# DEVELOPMENT OF THE FIELD BINDWEED BIOHERBICIDE; *PHOMOPSIS CONVOLVULUS*: SPORE PRODUCTION AND DISEASE DEVELOPMENT.

by

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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**PHOMOPSIS CONVOLVULUS.**

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## FOREWORD

This thesis is submitted in the form of original papers suitable for journal publications. The first section is a general introduction presenting the theory and previous knowledge on this topic. The next three sections represent the body of the thesis (each are a complete manuscript). The last section is a general discussion and a synthesis of the major conclusions. This thesis format has been approved by the Faculty of Graduate Studies and Research, McGill University, and follows the conditions outlined in the Guidelines Concerning Thesis Preparation, section 7 "Manuscripts and Authorship" which are as follows:

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Although all the work reported here is the responsibility of the candidate, the project was supervised by Dr. A.K. Watson, Department of Plant Science, Macdonald College of McGill University. The three manuscripts are co-authored by both Dr. A.K. Watson and Dr. R.D. Reeleder. For consistency and convenience, all manuscripts follow the same format. The copies that will be sent to the respective journals, however, will follow the requirements of each journal. The first, second, and third manuscripts are being submitted to the Canadian Journal of Microbiology, Canadian Journal of Plant Pathology, and Weed Science, respectively.

## ABSTRACT

M. Sc. Louise Morin Plant Science

**DEVELOPMENT OF THE FIELD BINDWEED BIOHERBICIDE; *PHOMOPSIS CONVOLVULUS* : SPORE PRODUCTION AND DISEASE DEVELOPMENT.**

*Phomopsis convolvulus* Ormeno, a foliar pathogen of field bindweed, is a good candidate to be developed as a bioherbicide. Large numbers of infective propagules were produced in shake-flask liquid fermentation with modified Richard's (V-8) medium and in solid-substrate fermentation with pearl barley grains. In complex liquid media, pycnidium-like structures were observed. Most conidia stored at -70°C remained viable and virulent for at least six months.

In controlled environment studies, a minimum of 18 hr of dew was required for severe disease development on inoculated plants. The addition of gelatin, Sorbo ™, or BOND ™ to the inoculum did not enhance the disease under various leaf wetness periods. A continuous dew period of 18 hr was superior to the cumulative effect of three interrupted 6 hr dew periods. Secondary inoculum was produced on diseased plants placed under moist conditions for 48 hr or more.

In greenhouse experiments, seedlings at the cotyledon and 3- to 5- leaf stage were severely diseased and killed when inoculated with $10^9$ conidia/$m^2$. This inoculum density adversely affected the regenerative ability of 4 wk old seedlings and established plants, but few plants were killed. Inoculation of the healthy regrowth from plants previously inoculated with the fungus resulted in much less disease symptoms than expected.

## RESUME

M. Sc. Louise Morin Phytologie

**DEVELOPPEMENT D'UN BIOHERBICIDE (PHOMOPSIS CONVOLVULUS) POUR LA REPRESSION DU LISERON DES CHAMPS: PRODUCTION DES SPORES ET DEVELOPPEMENT DE LA MALADIE.**

Phomopsis convolvulus Ormeno, un pathogène foliaire du liseron des champs, possède les charactéristiques essentielles pour l'élaboration d'un bioherbicide. Une grande quantité de propagules infectieuses sont produites dans un milieu de culture liquide (solution Richard modifiée [V-8]) et sur la surface de grains d'orge perlés. Dans les milieux liquides complex, on peut observer des structures ressemblant à des pycnidies. La plupart des conidies congelées à -70°C demeurent viables et virulentes pour au moins six mois.

Les études en environnement contrôlé démontrent qu'une période minimum de 18 hr de rosée est nécessaire, après l'inoculation, pour initier des infections sévères sur les plantes. L'addition de gélatine, de SORBO ou de BOND ne favorise pas l'infection par le pathogène sur des plantes exposées à différentes périodes de rosée. Les effects sur la maladie d'une période de rosée continue de 18 hr sont supérieurs aux effects cumulatifs de trois périodes interrompues de rosée de 6 hr. Des pycnidies et des conidies (inoculum secondaire) sont produites sur des plantes malades placées sous des conditions humides pour 48 hr ou plus.

Dans la serre, les plantules de liseron des champs (cotylédons seulement ou 3 à 5 feuilles) inoculées avec une densité de $10^9$ conidies/$m^2$ développent des symptômes foliaires

sévères et meurent rapidement. Cette même densité d'inoculum réduit la capacité des plantules agées et celle des plantes établies à développer de nouvelles pousses des racines, mais, seul un faible taux de mortalité est observé. La repousse des plantes ayant déjà été inoculées avec le champignon développe beaucoup moins de symptômes de la maladie suite à une deuxieme inoculation.

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# I. GENERAL INTRODUCTION

## General principles of biological control of weeds.

The presence, abundance, and distribution of plants depend on the climatic, edaphic, and biotic conditions that favor or suppress plant reproduction and spread (Andres 1977, 1981, Huffaker 1957, Wilson 1964). Agricultural practices intended to optimize crop production affect the microenvironment and often alter the plant community equilibrium resulting in serious weed problems. Intentional and accidental introduction of particular plant species into various areas of the world has led to greater aggressiveness and abundance of these plants, which have become economically important weeds (Andres et al., 1976). The disturbance, reduction, or absence of natural enemies associated with certain cultural practices and areas outside of a plant's natural distribution seriously limit the stress exerted on the new weed community (Andres, 1977).

Biological control of weeds refers to the deliberate use of exotic or native natural living enemies that attack, impair, and sometimes kill specific noxious weeds. This control measure reinforces natural stresses and offers a long-term suppression of the weed at an acceptable and subeconomic population level (Andres 1977, Huffaker 1957, Schroeder 1983, Wapshere 1982). Huffaker (1957) comments that biological control has

generally been considered when other weed control measures were inefficient in controlling highly troublesome weeds.

The use of biotic agents to control weeds has received increasing attention since the nineteenth century and particularly since World War II (Pemberton 1981, Schroeder 1983). The first reported successful example of biological control of a weed took place in India in 1863 when a South American insect Dactylopius ceylonicus Green was introduced to control the introduced cactus weed, Opuntia vulgaris Miller (Tyron 1910, cited by Pemberton 1981). In the early 1980's, it was estimated that 166 biological control projects against 64 different weed species have taken place in 41 countries. The United States, Canada, and Australia are the leaders in terms of numbers of projects (Pemberton, 1981).

According to Schroeder's (1983) terminology, biological weed control is divided into three different approaches: classical, conservation, and augmentation. The classical method is the most frequently employed and involves the introduction and establishment of one or several natural enemies from the weed's native range into the region where an exotic weed has become noxious. The conservation method, not widely used, involves the manipulation of the environment to promote and increase the effect of existing biocontrol agents in a particular area. The basis of the third strategy, the augmentative approach, is the periodical release or redistribution of native natural enemies to enhance their effect

on the target weed. The classical and augmentative tactics have also been termed inoculative and inundative strategies, respectively (Wapshere 1982).

Biological control of weeds based on the classical approach is a slow ecological process and is usually restricted to undisturbed areas where the introduced natural enemies have a better chance to become established in the new environment compared to disturbed habitats (Andres, 1977). On the other hand, the augmentative approach is considered a technological response to a particular weed problem (Wapshere, 1982).

Under various agricultural activities vertebrate herbivores, fish, phytophagous insects, mites, nematodes, parasitic plants, or plant pathogens have been evaluated and used as biocontrol agents (Andres 1977, Huffaker 1957). Arthropods have been widely and successfully employed as natural enemies in biological weed control programs. Julien et al. (1984) reported that out of a total of 499 worldwide releases of natural enemies of weeds, 488 involved insects. The great diversity of insects, the conspicuous damage they cause, their high rate of reproduction, and the ease of manipulation are some reasons explaining their predominance in the classical approach of biological control (Andres 1981, Schroeder 1983).

Successful weed biocontrol projects involving the use of insects include the control of Opuntia spp. by the introduced Cactoblastis moth in Australia (Haseler, 1981), Lantana camara L. by Teleonemia scrupulosa Stal. also in Australia (Harley et

al., 1979), Hypericum perforatum L. by the beetle Chrysolina quadrigemina (Suffrian), and Senecio jacobaea L. by the European moth Tyria jacobaea L. in the U.S.A. and Canada (Harris, 1974). The augmentative approach has not been extensively used with insects because of the difficulties of mass-rearing and storage (Wapshere, 1982).

During the past two decades plant pathogens have received more attention as potential biocontrol organims. Wilson's review in 1969 stimulated research involving plant pathogens to control weeds. The successful introduction of the rust Puccinia chondrillina Bubak & Syd. to control skeleton weed (Chondrillina juncea L.) in Australia (Hasan & Wapshere 1973) and Phragmidium violaceum (Schutz) Wint. for the control of Rubus sp. in Chile (Oehrens, 1977) represent examples of initial programs involving plant disease in the classical biological control strategy. Plant pathogens, however, have been mainly employed following the augmentative approach, which is often termed the bioherbicide tactic (Templeton & Smith, 1977).

Biological control of weeds is well documented and several detailed reviews have been published over the past thirty years (Huffaker 1957, Wilson 1964, Andres & Goeden 1971, Andres & Bennett 1975, Andres et al. 1976, Andres 1977, 1981, Batra 1981, Wapshere 1982, Schroeder 1983, Hokkanen 1985).

**The bioherbicide strategy of biological control of weeds.**

Under natural conditions several diseases of weeds remain endemic because of slight environmental constraints (Charudattan

1985, Holcomb 1982) or limited amounts of initial inoculum to initiate infection (Templeton *et al.* 1979, Templeton & Trujillo 1981) or both. In the bioherbicide strategy, these epidemiological constraints can be compensated for by massive inoculations of the pathogenic microorganisms in a way similar to chemical herbicides (Mortensen, 1986).

Many fungal pathogens, particularly the Deuteromycetes, are suitable for inundative use as opposed to other plant pathogens. Many fungi do not require wounds or insect vectors to penetrate and infect plant tissue, they are often highly host specific, and they multiply rapidly by producing stable infective propagules that can be stored easily (Templeton 1982 a, Scheepens & van Zon 1981). Mass-production of inoculum of the selected pathogen, formulation, and application of large numbers of infective propagules at the appropriate time for infection represent the main steps involved in the bioherbicide tactic leading to disease epidemics on the target weed population (Schroeder 1983, Charudattan 1985). When fungi are employed, Templeton et al. (1979) have been using the term mycoherbicide to characterize this strategy.

Potential candidates to be used as microbial herbicides should preferably be indigenous pathogens, highly virulent, genetically stable, relatively host specific, easily produced *in vitro*, and efficient in reducing weed infestations over a wide range of environmental conditions (Daniel *et al.* 1973, TeBeest 1985, Templeton & Smith 1977).

Three main steps are commonly followed in bioherbicide research: discovery, development, and deployment (TeBeest 1985, Templeton 1982 b). The discovery phase requires literature and herbarium searches of previously reported diseases on the target weed as well as field surveys at various times during the growing season in an attempt to collect, isolate, and identify promising biocontrol agents. Prior to the development phase, it is essential to ascertain the pathogenicity of the isolated organism using Koch's postulates and to patent the use of the specific pathogen (Saliwanchik, 1986). Subsequently, efficient methods for the production of virulent propagules are developed (Churchill, 1982), epidemiology studies are conducted under controlled environment conditions (TeBeest et al., 1978), and early efficacy testing is initiated in the greenhouse. The final deployment stage primarily deals with the development of an efficient formulation (Quimby & Fulghan 1986, Walker & Connick 1983), the evaluation of interactions with pesticides and surfactants (Khodayari & Smith 1988, Klerk et al. 1985), and the large-scale testing of the potential bioherbicide on the target weed under field conditions. Registration, large-scale production, marketing, and commercialization of the biological product are subsequently considered (Bowers 1982, Scher & Castagno 1986). An important part of the commercialization process is the patent protection for the prospective bioherbicide (Saliwanchik, 1986). Templeton (1982 b) emphasized that cooperation between the scientists of the public and private sector is essential to accelerate progress and success

with bioherbicides.

Biological weed control with mycoherbicides is a relatively young science. Only two fungal pathogens have been developed and registered as marketable bioherbicides in the United States. A short-lived liquid formulation of chlamydospores of the soil-borne fungus Phytophthora palmivora (Butl.) Butl. was registered under the commercial name DEVINE$^{TM}$ in 1981. This mycoherbicide is extremely effective in controlling milkweed vine (Morrenia odorata [H.& L.] Lindl), a serious weed of Florida citrus orchards (Ridings et al., 1978). Kenney (1986) reported that 95 to 100% control of the weed was still observed 6 to 8 years after the first and only application of DEVINE$^{TM}$.

COLLEGO$^{TM}$, a dry spore formulation of an anthracnose-inducing fungus Colletotrichum gloeosporioides (Penz.) Sacc. f.sp. aeschynomene, was registered in 1982 as a bioherbicide for northern jointvetch (Aeschynomene virginica L.) control in rice (Oryza sativa L.) and soybean (Glycine max [L.] Merr.) (Smith 1986, TeBeest & Templeton, 1985). It has been successfully marketed and excellent (90%) control of the weed is achieved when the product is used properly (Bowers 1986, Templeton 1986).

Several other potential bioherbicides have been investigated and could be registered in the near future, such as: Colletotrichum malvarum Braun & Casp. for the control of prickly sida (Sida spinosa L.) in rice and soybean fields (Kirkpatrick et al., 1982); Colletotrichum gloeosporioides f.sp. jussiaeae on winged waterprimrose (Jussieae decurrens [Walt.] D.C.) in rice (Boyette et al., 1979); Cercospora rodmanii Conway

on the aquatic weed waterhyacinth (Eichhornia crassipes [Mart.] Solms) (Charudattan 1986, Conway 1976); Alternaria cassiae Jurair & Khan on sicklepod (Cassia obtusifolia L.) (Walker & Boyette 1985, Walker & Riley 1982); Colletotrichum coccodes (Wallr.) Hughes on velvetleaf (Abutilon theophrasti Medik.) in soybean and corn (Zea mays L.) (Gotlieb et al. 1987, Wymore et al. 1988); Colletotrichum gloeosporioides f.sp. malvae on round-leaved mallow (Malva pusilla Sm.) (Mortensen 1988).

