established stands of *C. arvensis* under both controlled environment and field conditions (Vogelgsang *et al.* 1998b). However, disease development and weed control efficacy in experiments performed under controlled environment conditions were generally lower than in corresponding experiments carried out under field conditions. This is unusual in bioherbicide research. Typically, the high levels of disease obtained under laboratory conditions are difficult to reproduce in the field. The narrow environmental range of conditions that is often required to attain high levels of infection has commonly been cited to explain the disparity in results between laboratory and field trials (Watson & Wymore 1990).

The objective of this study is to gain additional knowledge about factors that might lead to different levels of disease by *P. convolvulus* under controlled environment conditions. Experiments presented here were designed to test the effect of moisture availability, inoculum production method, and planting substrate on disease development.

4.3 Materials and methods

4.3.1. Inoculum production of starter cultures

Single conidia isolates of *P. convolvulus* were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 4 °C. From these stock cultures, small pieces of mycelia were placed on 9-cm-diameter Petri dishes with PDA and incubated in the dark at 24 ± 1 °C. After 4 to 5 days, several mycelial plugs of 1-cm-diameter were transferred to PDA plates and incubated at room temperature (21 ± 2 °C) and 12 h day^{-1} near-ultraviolet light (F40 BLB Blacklight, General Electric Lighting, Cleveland, OH, USA). After 3 weeks, conidia were harvested by washing plates with 12 ml of sterile deionized water. Conidia density was then adjusted to 1×10^7 conidia ml^{-1} with the aid of a haemocytometer.

4.3.2. Preparation of granular barley inoculum

Twenty ml of deionized water were added to 20 g of pot barley grains (*Hordeum vulgare* L.) in 250 ml Erlenmeyer flasks and autoclaved (18 min, 100 kPa, 120 °C). Flasks were cooled to room temperature and inoculated with 1 ml of the previously prepared conidia suspension starter culture. Flasks were incubated at room temperature (21 ± 2 °C) and exposed to 12 h near-ultraviolet light day^{-1} and shaken every second day by hand in order to inhibit the substrate from clumping. Colonized barley grains were harvested after 3 weeks and milled using an electric coffee grinder (Braun® KSM 2, Lynnfield, MA, USA). The granules produced were air dried for 2 days and sieved, unless otherwise indicated, resulting in inoculum particles of < 710 µm diameter. In a previous study, inoculum of this size was found to have a high pre-emergence activity and a shelf-life of at least 6 months (Vogelgsang *et al.* 1994).

To determine conidia production and viability of inoculum produced on the barley grains, 1 g granule samples were routinely tested as described in Chapter 2.

4.3.3. Plant production

C. arvensis seeds (Valley Seed Co., Fresno, CA, USA) were washed under warm running tapwater for 2 h and soaked overnight in deionized water. Imbibed seeds were then incubated on moist paper towels in a glass Petri dish in the dark at 24 ± 1 °C for 24 to 36 h. Four germinated seeds having emerged radicles were sown at a depth of 3 cm into 10-cm-diameter plastic pots containing a commercial prepared potting medium (Pro-Mix™ BX, Les Tourbières Premier Ltée, Rivière-du-Loup, QC, Canada). Pots were placed in a growth chamber (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 $\mu Em^{-2}s^{-1}$).

4.3.4. General inoculation procedure and data collection

For each pot, 1 g of granules was manually spread onto the moistened soil surface. Pots were immediately covered with large transparent plastic bags and the ends tucked under the bottom of the pot. Bags did not need support and were not misted with water. Bags were removed after 6 days. Unless otherwise specified, inoculum applications were carried out 0, 1, and 2 days after sowing (DAS). Foliar necrosis was evaluated at 8 DAS using the following rating system: 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, and 4 = 76-100% necrosis (Ormeno-Nuñez *et al.* 1988a). Disease rating was performed for each plant or shoot and results pooled and averaged for each pot. Mortality was assessed 13 DAS by counting the number of seedlings with completely necrotic hypocotyls. Above-ground and root dry biomass were determined 13 and 14 DAS, respectively. Plants were cut at the soil line, roots were carefully removed from the potting medium and living tissues dried in paper bags for 4 days at 60 °C, and weighed. Biomass data were recorded as total biomass per pot.

4.3.5. Effect of moisture availability on disease severity

Following application of *P. convolvulus* granules on the soil surface of pots containing four pre-germinated *C. arvensis* seeds, two moisture regimes were compared. In one treatment, pots were covered with plastic bags and placed in a growth chamber under the same conditions that seedlings were grown. In order to simulate field conditions, plants subjected to the second treatment were placed for 8 h day^{-1} in a dark dew chamber (100% RH, 21 ± 1 °C) during the normal night period, and subsequently returned to the same growth chamber containing the other treatment, pots covered with plastic bags. Plants were subjected to the two moisture regimes for 6 consecutive days and assessed for disease severity, mortality, above-ground biomass, and root biomass as described previously.

4.3.6. Effect of inoculum production method on disease severity

In previously performed field trials, larger-sized incubation containers (1 L) were used, and the sieving procedure after harvest of fungal conidia from the colonized barley was replaced by a second grinding step (Vogelgsang *et al.* 1998b). In the present study, *P. convolvulus* inoculum was produced in 1 L screw cap jars (100 g pot barley, 80 ml water) and also in 250 ml Erlenmeyer flasks (20 g pot barley, 20 ml water). Three weeks after inoculation, half of the colonized barley grains from the jars and flasks were ground in a coffee grinder and sieved resulting in particles of < 710 µm. The other half was not sieved after the initial grinding in a coffee grinder, but was ground a second time using an electric meat grinder (Quaker City Mill, Model 4-E, Westinghouse, PA, USA). This process resulted in a mixture of larger (> 1 mm) and smaller-sized (150 µm) particles with approximately 70% of the particles being < 710 µm diameter. The four different inoculum treatments (250 ml flask, sieved; 250 ml flask, non-sieved; 1 L jar, sieved; 1 L jar, non-sieved) were applied 1 DAS. The experiment also included an uninoculated control treatment. Data collection was performed as described above.

4.3.7. Effect of planting substrate on disease severity

Two different planting substrates were evaluated; the commercial potting medium Pro-Mix and field soil, collected from a site adjacent (approximately 2 m) to a research plot area where field trials with this host-pathogen system had been carried out 4 to 5 months previous. Pro-Mix is characterized by 80% peat moss, equal parts of perlite and vermiculite, and a limestone adjusted pH of 6.0 whereas the Chicot fine sandy loam field soil is 70% sand, 20% silt, 10% clay, has a pH of 5.3, and 3% organic matter. Four pre-germinated *C. arvensis* seeds were sown in pots containing the respective soils, and treated with the granular inoculum as described in the general inoculation procedure.

4.3.8. Experimental design and data analysis

All three experiments were performed twice in a completely randomized design with four replicates per treatment. Mortality and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test (α = 0.05) (Steel & Torrie 1980). Disease ratings were compared using the Kruskal-Wallis one-way analysis of variance by ranks, followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel 1978). Due to significant interactions between main effects, data for each of the two moisture conditions and planting substrates were analyzed separately. In all experiments, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner *et al.* 1992).

4.4. Results

4.4.1. General

The amount of conidia produced as well as viability was similar for all experiments and methods of inoculum production. Consistently, 1 to 2 $\times$ 10^9 conidia g^{-1} granules were produced with 80-100% germination. *C. arvensis* seedlings typically emerged in all experiments 2 to 3 days after sowing.

4.4.2. Effect of moisture availability on disease severity

Disease symptoms were visible for all inoculum application dates under both moisture regimes. However, disease development and mortality were greatest for the delayed application treatments as well as pots covered with plastic bags (Table 4.1). Following inoculum application at 2 DAS, mortality of covered plants reached 56 and 88% in Trial 1 and Trial 2, respectively, whereas only 15 (Trial 1) and 6% (Trial 2) of seedlings were killed when pots were subjected to interrupted dew periods. Likewise, substantial shoot and root biomass reductions were achieved for all inoculation treatments, although the use of plastic bags to cover pots resulted in the greatest reductions [81 (Trial 1) and 94% (Trial 2) above-ground and 76 and 90% root biomass reduction (2 DAS treatment)] (Figure 4.1). In contrast, shoot and root biomass reductions of only 56 and 41% and 60 and 51%, respectively were obtained for the interrupted dew period treatment (2 DAS).

4.4.3. Effect of inoculum production method on disease severity

All granule applications resulted in similar disease development (Table 4.2). Differences in mortality and biomass reductions between varying inoculum production treatments were not significant. In Trial 1, the number of seedlings killed following fungal application ranged between 50 and 81%, whereas in Trial 2, efficacy was lower with mortality rates between 19 and 44% respectively (Table 4.2). Likewise, above-ground biomass in Trial 1 was reduced between 76 and 94%, while in Trial 2 reductions between 62 and 82% were obtained (Figure 4.2). Results for root biomass were similar.

4.4.4. Effect of planting substrate on disease severity

As in other experiments, seedling emergence occurred 2 to 3 DAS in both planting substrates, however, uninoculated plants emerging from the field soil were shorter and more robust compared with plants growing in peat moss (Figure 4.3A/C). Similarly, root biomass from uninoculated plants in field soil consisted of a tap root that was considerably larger and thicker (Figure 4.3B/D) but had

fewer secondary roots than seedlings in peat moss. When plastic bags were removed 6 DAS, soil particles covered by black pycnidia were commonly observed on the field soil surface. Foliar necrosis developed for all fungal treatments. Unexpectedly, plants in the uninoculated control treatment using field soil also showed some necrotic leaf spots (Table 4.3). Apparently, some *P. convolvulus* conidia moved from the field trial site to the adjacent area where the soil was collected. Effects of the inoculation treatments on peat moss grown seedlings were similar to those obtained in previously performed experiments and led to significant differences in mortality between the various application dates. For example, fungal application on peat moss 0 DAS resulted in a relatively low mortality (50 and 25%) compared with the same application 2 DAS (94% for both trials) (Table 4.3). However, this pattern was not observed for plants grown in field soil with mortality being less dependent on date of application. Fungal applications carried out on the same day that seeds were sown resulted in 81 and 56% seedling mortality. Biomass reductions followed the same trend as for disease incidence and mortality although no significant differences were obtained between application dates when compared by planting substrate. In Trial 1, inoculum application at 0 DAS on peat moss resulted in an above-ground and root biomass reduction of 62 and 55% whereas in Trial 2, 68 and 61% reductions were obtained, respectively. In contrast, inoculum application on the same day but on field soil, resulted in above-ground biomass reductions of 93% and 78% (Figure 4.3). With delayed application (2 DAS), efficacy of control was similar for both planting substrates, with 97 (or 99%) above-ground biomass reductions for plants grown in peat moss, and 100 (or 99%) reduction in biomass for plants grown in field soil. The relatively high biomass obtained following inoculation on field soil 1 DAS (Figure 4.3) was likely due to two *C. arvensis* seedlings not coming into contact with fungal inoculum and thus escaping the treatment.

4.5. Discussion

In view of several abiotic factors, this study examined the disease reaction of *C. arvensis* to a pre-emergence application of the selective fungal pathogen, *P. convolvulus* under controlled environment conditions. Disease development was substantially lower with a moisture regime of interrupted dew periods compared with a continuous moisture period provided by covering pots with a plastic bag. This confirms results from earlier studies, where post-emergence treatments with *P. convolvulus* were more effective when accompanied by 18 h constant dew compared with three interrupted 6-h dew periods (Morin *et al.* 1989a). Similarly, in preliminary experiments with pre-emergence treatments, symptom development was greater in trials with continuous plastic bag coverage than in those with interrupted dew periods (Vogelgsang *et al.* 1994). However, these experiments were conducted independently. Although the granular inoculum is assumed to provide greater protection from desiccation (Boyette & Walker 1985), reduced germination of conidia or penetration during interrupted dew periods may have led to lower disease development (Bashi & Rotem 1974). For both moisture regimes, timing of fungal application was crucial for disease severity. The level of control declined with increasing time interval between fungal application and actual emergence. Despite the granular formulation, a considerable amount of inoculum might have lost viability by lying on the soil surface. However, reduced infection rates under changing moisture availability and at different application times were not observed in field trials (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b). Consequently, moisture conditions are likely not a major factor contributing to the contradictory results under a controlled environment and in the field.

The inoculum production methods used in our study did not significantly affect disease development. Although conidia quantities were similar for all fungal treatments, larger amounts of conidial matrix were observed within the larger-sized incubation jars, possibly suggesting a greater protection of conidia from desiccation (Sparace *et al.* 1991). However, application of inoculum produced in larger-sized jars did not lead to greater symptom development. Given that in this

experiment, treatments were applied at single date (1 DAS), the protective effect of a more abundant conidial matrix might have been masked. Furthermore, *P. convolvulus* non-sieved inoculum, and thus less homogeneous granules, did not show a greater virulence compared with sieved inoculum. Therefore, the supposition that a mixture of smaller-sized particles serving as immediate infection sources, and larger-sized particles possibly being more persistent, could not be confirmed. A trial that would incorporate earlier application times (0 DAS) might, however, reveal such an effect.

The type of planting substrate used for growing *C. arvensis* seedlings had a strong influence on the performance of *P. convolvulus*. Disease in plants grown in the sandy loam field soil was more pronounced than in plants grown on the prepared peat moss, with the greatest difference observed for the early pre-emergence inoculum application. Similar results were found with spore suspensions of *Ascochyta caulina* applied onto different soil types for the control of *Chenopodium album* (Kempenaar *et al.* 1996). Control of the target weed was substantially lower when inoculum was applied to peat compared with applications on a sandy soil, especially at the lower spore densities ($< 10^6$ spores cm^{-2}). Factors such as particle size, pH, and available nutrients have been shown to favour or inhibit the survival and activity of fungal pathogens (Stotzky 1974, Paulitz & Baker 1987, Höpner & Alabouvette 1996, Rosskopf *et al.* 1996). Moreover, the presence of numerous micro-organisms in our field soil could have led to intense competition for resources (Waksman 1952), thus preventing *P. convolvulus* conidia from germinating rapidly and having an extensive period of saprophytic germ tube growth prior to invasion of host tissue. Likewise, fungistasis particularly microbial in origin, may have initially inhibited fungal germination on the field soil (Lockwood 1977). On the commercially prepared and sterilized peat moss medium, with presumably far fewer micro-organisms but higher moisture content, a substantial number of conidia might have germinated and perished before emergence of *C. arvensis*. Furthermore, the physical structure of the sandy loam is more compact with characteristically smaller pore sizes compared with peat moss. The presence and distribution of different pore sizes

have been shown to play a key role in determining the habitable space in some fungi which are often limited to colonizing pores of a given minimal diameter (Filip 1979). Furthermore, pore sizes also determine the water potential (Stotzky 1974) which might have been more favourable for fungal growth in the sandy loam compared with peat moss.

In field trials with *P. convolvulus*, applications of a granular formulation improved the capability of the fungus to withstand unfavourable weather conditions (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b). However, in this study, only the direct comparison of disease severity, mortality, and biomass reduction in treatments using field soil versus peat moss potting mixture provided some answers that could possibly explain the differences observed between experiments conducted in the field with those under a controlled environment. Experiments, incorporating a greater number of planting substrates and moisture levels may provide additional information as to the impact of these two critical factors on the performance of *P. convolvulus* under both controlled and field conditions.

Further research on soil-incorporation of granules, fungal persistence in soil, dose response, and effect under competitive cropping situations are in progress and should provide additional information as to the potential of *P. convolvulus* to be an effective biological agent against *C. arvensis*.

Table 4.1. Effect of moisture availability on foliar necrosis and mortality of *Convolvulus arvensis* caused by *Phomopsis convolvulus*. [a]

Moisture condition	Treatment[b]	Disease Rating [c]				Mortality (%)			
		Trial 1[d]		Trial 2		Trial 1		Trial 2	
Plastic	control	0.1(0.1)	a	0.1(0.1)	a	0 (0)	a	0 (0)	a
bags	0 DAS	1.1(0.1)	ab	1.7(0.3)	ab	0 (0)	a	13 (7)	a
	1 DAS	2.1(0.2)	b	2.4(0.5)	b	44 (12)	b	44 (19)	ab
	2 DAS	2.5(0.3)	b	3.2(0.4)	b	56 (12)	b	88 (7)	b
Dew	control	0.0(0.0)	a	0.1(0.1)	a	6 (6)	ab	0 (0)	a
(8 h day^{-1})	0 DAS	0.9(0.1)	ab	1.5(0.2)	b	0 (0)	a	13 (7)	a
	1 DAS	1.1(0.1)	b	1.6(0.4)	b	6 (6)	ab	25 (18)	a
	2 DAS	1.7(0.0)	b	1.3(0.2)	ab	15 (6)	b	6 (6)	a

[a] Trials were not combined because variances were not homogeneous. Disease rating data recorded after 8 days and mortality data after 13 days.