**The biology and control of Convolvulus arvensis L.**

Convolvulus arvensis L. (field bindweed, small-flowered morning glory, wild-morning glory, liseron des champs [Alex et al., 1980]) probably originated in Western Asia or Europe (Frankton & Mulligan 1974, Rosenthal 1983). It was introduced and reported in North America as early as 1739 (Wiese & Phillips, 1976) and was well established by 1900 (Maw, 1984). It is an important noxious weed throughout the world except in the tropics (Frankton & Mulligan, 1974), and is mostly found in temperate regions between latitudes 60° north and 45° south (Holm et al., 1977). Field bindweed infestations are encountered in most states of the United States, particularly in the west and midwest (Phillips 1978, Meyer 1978) and in most Canadian provinces (Alex, 1982). This weed is mainly prevalent along roadsides, in pastures, and cultivated lands (Frankton & Mulligan, 1974).

This perennial plant is included in the list of Class 1 Prohibited Noxious Weed Seeds of the Canadian Federal Seeds Act

(Weaver & Riley, 1982). It has been ranked the fourteenth most important weed in the United States (Jansen, 1972) and the twelfth most important weed on a worldwide basis (Holm et al., 1977). Recently, Convolvulus arvensis was considered by the members of the Weed Science Society of America as one of the 25 major weeds needing increased research emphasis (McWhorter & Barrentine, 1988).

Field bindweed belongs to the family Convolvulaceae. It is a creeping or twining perennial herb with an extensive root system. The leaves have the following major botanical characteristics: long-petioled, alternate, simple, glabrous or finely pubescent, ovate-oblong to saggitate, entire margin, 2-5 cm long. The botanical formula is K(5) C(5) A(5) G(2) (Hickey & King, 1981). The small pinkish-white funnel-shaped flowers and the two bracts attached to the peduncle are distinguishing characteristics of the species which is often confused with other members of the family, particularily Calystegia sepium (L.) R. Br. commonly named hedge bindweed (Weaver & Riley, 1982). The flowers are bisexual, usually self-incompatible (Mulligan & Findlay, 1970), borne singly or in pairs on long peduncles, and associated with five glabrous green sepals. The three-angled pear-shaped seeds (3-5 mm long) are brownish-gray, have a rough texture, and are produced in a two-valved capsule (Weaver & Riley, 1982). Hickey & King (1981) give a complete and good morphological description and Kennedy & Crafts (1931) published an excellent anatomical study of Convolvulus arvensis.

The survival of field bindweed is assured by sexual and vegetative reproduction. Seed production is variable but can be abundant (Roberts et al., 1982) and is estimated to be responsible for introduction of the weed in a new area (Weaver & Riley, 1982). Dormant field bindweed seeds may remain viable for more than 20 years (Timmons, 1949). The aggressiveness and competitive ability of this weed is primarily due to its ramified root system which favors rapid colonization and spreading of the infestation.

The weediness of Convolvulus arvensis is highly troublesome for agriculture. Established plants compete with crop species for light, water, nutrients, and dramatically reduce the available moisture in the soil (Wiese & Phillips 1976, Weaver & Riley 1982). During the dry summer months, yields of crops are significantly reduced (up to 75%) by heavy infestations (Phillips 1967, Schweizer et al. 1978). In California, the adverse effect of field bindweed in terms of yield losses have been estimated to be more than 25 million dollars annually (Rosenthal, 1983).

Eradication of field bindweed is extremely difficult principally because of its well-developed root system that can spread horizontally up to 8 m from the parent plant in one growing season sending new shoots from endogenous buds (Best 1963, Swan & Chancellor 1976). Root biomass density and carbohydrate reserves can be gradually decreased with frequent and intensive cultivation (Phillips, 1961) and effective control may be achieved in an average of 3-5 years (Derscheid et al.

1970, Wiese & Rea 1959). Combination of competitive crops (winter wheat, perennial forages) with tillage and application of postemergence herbicides may result in effective long-term suppression of established populations (Derscheid et al. 1970, Phillips 1967, Russ & Anderson 1960, Wiese & Rea 1959).

Excellent control is obtained on young seedlings by a single application of phenoxy herbicides(Muzik, 1970) but older plants generally survive by sending up shoots from root buds (Ogg 1975, Wiese & Lavake 1985). It may require two or more herbicide applications to obtain acceptable control of these plants (Anonymous, 1987). Application of systemic herbicides should coincide with maximum plant metabolic activity, allowing rapid movement of the chemical to the root system (Wiese & Rea, 1962). In Canada, several herbicides are available and recommended for the control of field bindweed: 2,4-D (several formulations), 2,4-DB [4 (2,4-dichlorophenoxy) butanoic acid], MCPA [(4-chloro-2-methylphenoxy) acetic acid], dicamba [3,6-dichloro-2-methoxybenzoic acid], bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide], and mecoprop [ (±) -2-(4-chloro-2-methylphenoxy) propanoic acid] for use in field crops and glyphosate [(N-phosphonomethyl) glycine] and MCPB [4-(4-chloro-2-methyl phenoxy) butanoic acid] in orchards, vineyards and non-crop areas (Anonymous, 1987).

Differences in susceptibility of field bindweed biotypes to 2,4-D (Whitworth 1964, Whitworth & Muzik 1967) and to glyphosate (DeGennaro & Weller, 1984 a.) have been reported. These biotypes were demonstrated to have morphological and reproductive

differences (DeGennaro & Weller, 1984 b.). Their variable responses to 2,4-D were apparently not correlated to morphological characteristics but rather to a physiological differences at the cell level (Whitworth & Muzik, 1967). Recently, a few growth regulators have been observed to improve herbicide efficiency in controlling this perennial in greenhouse and growth cabinet experiments. Field trials, however, were inconclusive (Lym & Humburg, 1987).

Rosenthal (1985) stated that conventional methods used for field bindweed control are not very effective in suppressing infestations and are particularly expensive. In California, this weed is more prevalent now than 20 years ago despite the intensive control programs (Rosenthal, 1983). Considering the seriousness of Convolvulus arvensis as a weed and the problems associated with traditional control methods, biological control has been considered a promising alternative to control and limit the spread of this pest.

In the early 1970's, a biological control program against field bindweed was established in North America. The classical approach was considered and a search for natural enemies of the weed was initiated in the European Mediterranean region (Rosenthal, 1981). Several insects, and a few mites and fungi were found associated with Convolvulus arvensis and its close relatives. The most studied species were the insects Galeruca rufa Germ. (Rosenthal & Carter, 1977), Tyta luctuosa Denis & Schiff. (Rosenthal, 1978), and Spermophagus sericeus Geof. , and the gall mite Aceria convolvuli Nal. (Rosenthal & Buckingham,

1982). These organisms were responsible for serious damage on the target weed but were not adequately host-specific because they fed and reproduced on the economically important sweet potato species and/or other native species of the genera Convolvulus and Calystegia (Rosenthal et al., 1983). Another insect, Noctuelia floralis Hb., from Pakistan (Baloch, 1977) demonstrated great potential as a biocontrol agent because of its feeding habits on field bindweed roots (Rosenthal & Buckhingham, 1982). Until recently, host specificity testing of this insect was still under way in the United States (Rosenthal, 1985).

North American surveys on the native natural enemies attacking Convolvulus and Calystegia spp. have reported several insects exerting some degree of damage on these plants (Balsbaugh et al. 1981, Mohyuddin 1969, Rosenthal et al. 1983). Their effect, however, seemed to be localized, insufficient, and too late in season to significantly reduce the infestations (Maw 1984, Rosenthal 1985).

A few fungal pathogens infecting field bindweed have been suggested as potential canditates for use within the scope of classical biocontrol but none of them, however, have been extensively studied: the rust Puccinia convolvuli (Pers.) Cast. and the powdery mildew Erysiphe convolvuli (DC) St-Amans (Hasan, 1974); Alternaria tenuissima (Kunz. ex Pers.) Wilt. (Rosenthal & Buckingham, 1982); Tecophora seminis-convolvulus (Dezm.) Lioro (Rosenthal et al., 1983).

The first research program involving the inundative biocontrol strategy (bioherbicide) on field bindweed was initiated in Quebec in 1984. A foliar fungal pathogen, Phomopsis convolvulus Ormeno, was isolated from lesions on field bindweed leaves and initial studies indicated the remarkable potential of this fungus for development as a bioherbicide (Ormeno-Nunez, 1987).

**The genus Phomopsis.**

The fungal species belonging to the genus Phomopsis cause a wide variety of disease symptoms (blight, rot, wilt, leafspot, canker) on several economically important plants (Westcott, 1979). This genus is classified in the order Sphaeropsidales of the class Coelomycetes (Deuteromycotina) (Hawksworth et al. 1983, Sutton 1977). In the mycological literature approximately 400 taxa have been described in Phomopsis (Sutton, 1980). The absence of lectotype species for the genus Phomopsis causes several taxonomic difficulties and a revision of the genus is required (Sutton, 1980). Most Phomopsis species are anamorphs (the imperfect or asexual stages) of Diaporthe sp., a member of the Ascomycetes (Ascomycotina) (Hawksworth et al., 1983).

Typical features of the genus Phomopsis include the dense floccose whitish mycelium, the pycnidial conidiomata, the production of two types of conidia (Muntanola-Cvetkovic et al., 1985), and the presence of honey-colored cirrhi under humid conditions. Sutton (1980) described the genus Phomopsis as follows:

> MYCELIUM immersed, branched, septate, hyaline to pale brown. CONIDIOMATA eustromatic, immersed, dark to dark-brown, separate or aggregated and confluent, globose, ampulliform or applanate, unilocular, multilocular or convoluted thick walled; walls of brown, thick or thin-walled textura angularis, often somewhat darker in the upper region, lined by a layer of smaller-celled tissue. OSTIOLE single or several in complex conidiomata, circular, often papillate. CONIDIOPHORE branched and septated at the base and above, occasionally short and only 1-2 septate more frequently multi-septate and filiform, hyaline, formed from the inner cells of the locular walls. CONIDIOGENOUS CELLS enteroblastic, phialidic, determinate, integrated, rarely discrete, hyaline, cylindrical, apertures apical on long or short lateral and main branches of the conidiophore, collarette, channel and periclinal thickening minute. CONIDIA of two basic types but in some species with intermediates between the two (C- or Gamma-conidia), A- or Alpha-conidia hyaline, fusiform, straight, usually biguttulate (one guttule at each end) but sometimes more guttules, aseptate; B- or Beta-conidia hyaline, filiform, straight or more often hamate (hooked at the apex), eguttulate, aseptate.

Several species of Phomopsis are the causal agents of diseases of ornamental plants, and horticultural, orchard, and field crops. Agrios (1978) considered Phomopsis as one of the most common imperfect fungi causing fruit and general diseases on plants. The diversity of Phomopsis spp. reported to incite various plant disease is vast. Some of the important plant diseases associated with Phomopsis are: The Diaporthe/Phomopsis disease complex of soybean including pod and stem blight, and seed decay caused by Phomopsis sojae Lehm. (Diaporthe phaseolorum [Cke. & Ell.] Sacc. var. sojae [Lehm.] Wehm.) (Hepperly & Sinclair, 1980) and stem canker induced by Diaporthe phaseolorum (Ck. & Ell.) Sacc. var. caulivora Ath. & Cald. and its Phomopsis asexual state (Backman et al., 1985); the sunflower stem disease incited by Phomopsis helianthi Munt.-

Cvet. et al. (Diaporthe helianthi Munt.-Cvet. et al.) (Mihaljcevic et al., 1982); the juniper blight induced by Phomopsis juniperivora Hahn. affecting several coniferous species in nurseries (Punithalingam & Gibson, 1973); the black root rot of greenhouse cucumber caused by Phomopsis sclerotioides van Kest. (Punithalingam & Holliday, 1975); the cane and leaf blight of grape induced by Phomopsis viticola (Sacc.) Sacc. (Willison et al., 1964); the stem canker and fruit rot of blueberries caused by Phomopsis vaccinii (Millholland & Daykin, 1983); the tuber dry rot of sweet potato in storage induced by Phomopsis batatis Hart. & Field (Harter & Field, 1912); the stem and pod blight of lupins caused by Phomopsis leptostromophormis (Kuh.) Bud. which is responsible for the poisoning of sheep that eat the infected plants (Ortazewski & Well, 1960).

A Phomopsis species was recently observed to produce distinctive foliar damage in the form of leafspots or anthracnose lesions on field bindweed plants. Since no previous record of Phomopsis infecting Convolvulus arvensis was found and on the basis of morphological characteristics and pathogenicity, the fungal pathogen was identified as a new species Phomopsis convolvulus Ormeno (Ormeno-Nunez, 1987). In Ormeno-Nunez's initial study, the etiology of the disease, the general conditions for disease development, and the host-range of the pathogen were established in an attempt to evaluate its possible use as a mycoherbicide. The results published by Ormeno-Nunez et

al. (1988) indicated that Phomopsis convolvulus can cause severe necrosis and blighting, and can significantly reduce the growth of field bindweed. The disease was most severe on seedlings when plants were inoculated with conidial suspensions of 5 X $10^6$ conidia/ml or more and subjected to a minimum of 12 hr of leaf wetness at 20° C. This report indicated that this fungal pathogen is as virulent as other fungi being investigated as bioherbicides and further assessments of the feasibility of developing and using Phomopsis convolvulus to control field bindweed were encouraged.