[b] 0 DAS, 1 DAS, 2 DAS: Application of 1 g of *P. convolvulus* granules pot^{-1} at 0, 1, or 2 days after sowing, respectively. Plants were subjected to the moisture regimes for 6 days.

[c] Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.

[d] Numbers in parentheses are the standard error of the mean. For each moisture regime, means in each column with the same letter are not significantly different, according to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating) or to Tukey's grouping ($\alpha = 0.05$) (mortality).

Table 4.2. Effect of inoculum production method on foliar necrosis and mortality of *Convolvulus arvensis* caused by *Phomopsis convolvulus*.[a]

Inoculum production [b] (Container size, processing)	Disease Rating [c]		Mortality (%)	
	Trial 1[d]	Trial 2	Trial 1	Trial 2
control	0.1 (0.1) a	0.0 (0.0) a	0 (0) a	0 (0) a
250 ml, sieved	3.4 (0.4) b	2.2 (0.4) b	81 (12) b	19 (12) ab
250 ml, non-sieved	2.5 (0.5) ab	2.1 (0.2) ab	50 (18) ab	19 (12) ab
1 l, sieved	2.9 (0.3) ab	2.2 (0.2) b	75 (10) b	44 (6) b
1 l, non-sieved	3.4 (0.4) b	2.0 (0.2) ab	75 (18) b	44 (6) b

[a,c,d] As for Table 4.1.

[b] 250 ml, 1 l: Production of *P. convolvulus* granules in 250 ml Erlenmeyer flasks or in 1 L jars, respectively. Sieved, non-sieved: After drying, inoculum was sieved (710 µm mesh size) or not sieved but ground a second time, respectively. One g of *P. convolvulus* granules pot^{-1} was applied to the soil surface 1 day after sowing.

Table 4.3. Effect of planting substrate on foliar necrosis and mortality of *Convolvulus arvensis* caused by *Phomopsis convolvulus.* [a]

Planting substrate	Treatment[b]	Disease Rating [c]				Mortality (%)			
		Trial 1[d]		Trial 2		Trial 1		Trial 2	
Peat moss	control	0.0(0.0)	a	0.0(0.0)	a	0 (0)	a	0 (0)	a
	0 DAS	2.0(0.4)	ab	1.8(0.4)	ab	50 (14)	b	25 (10)	a
	1 DAS	2.6(0.6)	b	3.5(0.3)	b	69 (16)	bc	88 (7)	b
	2 DAS	3.7(0.2)	b	3.7(0.2)	b	94 (6)	c	94 (6)	b
Field soil	control	0.4(0.1)	a	0.1(0.1)	a	6 (6)	a	0 (0)	a
	0 DAS	2.6(0.2)	ab	2.2(0.4)	ab	81 (6)	b	56 (12)	bc
	1 DAS	2.1(0.4)	ab	2.3(0.3)	ab	81 (12)	b	25 (14)	ab
	2 DAS	3.7(0.1)	b	3.7(0.2)	b	100 (0)	b	94 (6)	c

[a,b,c,d] As for Table 4.1. Analysis for each planting substrate separately.

Figure 4.1. Effect of moisture regime on (A/C) above-ground, (B/D) root biomass of *Convolvulus arvensis* after a pre-emergence application with *Phomopsis convolvulus*. (A), (B) and (C), (D) represent Trial 1 and Trial 2, respectively. Day 0, 1, 2 treatments refer to the application of 1 g of *P. convolvulus* granules pot^{-1} at 0, 1, or 2 days after sowing to the soil surface, respectively. Plants were subjected to the two moisture regimes for 6 consecutive days. Shoots and roots were harvested 13 and 14 days after sowing, respectively. Vertical bars indicate the standard error of the mean.

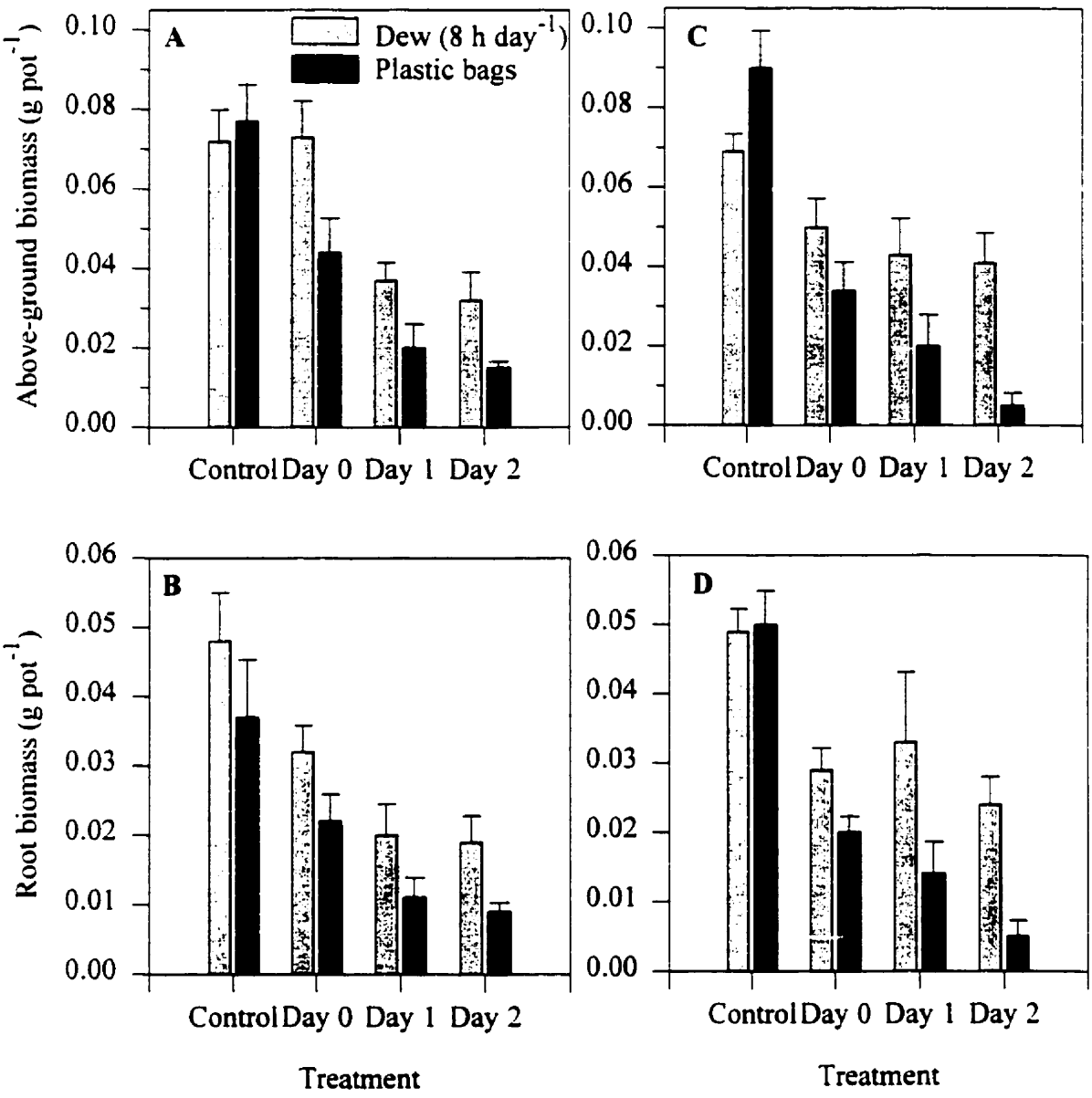

A
B
C
D
Dew (8 h day-1)
Plastic bags
Above-ground biomass (g pot-1)
Root biomass (g pot-1)
0.10
0.08
0.06
0.04
0.02
0.00
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Control
Day 0
Day 1
Day 2
Treatment

Figure 4.2. Effect of inoculum production method on *Convolvulus arvensis* biomass in (A) Trial 1 and (B) Trial 2. after a pre-emergence application with *Phomopsis convolvulus*. Two-hundred and fifty ml Erlenmeyer flasks and 1 L jars used as incubation containers are designated as 0.25 l and 1 l, respectively. S and NS refer to sieved (710 µm mesh size) and non-sieved inoculum, respectively. One g *P. convolvulus* granules pot^{-1} was applied 1 day after sowing to the soil surface. Shoots and roots were harvested 13, 14 days after sowing, respectively. Vertical bars indicate the standard error of the mean.

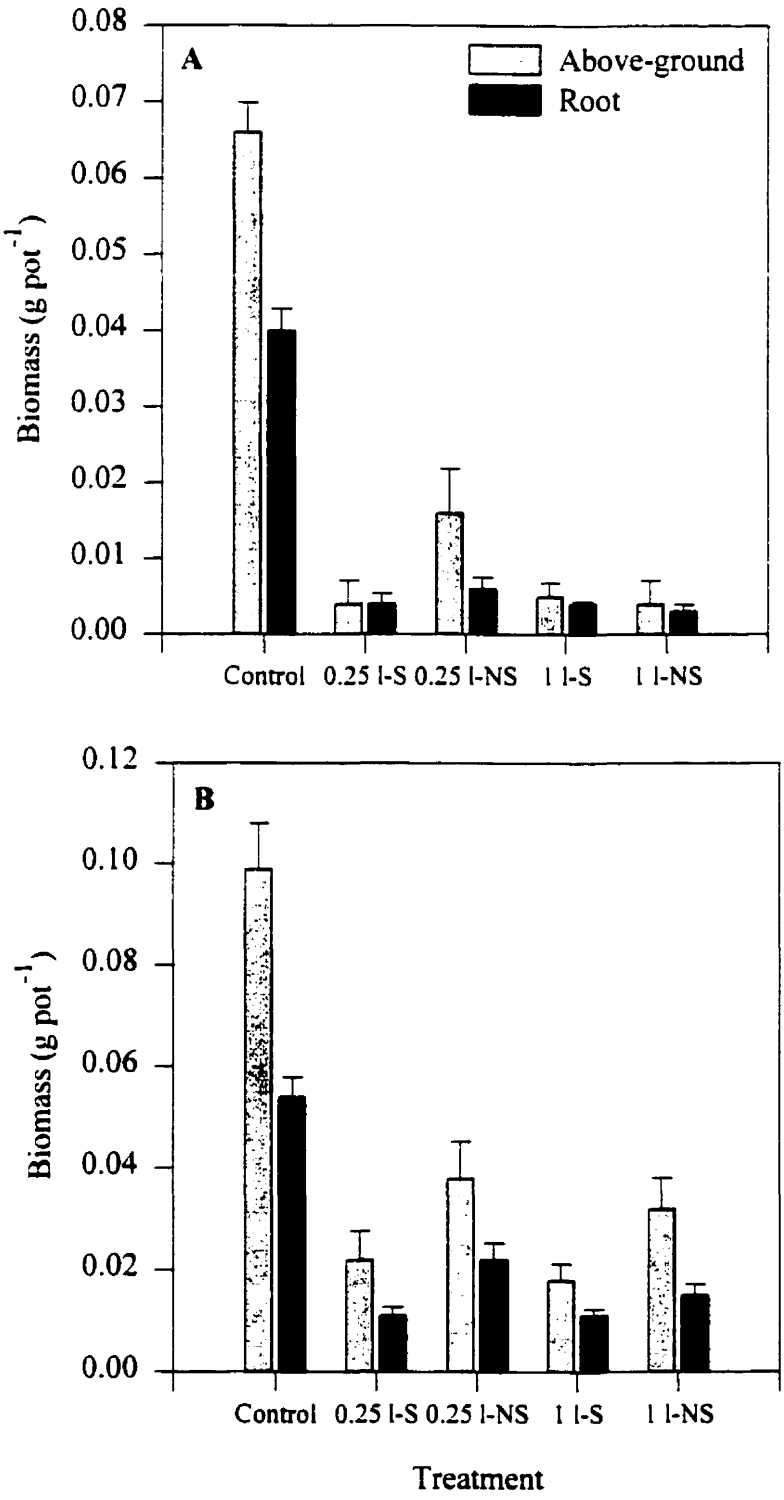
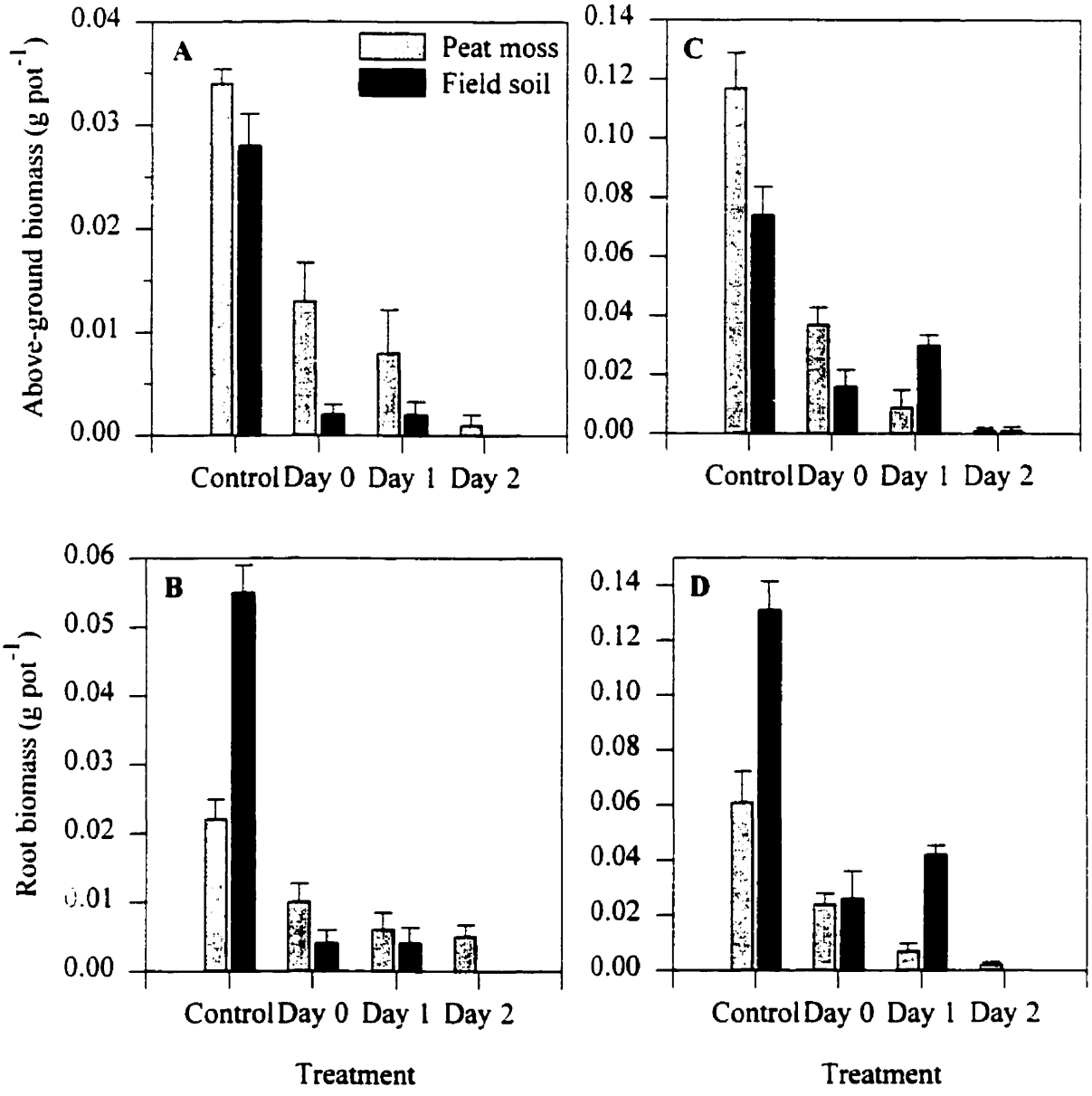

A
Above-ground
Root
Biomass (g pot-1)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Control
0.25 l-S
0.25 l-NS
1 l-S
1 l-NS
B
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Treatment

Figure 4.3. Effect of planting substrate on above-ground (A/C) and root biomass (B/D) of *Convolvulus arvensis* after a pre-emergence application with *Phomopsis convolvulus*. (A), (B) and (C), (D) represent Trial 1 and Trial 2, respectively. Day 0, 1, 2 treatments refer to the application of 1 g of *P. convolvulus* granules pot^{-1} at 0, 1, or 2 days after sowing, respectively. Shoots and roots were harvested 13, 14 days after sowing, respectively. Vertical bars indicate the standard error of the mean.


A
Peat moss
Field soil
Above-ground biomass (g pot-1)
0.04
0.03
0.02
0.01
0.00
Control Day 0 Day 1 Day 2
C
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control Day 0 Day 1 Day 2
B
Root biomass (g pot-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Control Day 0 Day 1 Day 2
Treatment
D
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control Day 0 Day 1 Day 2
Treatment

Connecting Text

C. arvensis is a troublesome weed in a number of important agricultural regions of the world. However, to date, the potential bioherbicide, *P. convolvulus*, has only been evaluated on two North American *C. arvensis* biotypes. In order to be considered as a truly effective biological control agent, the deleterious effects of this pathogen should not be limited to *C. arvensis* biotypes from a particular area but should cause disease in all members of the host. In this chapter, the occurrence of differential susceptibility in (a) various *C. arvensis* biotypes to foliar *P. convolvulus* post-emergence inoculum applications and (b) root stock material of two selected biotypes to pre-emergence inoculum applications of *P. convolvulus* will be evaluated.

Chapter 5. Susceptibility of *Convolvulus arvensis* biotypes to disease caused by the fungus *Phomopsis convolvulus*

5.1. Abstract

The susceptibility of *Convolvulus arvensis* biotypes from different geographic locations to disease caused by the fungal pathogen, *Phomopsis convolvulus* was evaluated. In a post-emergence application experiment, single excised plant shoots of *C. arvensis* collected from 11 different regions in North America and Europe were inoculated with *P. convolvulus* conidia. All *C. arvensis* biotypes showed similar disease reactions, however, plants originating in Canada (Québec) and Spain showed significantly greater disease development than plants from the USA (Montana, Iowa). In a separate pre-emergence application experiment, plants from two selected biotypes originating in Greece and the USA (Montana) were grown from root stock material and subjected to a granular formulation of *P. convolvulus* applied to the soil surface. The emerging shoots of both biotypes showed severe disease development and the fungal application on Greek and Montana biotypes reduced above-ground biomass 83 to 100% and 65 to 86%, respectively. Results of this study indicate that control of *C. arvensis* using *P. convolvulus* might be achieved in various geographic regions.

5.2. Introduction

Convolvulus arvensis L. (field bindweed) is a serious perennial weed in many important crops and is prevalent in temperate zones of Europe, West Asia, and North America (Holm *et al.* 1977). The move towards reduced cultivation or zero tillage in the past 15 years, has led to an increased prevalence of *C. arvensis* in many regions (Phillips *et al.* 1980). Effective control of *C. arvensis* using current methods including cultivation, crop rotation, and chemical herbicides (Derscheid *et al.* 1970) is often not possible due to the extensive root system, and strong competitiveness of this weed. In addition, variable susceptibility to several important herbicides has been demonstrated (Whitworth & Muzik 1967,

DeGennaro & Weller 1984, Duncan & Weller 1987, Kosinski & Weller 1989, Yerkes & Weller 1996, Westwood *et al.* 1997). *Phomopsis convolvulus* Ormeno, a foliar pathogen native to Canada, was first reported to infect *C. arvensis* plants in 1988 (Ormeno-Nuñez *et al.* 1988b). Since then, studies on host specificity, conidia mass production, storage, and efficacy of foliar applications have been carried out (Ormeno-Nuñez *et al.* 1988a,b, Morin *et al.* 1989a,b, Morin *et al.* 1990). In an attempt to overcome a relatively long dew period requirement during the germination and infection phases of the disease cycle, a granular pre-emergence formulation of *P. convolvulus* has been developed and been shown to be highly efficacious in suppressing *C. arvensis* seedlings as well as regrowth from established plants under both controlled environment and field conditions (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b).

C. arvensis is a troublesome weed in a number of important agricultural regions of the world. However, to date, the use of *P. convolvulus* as a potential bioherbicide has only been tested on *C. arvensis* biotypes originating from the USA (Montana) (e.g. Morin *et al.* 1989a, Vogelgsang *et al.* 1994) or Canada (Québec) (Ormeno Nuñez *et al.* 1988b). In order to be considered as a truly effective biological control agent, the deleterious effects of *P. convolvulus* should not only be limited to host plants found in a narrow geographic region but this impact should be evident in host plant biotypes from as many regions of the world as possible. Variations in *C. arvensis* morphological and physiological characteristics have been reported. Garcia-Baudin and Darmency (1979) observed intraspecific variations among *C. arvensis* populations with respect to leaf shape, flowering habit, seed weight, and soluble seed proteins when grown under similar conditions. Whitesides (1979) reported varying length and number of vines, number of leaves and flowers, and seed yield per plant in several *C. arvensis* ecotypes. In addition, biotype variations in floral characteristics, accumulation of shoot and root biomass, and flowering capacity have been demonstrated and linked to a differential susceptibility to chemical herbicides (e.g. DeGennaro & Weller 1984). Variations in disease susceptibility to biological control agents are possible

and have been observed in other host-pathogen systems (Shepherd 1995, Rayachhetry *et al.*, 1996, Okoli *et al.* 1997).

The objectives of this study were: (1) to determine the degree of disease susceptibility in *C. arvensis* biotypes originating from different regions of the world following foliar *P. convolvulus* post-emergence applications and (2) to evaluate differences in the disease response of plants from two selected biotypes grown from root stock material and subjected to granular pre-emergence applications of *P. convolvulus*.

5.3. Materials and methods

5.3.1. Inoculum production of starter cultures and for post-emergence experiments

Single conidia isolates of *P. convolvulus* were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 4 °C. From these stock cultures, small pieces of mycelia were placed on 9-cm-diameter Petri dishes with PDA and incubated in the dark at 24 ± 1 °C. After 4 to 5 days, several mycelial plugs of 1-cm-diameter were transferred to PDA plates and incubated at room temperature (21 ± 2 °C) and 12 h day $^{-1}$ near-ultraviolet light (F40 BLAB Blacklight, General Electric Lighting, Cleveland, OH, USA). After 3 weeks, conidia were harvested by washing plates with sterile deionized water and conidia density was adjusted to the desired level with the aid of a haemocytometer.

5.3.2. Granular inoculum production for pre-emergence experiments

Eighty ml deionized water were added to 100 g of pot barley grains (*Hordeum vulgare* L.) in 1 L screw cap jars and autoclaved (18 min, 100 kPa, 120 °C). Jars were cooled to room temperature and inoculated with 5 ml of the previously prepared conidia suspension at a density of 1×10^7 conidia ml^{-1}. Jars were incubated at room temperature (21 ± 2 °C) and exposed to 12 h near-ultraviolet light day^{-1} and shaken every second day by hand in order to prevent the substrate from clumping. Colonized barley grains were harvested after 3 weeks and milled

using an electric coffee grinder (Braun® KSM 2, Lynnfield, MA, USA). The granules produced were dried for 2 days and ground a second time with an electric meat grinder (Quaker City Mill, Model 4-E, Westinghouse, PA, USA). This resulted in a mixture of large and small-sized particles with approximately 70% of the particles being < 710 µm diameter. In an earlier study, inoculum of this size was found to have a high pre-emergence activity and a shelf-life of at least 6 months (Vogelgsang *et al.* 1994).

Granular inoculum was routinely tested for conidia levels and viability as described in Chapter 2. One gram granules contained between 3×10^8 to 1×10^9 conidia having > 90% viability.

5.3.3. Plant production

C. arvensis plant material was obtained from the following 11 regions: Canada-Ontario (CAN1), -Québec (CAN2), France-Nantes (F), Germany-Swabia (FRG1), -Brandenburg (FRG2), -Swabia (FRG3), Greece-Mandra (GR), Spain-Sevilla (E), UK-England (GB), USA-Montana (USA1), and -Iowa (USA2) (Table 5.1).

'CAN1' seeds were provided by Dr. J. E. Eckenwalder from the University of Toronto; 'CAN2' root stocks were collected on the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue; 'F' seeds were obtained from the Jardin Botanique de Nantes; 'FRG1' and 'FRG3' root stocks were collected at Stuttgart and Rastatt, respectively; 'FRG2' and 'GR' seeds were provided by the Botanical Garden in Berlin-Dahlem, Germany; 'E' seeds were provided by A. J. Pujadas Salvá at the Jardín Botánico de Córdoba; 'GB' seeds were obtained from J. Parsons at the Royal Botanical Gardens, Kew; seeds from 'USA1' and 'USA2' were purchased from Valley Seed Co., Fresno, CA.

For *C. arvensis* establishment, seeds were washed under warm running tapwater for 2 h and soaked overnight in deionized water. Imbibed seeds were then incubated on moist paper towels in a glass Petri dish in the dark at 24 ± 1 °C for 24 to 36 h. Five to 10 germinated seeds having emerged radicles were sown at a depth

of 3 cm into 22-cm-diameter plastic pots containing a mixture of sandy loam (Modugno-Hortibec Inc., St-Laurent, QC, Canada), potting medium (Pro-Mix™ BX, Les Tourbières Premier Ltée, Rivière-du-Loup, Québec), vermiculite (Vil Vermiculite Inc., Montréal, QC, Canada), and peat moss (Les Tourbières Premier Ltée) [3:3:2:1 (V/V/V/V)]. For root stock material, several root pieces bearing root buds were planted at a depth of 5 cm. Pots were placed in a greenhouse programmed at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod. After establishment, plants from both seed and root stock material were fertilized every second week with 200 ml of 20:20:20 (N:P:K, 3 g L^{-1}).

For post-emergence application experiments, single shoots of variable length (Table 5.1) and having 3 to 10 leaves per shoot were cut and placed in glass test tubes (15 × 125 mm) filled with deionized water. In order to prevent the shoots from sliding down and being partially submerged, stems were supported by a Parafilm® layer (American Can Company, Chicago, IL, USA) at the tube mouth.

For pre-emergence granular application experiments, two *C. arvensis* biotypes were selected. Based on the greatest morphological differences, plant material originating in the USA (Montana) and Greece were chosen. Plants from the USA possessed fairly thin, light green, and ovate-shaped leaves whereas leaves from *C. arvensis* plants of Greek origin were thicker, dark green, narrow, and lanceolate-shaped. For both biotypes, one 13- to 15-cm root piece bearing root buds was planted per 15.5-cm-diameter pot at a depth of 5 cm containing the same soil mixture as described above. Pots were subsequently placed in the greenhouse under original growing conditions.

5.3.4. Inoculation procedure

For post-emergence application experiments, tubes containing shoots from different origins were randomly placed in tube racks and inoculated with a conidia suspension at a density of 1×10^7 conidia m^{-2}, using a spray chamber (RIC, Research Instruments Mfg. Co., Ltd., Mandel Sci. Co., Ltd., Guelph, ON, Canada), equipped with a full-cone nozzle (Teejet GTO 0.7, Spraying Systems

Co., Wheaton, IL, USA), 200 kPa air pressure, a speed of approximately 1 kph, and a spray volume of 500 L/ha. The conidia density chosen was less than required (i.e. 1×10^8 to 1×10^9 conidia m^{-2}) for optimal disease development and high mortality (Morin *et al.* 1989a) in order to observe a range of responses to the fungal treatment. An equal number of shoots was sprayed with deionized water and served as controls. Immediately after inoculation, tube racks were placed in a dew chamber (24 °C, 100% RH) for 24 hours and subsequently transferred to a growth chamber (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 $\mu Em^{-2}s^{-1}$). Since *C. arvensis* biotypes were not all available at the same time, post-emergence experiments were performed in two sets. In both sets, plant material from the USA (Montana) served as a standard since plants originating from these seeds had also been used in earlier studies to evaluate the *P. convolvulus* pathogen (e.g. Morin *et al.* 1989a, Vogelgsang *et al.* 1994).

For pre-emergence application experiments, 2.5 g granular inoculum was spread onto the soil surface of 15.5-cm-diameter pots as evenly as possible by hand. The dose selected was based on preliminary studies that had made use of smaller pots (10-cm-diameter) and a dose of 1 g (Vogelgsang *et al.* 1994). In trials 1 and 2, inoculum was applied 8 and 7 days after planting, respectively. Following inoculation, pots were subjected to irrigation using a system that was designed to simulate natural precipitation. Five pots were placed randomly in circles (60- to 70-cm-diameter) beneath each of four nozzles attached 1.3 m above the greenhouse bench (Orbit Shrub Head Nozzle, Model 54009, full spray pattern 3.7 m, 7.6 L min^{-1} at an applied pressure of 140 kPa, Orbit Sprinklers, Bountiful, UT, USA) (Figure 5.1). Irrigation was carried out 12 hours per night for 11 days. The duration of one irrigation cycle was 15 min, with 1 min irrigation sequence followed by a 14 min pause. In order to avoid conidia splash dispersal, pots containing uninoculated control plants were separated from pots subjected to the granular surface inoculation by a transparent plastic sheet vertically attached to the

centre of the greenhouse bench. The sheet was removed after 11 days (completion of irrigation period) and pot location subsequently re-randomized.

5.3.5. Assessment of efficacy

For post-emergence experiments, foliar necrosis was evaluated 7 days after inoculation (DAI) using the following rating system: 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, and 4 = 76-100 % necrosis (Ormeno-Nuñez *et al.* 1988a). Disease rating was performed for each shoot and results averaged for each plant origin. Re-isolations were attempted for inoculated shoots. Leaves showing symptoms were excised, soaked in 70% ethanol for a few seconds, rinsed in sterile, deionized water, and 1-cm^2 diseased sections were cut using a flame-sterilized scalpel. Leaf sections were then immersed for 2 min in 1% sodium hypochlorite, subsequently rinsed in sterile water, and placed on sterile filter paper to dry. Leaf sections were placed on ½ strength PDA plates. Advancing edges from growing colonies were transferred to full strength PDA, placed under near UV-light for 12 h day^{-1}, and maintained for 4 weeks.

For pre-emergence experiments, disease rating was performed 10 DAI on emerged shoots. Above-ground and root biomass were determined 21 and 22 days after planting, respectively. Plants were cut at the soil line, roots carefully removed from the potting medium and living tissues dried in paper bags for 4 days at 60 °C, and weighed. Above-ground biomass was recorded as total biomass per pot whereas for root biomass, both entire root material and new growth was determined. In addition, the shoot/bud ratio was assessed by comparing the number of emerged shoots with the number of root buds observed.

5.3.6. Experimental design and data analysis

All experiments were performed twice and set-up in a completely randomized design. Post- and pre-emergence experiments had four and five replicates per treatment, respectively. Disease ratings were compared using the Kruskal-Wallis one-way analysis of variance by ranks, followed by a multiple-comparison

procedure to evaluate differences between treatment means (Daniel 1978). Shoot/bud ratio, shoot number, and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test ($\alpha = 0.05$) (Steel & Torrie 1980). In both post- and pre-emergence experiments, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner *et al.* 1992).

5.4. Results

5.4.1. Post-emergence experiment

Disease symptoms developed on shoots of all *C. arvensis* biotypes inoculated with *P. convolvulus* (Table 5.2). Necrotic spots on leaves and leaf tips were visible 2 to 3 days after inoculation. Subsequently, these diseased areas expanded to anthracnotic symptoms on leaves and several stems. Severe disease development often resulted in the complete wilting of treated shoots. In the first set of shoots tested, the disease reaction was not alike for all biotypes, but differences were not significant ($P > 0.15$) and results for the two trials were inconsistent (Table 5.2). In the second set of shoots tested, the disease reactions for the Canadian (Québec) and Spanish biotypes were significantly greater than the disease reaction for the USA (Montana, Iowa) biotypes (Table 5.2). For both sets, symptoms were less severe in the second trial. Except for the slightly lower disease reaction observed for the French biotype having thick, densely-haired leaves, morphological leaf variation between the different biotypes did not appear to play a major role in the disease response to *P. convolvulus*. Re-isolation of the pathogen from leaf sections of tested plants resulted in development of *P. convolvulus* mycelia, pycnidia and conidia.

5.4.2. Pre-emergence experiment

Most shoots began to emerge 7 to 8 DAS, however emergence continued until the end of the experiment. The number of shoots produced was highly variable

with 0 to 7 shoots pot^{-1} for inoculated plants, and 4 to 9 shoots pot^{-1} for controls, respectively. In both trials, maximum temperatures reached 36 °C (Figure 5.2).