The research presented here built upon the previous knowledge and further ascertained the potential of Phomopsis convolvulus as a mycoherbicide. A more practical approach, using known rates of infective propagules instead of inoculation to runoff, was considered because it allows an easier translation of laboratory and greenhouse results to the field. The main objectives of this project were to evaluate various systems to mass-produce virulent spores, to determine the viability of the spores under various conditions of storage, to complete studies on the biology of the pathogen and thereby define more accurately the optimum conditions for infection and disease development, and to maximize disease expression on field bindweed seedlings and established plants to achieve efficient weed control.

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## II. CONIDIA PRODUCTION OF *PHOMOPSIS CONVOLVULUS*, A POTENTIAL BIOHERBICIDE FOR FIELD BINDWEED CONTROL.

### INTRODUCTION

Field bindweed (*Convolvulus arvensis* L.) is an important, troublesome, perennial weed in many agricultural areas of the world as well as most Canadian provinces (Weaver & Riley, 1982). A foliar pathogen of field bindweed, *Phomopsis convolvulus* Ormeno was isolated in 1984 (Ormeno-Nunez, 1987) and is being investigated as a possible bioherbicide. Controlled environment and greenhouse studies have demonstrated that *Phomopsis convolvulus* has great potential for use as a biological herbicide because it is effective in reducing field bindweed growth and it is highly host specific.

As with other bioherbicides, large amounts of infective propagules of this fungus are required for its use as a bioherbicide. Production methods for large quantities of spores should be economical, relatively simple, require no special equipment or handling, and the inoculum produced should retain its viability for long storage periods (Hildebrand & McCain, 1978). Several methods and media have been used to produce sufficient amounts of inoculum of various fungi studied or used as bioherbicides. Liquid cultures in shake flasks or small

fermentation vessels supported sporulation in vitro of Colletotrichum gloeosporioides (Penz.) Sacc. f.sp. aeschynomene (Daniel et al., 1973), Colletotrichum coccodes (Wallr.) Hughes (Wymore et al., 1988), and Fusarium solani App. & Wr. f.sp. cucurbitaceae Snyd. & Hans. (Boyette et al., 1984) in modified Richard's medium with V-8 juice. This technique has been used for mycelium production of Alternaria macrospora Zimm. and A. cassiae Jurair & Khan which was induced to sporulate under controlled environmental conditions (Walker, 1980, 1982). Amended natural solid substrate diffusates (Hildebrand & McCain, 1978) and cornmeal-soyflour-sucrose liquid (Walker & Riley, 1982) have also been used as media.

Inexpensive agricultural products such as wheat, barley, and soybean are commonly screened for economic production of microbial pesticides (Miller & Churchill, 1986). Cornmeal/sand medium has been used to produce fungus-infested granules of Fusarium solani f.sp. cucurbitae (Boyette et al., 1984).

In addition to the production of large quantities of viable spores for biological control, optimum conditions for storage of spores must also be determined for a biocontrol pathogen (Soper & Ward, 1981). Various storage techniques for bioherbicides have been reported: wet (Daniel et al., 1973) and dried spores (Walker, 1980) at approximately 4°C, air-dried spores in plastic bags at room temperature (Hildebrand & McCain, 1978), freeze-dried spores in liquid nitrogen at -10°C (Walker, 1980), pellets of mycelium-sodium alginate-clay (Walker & Connick, 1983), and dry granules of vermiculite-mycelium-spores in plastic bags at

4°C (Walker, 1981).

Field testing of P. convolvulus as a potential bioherbicide for the control of field bindweed requires large quantities of inoculum. The agar plate technique developed by Ormeno-Nunez (1987) was appropriate for initial experiments but it was time-consuming and expensive to produce inoculum for larger-scale experiments. It was then necessary to improve spore production methods. The objectives of this study were to evaluate solid agricultural products and commonly used complex or defined liquid media for spore production of Phomopsis convolvulus and to evaluate the effectiveness of various short-term and long-term storage techniques of conidia.

## MATERIALS AND METHODS

**Seed inoculum preparation.** Stock cultures of the original single-conidium isolate of P. convolvulus were established on potato carrot agar slants in small glass vials, and were maintained under mineral oil at 4°C (Tuite, 1969). Small pieces of mycelium from the stock culture were transferred to fresh potato dextrose agar (PDA) in petri dishes (9 cm diameter). Plate cultures were incubated at room temperature (approx. 21°C) with 12-14 hr of fluorescent light for 5-7 days. Agar disks with mycelium (6 mm diameter) from the margin of these young colonies were used to seed liquid media in some experiments.

Some cultures growing on PDA were incubated under conditions described above for 45-60 days until the appearance of conidial droplets oozing from pycnidia embedded in the mycelial mat. The surface of each colony was then flooded with 5-10 ml of sterile deionized water and flushed several times with this water using a 5 ml sterile syringe (Becton-Dickinson, Rutherford, NJ). This technique allowed removal and suspension of most conidia. Fifty to forty single droplets of this conidial suspension were deposited on petri plates containing 15-20 ml half strength PDA (12 g/L potato dextrose broth, 15 g/L Difco Bacto agar and 100 ml/L of bindweed decoction) (Ormeno-Nunez et al., 1988). After 2-3 wk incubation at room temperature conidia had germinated and produced small colonies with little mycelial growth but extensive production of pycnidia from which conidia in mucilaginous matrix were extruding. Agar disks (6 mm diameter) from these circular pycnidial areas were used to seed liquid media in some experiments.

In the solid substrate fermentation experiments and in some treatments of liquid fermentation experiments, conidia suspensions were used to seed the growth media. Half strength PDA plates covered with pycnidia and conidia were flooded and flushed with 10-15 ml sterile deionized water using a syringe. Conidial density was determined with the aid of a haemocytometer, adjusted to the desired density with sterile water, and 1 ml of this conidial suspension was used to seed media.

Phomopsis convolvulus produces two types of conidia in culture, alpha- and beta-conidia. Alpha-conidia are biguttulate, oblong amerospores and beta-conidia are filiform stylospores (Ormeno-Nunez, 1987). In the present experimental work, the term conidium(a) refers exclusively to the alpha-conidia because beta-conidia fail to germinate and are not considered to be part of the infection process (Ormeno-Nunez, 1987).

**Assessment of germination.** Droplets (50 µl) of conidia suspensions (2 X $10^5$ conidia/ml) in deionized water and in 0.01% potato dextrose broth were placed on 1.5% water agar disks (20 mm diameter), allowed to air dry for 5-10 min, covered with a cover-slip, and incubated in petri dishes in the dark at 24°C for 24 hr. Prior to observation, germinating conidia were killed and stained with lactophenol-cotton blue stain (Tuite, 1969). Conidia were considered to have germinated when the length of the germ tube was greater than the width of the conidium. Each treatment replicate had two sample units (agar disks) for each conidial suspension. Using a compound microscope at 500X magnification, several random fields of view were observed per sample unit until a total of 100 conidia were assessed for germination.

**Pathogenicity test.** Field bindweed seeds (Valley Seed Co., Fresno, Ca) were washed under warm running tap water for 2-3 hr and soaked in deionized water for approximately 24 hr. Imbibed seeds were germinated on moist filter papers in glass Petri

dishes at 24°C for 24-36 hr in the dark (radicle 5-10 mm in length). Germinated seeds were sown in potting medium (Pro-Mix BX, Premier Brands, Inc., New Rochelle, NY) in 10-cm plastic pots (4 seeds/pot) and grown in controlled environment chambers (23/18°C day/night temperature, 15-hr photoperiod, 250 $uEm^{-2}{}_{s}{}^{-1}$). Three- to five-leaf stage plants (thinned to 3 plants/pot) were inoculated with conidia from the various experiments at a density of $10^9$ conidia/$m^2$ using a spray chamber (Research Instrument Manufacturing Co. Ltd, Guelph, Ontario) with a full cone nozzle (TG 0.7), 200 kPa air pressure, a speed of 0.85 kph, and a spray volume of 500 L/ha. Plants were incubated in a dark dew chamber (Percival, Model E-54UDL, Boone, Iowa) for 24 hr at 20°C and subsequently transferred to growth cabinets at the original conditions. Disease severity was evaluated 1 wk after inoculation using a rating system based on a scale from 0 to 4 where 0 = no visible symptoms and 4 = ¨ 75% necrosis (Ormeno-Nunez et al., 1988). Dry weight of above-ground biomass was determined 2 wk after inoculation by cutting the plants at soil level and drying living tissue in paper bags for 4-5 days at 60°C.

**Solid substrate fermentation.** The following agricultural products: pearl barley grains, flax seeds, oat bran, wheat bran, green lentils, and field bindweed foliage, were screened as solid substrates for P. convolvulus conidia production. For each substrate, 10 g of the substrate were moistened with 20 ml of

deionized water in a 250 ml Erlenmeyer flask and autoclaved for 17 min (100 KPa and 120°C). Flasks of cooled medium were shaken by hand and seeded with 1 ml of a suspension of $10^7$ conidia/ml under aseptic conditions. Inoculated flasks were incubated on a laboratory bench at room temperature for 15 days. Flasks were shaken by hand every 2-3 days during the first week of incubation to prevent aggregation of solid particles and to improve aeration (Mudgett, 1986). Conidia from solid media were harvested by adding 50 ml of deionized water to each flask, shaking the flasks on a rotary shaker at 250 rpm for 5-10 min, and pouring the contents through a soil sieve (250 um, Fisher Scientific) lined with two layers of cheesecloth. Conidia production was determined with the aid of a haemocytometer and percent germination of the conidia was evaluated as above.

Additional experiments were designed to maximize conidia production in solid substrate fermentation using pearl barley. General methodology followed the previously described procedures unless otherwise indicated. Production of conidia for a second harvest was evaluated by performing the first harvest under sterile conditions, washing the substrate three times with sterile water, incubating the flasks for an additional 2 wk and performing the second harvest. Conidia production and percent germination were assessed as above.

Long-term viability and virulence of the conidia produced over time with the pearl barley system were assessed. Pearl barley grains seeded with conidia were maintained on the laboratory bench for 16 wk. Every 2 wk during this period,

conidia from 4 replicate flasks were harvested to evaluate their germinability and pathogenicity.

The pearl barley production system was optimized by determining the moisture content and quantity of grains needed for maximum production of conidia. Ten and twenty g of pearl barley substrate, moistened with 10, 20 or 30 ml of deionized water were tested in a factorial design. Percent moisture content of the grains after autoclaving was evaluated on a wet weight basis using the following formula: (g $H_2O$ / g wet pearl barley) X 100. Various conidial densities of liquid seed culture inoculum ($10^5$, $10^6$, $10^7$ and $10^8$ conidia/ml) were evaluated for their effect on final production of conidia.

**Liquid fermentation.** The shake-flask technique using a rotary shaker (250 rpm) was used to screen various complex and defined liquid media for submerged production of conidia of P. convolvulus. Three sources of seed inoculum (6 mm diameter agar disk with mycelium cut at the margin of a P. convolvulus colony, 6 mm diameter agar disk with pycnidia and conidial matrix, and 1 ml of $10^7$ conidia/ml suspension) were used to seed 250 ml Erlenmeyer flasks containing 100 ml of the following sterile liquid media: modified beef peptone medium (10g dextrose, 4 g nutrient broth, 11 g Bacto-peptone, 1000 ml $H_2O$), Tochinai solution (10 g peptone, 0.5 g $KH_2PO_4$, 0.25 $MgSO_4 \cdot 7H_2O$, 20 g maltose, 1000 ml $H_2O$), Richard's solution (10 g $KNO_3$, 5 g $KH_2PO_4$, 2.5 g $MgSO_4 \cdot 7H_2O$, 0.02 g $FeCl_3$, 50 g sucrose, 1000 ml

$H_2O$) (Tuite, 1969), half strength PDB solution (12 g potato dextrose broth, 1000 ml $H_2O$), Czapek dox solution (35 g of Czapek dox broth, 1000 ml $H_2O$) and modified Richard's (V-8) medium (Walker, 1980). Inoculated flasks were incubated on a rotary shaker for 2 wk under laboratory conditions as described previously. Successful medium/seed inoculum combinations were determined by microscopic examination of liquid media for the presence of conidia.

Only the combinations, in which conidia were produced in large amounts, were harvested by filtration through the soil sieve and cheesecloth (as described above). The fungal material (hyphae and pycnidia) remaining on the cheesecloth and inside the flask was rinsed with 50 ml of water. Conidia production was determined with the aid of a haemocytometer. The conidial suspension was centrifuged with a gravity force of 6300g for 10 min., the supernatant was dicarded, and the conidia pellet was resuspended in water prior to the germination test.

Liquid fermentation with Richard's (V-8) medium was further investigated. The optimum density of liquid seed culture inoculum ($10^5$, $10^6$, $10^7$, and $10^8$ conidia/ml) was determined following the previously described procedures. The effects of modifications of Richard's (V-8) medium recipe (omission of V-8 juice or increased $KNO_3$ content from 10 to 50 g per liter) on final production of conidia were also evaluated. In these tests the liquid media were seeded with agar disks covered with pycnidia. Conditions of incubation and harvesting procedures were similar to the previous experiments.

**Overall comparison of conidia production methods.** Liquid and solid substrate fermentation methods to produce P. convolvulus conidia were compared to the agar plate method used by Ormeno-Nunez (1987). Richard's (V-8) liquid, pearl barley grains, and half strength PDA plates were seeded with the same conidial suspension ($10^7$ conidia/ml) (harvested from half strength PDA plates) using the methodology described for each method. Germinability and pathogenicity of conidia produced were evaluated.

**Cold storage of conidia suspended in water.** One month after pearl barley grains were seeded with P. convolvulus, conidia were harvested with sterile deionized water (see above procedure). Inoculum density was determined with the aid of a haemocytometer and adjusted to 2 X $10^7$ conidia/ml with sterile water. Twenty sterile test tubes (16 X 150mm, Kimax brand, Fisher Scientific) with screw caps were filled with 10 ml of the conidial suspension and stored in a refrigerator at 4°C. Four replicate test tubes were randomly selected every day for 5 consecutive days. Germinability and pathogenicity of conidia were evaluated.