During the irrigation period, droplets of fungal matrix were observed on the soil surface of inoculated pots, and emerging shoots from the two biotypes rapidly developed severe necroses so that by 10 DAI, the number of shoots with fully developed leaves was considerably lower than for uninoculated controls (Table 5.3). Similarly, the shoot/root bud ratio was greater for uninoculated controls compared with inoculated plants (Table 5.3), and significant biomass reductions were achieved for both biotypes tested (Figure 5.3, Figure 5.4). In the two trials, above-ground biomass reductions for plants from Greece ranged from 83 to 100% whereas the biomass from plants originating in the USA (Montana) was reduced by 65 to 86%, compared with uninoculated controls. Root biomass reductions were less uniform than for above-ground biomass. For example, total root biomass of the Greek biotype was reduced by 40 to 52% whereas reductions for the USA (Montana) biotype ranged from 9 to 46% (Figure 5.5). However, biomass reductions for new root growth were similar for both biotypes (56 to 72% for the Greek biotype and 58 to 70% for the USA biotype) (Figure 5.5).

5.5. Discussion

In this study, the susceptibility of *C. arvensis* biotypes collected from various geographical regions to disease caused by the fungal pathogen *P. convolvulus* was evaluated. Following a post-emergence foliar inoculum application on single excised shoots, disease development was generally similar for all biotypes tested. However, a Canadian (Québec) and a Spanish biotype were more susceptible than a USA biotype. Thus, despite the wide range of morphological attributes observed in the *C. arvensis* biotypes selected, such physical features generally appear to play a minor role in disease susceptibility to the pathogen. It should be noted however, that the conidia rate applied (1×10^7 conidia m^{-2}) was too low to result in mortality of treated shoots (Morin *et al.* 1989a), thus different responses might have been obtained had higher conidia rates been used.

The overall lower disease incidence in the second trial might have been caused by insufficient coverage during spraying and/or by lower conidia viability. In addition, the second trial was performed using more mature plants than was the case for the first trial, hence, leaves of these older plants might have developed thicker cuticles possibly resulting in altered *C. arvensis* defence mechanisms (Martin 1965).

Application of *P. convolvulus* conidia suspensions on excised *C. arvensis* shoots provided a fast and effective method to assess host plant susceptibility of a relatively large number of biotypes. However, caution must be used in interpreting such results since data obtained may not be representative of disease reactions that would be observed on intact, perennial plants of *C. arvensis*. Consequently, pre-emergence granular applications were carried out using established *C. arvensis* plants from cloned root stocks. High disease incidence and excellent biomass reductions were observed for the two selected biotypes, one from Southern Europe (Greece) and the other from North America (Montana, USA). In addition to large above-ground biomass reductions, new root growth of both biotypes was also substantially reduced. These findings differ to some degree from earlier controlled environment studies using pre-emergence applications of *P. convolvulus* where it had been difficult to consistently reproduce high levels of weed suppression, especially when established plant material was used (Vogelgsang *et al.* 1998a,b). Fungal application in these preliminary studies was typically followed by a moisture treatment using either a dew chamber, or covering pots with plastic bags. However, the development of a disease epidemic is also dependent upon an adequate distribution of the pathogen near its host. In this study, the irrigation system was designed to simulate natural rainfall, thus likely providing both sufficient moisture as well as effective splash dispersal. Consequently, dissemination of the fungal inoculum on the soil surface might have been enhanced (Fitt *et al.* 1989, Madden 1992). In addition to determining susceptibility of geographically diverse *C. arvensis* biotypes to *P. convolvulus* infection, this

experiment also suggests a more suitable methodology for carrying out such trials under controlled environment conditions.

Following pre-emergence granular application, air temperatures in the greenhouse were relatively high (> 30 °C). Nonetheless, the granular formulation of *P. convolvulus* used was capable of performing well under these conditions. Similar findings where the efficacy of granular pre-emergence applications was superior to post-emergence foliar sprays has been demonstrated in other weed-pathosystems (Walker 1981, Walker & Connick 1983, Boyette & Walker 1985, Weidemann & Templeton 1988).

The results of our study indicate that despite obvious morphological variations amongst the *C. arvensis* biotypes investigated, limited differential susceptibility to *P. convolvulus* was observed. However, it should be noted that the geographical range from which plant material was collected was rather restricted. Hence, trials that will make use of plant material from other regions of the world (e.g. South America, Australia, Asia) as well as including a greater number of test plants could provide more accurate information about the genetic diversity of *C. arvensis* host plants to infection by *P. convolvulus*. Moreover, in this study, all *C. arvensis* biotypes were grown under controlled environment, so that little can be implied about the stability or genetic heredity of various morphological characteristics under field conditions. Therefore, *in situ* studies would be required to fully evaluate the efficacy of *P. convolvulus* to control *C. arvensis* natural field populations in different geographical regions of the world.

Table 5.1. Leaf and shoot characteristics of *Convolvulus arvensis* biotypes evaluated.

Origin[a]	Blade length (cm)	Leaves shoot^{-1}	Leaf description
USA1	1.2 - 2.8	4 - 10	light green, ovate, saggitate or hastate, slender
CAN1	1.3 - 2.8	5 - 7	oblong, hastate
F	1.2 - 2.3	5 - 8	dark green, ovate, saggitate, densely haired
FRG1	1.6 - 2.9	5 - 8	oblong, saggitate
FRG2	1.9 - 3.9	4 - 6	ovate to oblong, saggitate
FRG3	1.5 - 2.6	5 - 8	narrow, ovate to lanceolate, saggitate
GB	1.1 - 1.9	5 - 8	light green, oval, hastate, slender
GR	1.6 - 2.5	3 - 8	dark green, narrow, lanceolate, saggitate, long lobes
CAN2	2.5 - 3.6	5 - 9	ovate to lanceolate, hastate, long lobes, hairy
E	1.5 - 2.5	4 - 6	oval, hastate, slender
USA2	2.5 - 3.4	6 - 8	ovate to oval, saggitate or hastate

[a] Plant origins USA1: USA-Montana; CAN1: Canada-Ontario; F: France-Nantes; FRG1, 2, 3: Germany-Swabia, -Brandenburg; -Swabia; GB: UK-England; GR: Greece-Mandra; CAN2: Canada-Québec; E: Spain-Sevilla; USA2: USA-Iowa.

Table 5.2. Disease reaction of various *Convolvulus arvensis* biotypes to the post-emergence application of *Phomopsis convolvulus*. [a]

Set 1 [b]			Set 2				
Origin [c]	Trial 1	Trial 2	Origin [c]	Trial 1		Trial 2	
USA1 [d]	1.8 (0.3)	1.0 (0.0)	USA1 [d]	1.0 (0.0)	a	1.0 (0.0)	a
CAN1	2.5 (0.5)	1.0 (0.0)	CAN2	2.8 (0.5)	bc	2.0 (0.4)	c
F	1.5 (0.3)	0.8 (0.3)	E	2.8 (0.3)	c	1.5 (0.3)	bc
FRG1	2.5 (0.3)	1.0 (0.0)	USA2	1.3 (0.3)	ab	1.3 (0.3)	ab
FRG2	1.5 (0.3)	1.0 (0.0)					
FRG3	2.8 (0.5)	1.3 (0.3)					
GB	1.8 (0.3)	1.0 (0.0)					
GR	3.0 (0.4)	0.8 (0.3)					

[a] *C. arvensis* shoots were sprayed at a rate of 1×10^7 conidia m^{-2}. Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.

[b] Experiments were performed in two sets based on the availability of plant material. For Sets 1 and 2: No significant differences, or means in each column with the same letter are not significantly different, respectively, according to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure (P = 0.15). Numbers in parentheses are the standard error of the mean.

[c] Plant origins as for Table 5.1.

[d] USA1 plant material was used as a standard.

Table 5.3. Disease susceptibility of two *Convolvulus arvensis* biotypes to the pre-emergence application of *Phomopsis convolvulus*.

	Trial 1 [a]			Trial 2		
Origin [b]/ Treatment	Disease rating [c, d]	Shoots pot^{-1}	Shoot/ bud ratio	Disease rating [c, d]	Shoots pot^{-1}	Shoot/ bud ratio
USA/control	0.0 a	7.8 a	1.1 a	0.0 a	6.2 a	0.9 a
	(0.1)	(0.5)	(0.1)	(0.0)	(1.0)	(0.1)
USA/inoculated	1.4 b	1.2 b	0.2 c	3.0 b	3.4 a	0.4 b
	(0.4)	(0.5)	(0.1)	(0.6)	(1.4)	(0.2)
GR/control	0.0 a	6.6 a	0.7 b	0.0 a	5.2 a	0.7 a
	(0.0)	(0.8)	(0.1)	(0.0)	(0.4)	(0.0)
GR/inoculated	2.2 b	1.0 b	0.1 c	3.0 b	4.2 a	0.4 b
	(0.7)	(0.3)	(0.0)	(0.6)	(0.9)	(0.1)

[a] Trial 1, 2: 2.5 g of *P. convolvulus* inoculum granules pot^{-1} were applied to the soil surface 8, 7 days after planting, respectively.

[b] USA, GR: USA-Montana, Greece-Mandra, respectively.

[c] Disease rating data were recorded 10 after inoculation (DAI); shoot/bud ratio was determined 22 days after planting. Disease rating scale as for Table 5.2.

[d] Means in each column with the same letter are not significantly different according to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating) or to Tukey's grouping ($\alpha = 0.05$) (shoots, shoot/bud ratio). Numbers in parentheses are the standard error of the mean.

Figure 5.1. Irrigation system used during the pre-emergence experiment evaluating two *Convolvulus arvensis* biotypes. Five pots were placed randomly in circles beneath each of four nozzles attached above the greenhouse bench. In order to avoid conidia splash dispersal, pots containing uninoculated control plants were separated from pots subjected to the granular surface inoculation by a transparent plastic sheet.

Figure 5.2. Air temperatures (solid line: mean, dotted lines: minimum, maximum) in greenhouse during (A) Trial 1, (B) Trial 2. Grey shaded areas refer to irrigation period.

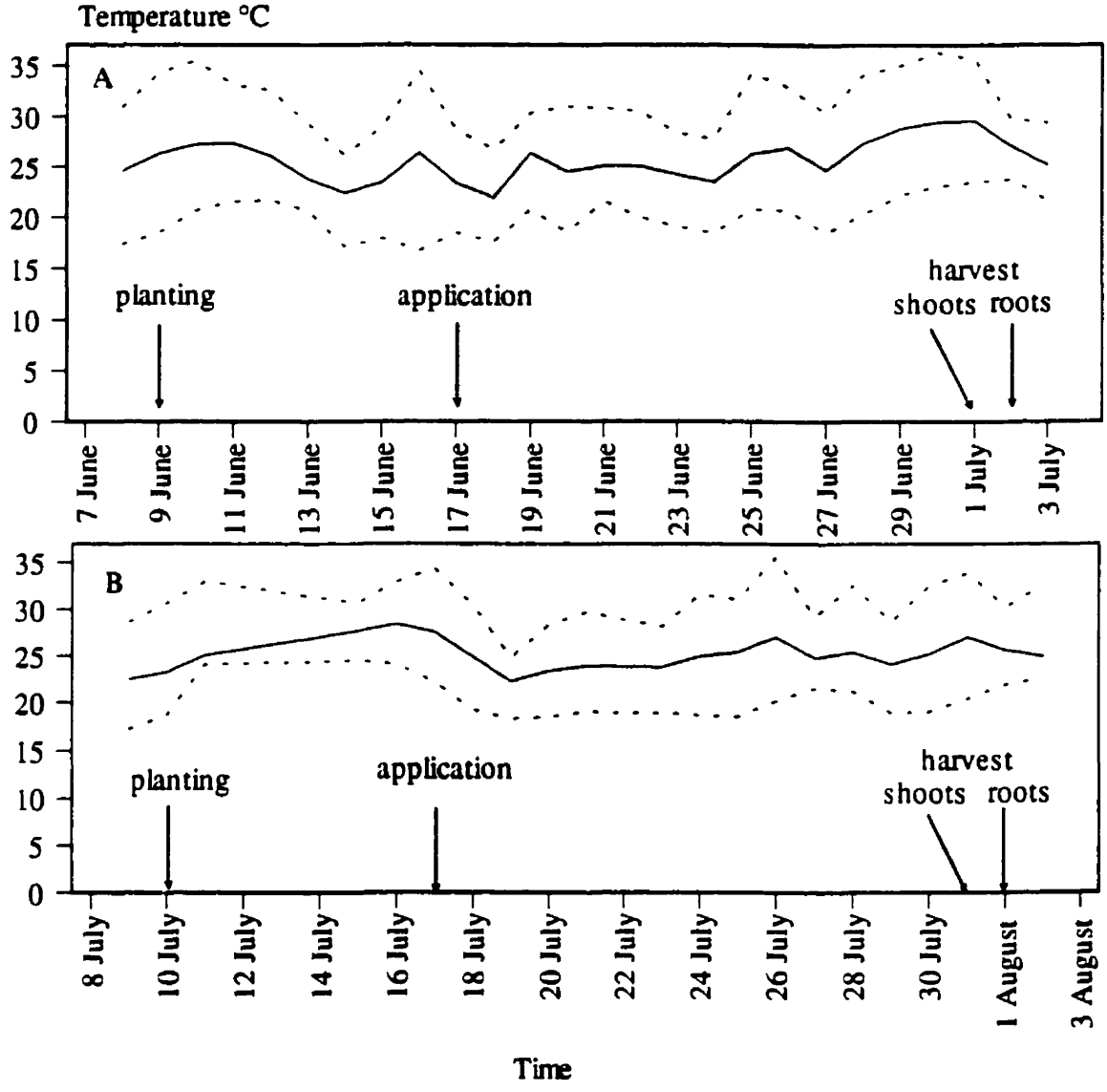
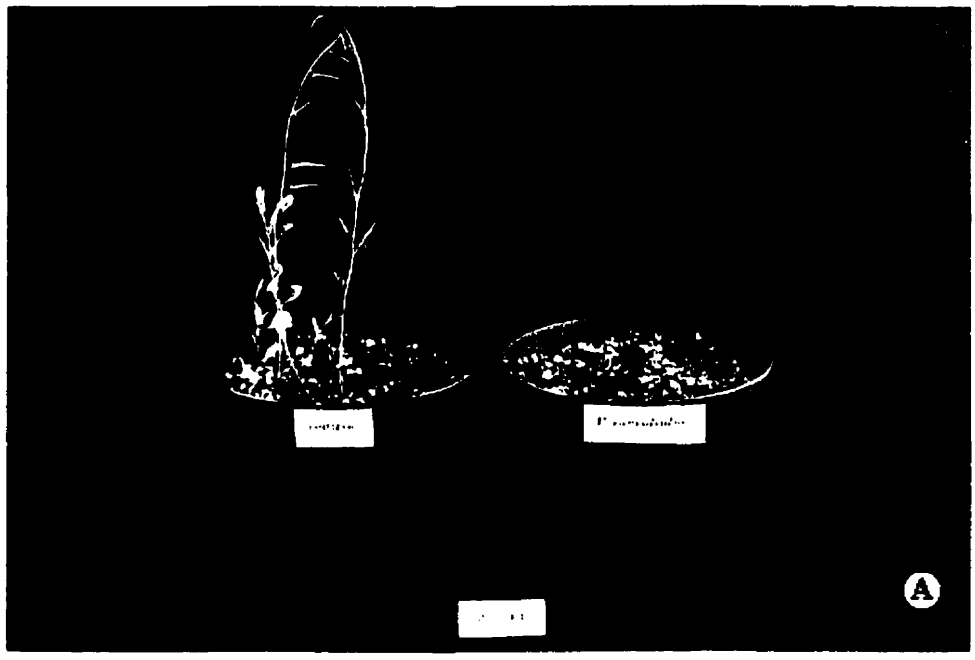

Temperature °C
A
35
30
25
20
15
10
5
0
planting
application
harvest
shoots roots
7 June
9 June
11 June
13 June
15 June
17 June
19 June
21 June
23 June
25 June
27 June
29 June
1 July
3 July
B
35
30
25
20
15
10
5
0
planting
application
harvest
shoots roots
8 July
10 July
12 July
14 July
16 July
18 July
20 July
22 July
24 July
26 July
28 July
30 July
1 August
3 August
Time

Figure 5.3. Effect of pre-emergence application of *Phomopsis convolvulus* on (A) Greece, (B) USA-Montana *Convolvulus arvensis* biotypes. Seven days after planting (DAP), 2.5 g of *P. convolvulus* granules were spread onto the soil surface. At day of harvest (21 DAP) from left to right: uninoculated control, inoculated.