**Low temperature storage of conidia.** Two weeks after pearl barley grains were seeded with P. convolvulus, conidia were harvested with a 10% (w/v) sucrose solution and the conidial density was adjusted to 2 X $10^8$ conidia/ml by adding more sucrose solution. One ml aliquots of this suspension were distributed to ninety-

six 4.5 ml plastic cryovials (Simport Plastics, Beloeil, Quebec). Half of the cryovials were placed in a freezer at -10°C and the remaining ones were frozen at -70°C in an ultracold mechanical freezer. Once a month for 8 months (or less if frozen conidia lost their viability), four replicate vials were selected at random from each freezer. Conidia-sucrose suspensions were thawed at room temperature and densities were adjusted to $2 \times 10^7$ conidia/ml with deionized water to test germination and pathogenicity of the conidia.

**Data analyses.** A completely randomized design with 3 or 4 replicates was used for all experiments. All experiments were performed twice. Count and percentage data were transformed with logarithmic, square root, or arcsin transformations (Steel & Torrie, 1980) prior to an analysis of variance (ANOVA). Data were subjected to polynomial regression when appropriate. Results were pooled after testing for homogeneity of variances using Bartlett's test (Steel & Torrie, 1980) and also when no significant difference due to the experiment was detected. Differences of treatment means were established with a Fisher protected LSD test ($\alpha$=0.05) (Steel & Torrie, 1980). Disease ratings were compared by the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel, 1978).

## RESULTS

**Solid substrate fermentation.** Prelimary experiments indicated that various agricultural products such as barley, wheat, and oat straw or soybean seeds allowed extensive mycelial growth but no conidia were produced (S. Monette, personal communication). Other solid substrates promoted conidia production to various extents (Table 1). Pearl barley grains produced the maximum number of conidia per gram of substrate. This may be due to its large surface area, structure retention, and lack of particle agglomeration in which vegetative mycelial growth was restricted and large numbers of pycnidia were produced. Moistened small oat bran particles and flax seeds with a sticky coating aggregated at the bottom of the flasks, reducing the surface area available for development of pycnidia. These solid media as well as green lentils allowed more mycelial growth than wheat bran, field bindweed foliage, and pearl barley grains. The structure of the field bindweed foliage was partly disintegrated by excess water and autoclaving, and this appeared to restrict fungal growth.

Conidia produced on these solid substrates were viable with maximum germination in water occurring with conidia produced on oat bran and pearl barley with significantly ($\alpha$=0.05) lower germination of conidia produced on other substrates (Fig. 1). When conidia were suspended in 0.01% PDB prior to the germination test, no significant differences ($\alpha$ =0.01) were detected between conidia produced on the various substrates.

**Table 1.** Effect of various solid media on quantity of conidia produced by P. convolvulus after 15 days growth at room temperature. [y]

| Solid substrate | Conidia / g of product X 10⁸ |
|---|---|
| pearl barley grains | 4.8 a [z] |
| oat bran | 1.5 b |
| lentil seeds | 1.5 b |
| flax seeds | 1.3 b |
| wheat bran | 1.4 b |
| field bindweed foliage | 0.3 c |

[y] Results are from pooled experiments.

[z] Means followed by the same letter in a column are not significantly different at $\alpha$=0.05, according to the LSD test.

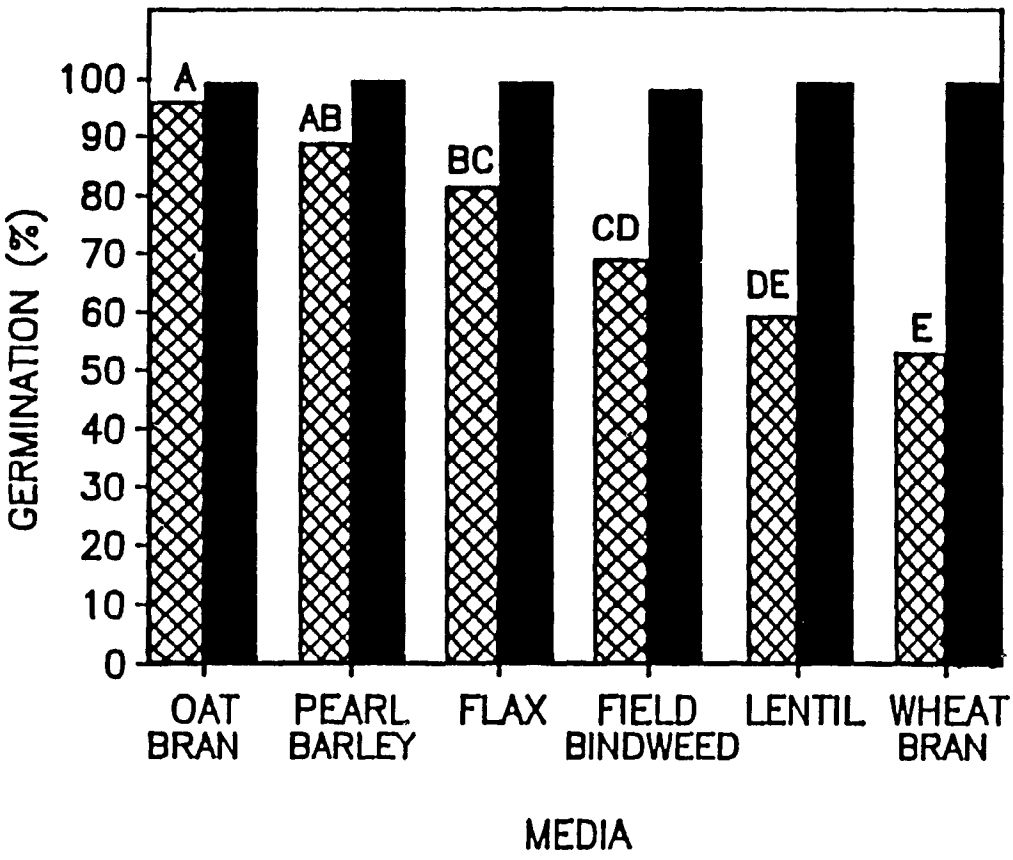


**Figure 1.** Germination of conidia produced on different solid media (24 hr incubation at 24°C). Bars represent mean % germination of 3 replicates (2 sample units/rep) of 100 conidia in $H_2O$ (▨) or in 0.01% PDB (■). For germination in water, bars over which the same letter appears are not significantly different at $\alpha$=0.05, according to the LSD test. There was no significant ($\alpha$=0.01) difference for germination in 0.01% PDB. Results are from pooled experiments.

The pearl barley production system was demonstrated to be very efficient in terms of number and quality of the conidia produced, and was further investigated. Distinct immature pycnidia, covering the surface of pearl barley grains, were observed 3 to 4 days after seeding with conidia. Small concentrated droplets of water-soluble mucilage (conidial matrix) containing large numbers of conidia appeared at the ostioles of pycnidia approximately 10 days after seeding (Fig.2A).

Two harvests of conidia from the pearl barley system permitted the recovery of large numbers of viable conidia (Table 2). The first harvest, performed after 2 wk of incubation, produced the higher yield of conidia. The second harvest yielded one-tenth the number of conidia obtained from the first harvest, but this was still considered to be a high level of production.

The conidia produced in mucilagenous matrix by pycnidia on the surface of pearl barley grains remained viable for up to 8 wk after seeding of the solid substrate (Fig.3). Subsequently, a slight but significant ($P=0.0001$) decrease in germinability was observed in both experiments, which corresponded to a significant ($P=0.0001$) increase in dry weight of above-ground biomass of inoculated field bindweed in one experiment. This increase in dry weight of foliage was associated with a significant ($\alpha=0.01$) decrease in disease ratings (Appendix A, Table 1).

**Figure 2.** Production of conidia on solid substrate and in liquid culture. **A:**Mature pycnidia of Phomopsis convolvulus covering the surface of pearl barley grain. Dense conidial matrix is oozing out from pycnidial neck (arrow). Bar equals 1 mm. **B to F:** Growth of the fungus and development of pycnidium-like structures under submerged conditions in modified Richard's (V-8) medium. Bar equals 100 µm for B and C, and equals 50 µm for D, E, and F. **B:** Mycelial strands produced 48 hr after seeding liquid medium with conidia. **C:** Young pycnidium-like structures appearing after 72 hr (arrows). **D:** Pycnidium-like structures extruding newly produced conidia (arrow) after 72 hr. **E:** Mature pycnidium-like structures after 96 hr. **F:** Large pycnidium-like structure full of conidia after 168 hr.


A
B
C
D
E
F

**Table 2.** Production of conidia on pearl barley grains. [w]

| Harvest [x] | Conidia / g. of pearl barley grains X $10^8$ | Germination of conidia in 0.01% PDB (%) |
|---|---|---|
| 1 | 7.10 [y] | 98.94 [z] |
| 2 | 0.78 | 99.50 |

[w] Results are from pooled experiments.

[x] Harvest 1 = first harvest of flasks, 2 wk after seeding the solid substrate with conidia. Harvest 2 = second harvest of the original flasks, 2 wk after the first harvest.

[y] Significant difference between treatments at $\alpha$=0.05, according to the F-test.

[z] No significant difference between treatment at $\alpha$=0.05, according to the F-test.

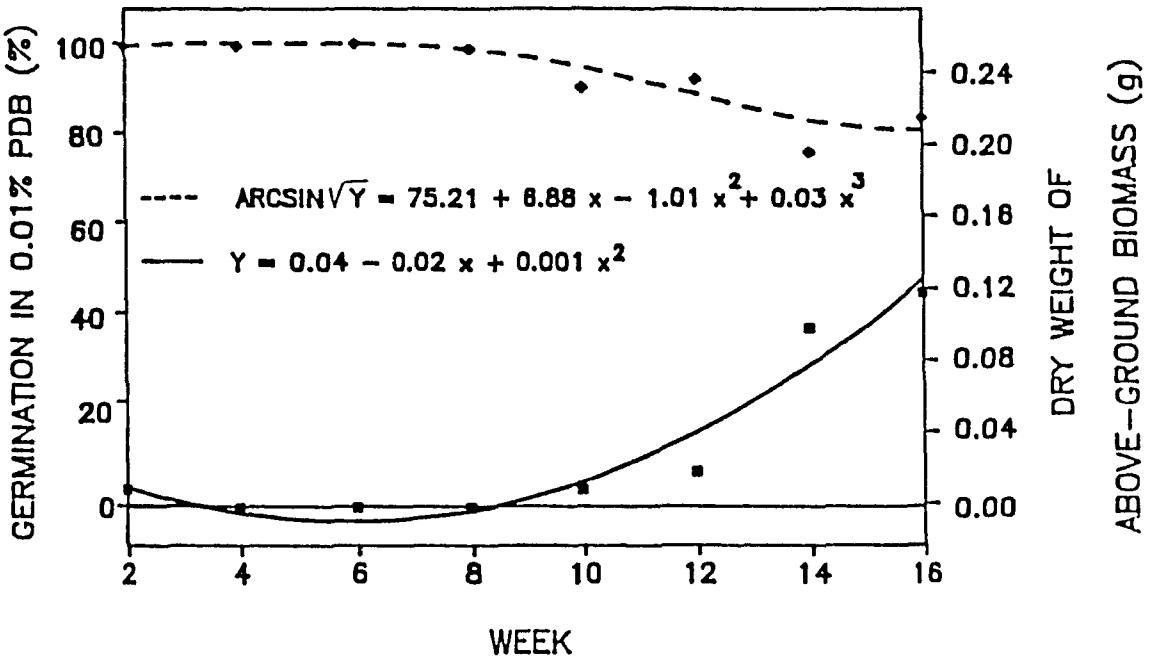


**Figure 3.** Viability and virulence of P. convolvulus conidia produced on pearl barley grains over time. Experiments were not combined because a significant difference (P=0.007) and no homogeneity of variances were found for % germination and dry weight data, respectively. Similar trends, however, were observed for both experiment. Results from one experiment are presented. The dashed line ( — — - ) represents germination in 0.01% PDB, and the solid line ( ——— ) represents the dry weight of above-ground biomass of field bindweed. Regression parameters for % germination data were estimated following arcsin transformation. In the regression equations Y = percent germination or g of above-ground biomass and x = no. of weeks. F-statistics for both model were highly significant (P=0.0001). Data points represent means of 4 replicates (2 sample units/replicate) for both dependant variables.

A combination of 20 g pearl barley grains and 20 ml $H_{20}$ in the 250 ml flask system produced the maximum number of conidia (Table 3). Production of pycnidia was closely related to the volume of the flask occupied by the grains and the grain moisture content. Low moisture content (31%, 20 g grains + 10 ml water) restricted growth and sporulation and high moisture content (72%, 10 g grains + 30 ml water) caused the grains to become gummy and to aggregate, resulting in extensive mycelial growth and limited conidia production.

The optimum seed culture inoculum densities to seed pearl barley grains were $10^7$ and $10^8$ conidia/ml (Fig. 4). Lower densities significantly (P=0.0001) reduced the final yield of conidia.

**Liquid fermentation.** Complex media composed of a natural plant substrate and defined chemicals (modified Richard's [V-8], half strength PDB), supplied essential nutrients in a balance which favored maximum production of conidia for those media tested (Table 4). Defined liquid media composed of commercially available chemicals, such as Richard's solution and Czapek Dox, allowed mycelium formation but resulted in poor sporulation. Peptone-based complex media like modified beef peptone and Tochinai solution did not support sporulation under submerged liquid conditions.

Agar disks with pycnidia and conidial suspensions provided initial seed inoculum in the form of individual conidia

**Table 3.** Moisture content and production of conidia for two quantities of pearl barley grains moistened with three different volumes of water in 250 ml Erlenmeyer flasks. [x]

| Quantity of pearl barley grains (g) | Volume of $H_2O$ (ml) | | |
|---|---|---|---|
| | 10 | 20 | 30 |
| | Conidia / flask X $10^8$ | | |
| 10 | 6.0 a [y] (46)[z] | 7.1 a (64) | 3.4 b (72) |
| 20 | 0.4 a (31) | 12.0 b (47) | 6.4 c (58) |

[x] Results are from pooled experiments.

[y] Means followed by the same letter in a row are not significantly different at $\alpha$=0.05, according to the LSD test.

[z] Percent moisture content of pearl barley grains after autoclaving.

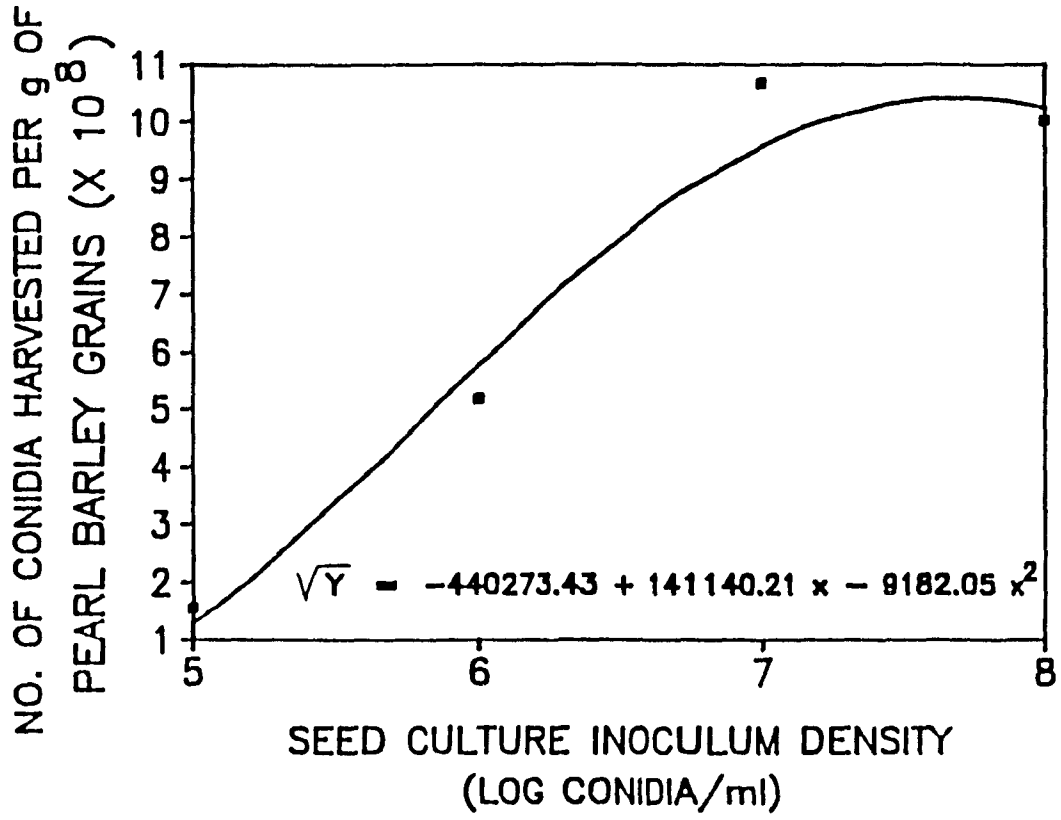


**Figure 4** Effect of quantity of conidia used to inoculate pearl barley grains on the final production of conidia. Data points represent means of 6 replicates. Regression parameters were estimated following square root transformation of the dependant variable. In the regression equation Y = no. of conidia and x = log of seed culture inoculum density. F-statistic for the model was highly significant (P=0.0001). Results are from pooled experiments.

**Table 4.** Effect of various liquid media and types of inoculum on production of conidia by *P. convolvulus*. y

| Liquid media | Type of seed inoculum: Mycelium agar disk | Pycnidia agar disk | Conidia suspension ($10^7$ conidia/ml) |
|---|---|---|---|
| modified Richard's (V-8) | ++ z | +++ | +++ |
| Richard soln. | - | - | + |
| modified beef peptone | - | - | - |
| Tochinai soln. | - | - | - |
| half strength PDB | - | ++ | ++ |
| Czapek dox | - | + | + |

y Results are from pooled experiments.

z Microscopic observation of media 2 wk after seeding: Index of conidial density; - = no conidia present; + = few conidia; ++ = some conidia; +++ = many conidia.

germinating readily and forming small colonies when seeded in the nutritive solutions. Seed inoculum consisting of hyphae resulted in the formation of a dense hyphal pellet.

Modified Richard's (V-8) medium seeded with conidial suspensions or seeded with agar disks covered with pycnidia supported maximum production of conidia (Table 5). Dark, pear-shaped structures (Fig.2C,E) externally similar to pycnidia produced on solid substrates were observed 3 to 4 days after seeding complex liquid media (without peptone) with seed inoculum consisting of mature pycnidia or conidia. When mycelium was used to seed modified Richard's (V-8) medium, pycnidium-like structures first appeared on the surface of the hyphal pellet after 7 days.

Blastospores or binary fission spores were not observed. Conidia were produced in the pycnidium-like structures, extruded from the ostioles and released into the surrounding medium (Fig. 2D). No significant differences ( =0.05) were found between germination (in $H_2O$ or PDB) of conidia produced in half strength PDB and modified Richard's (V-8) media seeded with various types of seed inoculum. For these combinations, average values for germination in $H_2O$ and PDB ranged from 79 to 92% and 94 to 98% respectively.

A negative relationship (P=0.0001) was demonstrated between seed culture inoculum density and the yield of conidia in modified Richard's (V-8) liquid culture (Fig.5). Use of modified Richard's medium without V-8 juice resulted in a seven fold reduction in numbers of conidia produced (Table 6). Decreasing

**Table 5.** Quantities of conidia produced in the successful combinations of liquid medium/type of seed inoculum. x

| Liquid media | Type of seed inoculum: Mycelium agar disk | Pycnidia agar disk | Conidia suspension ($10^7$ conidia/ml) |
|---|---|---|---|
| | Conidia / ml X $10^6$ | | |
| modified Richard's (V-8) | 0.38 d y | 7.17 a | 4.98 ab |
| half strength PDB | _ z | 3.31 bc | 2.08 c |

x Results are from pooled experiments.

y Means followed by the same letter are not significantly different at $\alpha$=0.05, according to the LSD test.

z No conidia were produced in this treatment.

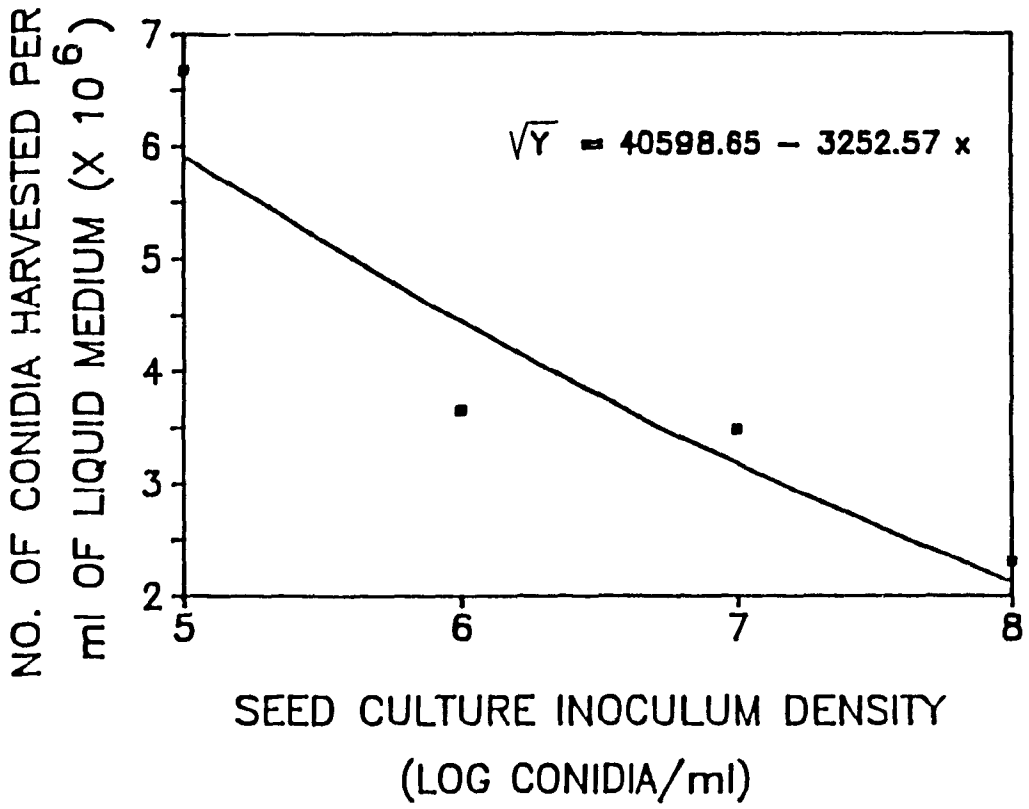


**Figure 5.** Effect of seed culture inoculum density on the total production of conidia in 100 ml of Richard's (V-8) liquid medium. Data points represent means of 6 replicates. Regression parameters were estimated using the square root transformation of the dependant variable. In the regression equation Y = no. of conidia and x = log of seed culture inoculum density. F-statistic for the model was highly significant (P=0.0001). Results are from pooled experiments.

**Table 6.** Effect of modifications of the Richard's (V-8) liquid medium on final production of conidia. x

| Modified Richard's medium recipe | Conidia / ml of medium X 106 |
|---|---|
| standard recipe + V-8, C:N y = 1:1 | 7.29 a z |
| modified recipe + V-8, C:N = 1:5 | 0.00 b |
| modified recipe - V-8, C:N = 1:1 | 0.87 c |

x Results are from pooled experiments.

y C:N = carbon:nitrogen ratio; for 1:5, $KNO_3$ content in the medium was increased 5-fold.

z Means followed by the same letters in the column are not significantly different at $\alpha = 0.05$, according to the LSD test.

the carbon to nitrogen ratio (from 1:1 to 1:5) in modified Richard (V-8) medium inhibited sporulation when measured after 2 wk incubation. Pycnidium-like structures, however, were present in the high C:N ratio medium.

**Comparison of conidia production methods.** Conidia produced in submerged culture were morphologically similar to conidia produced on solid media (pearl barley and half strength PDA plate). Viabilities of the conidia produced on agar and in liquid culture (modified Richard's [V-8] medium) were similar (Table 7). Germination of conidia produced on pearl barley grains was slightly lower ( =0.05) than in other production systems. No differences in pathogenicity, however, were detected.

**Storage of conidia.** Conidia in water were stored in a refrigerator at 4°C for 2 to 3 days without any adverse effect on germinability, but storage for up to 5 days reduced germinability (Fig.6). This gradual loss of conidia viability after 3-5 days storage at 4°C was correlated with a significant (P=0.0001) decrease in disease incidence in one experiment (Table 8). In the second experiment, storage of conidia for 5 days did not cause a significant decrease in disease incidence. No conidia were observed to germinate during the cold period.

Low-temperature (-10°C) storage in 10% sucrose solution reduced germinability and subsequent disease development (Table 9). Conidia frozen at -10°C in sucrose for 30 days were not viable in germination tests. The infrequent lesions produced on

**Table 7.** Comparison of methods to produce conidia of *P. convolvulus*. [v]

| Media | Conidia / plate or flask X $10^9$ | Germination of conidia in 0.01% PDB | Disease rating [w] | Dry weight of above-ground biomass (g) |
|---|---|---|---|---|
| half strength PDA plate | 0.38 a [x] | 99.2 a [x] | 3.8 [y] | 0.03 [z] |
| pearl barley grains | 6.91 b | 96.8 b | 3.5 | 0.08 |
| modified Richard's (V-8) liquid | 0.25 a | 99.3 a | 3.6 | 0.06 |

[v] Results are from pooled experiments.

[w] Ratings: 0 = no visible symptoms; 1 = < 25% necrosis; 2 = 25%-50% necrosis; 3 = 51-75% necrosis; 4 = > 75% necrosis.

[x] Means followed by same letter in the same column are not significantly different at $\alpha$=0.05, according to the LSD test.

[y] No significant difference among treatments at the 0.15 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks.

[z] No significant difference among treatments at $\alpha$=0.05, according to the F-test.

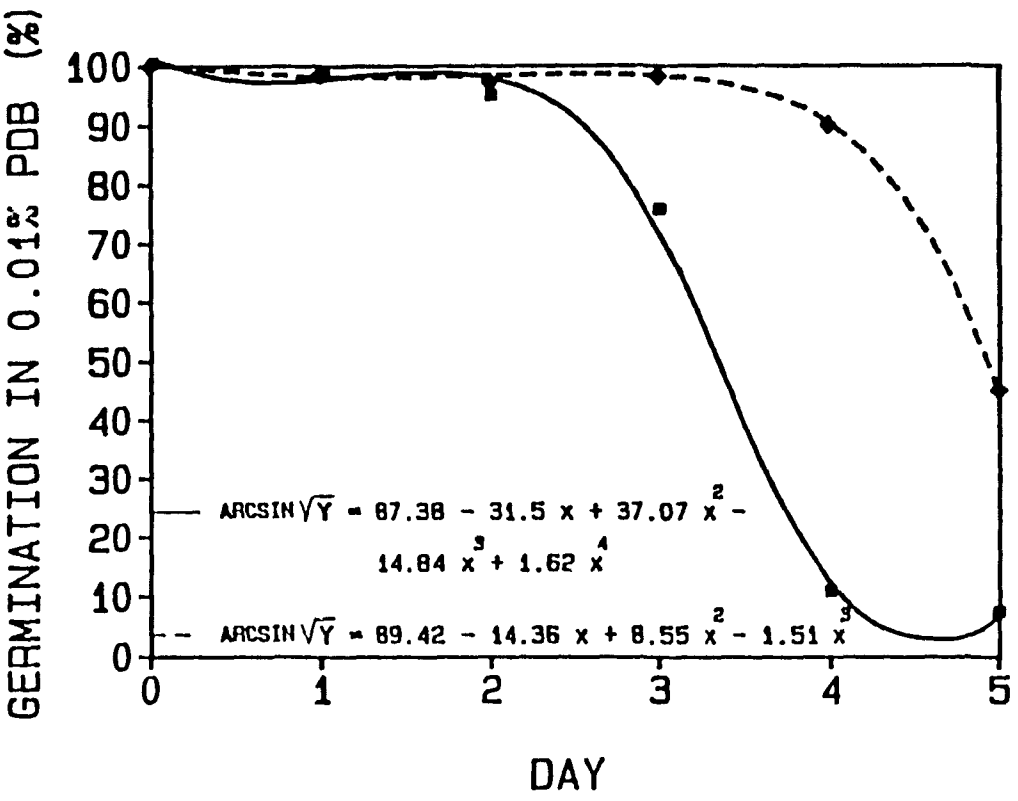


**Figure 6.** Effect of short-term cold (4°C) storage in water on germinability of P. convolvulus conidia. Experiments were not combined because a significant difference (P=0.0001) was detected. First and second experiments are represented by ---- and ———, respectively. Data points represent means of 4 replicates with 2 sample units each. Regression parameters were estimated following arcsin transformation of the dependant variable. In the regression equations Y = percent germination and x = no. of days. F-statistic for the model of each experiment was highly significant (P=0.0001).