A

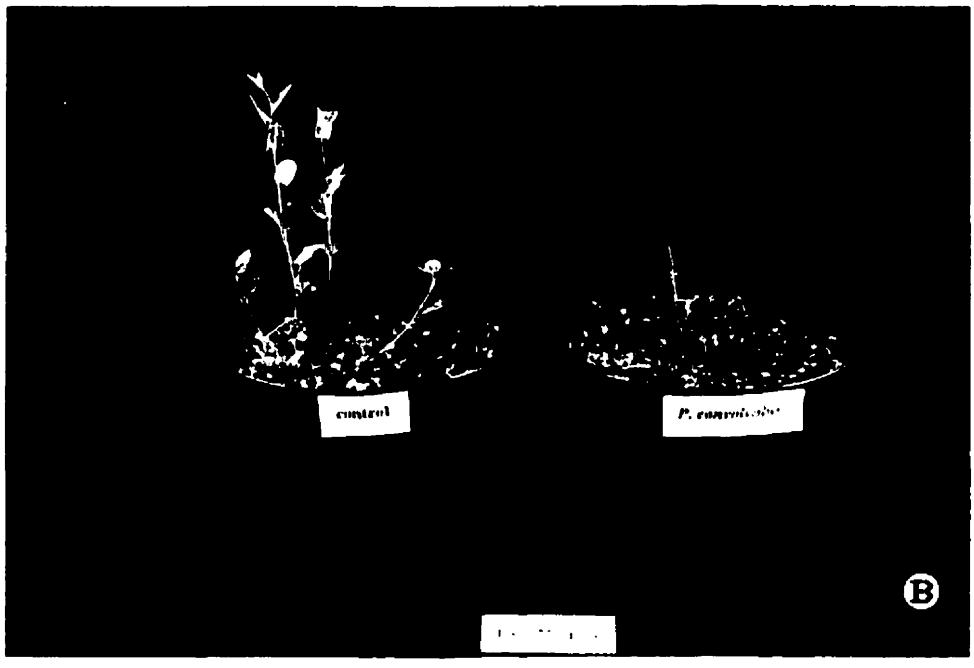

control
B

Figure 5.4. Effect of pre-emergence application of 2.5 g of *Phomopsis convolvulus* inoculum granules applied onto the soil surface of 15-cm-diameter pots 8 (Trial 1) or 7 (Trial 2) days after planting on above-ground biomass of two *Convolvulus arvensis* biotypes in (A) Trial 1, (B) Trial 2. Plant material was harvested 21 days after planting. Vertical bars indicate the standard error of the mean.

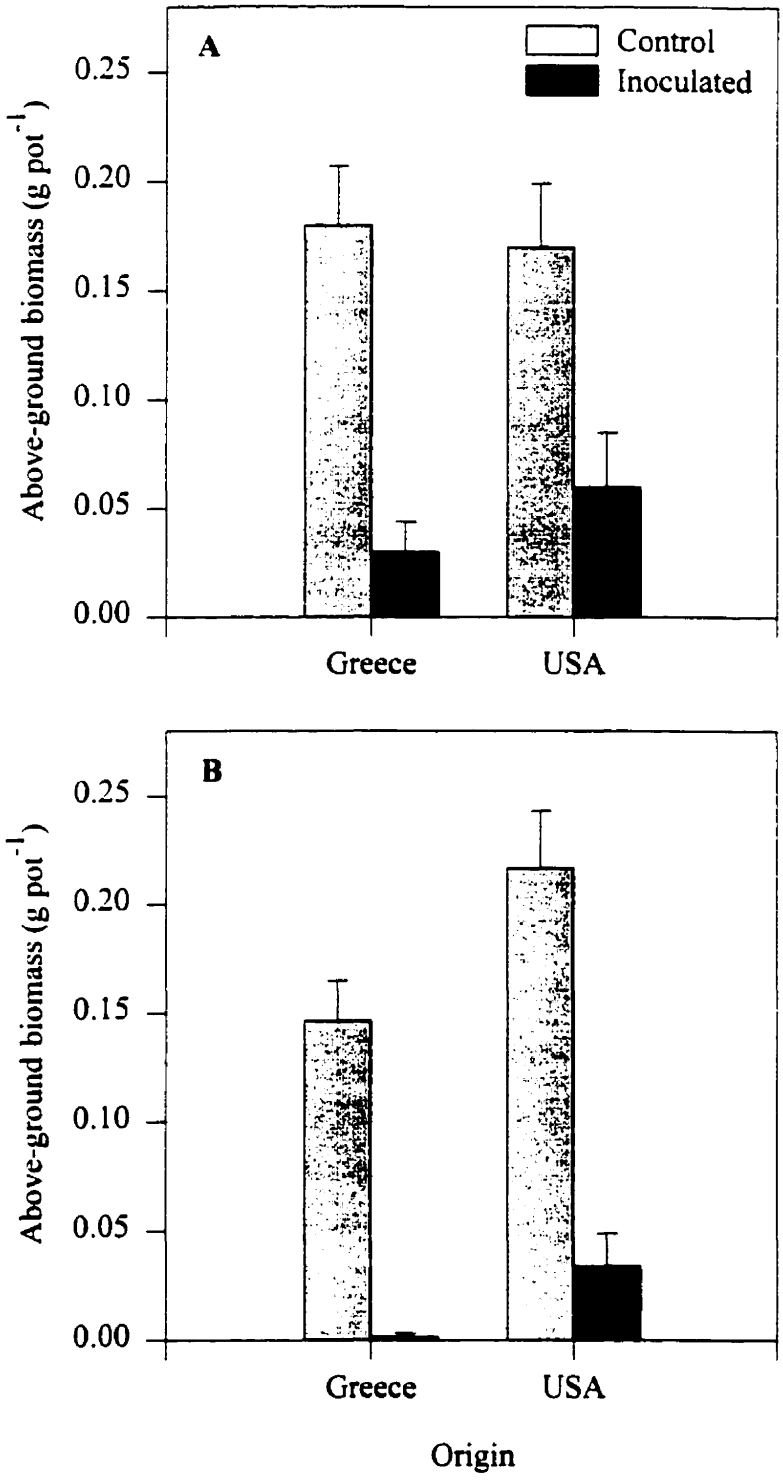
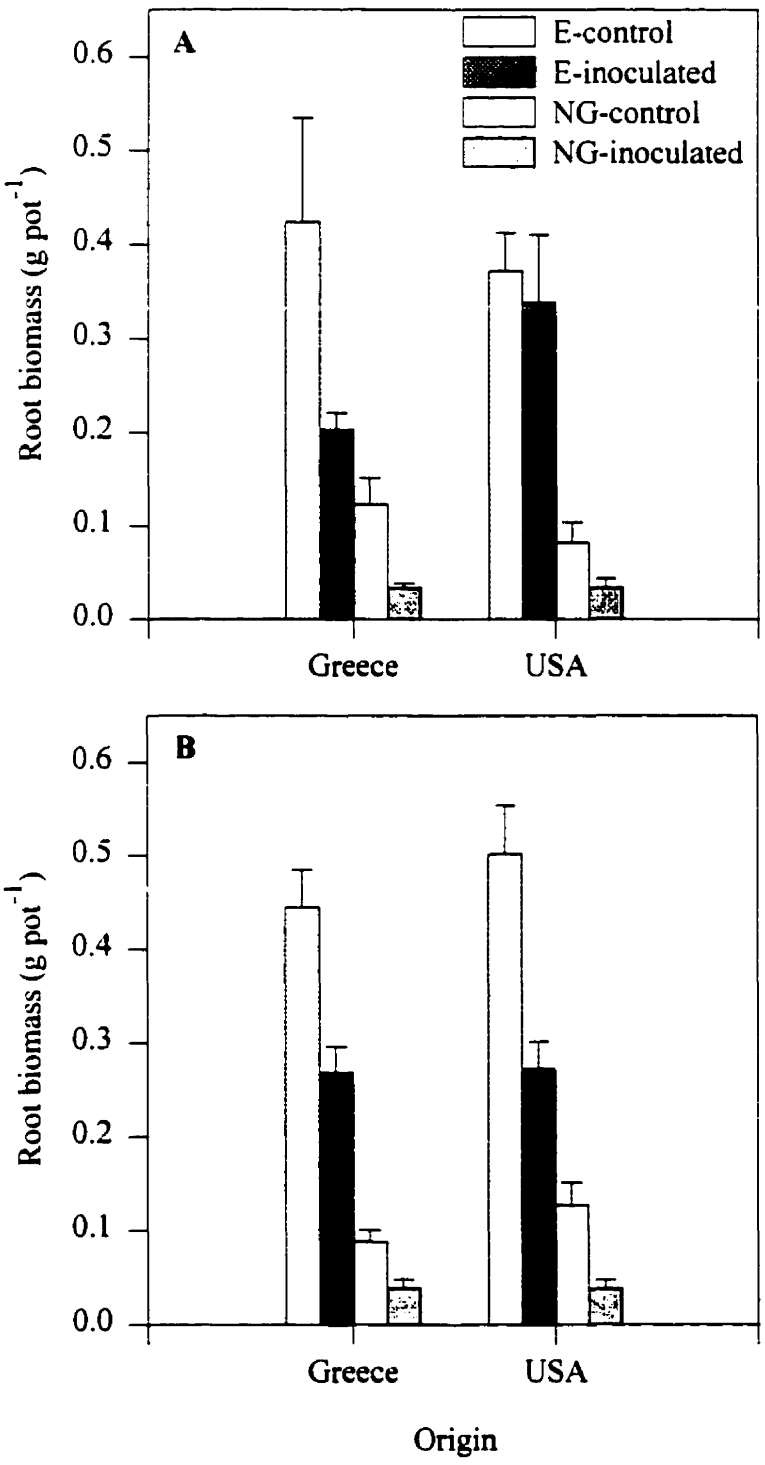

A
Control
Inoculated
Above-ground biomass (g pot-1)
0.25
0.20
0.15
0.10
0.05
0.00
Greece
USA
B
Above-ground biomass (g pot-1)
0.25
0.20
0.15
0.10
0.05
0.00
Greece
USA
Origin

Figure 5.5. Effect of pre-emergence application of *Phomopsis convolvulus* on root biomass of two *Convolvulus arvensis* biotypes in (A) Trial 1, (B) Trial 2. E, NG refer to biomass of entire roots and new root growth, respectively. Application dates and dose as for Figure 5.4. Roots were harvested 22 days after planting. Vertical bars indicate the standard error of the mean.


A
B
E-control
E-inoculated
NG-control
NG-inoculated
Root biomass (g pot-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Greece
USA
Origin

Connecting Text

Field applied granules of *P. convolvulus* can maintain viability and virulence for several days, however, the precise onset of *C. arvensis* emergence can be highly variable, rendering the decision of when to optimally apply the fungal inoculum difficult. Moreover, to date, the efficacy of *P. convolvulus* has only been assessed in *C. arvensis* monoculture populations. A number of studies have demonstrated the more detrimental effect of fungal pathogens on their hosts when grown in mixture with a crop than when grown in pure stands. In the following chapter, the pre-emergence efficacy of *P. convolvulus* inoculum to control *C. arvensis* plants when applied at different dates will be determined as well as the impact of *P. convolvulus* inoculation on *C. arvensis* performance under competitive cropping situations evaluated.

Chapter 6. Effect of application timing and competitive cropping conditions on efficacy of *Phomopsis convolvulus* to control *Convolvulus arvensis*

6.1. Abstract

The efficacy of the pre-emergence applied fungal pathogen, *Phomopsis convolvulus*, to control established *Convolvulus arvensis* was examined at different application dates. Furthermore, control of *C. arvensis* in pure stands was compared with application in mixture with spring wheat (*Triticum aestivum* L.). In a spring field trial conducted over two growing seasons, a granular barley formulation of the fungal inoculum was applied onto the soil containing established *C. arvensis* root stocks. In the first year of the trial, overwintering and emergence of *C. arvensis* plants was poor and insufficient data for appropriate statistical analyses was obtained. In the second year of the trial, overwintering of *C. arvensis* was successful, however, the onset of shoot emergence was earlier than expected, so that only the first inoculum application treatment was truly a pre-emergence application treatment. Nevertheless, above-ground biomass reductions for all fungal inoculation treatments ranged between 26 and 43%. In the second field trial, efficacy of *P. convolvulus* was enhanced in the presence of spring wheat and above-ground biomass of inoculated *C. arvensis* plants was reduced by 98% compared with inoculated *C. arvensis* plants grown in pure stand. However, no significant differences in spring wheat yield were found between *P. convolvulus*-inoculated plots and uninoculated control plots.

6.2. Introduction

Convolvulus arvensis L. (field bindweed) is a serious perennial weed in a number of important crops throughout the world (Holm *et al.* 1977). The move towards reduced cultivation or zero tillage in the past 15 years, has led to an increased prevalence of *C. arvensis* (Phillips *et al.* 1980). Effective control of this weed using current methods including cultivation, crop rotation, and chemical herbicides (Derscheid *et al.* 1970) is often not possible due to its extensive root

system, high competitiveness, and variable susceptibility to several important herbicides (DeGennaro & Weller 1984; Kosinski & Weller 1989; Yerkes & Weller 1996). The occurrence of a foliar pathogen, *Phomopsis convolvulus* Ormeno on *C. arvensis* plants, was first reported by Ormeno-Nuñez in 1988 (Ormeno-Nuñez *et al.* 1988b). Since then, studies on host specificity, conidia mass production, storage, and efficacy of foliar treatments have been carried out (Ormeno-Nuñez *et al.* 1988a,b, Morin *et al.* 1989a,b, Morin *et al.* 1990). In an attempt to overcome the pathogen's requirement for a long dew period during the germination and infection phases, a granular pre-emergence formulation of *P. convolvulus* has been developed and tested. Inoculum produced on pot barley grains and applied onto the soil surface has effectively controlled *C. arvensis* seedlings and established populations under both controlled environment and field conditions (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b).

In previous studies, most pre-emergence applications of *P. convolvulus* inoculum have been carried out during the summer months (July, August). However, depending on local climatic conditions, *C. arvensis* shoot emergence from established stands usually starts in early- to mid-May (Weaver & Riley 1982). The exact onset of shoot emergence in *C. arvensis* cannot be easily predicted, especially if spring weather conditions are highly variable. This might render the decision of when to optimally apply the fungal inoculum difficult. Similarly, timing of pre-emergence applications may be compromised because of cool and wet weather conditions typical at this time of the year in Québec. However, recent research has shown that field applied granules of *P. convolvulus* can remain viable and virulent for several days under a wide range of environmental conditions (Vogelgsang *et al.* 1994, Vogelgsang *et al.* 1998b).

Previous controlled environment and field work has focused on efficacy of *P. convolvulus* only within pure stands of the host plant. As has recently been cautioned by DiTommaso *et al.* (1996), assessing the efficacy of potential biocontrol agents in host monoculture populations may result in significantly different responses than might be obtained when the target weed is grown in the

presence of a competitor crop. Indeed, a number of studies have demonstrated the more detrimental effect of fungal pathogens on their hosts when grown in mixture with a crop than when grown in pure stand (Groves & Williams 1975, Paul & Ayres 1987b, DiTommaso *et al.* 1996). Hence, the objectives of this field study were to: (1) determine the pre-emergence efficacy of *P. convolvulus* inoculum to control *C. arvensis* when applied at different dates, and (2) evaluate the impact of *P. convolvulus* on *C. arvensis* performance under a competitive field cropping situation.

6.3. Materials and methods

6.3.1. Study sites and experimental design

Experiment 1 was performed at the Horticulture Research Centre of Macdonald Campus, Ste-Anne-de-Bellevue, QC, Canada. The soil type was a Chicot fine-sandy loam with 70% sand, 20% silt, 10% clay, a pH of 5.3 and 3% organic matter. A randomized complete block design (RCBD) with five blocks was used. Blocks were arranged so that their length was perpendicular to a slight slope within the field site. In total, 25 0.25 m^2 plots were established each year. The distance between plots and experimental blocks was 0.5 m and 1 m, respectively. In an attempt to reduce rain-splash dispersal of inoculum, garden edgings of 15 cm height were placed between experimental plots, leaving approximately a 9 to 10 cm barrier above the ground.

Experiment 2 was carried out at the Emile Lods Agronomy Research Centre of Macdonald Campus. The soil type was a St. Bernard fine sandy-loam (orthic melanic brunisol) with a pH of 6.8 and 3% organic matter. A RCBD with four blocks was used, and in total, 20 2 m^2 (1 × 2 m) plots were established.

6.3.2. Inoculum and plant production

Unless otherwise stated, granular *P. convolvulus* inoculum and *C. arvensis* plants were produced as described in Chapter 2 for field trials. For Experiment 1, fungal granules were prepared at 1-week intervals 3 weeks before application. For

experiments 1 and 2, 1 g of granular inoculum produced contained 1×10^9 (1996), 8×10^7 to 6×10^8 (1997), and 8×10^8 conidia, respectively. Viability based on germination data was > 85 (Experiment 1) and 96% (Experiment 2).