**Table 8.** Effect of short-term cold (4°C) storage in water on pathogenicity of *P. convolvulus* conidia.

| Duration of storage (days) | Disease rating x | |
|---|---|---|
| | Exp 1 | Exp 2 |
| 0 | 4.0 y | 4.0 a z |
| 1 | 4.0 | 3.8 ab |
| 2 | 4.0 | 4.0 a |
| 3 | 3.8 | 2.9 ab |
| 4 | 4.0 | 1.5 b |
| 5 | 3.7 | 1.0 b |

x Ratings : 0 = no visible symptoms; 1 = $<$25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$75% necrosis.

y No significant difference among treatments in the column at the 0.15 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks.

z Means followed by the same letter in the column are not significantly different at the 0.15 experiment wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparaison procedure.

**Table 9.** Effect of low-temperature (-10°C) storage on *P. convolvulus* conidia suspended in 10% sucrose solution. [w]

| Duration of storage (days) | Germination of conidia in 0.01% PDB (%) [x] | | Disease rating [y] [z] | | Dry weight of above-ground biomass (g) [x] | |
|---|---|---|---|---|---|---|
| | Exp 1 | Exp 2 | Exp 1 | Exp 2 | Exp 1 | Exp 2 |
| 0 | 98.5 | 98.9 | 3.9 | 4.0 | 0.02 | 0.00 |
| 30 | 0.0 | 0.0 | 1.0 | 1.2 | 0.68 | 0.23 |

[w] Experiments were not combined because variances were not homogenous for parametric data.

[x] Significant difference among treatments at $\alpha$=0.05, according to the F-test.

[y] Ratings: 0 = no visible symptoms; 1 = $<$25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$75% necrosis.

[z] Significant difference among treatments at $\alpha$=0.05, according to the Median test.

field bindweed plants were probably due to the few conidia that survived the cold treatment or thawing process. Low-temperature storage at -70°C and subsequent thawing at room temperature reduced germination in PDB after 5-6 months (Fig.7). No significant differences ($\alpha$=0.05) in pathogenicity, however, were detected among these conidia stored at -70°C.

## DISCUSSION

Several methods to produce P. convolvulus conidia were discovered. Solid agar substrate fermentation used by Ormeno-Nunez et al. (1988) is time consuming and expensive, and was abandoned for large-scale production of conidia for field studies. Solid substrate fermentation utilizing crude agricultural commodities is commonly used for mass production of spores which can be used to transform organic compounds (Mudgett, 1986) and for microbial insecticides (Soper & Ward, 1981). Natural solid substrates pretreated by pearling (grinding off of the outer portions of kernel), crushing, or steaming are preferred over untreated natural substrates. These pretreatments make chemical constituents more accessible and the physical structure more favorable for mycelial penetration (Mudgett, 1986). Differences in availability of nutrients, moisture content, surface area, vitamins and other growth factors may be responsible for the variable sporulation response of P. convolvulus on the solid media tested. Previous studies on P. convolvulus indicated that solid agar media rich in

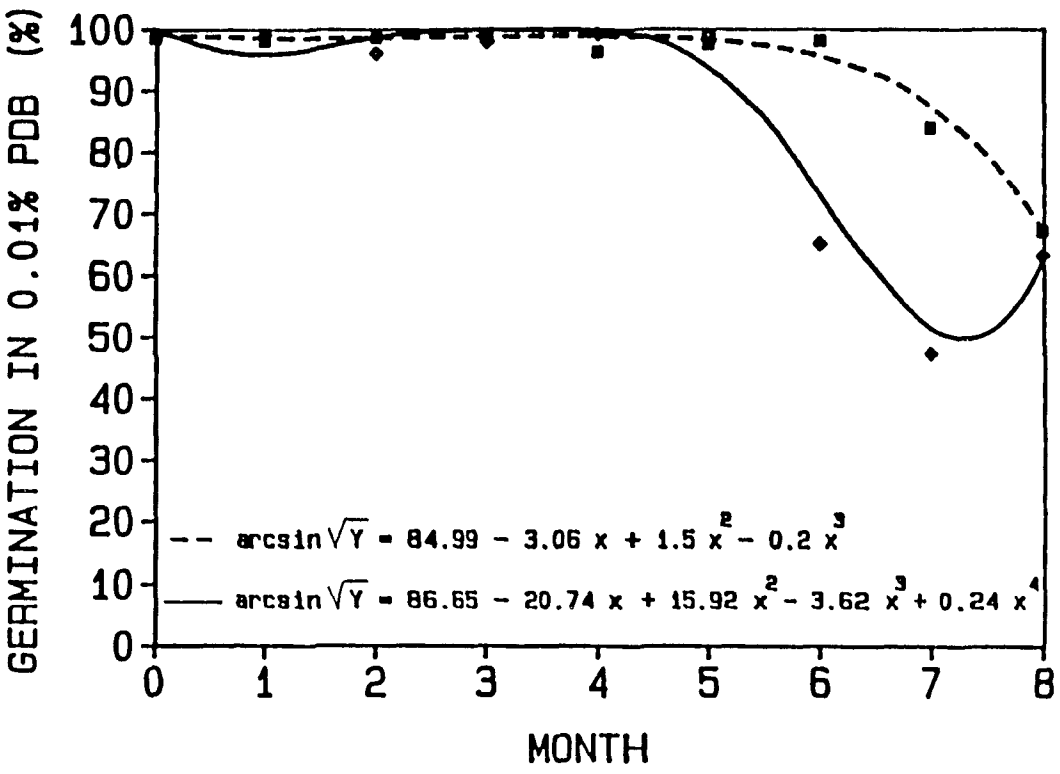


**Figure 7.** Effect of low-temperature (-70°C) storage on *P. convolvulus* conidia suspended in 10% sucrose solution. Experiments were not combined because variances were not homogenous. First and second experiments are represented by---- and ——, respectively. Data points represent means of 4 replicates with 2 sample units each. Regression parameters were estimated following arcsin transformation. In the regression equations Y = percent germination and x = no. of months. F-statistic for the model of each experiment was highly significant (P=0.0001).

carbohydrates (e.g. PDA) greatly enhance vegetative growth while low carbohydrate media (e.g. half strength PDA) support limited mycelial growth followed by rapid differentiation into pycnidia (Ormeno-Nunez, 1987). Limited diffusion of nutrients on solid materials is known to favor differentiation and sporulation of some fungi (Mudgett, 1986).

Germination in water of P. convolvulus conidia produced on the various solid media was different. Cochrane (1966) suggested that during fungal growth and sporulation, the accumulation of reserve materials in conidia differed depending on the type of medium involved. Also, various amino acids carried over from the growth medium may lower the requirement for exogenous nutrients. Low germination of conidia in water compared to 0.01% PDB was not surprising since some of the relatively short-lived asexual spores of the Fungi Imperfecti often require exogenous carbon, nitrogen, or other compounds (Cochrane, 1966).

The conidia yield from one flask containing 10 g of pearl barley at 64% moisture content (at time of seeding) was equal to the yield of approximately 18 agar plates. Pearl barley grains covered with P. convolvulus pycnidia yielded 5 to 7 X $10^8$ conidia/g of substrate. This result was superior to Fusarium solani infested-cornmeal sand granules which produced 1.6 X $10^7$ propagules/g of substrate (Boyette et al., 1984).

Most conidia of P. convolvulus produced in pycnidia on the surface of pearl barley grains were viable for at least 4 months. The mucilagenous matrix extruded from pycnidia during

the release of conidia may be responsible for this prolonged conidia survival. Louis & Cook (1983) demonstrated the adverse effect of drying on matrix-free spores of Sphaerellopsis filum (Biv-Bern:Fr) Sutton. The same phenomenon was observed with spores of Colletotrichum graminicola (Ces.) Wils. which completely lost their viability within 48 hr when exposed to low relative humidities after removal of the matrix (Nicholson & Moraes, 1980). These authors suggested that spore matrix has a role in protection of spores against desiccation.

The prolonged survival of P. convolvulus conidia produced on pearl barley suggests the potential for application of pycnidia-bearing pearl barley grains to the field in a manner similar to mycelial-vermiculite and mycelial-alginate granules of Phyllosticta sp. proposed by Walker (1981) and Walker & Connick (1984). Walker (1983) suggests that such granular formulations are suitable because pycnidia (and possibly also the conidial matrix) would help protect the conidia from adverse environmental conditions. P. convolvulus pycnidia formed on pearl barley surface were shown to produce conidia over an extended period of time (about one month), with a peak of production at 2 wk after seeding of the substrate. This characteristic may be favorable under field conditions, allowing multiple releases of conidia over time.

Production of conidia on solid substances is time consuming, labor intensive, prone to contamination, and may be uneconomical (TeBeest, 1985, Thomas et al., 1987). Submerged

production techniques are favored since the expertise and technology are available, and because scale-up of the process is relatively easy (Churchill, 1982). Some fungi investigated as potential mycoherbicides, however, such as Cercospora rodmanii Conway (Kenney et al., 1976) and Alternaria spp. (Walker, 1981,1982), do not sporulate in liquid culture. Other fungi like Colletotrichum spp. produce few true conidia but abundant binary fission spores and blastospores under submerged culture conditions (Churchill, 1982). The latter types of spores are often reported to be more labile, unstable, and difficult to preserve even though they are infectious (Kenney et al. 1976, Van Winkelhoff & McCoy 1984). Formation of pycnidium-like structures by P. convolvulus in complex liquid media (half strength PDB and modified Richard's [V-8]) was surprising, and countered the widespread belief that pycnidial fungi had an absolute requirement for solid attachment for formation of fruiting bodies and abundant conidia production.

Culture media made from plant materials are rarely deficient in complex organic compounds, such as vitamins. Hildebrand & McCain (1978) explain that the various effects of natural plant diffusates on chlamydospore formation of Fusarium oxysporum Schl. f.sp.cannabis Noviello & Snyder is probably related to their different nutritional composition. Possibly for the same reason, addition of V-8 juice in modified Richard's solution was responsible for extensive growth of P. convolvulus. In this medium, mycelial differentiation decreased and no

conidia were extruded from pycnidia (2 wk after seeding of medium) when the nitrate concentration was increased (C:N ratio changed to 1:5). The importance of the carbon/nitrogen ratio for optimum production of some fungi has been discussed by several researchers (Churchill 1982, Soper & Ward 1981, Thomas et al. 1987, Van Winkelhoff & McCoy 1984, Vezina et al. 1965). Peptone, a complex nitrogen source, did not favor production of P. convolvulus conidia in liquid culture. Media containing peptone, neopeptone, tryptone, or yeast extract have prevented conidia production of the entomopathogenic fungus Beauveria bassiana (Bals) Vuill. (Thomas et al., 1987). Blastospores, however, were produced in these liquid media. Thomas et al. (1987) reported that a carbon to nitrate ratio of 5:1 promoted conidiation in B. bassiana. This finding differs from P. convolvulus which produced low numbers of conidia in defined liquid media with such a high C:N ratio (Richard's solution, Czapek Dox).

The yield of Phomopsis conidia in modified Richard's (V-8) medium was 10-100 times less than other submerged cultures of fungi investigated as bioherbicides (Boyette et al. 1984, 1979, Wymore et al. 1987, Churchill 1982). Virulence and germinability of the submerged conidia, however, was comparable to conidia produced on solid substrates.

Adjustment of inoculum size for maximum submerged sporulation of filamentous fungi is suggested by Vezina et al. (1965). They reported that increased inoculum size allows the highest sporulation of Septomyxa affinis (Sherbakoff)

Wollenweber in modified Czapek's solution. In the present study the opposite was observed. Using a high density of conidia to seed liquid cultures adversely affected the subsequent yield of conidia. Large numbers of germinating spores (per ml of medium) may have rapidly depleted carbon source and thus lowered the C:N ratio, resulting in inhibition of pycnidia formation as discussed earlier. Excessive production of secondary metabolites by fungal mycelium, low oxygen exchange, and unfavorable pH are other possible explanations for reduced conidiation.

P. convolvulus conidia suspended in water can be stored at 4°C for about the same period (2-4 days) as the northern jointvetch pathogen, Colletotrichum gloeosporioides f.sp. aeschynome (Daniel et al., 1973). Hydrated conidia are believed to be more metabolically active (Thomas et al., 1987) and depletion of nutrient reserves to sustain conidial metabolism may be responsible for limited survival in water at 4°C. Prolonged survival of most conidia frozen at -70°C was probably due to the balanced effects of the cryoprotective agent (sucrose), and rapid rate of cooling of the mechanical freezer (1 to 2°C/min) that prevented intracellular ice formation and restricted conidia dehydration (Mazur, 1968).

Several methods and media have been described to produce sufficient quantities of P. convolvulus conidia. Submerged liquid culture offers significant avantages over solid substrate fermentation for future industrial scale-up. Optimization of nutrient balance, pH, temperature, and aeration of the liquid

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Several methods and media have been described to produce sufficient quantities of P. convolvulus conidia. Submerged liquid culture offers significant avantages over solid substrate fermentation for future industrial scale-up. Optimization of nutrient balance, pH, temperature, and aeration of the liquid

fermentation process will probably improve spore yields. Low-temperature storage of conidia was adequate and permits preparation in advance of inoculum to be used in large field experiments.

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## CONNECTING TEXT

A large quantity of infective inoculum is a primary requirement for studies of the potential of an organism to be used as a bioherbicide. Various systems to produce Phomopsis convolvulus inoculum were developed and they permitted the completion of several large experiments on the effect of the fungus on Convolvulus arvensis.

To complete the work of Ormeno-Nunez (1987), more research was required to describe precisely the main environmental factors affecting the initiation, development, and severity of the disease on field bindweed plants. The presence of free water and the amount of inoculum were primarily considered since they represent the major limiting factors of most bioherbicides under natural conditions. The great importance of leaf wetness duration in the epidemiology of the disease was emphasized and several experiments were designed to evaluate its impact on disease expression.

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Ormeno-Nunez, J. 1987. Phomopsis convolvulus sp. nov., a foliar pathogen of field bindweed (Convolvulus arvensis) and its potential as a mycoherbicide. Ph. D. thesis. McGill University, Ste-Anne-de-Bellevue, Canada.

## III. EFFECT OF LEAF WETNESS AND OTHER LEAF SURFACE FACTORS ON *PHOMOPSIS CONVOLVULUS* INFECTION AND DISEASE DEVELOPMENT ON FIELD BINDWEED.

### INTRODUCTION

Field bindweed (*Convolvulus arvensis* L.) is a noxious and economically important weed associated with 32 different annual and perennial crops in more than 44 countries (Holm *et al.*, 1977). An extensive, long term program involving traditional control measures is usually required to reduce populations of field bindweed to acceptable levels (Derscheid *et al.* 1970, Weaver & Riley 1982). Since the early 1970's, considerable interest in biological control of field bindweed has developed in North America (Rosenthal, 1981). Recently a foliar pathogen, *Phomopsis convolvulus* Ormeno, was reported to incite leaf spots and anthracnose lesions on field bindweed and demonstrated great potential for use as a bioherbicide (Ormeno-Nunez *et al.*, 1988). The optimum conditions for infection and disease development were established under controlled environments by Ormeno-Nunez *et al.* (1988). A minimum of 12 hr of free water on the foliage at 20°C was necessary to allow extensive disease development on plants inoculated with 5 X $10^6$ spores/ml or more.

Several constraints on a disease may retard or suppress an epidemic. Unfavorable environmental conditions, the genetic makeup of host and pathogen, and/or the amount of inoculum present may restrain disease development (Holcomb, 1982). Nearly all pathogens of above-ground parts of plants require a film of free moisture on the plant surface at early stages of infection (Colhoun, 1973). The presence of free water during P. convolvulus conidia germination, elongation of germ-tube, appressoria formation, and penetration is essential for symptom development. Further investigations were required to improve, extend, and supplement the work of Ormeno-Nunez et al. (1988). The study presented here describes more accurately the relationship between moist conditions and this particular disease of field bindweed.

An understanding of the epidemiology of disease is essential to manipulate pathogens for the biological control of weeds (Holcomb 1982, Tebeest 1985). The main purpose of this research was to determine the effect of moist conditions on sporulation of P. convolvulus on diseased field bindweed plants. Effects of short wet periods interrupted by dry periods and the influence of inoculum density and spray additives under various periods of leaf wetness on disease expression were also studied.

## MATERIALS AND METHODS

**Inoculum production.** Small pieces of agar with mycelium from the stock culture (single-conidium isolate of P. convolvulus grown

on potato carrot agar slants in small vials and maintained under mineral oil at 4°C) (Tuite, 1969) were transferred to fresh potato dextrose agar (PDA) in Petri dishes (9 cm diameter). Plate cultures were incubated at room temperature (approx. 21°C) with 12-14 hr of fluorescent light for 45-60 days. Cultures at this stage had an abundance of conidial droplets oozing from pycnidia embedded in the mycelial mat. Using a 5 ml sterile syringe (Becton-Dickinson, Rutherford, NJ), the surface of each colony was flushed several times with 5-10 ml of sterile deionized water until most conidia were removed and suspended in water. Forty to fifty single droplets of this conidial suspension were deposited on half strength PDA plates (12 g/L potato dextrose broth, 15 g/L Difco Bacto agar and 100 ml/L of bindweed decoction) (Ormeno-Nunez et al., 1988). Plates were sealed with Parafilm $^{R}$ (American Can Company, Greenwich, CT) and incubated for 2-3 wk at room temperature. After this period, numerous pycnidia developed and produced large masses of conidia in mucilagenous matrix. These plates were flooded and flushed with 10-15 ml sterile deionized water using a syringe. One ml of this conidia suspension was used to seed 10 g of pearl barley grains moistened with 20 ml of $H_2O$ in a 250 ml Erlenmeyer flask and autoclaved for 17 min (100 kPa and 120°C). After 2 wk incubation at ambient conditions, conidia were harvested by adding 50 ml of deionized water to the flask, shaking the flask on a rotary shaker at 250 rpm for 5-10 min., and pouring the contents through a soil sieve (250 um, Fisher Scientific) lined

with two layers of cheesecloth. Inoculum density was determined with the aid of a haemacytometer and adjusted to the desired density with water.

**Plant production.** Field bindweed seeds (Valley Seed Co., Fresno, CA) were washed under warm running tap water for 2-3 hr and soaked in deionized water for approximately 24 hr. Imbibed seeds were germinated on the surface of moist filter papers in glass Petri dishes at 24°C for 24-36 hr in the dark. Four germinated seeds (radicle 5-10 mm in length) were sown in potting medium (Pro-Mix Bx, Premier Brands, Inc. New Rochelle, NY) in 10-cm plastic pots and grown in controlled environment chambers (Conviron, Model E-15, Controlled Environments, Winnipeg, Man.) (23/18°C day/night temperature, 15-hr photoperiod, 250 $uEm^{-2}s^{-1}$). After 6-9 days plants were thinned to three seedlings per pot.

**General inoculation procedures and data collection.** Three- to five-leaf stage plants were inoculated with conidia suspended in 0.1% (w/v) gelatin solution (unless otherwise indicated) at the various densities specified for each experiment, using a spray chamber (Research Instrument Manufacturing Co. LTD, Guelph, Ontario) with a full cone nozzle (TG 0.7), 200 kPa air pressure, a speed of 0.85 kph, and a spray volume of 500 L/ha. Plants were incubated in a dark dew chamber at 20°C (Percival, Model E-54UDL, Boone, Iowa) for the desired period of time and subsequently transferred to a growth cabinet at the original conditions.

Disease severity was evaluated 1 wk after inoculation using a rating system based on a scale from 0 to 4 where 0 = no visible symptoms and 4 = >75% necrosis (Ormeno-Nunez et al., 1988). Seedling mortality and dry weight of above-ground biomass and roots were determined 2 wk after inoculation. Seedling mortality was evaluated by determining the number of individual plants that were completely necrotic, did not show any regrowth, and had a discolored and damaged hypocotyl. Dry weight of above-ground biomass was determined by cutting the plants at the soil level and drying living tissue in paper bags for 4-5 days at 60°C. Root biomass was determined by soaking the root masses in water for approximately 5 min., washing them with running water, and drying them in paper bags for 4-5 days at 60°C. Disease rating and mortality were evaluated for each plant and results were pooled and averaged for each pot. Dry weight of foliage and roots were recorded as total weight per pot.

**Effect of moist conditions on sporulation of P. convolvulus on infected leaves.** Thirty-two pots of field bindweed seedlings were inoculated with $10^9$ conidia/$m^2$, placed in the dew chamber for 18 hr and incubated for 6 days in the controlled environment chamber. Pots were then covered with small plastic bags to provide a humid environment around the infected plants. Every day for 7 days, 4 replicate pots were selected randomly. Three heavily infected leaves of similar size were removed from each pot, placed in a vial, and soaked in 3 ml deionized water for 5

min. The vials were lightly shaken by hand before an aliquot of the water was sampled. The number of conidia present in the water was determined with the aid of a haemacytometer.

**Effect of inoculum density and leaf wetness duration on disease development.** Field bindweed seedlings were inoculated with 0, $10^6$, $10^7$, $10^8$, $10^9$, and $10^{10}$ conidia/$m^2$, placed in the dew chamber for 6, 12, 18, and 24 hr, incubated in the growth cabinet, and assessed for disease severity, mortality, and dry weight of above-ground biomass and roots as described above.

**Cumulative effect of short wet periods interrupted by dry periods on disease development.** Field bindweed seedlings were inoculated with $10^9$ conidia/$m^2$ and subjected to one of the following wet/dry regimes: 1) 0 hr leaf wetness period (LWP), 2) 6 hr LWP, 3) 6 hr LWP, 18 hr dry period (DP), and 6 hr LWP (6 + 6), 4) 6 hr LWP, 18 hr DP, 6 hr LWP, 18 hr DP, and 6 hr LWP (6 + 6 + 6), 5) 18 hr LWP. The wet period (in dark dew chamber) corresponded to the presence of free water on the foliage and the dry period was either at 80-83% ambient air relative humidity (RH) or 97-100% RH. Two small humidity chambers covered with transparent plastic bags were placed in one growth cabinet and provided two different environments during the dry period. For the 97-100% RH environment the plastic tray in the chamber was filled with deionized water and the plastic bag was tightly closed. For the 80-83% RH environment, the plastic bag was left open and the tray empty. An electronic psychrometer (Model 90,

Yellow Springs Instrument Co., Yellow Springs, Ohio) was used to measure relative humidity in each chamber once a day. After the 3-day period, plants were removed from the humidity chambers and placed in the same growth cabinet for the remaining 11-day incubation period. Disease severity rating, mortality, and dry weight of above-ground biomass and roots were assessed.

**Effect of various additives and leaf wetness durations on disease development.** Field bindweed seedlings were inoculated with conidia suspensions ($10^9$ conidia/$m^2$) mixed with three additives at two concentrations: 1 and 2% (w/v) gelatin (BDH Chemicals, Toronto, Ont.), 20 and 30% SORBO TM (v/v) (64% sorbitol, Atkemix Inc., Brantford, Ont.), and 0.74 and 1.48 L/ha BOND TM (carboxylated synthetic latex, Loveland Industries Inc., Loveland, Colorado). Following inoculation, plants were subjected to 12, 14, 16, or 18 hr of leaf wetness in a dew chamber and incubated in the growth cabinet until assessment of disease severity, mortality, and dry weight of above-ground biomass.

**Data analysis.** A completely randomized design with 3 or 4 replicates per treatment was used for all experiments. All experiments were performed twice. Numbers of conidia recovered from infected leaves and mortality data were transformed with the square root and arcsin transformation, respectively, prior to analysis (Steel & Torrie, 1980). Factorial experiments were

analyzed with a factorial analysis of variance (ANOVA) considering the effect of each factor individually and their interaction. Polynomial regression analysis was performed when appropriate on the parametric data. Experimental results for the two trials of each experiment were not pooled because homogeneity of variances using Barlett's test was not observed (Steel & Torrie, 1980), or a significant difference due to the experiment was detected, or both. Results from only one experiment are presented when a similar trend was observed in the other. Differences between treatment means were established with a Tukey's W test ($\alpha$=0.05) (Steel & Torrie, 1980). Disease ratings were compared by the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure to evaluate differences between treatment means or by the Median test for two independant samples (when appropriate) (Daniel, 1978).