For the 1996 trial of Experiment 1, five *C. arvensis* plants with 10-15 cm-long roots were transplanted in August 1995 from neighbouring field experiments into each of the 25 0.25m^2 plots and watered as needed. However, due to poor establishment and heavy infestation by tortoise beetles (*Chelymorpha* sp.), emergence in the following spring was extremely low. Hence, for the 1997 trial, plant production was modified. *C. arvensis* seeds (Valley Seed Co., Fresno, CA, USA) were soaked for 20 s in near boiling water and incubated on moist paper towels for 24 to 36 h at 30 ± 1 °C in the dark. Approximately 200 imbibed seeds were sown in June 1996 in each 0.25 m^2 plot at a depth of approximately 5 cm as evenly as possible by hand. During early plant establishment, plots were watered as required. Two weeks after sowing, plant density in each plot was adjusted by thinning to a maximum of 50 plants $plot^{-1}$. All other weed species within plots were removed by hand throughout the trial. Following the first frost in mid-October, all above-ground biomass was cut at ground level to avoid fungal applications on senesced, withered shoots.

In Experiment 2, spring wheat (cultivar 'SS Blomidon') was sown (8 May 1997) at a rate of approximately 120 kg ha^{-1} in rows 20 cm apart using a mechanical seeder. The experimental plot size was 1 × 2 m (i.e. consisting of five wheat rows) and spacing between plots and blocks was 2 m. Five spring wheat border rows (1 m width) were sown as well. Two days after planting, 400 imbibed *C. arvensis* seeds were sown as evenly as possible by hand in between spring wheat rows in 0.64 m^2 (80 cm × 80 cm) subplots. Care was taken not to disturb spring wheat rows. No irrigation was carried out during this trial. Outside the 1 × 2 m mixture plots or within *C. arvensis* monoculture plots, all emerging wheat seedlings were removed by hand.

6.3.3. Inoculation procedure

Experiment 1 was designed as a single-factor experiment involving four different inoculum application dates and one uninoculated control. Following snow melt in each year of the trial and prior to fungal application, the soil surface of experimental plots was broken up using a hand-cultivator in order to avoid wind drift of granular inoculum on an otherwise heavily compacted soil. Thirty g of granular inoculum was uniformly spread by hand on the soil surface. In preceding years, the approximate emergence of natural *C. arvensis* populations occurred near the end of May to beginning of June. Consequently, fungal treatments in 1996 and 1997 were conducted at weekly intervals with the first and last application carried out on the 8/7 and 29/28 of May, respectively. Uninoculated plots served as controls.

Experiment 2 was designed as a three-factor experiment incorporating the presence or absence of crop (spring wheat), weed (*C. arvensis*), and pathogen (*P. convolvulus*). The following treatments (TRT) were evaluated:

TRT	Crop	Weed	Pathogen
(1)	+	+	+
(2)	+	+	-
(3)	+	-	-
(4)	+	-	+
(5)	-	+	+

Five days after sowing (DAS) of *C. arvensis* (15 May), 65 g of inoculum granules were spread onto the soil surface of subplots as evenly as possible by hand. At time of inoculum application, neither spring wheat nor *C. arvensis* seedlings had emerged.

For both experiments, applications were carried out late in the afternoon. Air temperature and precipitation data were obtained from the McGill Meteorological Observation Centre (1.5 km away from field site).

6.3.4. Assessment of efficacy

In Experiment 1, overall foliar necrosis was evaluated at harvest using the following rating system: 0 = no visible symptoms, 1 = less than 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis and 4 = greater than 75% necrosis (Ormeno-Nuñez *et al.*, 1988a). Disease rating was based on a per plot rather than on individual shoot basis. In 1996 and 1997, above-ground biomass per plot was determined on the 27 and 12 of June, respectively. Plants were cut at the soil line, and living tissues dried in paper bags for 4 days at 60 °C, and weighed. Biomass was recorded as total biomass per plot.

In Experiment 2, foliar necrosis was evaluated 20, 27, and 34 DAS of *C. arvensis*. Mortality was assessed 41 DAS by counting the number of seedlings with completely necrotic hypocotyls. Disease rating and mortality were determined for each plant, results pooled, and averaged for each plot. *C. arvensis* above-ground dry biomass was determined on 21 August (104 DAS). Spring wheat was harvested from subplots 1 day after harvest of *C. arvensis* (107 DAS of spring wheat). Both spring wheat above-ground biomass and grain weight per plot were recorded. The number of fully developed heads per subplot was also determined.

6.3.5. Statistical analysis

Experiment 1 was performed twice and Experiment 2 once. Mortality and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test (α = 0.05) (Steel & Torrie 1980). Disease ratings were compared using the Friedman Test, followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel 1978). In Experiment 1, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner *et al.* 1992).

6.4. Results

6.4.1. Experiment 1

6.4.1.1. 1996

C. arvensis shoot emergence was very low with only 11 out of 25 plots showing any growth. In some of the remaining plots, emergence began between the third and fourth application treatments (22/29 May), but in others, no shoots emerged until the second week of June. Throughout the inoculum application period, temperature was extremely variable while precipitation was more constant (Figure 6.1A). Average temperatures ranged between 3 and 20 °C during the application period, with night frost occurring 4 days following the first fungal application.

Due to insufficient numbers of observations, data were not statistically analysed. Nonetheless, typical disease symptoms on *C. arvensis* shoots were observed indicating some persistence of soil applied *P. convolvulus* granules (Table 6.1).

6.4.1.2. 1997

In contrast to 1996, *C. arvensis* emergence from overwintering root stocks occurred in all experimental plots. Emergence began earlier than expected (i.e. between the first and second fungal application (7/14 May)). Consequently, a pre-emergence treatment was carried out only for the first application date, whereas some inoculum granules for the remaining applications were also scattered onto *C. arvensis* foliage. Weather conditions throughout the 1997 application period were generally cool with little precipitation (Figure 6.1B). Average air temperatures during the inoculum application period ranged between 5 and 17 °C.

All plants subjected to the granular inoculum showed disease symptoms, but at very low levels (Table 6.2). However, above-ground biomass reductions were significant for the first three fungal application dates, reaching as high as 43% (second application) compared with uninoculated controls.

6.4.2. Experiment 2

Spring wheat and *C. arvensis* seedlings began to emerge 9 and 6 DAS, respectively. Due to a technical error in using the seeder, emergence of spring wheat was very heterogeneous throughout the experimental area, making it necessary to transplant some seedlings collected from spring wheat border rows by hand into experimental plots (22 May). No precipitation was received for more than 2 weeks after fungal application, and night temperatures dropped as low as 4 °C (Figure 6.2).

Initial efficacy of the fungal inoculum was very low (Table 6.3), however, with rising temperatures and rainfall 3 weeks after sowing, disease symptoms became more apparent, especially in mixed stands with spring wheat. In contrast, disease incidence in inoculated *C. arvensis* plants grown in monoculture remained insignificant. Similarly, mortality of inoculated *C. arvensis* in mixture reached 22% whereas only 9% mortality was recorded in monoculture (Table 6.3). Moreover, the above-ground biomass of inoculated *C. arvensis* plants in mixture was reduced by 86% compared with uninoculated controls (Figure 6.3, Figure 6.4A).

Above-ground biomass of wheat plants grown in mixture with uninoculated *C. arvensis* was reduced by 27% compared with biomass of plants within a weed-free stand (Figure 6.4B). When grown in mixture with diseased *C. arvensis*, wheat yield reduction was only slightly lower (20%) than for the uninoculated treatment. Similarly, the number of heads and grain weight of spring wheat were lowest in mixture plots with uninoculated *C. arvensis*, greater in mixture plots with diseased *C. arvensis*, and highest in weed-free plots (Table 6.4). However, differences were not significant ($P > 0.05$) for any of the parameters measured. Application of fungal granules in pure stands of spring wheat had no significant effect on growth or yield.

6.5. Discussion

In the present study, the efficacy of pre-emergence applied *P. convolvulus* to control *C. arvensis* was examined for different inoculum application dates and in

the absence or presence of a crop. In the 1996 trial of the first experiment, insufficient data were obtained due to poor overwintering of established plants resulting in low overall emergence. Nevertheless, disease symptoms and biomass reductions for inoculated *C. arvensis* plants were observed for all application dates. For 1997, plant establishment improved, but surprisingly emergence onset from *C. arvensis* root stocks within experimental plots occurred between the first and second application dates (7/14 May). The difference between emergence in natural field and experimental *C. arvensis* populations might have been caused by shading from crops and/or other weeds in natural stands, likely delaying growth of overwintering root buds. In experimental fields however, the lack of competition from other plants could have resulted in earlier shoot development. Although emergence of shoots continued until the termination of the experiment, only the first application date (May 7) was considered to be a true pre-emergence treatment. In the remaining three inoculum applications, granules were scattered on emerged *C. arvensis* foliage, which might have resulted in loss of granular inoculum and/or decreased efficacy of the fungal pathogen. Moreover, weather conditions during the 1997 inoculum application period were cool with little precipitation, thus likely reducing disease incidence and subsequent *C. arvensis* suppression.

The presence of a spring wheat crop within a *C. arvensis* population had a strong effect on the efficacy of *P. convolvulus*. In general, disease development in this experiment was delayed, possibly due to the cool, dry weather conditions prevailing shortly after fungal application. However, when grown in mixture with spring wheat, *C. arvensis* growth was substantially suppressed. Within mixed stands of weed and crop, free moisture availability might have been prolonged due to the altered microclimate (i.e. higher humidity due to shading from taller wheat plants) possibly leading to greater disease severity. Similarly, higher plant densities in mixed stands are more favourable to the reproduction and spread of fungal pathogens (Hasan & Ayres 1990). In addition, competition from the neighbouring crop might have provided additional stress for inoculated *C. arvensis* plants.

Indeed, Paul and Ayres (1987a,b) have shown that fungal infection and abiotic stress (i.e. competition from a crop) act additively, resulting in greater damage to the host plant. In contrast, growth of *C. arvensis* plants in monoculture was greater by the time infection was detected, resulting in lower *P. convolvulus* efficacy. The substantial differences in disease incidence observed between inoculated and uninoculated mixture plots were not reflected in greater spring wheat yields. It is possible that intense weed competition had already occurred by the time *C. arvensis* plants had been infected by *P. convolvulus*. A repeat of this experiment as well as trials that will make use of various plant densities may provide additional information as to the importance of the weed-crop-pathogen interactions observed in this preliminary field trial.

The results of this study indicate that timing of pre-emergence *P. convolvulus* inoculum application may be critical for effective control of *C. arvensis*. Furthermore, weed suppression may be greatly enhanced in the presence of crop competition.

Table 6.1. Effect of different *Phomopsis convolvulus* application dates in 1996 on *Convolvulus arvensis* above-ground biomass. [a]

	Above-ground biomass (g plot^{-1})				
	Blocks				
Treatment [b]	I	II	III	IV	V
Control	n.a.[c]	0.07	n.a.	16.05	23.73
A1	3.49	n.a.	n.a.	n.a.	1.70
A2	n.a.	n.a.	n.a.	2.43	0.03
A3	1.03	n.a.	n.a.	0.75	8.87
A4	n.a.	n.a.	n.a.	0.06	n.a.

[a] No statistical analysis was carried out due to a large portion of missing data.

[b] A1, A2, A3, A4: Application of 30 g of *P. convolvulus* granules on 0.25 m^2 plot^{-1} on 8, 15, 22, 29 May 1996, respectively. *C. arvensis* plants were harvested on 27 June 1996.

[c] n.a.: missing data = no emergence of *C. arvensis*.

Table 6.2. Effect of different *Phomopsis convolvulus* application dates in 1997 on *Convolvulus arvensis* disease severity and above-ground biomass.

Treatment [a]	Disease rating [b]			Above-ground biomass ($g\ plot^{-1}$)		
Control	0.2	(0.2)	a	136.8	(12.5)	a
A1	1.0	(0.0)	b	88.6	(9.1)	b
A2	1.0	(0.0)	b	77.4	(7.5)	b
A3	1.0	(0.0)	b	88.2	(8.3)	b
A4	1.0	(0.0)	b	101.4	(9.4)	ab

[a] A1, A2, A3, A4: Application of 30 g of *P. convolvulus* granules on 0.25 m^2 $plot^{-1}$ on 7, 14, 21, 28 May 1997, respectively. *C. arvensis* plants were harvested on 12 June 1997.

[b] Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis. Numbers in parentheses are the standard error of the mean. Means in each column with the same letter are not significantly different, according to the Friedman two-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating) or to Tukey's grouping ($\alpha = 0.05$) (biomass).

Table 6.3. Effect of *Phomopsis convolvulus* on disease severity and mortality of *Convolvulus arvensis* in pure stands or in mixture with spring wheat. [a]

Treatment [b]	Disease rating [c] 28 May	4 June	11 June	Mortality (%)
Ta - Ca	0.10 (0.02) a	0.21 (0.05) a	0.20 (0.05) a	0.8 (0.8) a
Ca - Pc	0.21 (0.02) b	1.10 (0.09) ab	1.33 (0.08) ab	8.5 (3.2) a
Ta - Ca - Pc	0.23 (0.02) b	1.24 (0.04) b	1.84 (0.11) b	22.3 (2.9) b

[a] *C. arvensis* and spring wheat were sown on 8, 11 May, respectively. Application of 65 g *P. convolvulus* granules per 0.64 m^{-2} plot on 15 May. Mortality was determined on 18 June.

[b] Ta, Ca, Pc refer to *Triticum aestivum*, *Convolvulus arvensis*, *Phomopsis convolvulus*, respectively.

[c] Disease rating scale as for Table 6.2. Numbers in parentheses are the standard error of the mean. Means in each column with the same letter are not significantly different, according to the Friedman test followed by a multiple comparison procedure (P = 0.15) (disease rating) or to Tukey's grouping (α = 0.05) (mortality).

Table 6.4. Effect of *Phomopsis convolvulus* and *Convolvulus arvensis* on spring wheat yield. [a]

Treatment [b]	Heads subplot^{-1}	Total grain weight (g subplot^{-1})	Grain weight head^{-1} (g)
Ta - Ca	165.3 (10.1) [c]	77.6 (13.0)	0.46 (0.06)
Ta - Ca - Pc	182.8 (5.1)	84.6 (11.4)	0.46 (0.04)
Ta - Pc	187.0 (12.8)	102.9 (17.6)	0.55 (0.09)
Ta	210.5 (15.1)	108.4 (20.3)	0.51 (0.09)

[a, b] As for Table 6.3. Spring wheat was harvested on 22 August.

[c] Numbers in parentheses are the standard error of the mean. No significant differences for all parameters according to Tukey-grouping ($\alpha = 0.05$).

Figure 6.1. Precipitation and air temperatures during Experiment 1 in (A) 1996, (B) 1997 field trials. Temperatures are daily means.

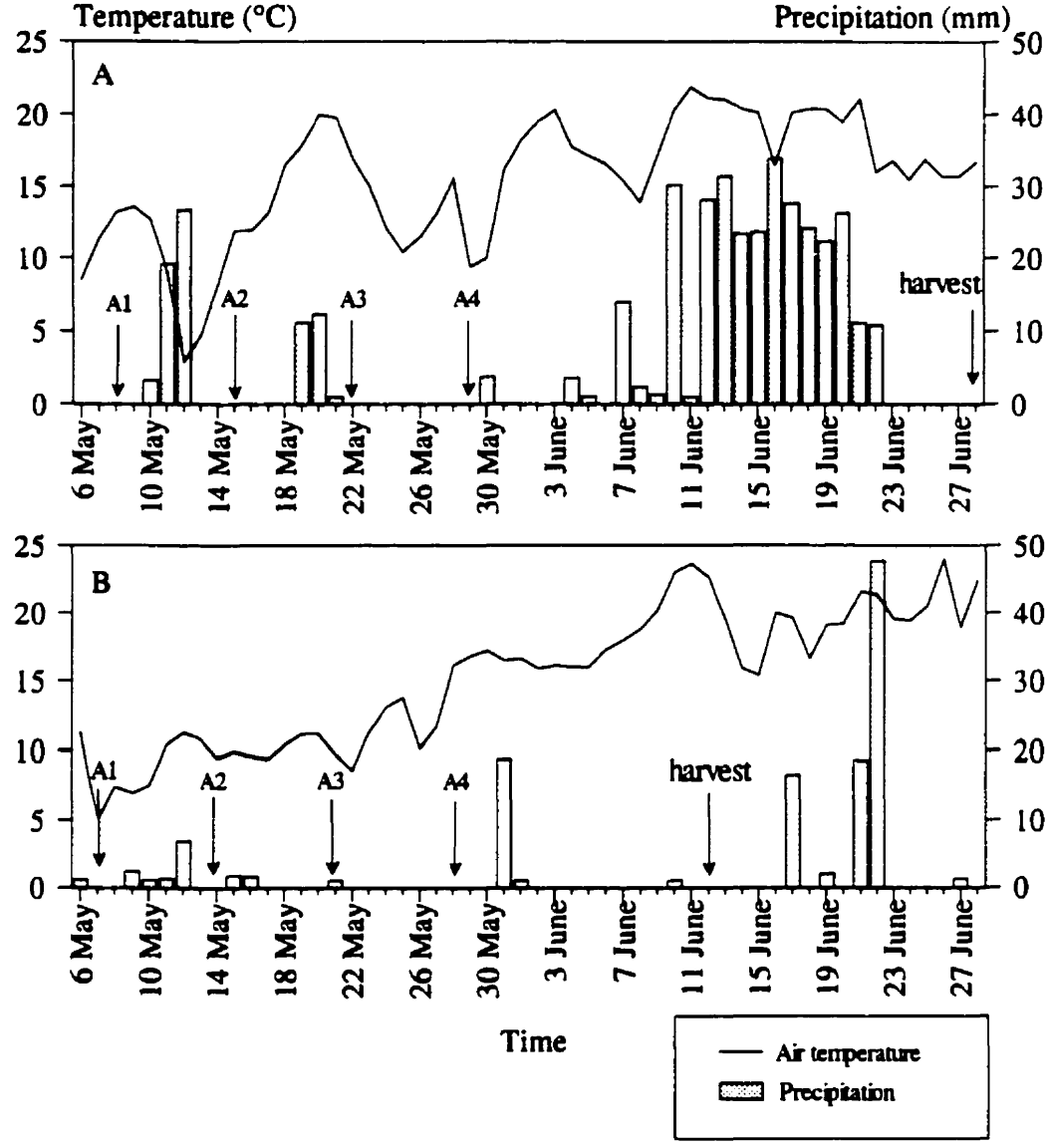

Temperature (°C)
Precipitation (mm)
A
B
A1
A2
A3
A4
harvest
6 May
10 May
14 May
18 May
22 May
26 May
30 May
3 June
7 June
11 June
15 June
19 June
23 June
27 June
Time
Air temperature
Precipitation

Figure 6.2. Total precipitation and mean air temperatures at 7-day intervals from sowing (08-10 May) to harvest (20-21 August) during Experiment 2. Spring wheat and *Convolvulus arvensis* seedling emergence occurred 9 and 6 days after sowing, respectively.

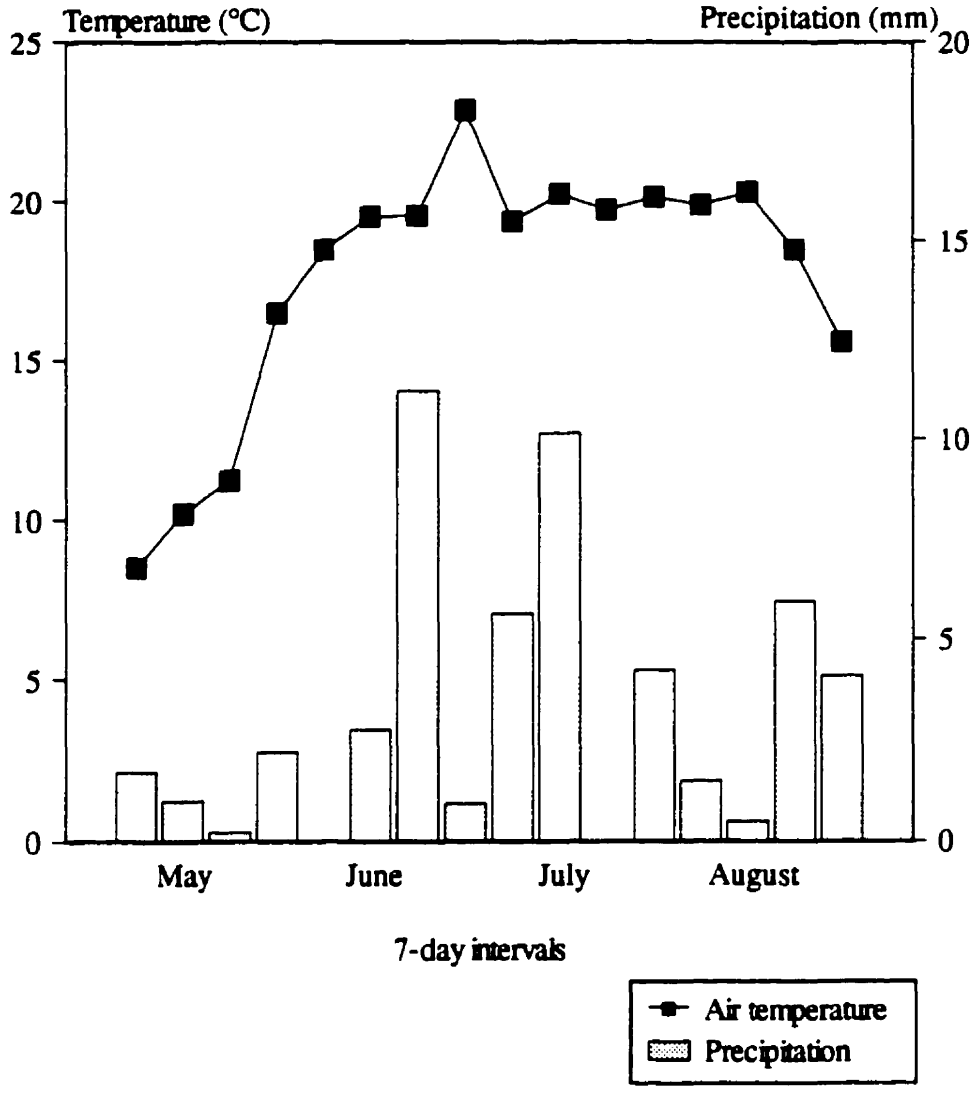

Temperature (°C)
Precipitation (mm)
25
20
15
10
5
0
20
15
10
5
0
May
June
July
August
7-day intervals
Air temperature
Precipitation

Figure 6.3. Effect of *Phomopsis convolvulus* on *Convolvulus arvensis* in spring wheat. Thirty g of *P. convolvulus* granules were applied 5 days after sowing (DAS) of *C. arvensis*. At day of harvest (104 DAS): (A) uninoculated control, (B) inoculated.


A

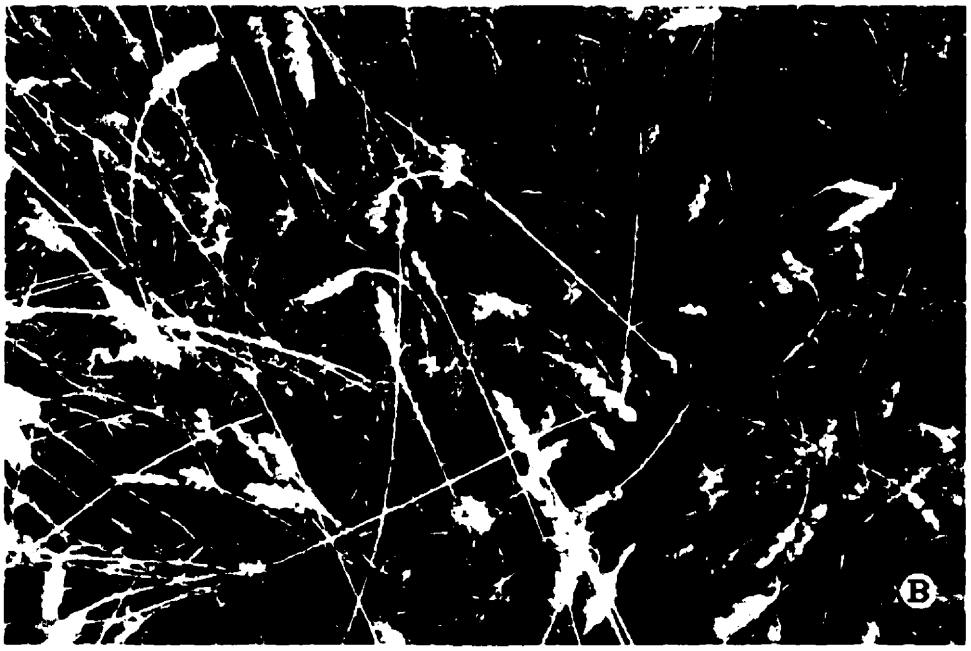
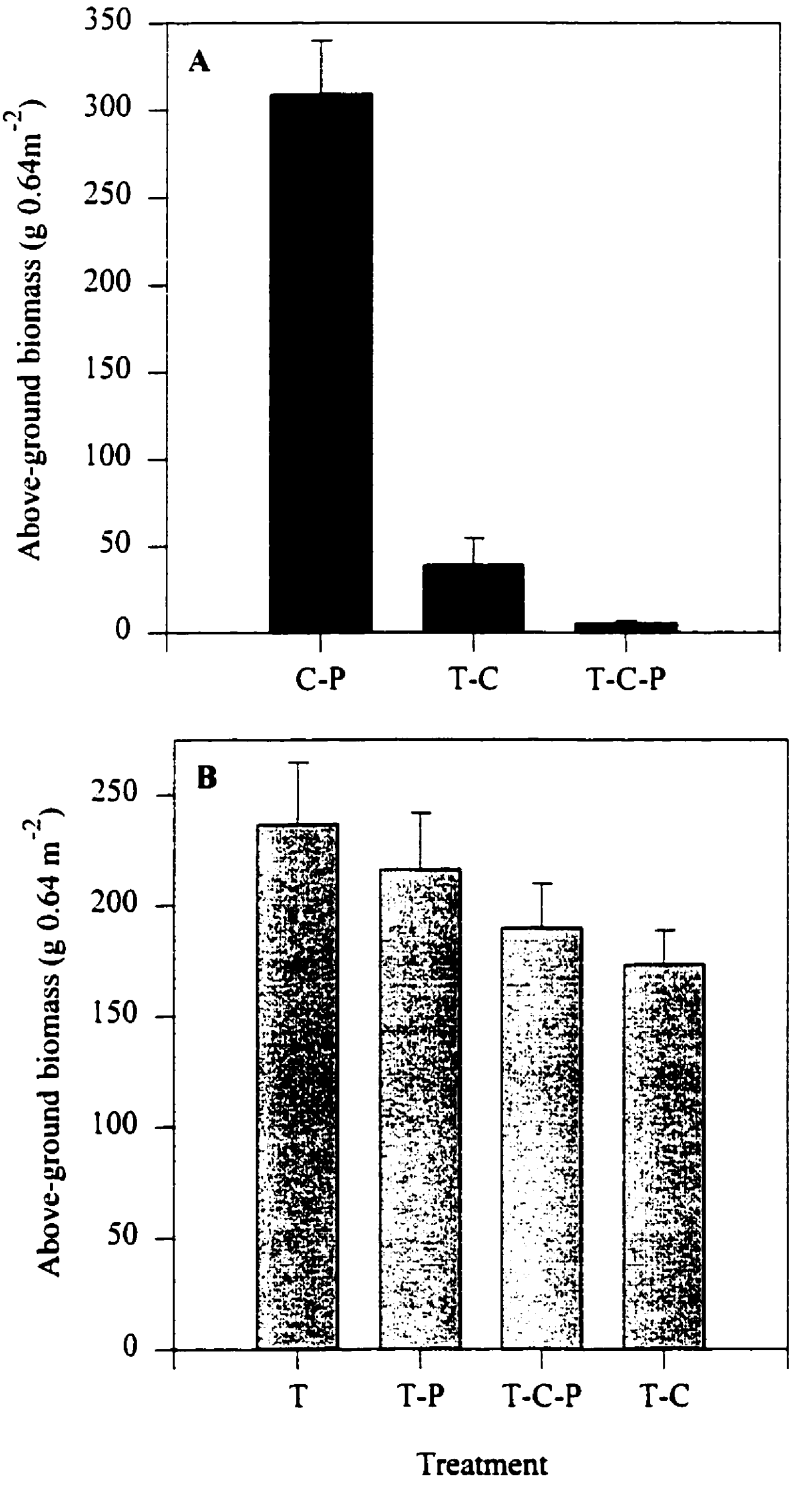

B

Figure 6.4. Effect of pre-emergence field application of *Phomopsis convolvulus* on (A) *Convolvulus arvensis* and (B) spring wheat above-ground biomass in monoculture or mixture. T, C, P: *Triticum aestivum*, *Convolvulus arvensis*, *Phomopsis convolvulus*. Sixty-five g of *P. convolvulus* granules were applied on 0.64 m^2 $plot^{-1}$ 4 days after sowing. Vertical bars indicate the standard error of the mean.


A
Above-ground biomass (g 0.64m-2)
350
300
250
200
150
100
50
0
C-P
T-C
T-C-P
B
Above-ground biomass (g 0.64 m-2)
250
200
150
100
50
0
T
T-P
T-C-P
T-C
Treatment

Chapter 7. General Conclusions

The research reported in this thesis investigated the pre-emergence potential of the fungal pathogen, *Phomopsis convolvulus*, to suppress *Convolvulus arvensis*. Experiments were designed to gain additional information on how to render applications of this potential bioherbicide practically and economically feasible while achieving high levels of weed control.

This study showed that a granular formulation of *P. convolvulus* applied in the field was capable of withstanding unfavourable weather conditions, thus eliminating the requirement for long dew periods or moderate ambient temperatures during the infection phase in order to achieve high efficacy.

Under field conditions, granular applications of *P. convolvulus* resulted in dramatic *C. arvensis* biomass reductions of both seedlings and plants from established stands. This finding is important given that, intact or fragmented roots are the major source from which above- and below-ground biomass develop.

The level of weed control obtained following field applications is often largely dependent on environmental conditions, however, infective propagules of soil applied bioherbicides might be better protected by incorporating inoculum into the soil. Under field conditions, pre-emergence surface applications of *P. convolvulus* provided better control of *C. arvensis* compared with inoculum that was soil incorporated, thus reducing the number of steps necessary for application.

The high dose of *P. convolvulus* applied to the soil in earlier studies represented a practical and economical constraint for field applications. When rates of soil applied granules were reduced to one third of the original dose (10 g 0.25 m^{-2}) disease development and subsequent control of *C. arvensis* were unaffected. However, further inoculum dose reductions should be evaluated in order to decrease input costs of granule applications.

Efficacy of *P. convolvulus* was generally lower under controlled environment conditions than in the field, an unusual phenomenon in bioherbicide research. Experiments designed to evaluate the impact of moisture availability, inoculum

production method, and planting substrate on disease development showed a strong influence of the planting substrate used for growing *C. arvensis* seedlings. Disease in plants grown in a sandy loam field soil was more pronounced than for plants grown in a commercially prepared peat moss medium. Experiments that will make use of a greater number of planting substrates may provide additional information as to the impact of this critical factor on the performance of *P. convolvulus*.

The efficacy of *P. convolvulus* should not only be limited to host plants found in a narrow geographic region but this impact should be evident in host plant biotypes from as many regions of the world as possible. Therefore, the susceptibility of morphologically variable *C. arvensis* biotypes from different geographic locations to disease caused by *P. convolvulus* was evaluated. Following foliar *P. convolvulus* post-emergence applications on single excised shoots, differences in disease reaction of 11 *C. arvensis* biotypes were minor. Pre-emergence fungal applications on established plants from cloned root stocks of two selected *C. arvensis* biotypes (Greece, USA), resulted in equally high disease incidence and excellent weed control. Consequently, P. *convolvulus* may be used for control of various *C. arvensis* biotypes, but further research and government approval is required before this biotic agent can be introduced into other countries.

Timing of *P. convolvulus* pre-emergence applications may be critical for effective control of *C. arvensis*. Thus, *P. convolvulus* was applied at different dates onto soil where root stocks of overwintered, established *C. arvensis* plants were located. Disease symptoms and biomass reductions of inoculated *C. arvensis* were observed for all application dates, however, insufficient data were obtained in one of the field years due to poor emergence.

A number of bioherbicide studies have demonstrated the greater detrimental effect of fungal pathogens on their hosts when grown in mixture with a crop than when grown in pure stand. Therefore, control of *C. arvensis* in pure stands was compared with control when grown in mixture with spring wheat. Although

differences in spring wheat yield were minor, efficacy of *P. convolvulus* was greater in the weed-crop mixture than for inoculated *C. arvensis* plants grown in pure stand.

Chapter 8. Contributions to Knowledge

The following are considered to be key contributions to original knowledge arising from the research described in this thesis:

1. This is the first report of pre-emergence soil applications of *Phomopsis convolvulus* on both *Convolvulus arvensis* seedlings and regrowth from established plants under field conditions, indicating effective control of both growth stages.

2. Findings in this study demonstrated that under field conditions, surface applications of *P. convolvulus* provide better control of *C. arvensis* compared with inoculum that is incorporated into the soil.

3. Reduced rates of *P. convolvulus* granules (10 g 0.25 m^{-2}) applied to the soil had a similar effect on disease development and subsequent control of *C. arvensis* in both controlled environment and field trials as relatively greater inoculum rates (i.e. 30 g 0.25 m^{-2}).

4. Under controlled environment conditions, the type of planting substrate used for growing *C. arvensis* seedlings had a strong influence on the performance of the fungal pathogen, *P. convolvulus*. Disease in plants grown in a sandy loam field soil was more pronounced than in plants grown in a peat moss medium.

5. This is the first study to have examined the susceptibility of *C. arvensis* biotypes from various geographical regions to the fungus *P. convolvulus*, indicating limited differential susceptibility despite important morphological variations among some of the biotypes investigated.

6. This study demonstrated that timing of *P. convolvulus* inoculum granule applications is crucial if effective control of overwintered *C. arvensis* is to be achieved.

7. This is the first report of *P. convolvulus* being evaluated under competitive field cropping conditions using spring wheat (*Triticum aestivum* L.). Efficacy of *P. convolvulus* was greatly enhanced in the weed-crop mixture compared with inoculated *C. arvensis* plants grown in pure stand.

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Appendix 1.1. Fungal pathogens associated with *Convolvulus arvensis* collected in Germany

Collection of plant material, isolation of pathogens, and transportation

These studies were carried out in collaboration with the Institut für Phytomedizin, Universität Hohenheim, Germany in spring 1994. At 7 locations in South-West Germany, diseased *C. arvensis* plant parts were collected. Leaves and stems were placed between paper towels in plastic bags and kept in a cooler until returning to the laboratory in Hohenheim. Immediately upon arrival, plant material was stored in a cold room (4 °C) in the dark for 1 to 2 days.

Leaves and stems with disease symptoms were excised, soaked in 70% ethanol for a few seconds, rinsed in sterile, deionized water, and 1-cm^2 tissue sections were cut using a flame-sterilized scalpel. Tissue sections were then immersed for 2 min in 1% sodium hypochlorite, subsequently rinsed in sterile water and placed on sterile filter paper to dry. Leaf sections were transferred into Petri dishes containing half strength potato dextrose agar (PDA; Difco, Detroit, MI, USA). Plates were placed in an incubator (Ehret Co., Emmendingen, Germany) at 25 °C in the dark. Advancing edges from growing colonies were transferred to full strength PDA, and incubated for 4 weeks at room temperature (21 ± 2°C) and 12 h day^{-1} near-ultraviolet light (F26W-BLB, Sylvania Co., Japan).

For transportation to Canada, 140 fungal isolates were transferred into test tubes containing V8-juice agar amended with 300 mg chloramphenicol L^{-1}. Cultures were then sent by air freight with the appropriate import permit to the quarantine laboratory on the Macdonald Campus of McGill University.

Storage and pathogenicity tests

Upon arrival, fungal isolates were placed in long-term storage in either glass bottles (2 × 7 cm) containing half strength PDA at 4 °C or in glass test tubes (1.5 × 12.5 cm) containing a soil mixture at room temperature. A loamy sand was passed through a soil sieve (mesh size 2 mm) and mixed with 1% (w/w) ground rolled

oats. Tubes were filled three quarters of their length with the soil/flour mixture and 3 ml destilled water were added. Tubes were sealed with a cotton plug, autoclaved twice for 45 min with 48 h in between the autoclaving cycles, and inoculated.

For pathogenicity tests of the German isolates, four pre-germinated seeds were sown at a depth of 3 cm into 10-cm-diameter plastic pots containing a commercial prepared potting medium (Pro-Mix™ BX, Les Tourbières Premier Ltée, Rivière-du-Loup, QC, Canada). Pots were placed in a growth chamber (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 $\mu Em^{-2}s^{-1}$). Small pieces of mycelia from agar slants or colonized soil granules were placed in Petri dishes containing PDA or V8-juice agar and incubated for 4 weeks at room temperature (21 ± 2 °C) and 12 h day^{-1} near-ultraviolet light (F40 BLAB Blacklight, General Electric Lighting, Cleveland, OH, USA). Mycelia and/or spores were harvested by flooding the plates with 15 ml deionized water and scraping the colony surface with a spatula. Resulting suspensions were poured through a plastic funnel lined with two layers of cheesecloth and the filtrate paint brushed on 2-week old *C. arvensis* seedlings (3-5 leaf stage). Inoculated plants were placed in a dew chamber (24 °C, 100% RH) for 24 hours and subsequently transferred to a growth chamber under the original conditions. Plants were examined for disease symptoms weekly for 2 weeks. Koch's postulates were verified for those fungal isolates which repeatedly produced disease symptoms. After 3 to 4 weeks, leaf or stem sections were surface sterilized and placed on PDA plates as described above. The characteristics of the re-isolated fungal colonies were compared with those from original colonies that had been used for initial inoculation of *C. arvensis*. Fungi, that were non-pathogenic to *C. arvensis* were discarded.

Out of the 140 isolates, 101 were tested for pathogenicity. The remaining isolates showed either no or insufficient growth on both media used. Among 19 isolates that repeatedly produced disease symptoms, isolate VI 2/2, a *Bipolaris* species, was the most pathogenic (Appendix 1.2, Figure A1).

Appendix 1.2. Cultural characteristics on V8-agar, and resulting disease symptoms of German fungal isolates pathogenic to *Convolvulus arvensis*.

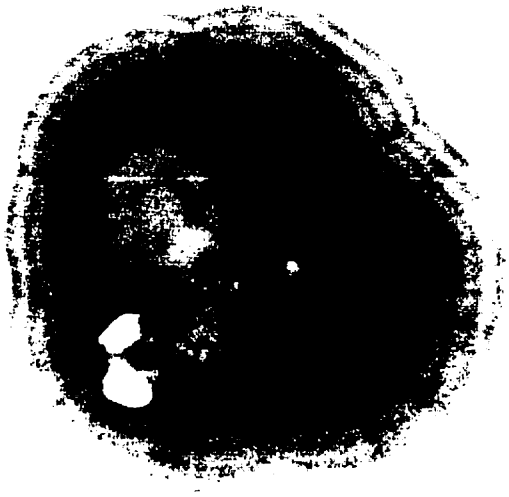
Isolate [a]	Genera	Cultural characteristics	Disease symptoms[b]
II 2/1	*Phoma*	rose mycelia, pycnidia with multiple ostioles, hyaline, cylindrical-oblong spores (5 × 3 µm)	CN
II 4/1	*Colletotrichum*	black acervuli, hyaline, falcate spores (18 × 3 µm)	PL
IV 1a/1	*Fusarium*	orange-beige and pink mycelia, clavate, septate macroconidia (38-43 × 5 µm)	IL, LD, PL
IV 1a/3	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (20-35 × 8-13 µm)	IC, IL, NL, NT, PL
IV 2a/4	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (30-38 × 13-20 µm)	IL
IV 2b/1	*Fusarium*	orange-light brown and pink mycelia, clavate, septate macroconidia (38-43 × 5 µm)	IL, PL
IV 2b/2	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (20-30 × 10-13 µm)	IL, LD
IV 2b/10	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (25-35 × 10-15 µm)	NT, PL
IV 2b/12	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (20-33 × 8-13 µm)	LD, NT, PL
IV 2b/13	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (20-43 × 13-15 µm)	IC, IL
IV 2c/2	*Fusarium*	orange-cream mycelia, orange sporodochia, clavate, septate macroconidia (25-38 × 4-5 µm)	LD, IL, PL
V 5	*Colletotrichum*	grey mycelia, black acervuli, beige or rose sporodochia, hyaline, cylindrical spores (5 × 3 µm)	PL
VI 2/2	*Bipolaris*	black culture with spotted rose mycelia, phaeo-, fusiform, spindle-shaped phragmo-spores (45-65 × 15-23 µm)	LD, IL, PL

Appendix 1.2. continued

Isolate [a]	Genera	Cultural characteristics	Disease symptoms[b]
VI 2/4	*Fusarium*	beige-cream mycelia, orange sporodochia, clavate, septate macroconidia (43-55 × 3-4 µm)	IL, PL
VI 2/7	*Alternaria*	grey to black mycelia, phaeo-, dictyospores (25-40 × 13 µm)	IL
VI 2/10	*Bipolaris*	black culture with spotted rose mycelia, phaeo-, fusiform, spindle-shaped phragmo-spores (45-73 × 15-23 µm)	LD, LMN, PC, PL
VI 2/13	*Alternaria*	grey to black or dark green mycelia, phaeo-, dictyospores (30-38 × 10-13 µm)	PL
VI 2/14	*Bipolaris*	black culture with spotted rose mycelia, phaeo- fusiform, spindle-shaped phragmospores (33-50 × 10-15 µm)	LMN, PC
VII 1/1	*Colletotrichum*	grey-white mycelia, black acervuli with setae, hyaline, cylindrical or allantoid spores (4-5 × 2-3 µm)	LMN, PL

[a] II: Zainingen, Swabian Albs; Swabia; IV: Rastatt, Swabia; V: Offenburg, Black Forest; VI: Schramberg, Black Forest; VII: Ballendorf, Rhineland-Palatinate.
[b] CN: concentric shaped lesions; IL: irregular shaped lesions LD: leaf deformations; LMN: leaf margin necrotic; NT: necrotic tip; PC: pinpoint chloroses; PL: pinpoint lesions.

Figure A1. (A) Two-week old culture on V8-agar and (B) spores of a *Bipolaris* isolate pathogenic to *Convolvulus arvensis*.



A

Appendix 2.1. Effect of pre-emergence applied *Phomopsis convolvulus* on disease severity and growth of *Convolvulus arvensis* seedlings and established plants. Trial 2 [a]

Plant stage	Treatment [b]	Above-ground biomass (g pot^{-1}) [c]		Root biomass (g pot^{-1})		Disease rating [d]	
Seedlings	Control	0.074 (0.014)	a	0.044 (0.008)	a	0.13 (0.13)	a
	0 DAS	0015 (0.004)	b	0.008 (0.001)	b	2.75 (0.18)	ab
	1 DAS	0.011 (0.008)	b	0.011 (0.004)	b	3.19 (0.59)	b
	2 DAS	0.001 (0.001)	b	0.005 (0.001)	b	3.81 (0.19)	b
Established	Control	0.501 (0.057)	a	1.387 (0.210)	a	0.03 (0.03)	a
plants	0 DAS	0.456 (0.076)	ab	1.634 (0.176)	a	0.75 (0.07)	ab
	1 DAS	0.227 (0.042)	b	1.324 (0.196)	a	1.02 (0.18)	b
	2 DAS	0.213 (0.076)	b	1.074 (0.095)	a	1.24 (0.20)	b

[a] Trials were not combined because variances were not homogeneous.

[b] 0 DAS, 1 DAS, 2 DAS: Application of 1 g of *P. convolvulus* granules plot^{-1} at 0, 1, 2 days after sowing, respectively.

[c] Numbers in parentheses are the standard error of the mean. For each plant stage, means in each column with the same letter are not significantly different, according to Tukey's grouping ($\alpha = 0.05$) (biomass) or the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure ($P = 0.15$) (disease rating).

[d] Disease rating scale is 0 = no visible foliar symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.

Appendix 2.2A. Analysis of variance for the effect of plant stage and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 1

Source	d.f.	Mean square	F value	Significance level
Plant stage (P)	1	6.1390	514.90	0.0001
Application (A)	3	0.1276	10.70	0.0001
P x A	3	0.0657	5.51	0.0050
Error	24	0.0119		

Appendix 2.2B. Analysis of variance for the effect of plant stage and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 2

Source	d.f.	Mean square	F value	Significance level
Plant stage (P)	1	0.8392	99.98	0.0001
Application (A)	3	0.0628	7.48	0.0011
P x A	3	0.0324	3.86	0.0219
Error	24	0.0084		

Appendix 2.3A. Analysis of variance for the effect of plant stage and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. Trial 1

Source	d.f.	Mean square	F value	Significance level
Plant stage (P)	1	529.4745	20.95	0.0001
Application (A)	3	99.4484	3.93	0.0169
P x A	3	40.6158	1.61	0.2071
Error	32	25.2742		

Appendix 2.3B. Analysis of variance for the effect of plant stage and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. Trial 2

Source	d.f.	Mean square	F value	Significance level
Plant stage (P)	1	31.8087	32.90	0.0001
Application (A)	3	51.9235	53.71	0.0001
P x A	3	24.2043	25.04	0.0001
Error	32	0.9667		

Appendix 3.1A. Analysis of variance for the effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 1

Source	d.f.	Mean square	F value	Significance level
Application	3	0.0133	39.72	0.0001
Error	12	0.0003		

Appendix 3.1B. Analysis of variance for the effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 2

Source	d.f.	Mean square	F value	Significance level
Application	3	0.0085	21.75	0.0001
Error	12	0.0004		

Appendix 3.2A. Analysis of variance for the effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. 1996 Trial 1

Source	d.f.	Mean square	F value	Significance level
Application	2	5.1374	18.23	0.0002
Error	12	0.2818		

Appendix 3.2B. Analysis of variance for the effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. 1996 Trial 2

Source	d.f.	Mean square	F value	Significance level
Application	2	29.5861	244.10	0.0001
Error	12	0.1212		

Appendix 3.2C. Analysis of variance for the effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. 1997 Trial 1

Source	d.f.	Mean square	F value	Significance level
Application	2	0.3970	9.71	0.0031
Error	12	0.0409		

Appendix 3.2D. Analysis of variance for effect of surface applied versus soil incorporated *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under field conditions. 1997 Trial 2

Source	d.f.	Mean square	F value	Significance level
Application	2	1.6448	38.97	0.0001
Error	12	0.0422		

Appendix 4.1A. Analysis of variance for the effect of moisture availability and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 1

Source	d.f.	Mean square	F value	Significance level
Moisture (M)	1	0.0020	9.48	0.0051
Application (A)	3	0.0044	21.16	0.0001
M x A	3	0.0004	2.11	0.1249
Error	24	0.0002		

Appendix 4.1B. Analysis of variance for the effect of moisture availability and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 2

Source	d.f.	Mean square	F value	Significance level
Moisture (M)	1	0.0014	6.43	0.0182
Application (A)	3	0.0050	23.27	0.0001
M x A	3	0.0012	5.52	0.0050
Error	24	0.0002		

Appendix 4.2A. Analysis of variance for the effect of inoculum production method and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 1

Source	d.f.	Mean square	F value	Significance level
Inoculum	4	0.0028	50.16	0.0001
Error	15	0.0001		

Appendix 4.2B. Analysis of variance for the effect of inoculum production method and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 2

Source	d.f.	Mean square	F value	Significance level
Inoculum	4	0.0044	25.71	0.0001
Error	15	0.0002		

Appendix 4.3A. Analysis of variance for the effect of planting substrate and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 1

Source	d.f.	Mean square	F value	Significance level
Substrate (S)	1	0.0003	13.68	0.0011
Application (A)	3	0.0015	64.57	0.0001
S x A	3	0.0000	1.52	0.2345
Error	24	0.0000		

Appendix 4.3B. Analysis of variance for the effect of planting substrate and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass under controlled environment. Trial 2

Source	d.f.	Mean square	F value	Significance level
Substrate (S)	1	0.0009	5.09	0.0334
Application (A)	3	0.0135	77.14	0.0001
S x A	3	0.0015	8.69	0.0004
Error	24	0.0002		

Appendix 5.1A. Analysis of variance for the effect of biotype and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass. Trial 1

Source	d.f.	Mean square	F value	Significance level
Biotype (B)	1	0.0005	0.18	0.6766
Application (A)	1	0.0824	27.51	0.0001
B x A	1	0.0031	1.04	0.3224
Error	16	0.0030		

Appendix 5.1B. Analysis of variance for the effect of biotype and application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass. Trial 2

Source	d.f.	Mean square	F value	Significance level
Biotype (B)	1	0.0113	7.18	0.0164
Application (A)	1	0.1405	89.06	0.0001
B x A	1	0.0026	1.62	0.2214
Error	16	0.0016		

Appendix 6.1. Analysis of variance for the effect of timing of *Phomopsis convolvulus* application on *Convolvulus arvensis* above-ground biomass.

Source	d.f.	Mean square	F value	Significance level
Timing	4	2655.7600	5.88	0.0027
Error	20	451.8600		

Appendix 6.2. Analysis of variance for the effect of competition and/or application of *Phomopsis convolvulus* granules on *Convolvulus arvensis* above-ground biomass.

Source	d.f.	Mean square	F value	Significance level
Treatment	2	110975.1221	70.21	0.0001
Error	9	1580.5551		