## RESULTS

**Conidia production on diseased field bindweed leaves.** Pycnidia and newly produced conidia were detected after 2 days on heavily diseased field bindweed plants placed under moist conditions (Fig. 2a). The number of conidia washed from the leaves increased with increasing length of the moist period and reached a plateau in one experiment but continued to increase in the other experiment (Fig. 1). P. convolvulus did not produce pycnidia on diseased plants in the absence of additional moist

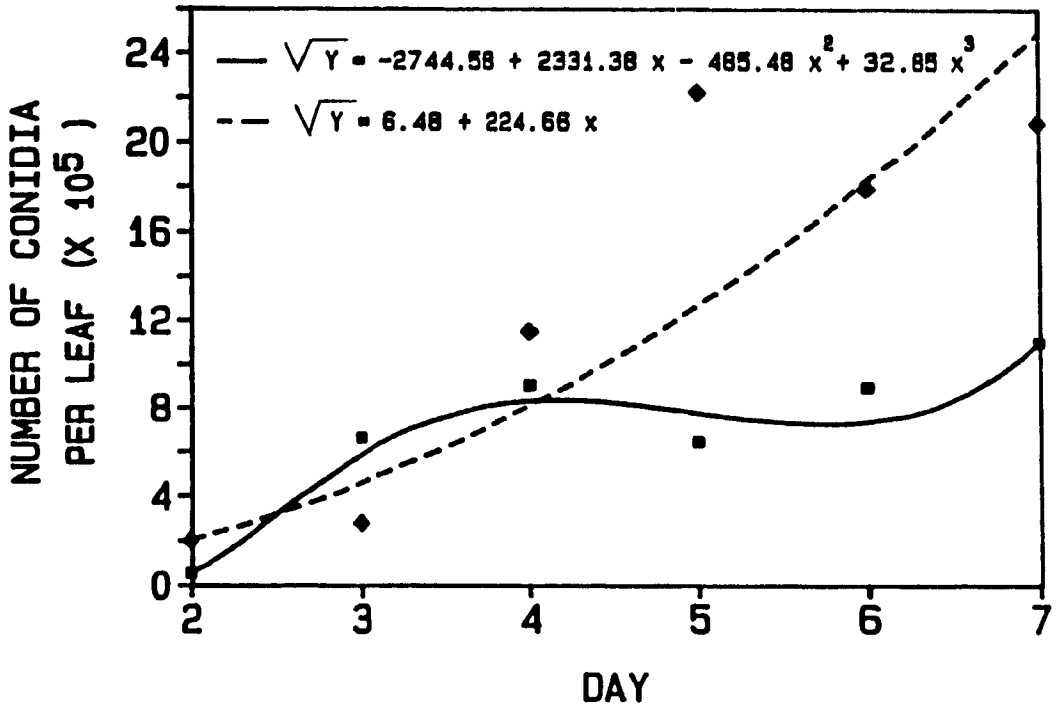
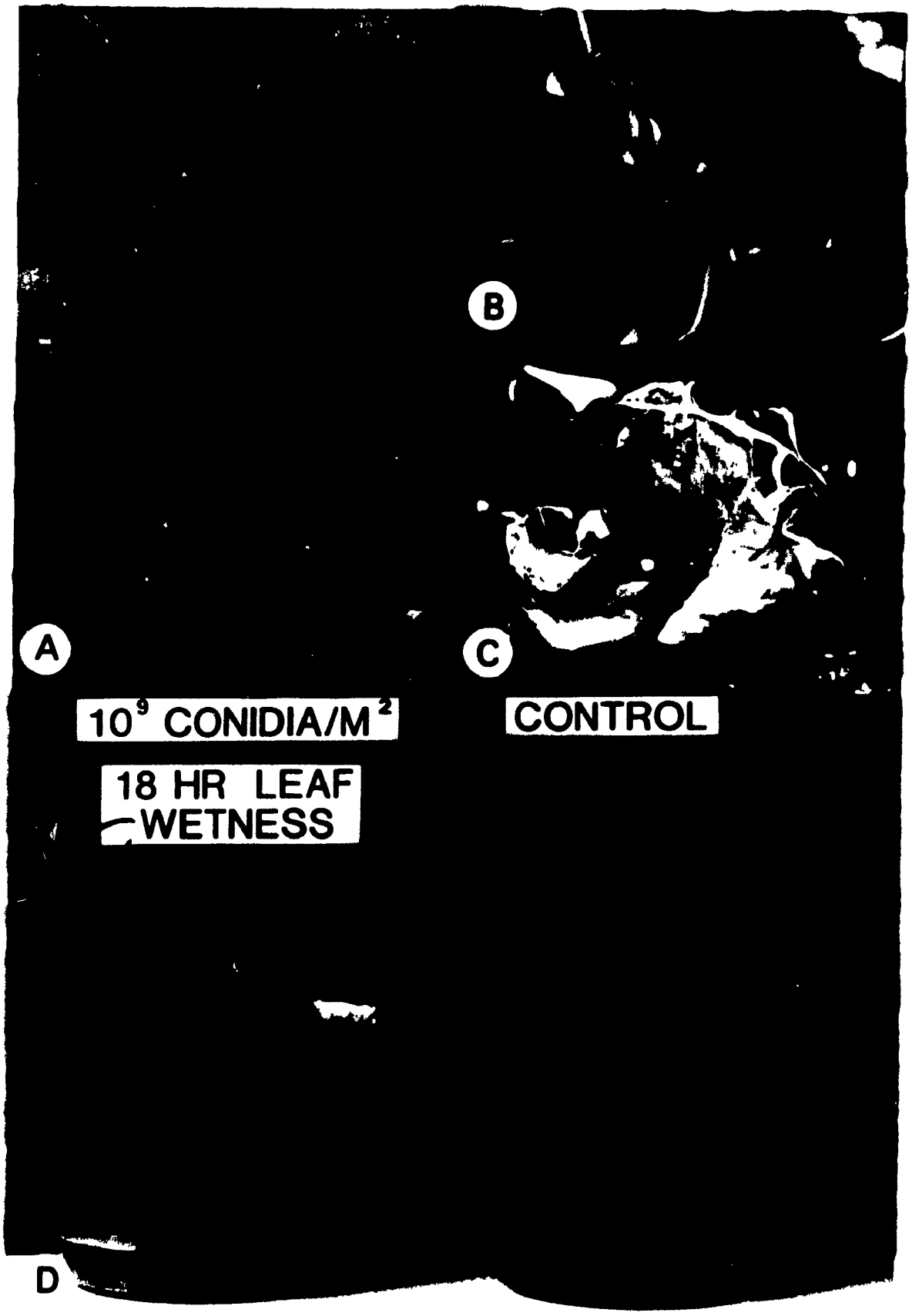



**Figure 1.** Effect of moist conditions on sporulation of P. convolvulus on infected leaves. Experiments were not combined because variances were not homogenous. First and second experiments are represented by ---- and —— respectively. Data points represent means of 4 replicates with 3 sample units each. Regression parameters were estimated following square root transformation. In the regression equations Y = no. of conidia per leaf and x = no. of days. F-statistic for the model of each experiment was highly significant (P=0.0001).

**Figure 2.** Effect of leaf wetness and inoculum density on disease expression on field bindweed. **A.** Pycnidia produced on a diseased field bindweed plant incubated for 5 days under moist conditions. Bar equals 500 µm. **B.** Type of lesions observed on plants inoculated with $10^7$ conidia/$m^2$ and subjected to 18 hr leaf wetness. **C.** Heavily infected plant inoculated with $10^9$ conidia/$m^2$ and subjected to 18 hr leaf wetness. **D.** Non-inoculated (control) field bindweed seedlings (right) and seedlings inoculated with $10^9$ conidia/$m^2$ and subjected to an 18 hr leaf wetness (left).


B
A
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$10^9$ CONIDIA/$M^2$
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18 HR LEAF WETNESS
D

periods. A few pycnidia, however, were sometimes observed on diseased plant parts (bases of stems, lower leaves) in contact with the moist potting medium.

**Effect of inoculum density and leaf wetness duration on disease development.** On field bindweed seedlings, P. convolvulus symptoms differed depending on the inoculum density, leaf wetness duration, or both. When low inoculum densities ($10^6$ and $10^7$ conidia/$m^2$) were applied to plants, a few, scattered, small lesions (1mm diameter) were observed on leaves within the first week after inoculation (Fig. 2B). Short leaf wetness periods ($<12$ hr) resulted in the same type of symptoms. A high inoculum density ($10^9$ conidia/$m^2$) and a long leaf wetness period ($>18$ hr) were ideal conditions for the development of numerous pinpoint lesions covering leaves, stems, and petioles and causing extensive blighting and necrosis on the plants (Fig. 2C).

In controlled environment studies, $10^9$ conidia/$m^2$ was the optimum inoculum density since it caused maximum disease of field bindweed seedlings subjected to 12 hr leaf wetness period or more after inoculation (Table 1, Figs. 2D, 3). A higher inoculum density ($10^{10}$ conidia/$m^2$) did not improve the efficacy of P. convolvulus. This result may have been due to technical difficulties caused by the presence of a large amount of the thick mucilagenous matrix in the conidia suspension, which was difficult to apply with the full cone agricultural nozzle and resulted in uneven coverage. An extended leaf wetness period

Table 1. Effect of leaf wetness duration and inoculum density on disease severity and mortality of field bindweed. [w]

| Inoculum density (log conidia/$m^2$) | Leaf wetness duration (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | 6 | 12 | 18 | 24 |
| | Disease rating [x] | | | | Mortality (%) [y] | | | |
| uninoculated control | 0.0 a/e [z] | 0.0 a/e | 0.0 a/e | 0.0 a/e | 0 | 0 | 0 | 0 |
| 6 | 0.0 a/e | 0.0 a/e | 0.2 a/e | 0.6 a/e | 0 | 0 | 0 | 0 |
| 7 | 0.0 a/e | 0.4 ab/ef | 1.2 ab/f | 1.4 ab/f | 0 | 0 | 0 | 0 |
| 8 | 0.0 a/e | 1.3 ab/ef | 2.4 ab/f | 3.2 ab/f | 0 | 0 | 8.3 | 16.5 |
| 9 | 0.1 a/e | 1.8 b/ef | 3.9 b/f | 3.8 b/f | 0 | 0 | 83.0 | 83.0 |
| 10 | 0.1 a/e | 2.1 b/ef | 3.5 b/f | 4.0 b/f | 0 | 0 | 58.0 | 100.0 |

[w] Experiments were not combined because variances were not homogenous for parametric data. Similar results, however, were observed for both experiments. Results from one experiment are presented.

[x] Ratings: 0 = no visible symptoms; 1 = $<$25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$75% necrosis.

[y] For mortality data, inoculum densities of 0, $10^6$, $10^7$ conidia/$m^2$ and leaf wetness durations 6 and 12 hr were not included in the analysis because no variance was detected over all levels of each factor for each treatment. No significant difference (P=0.0708) between 18 and 24 hr leaf wetness durations was observed and a significant (P=0.0001) effect was detected among the levels of inoculum density tested, according to the F-test.

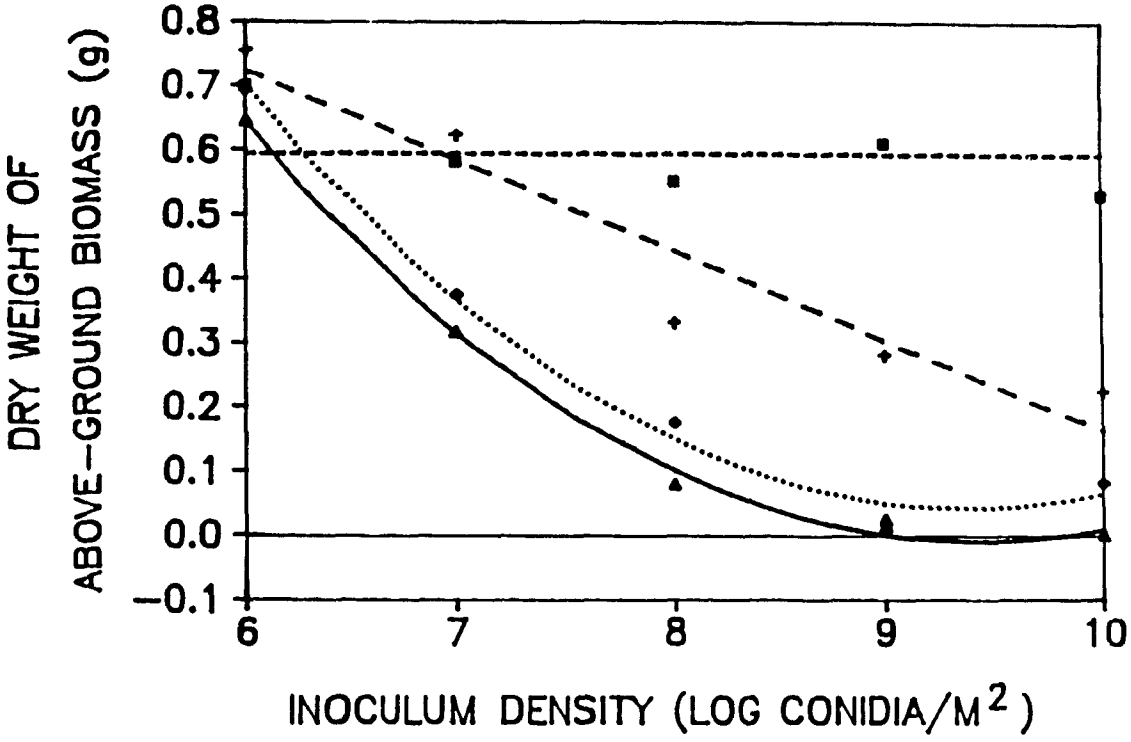
[z] Means followed by the same letter in a column (a,b) or in a row (e,f) are not significantly different at the 0.10 and 0.15 experiment-wise error rate respectively, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

**Figure 3.** Effect of leaf wetness duration and inoculum density on field bindweed. Experiments were not combined because variances were not homogenous. Similar trends, however, were observed for both experiments. Results from one experiment are presented. Data points represent means of 4 replicates.

A) Dry weight of above-ground biomass versus inoculum density at 4 levels of leaf wetness duration. There was no significant effect of 6 hr leaf wetness period ( ------ ) over all levels of inoculum density. Regression equation for 12 hr leaf wetness period ( — — — ): $Y = 1.56 - 0.14x$, for 18 hr ( ····· ): $Y = 5.16 - 1.09x + 0.06x^2$, and for 24 hr ( ——— ): $Y = 4.97 - 1.06x + 0.06x^2$ where Y = g of above-ground biomass and x = log of inoculum density. F-statistic for the model of each level of leaf wetness period was significant (P=0.0001).

B) Dry weight of above-ground biomass versus leaf wetness duration at 6 levels of inoculum density. There was no significant effect of control 0 conidia/$m^2$ ( —— ) and inoculum density of $10^6$ conidia/$m^2$ ( —·— ) over all levels of leaf wetness period. Regression equation for inoculum density of $10^7$ conidia/$m^2$ ( — — — ): $Y = 0.73 - 0.02x$, for $10^8$ conidia/$m^2$ ( ·····. ): $Y = 0.68 - 0.03x$, for $10^9$ conidia/$m^2$ ( ····· ): $Y = 1.18 - 0.11x + 0.01x^2$, and for $10^{10}$ conidia/$m^2$ ( ——— ): $Y = 0.92 - 0.08x + 0.002x^2$ where Y = g of above-ground biomass and x = hr of leaf wetness. F-statistic for the model of each level of inoculum density was significant ($P<0.001$).

A)


DRY WEIGHT OF
ABOVE-GROUND BIOMASS (g)
0.8
0.7
0.6
0.5
0.4
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6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M 2 )

B)

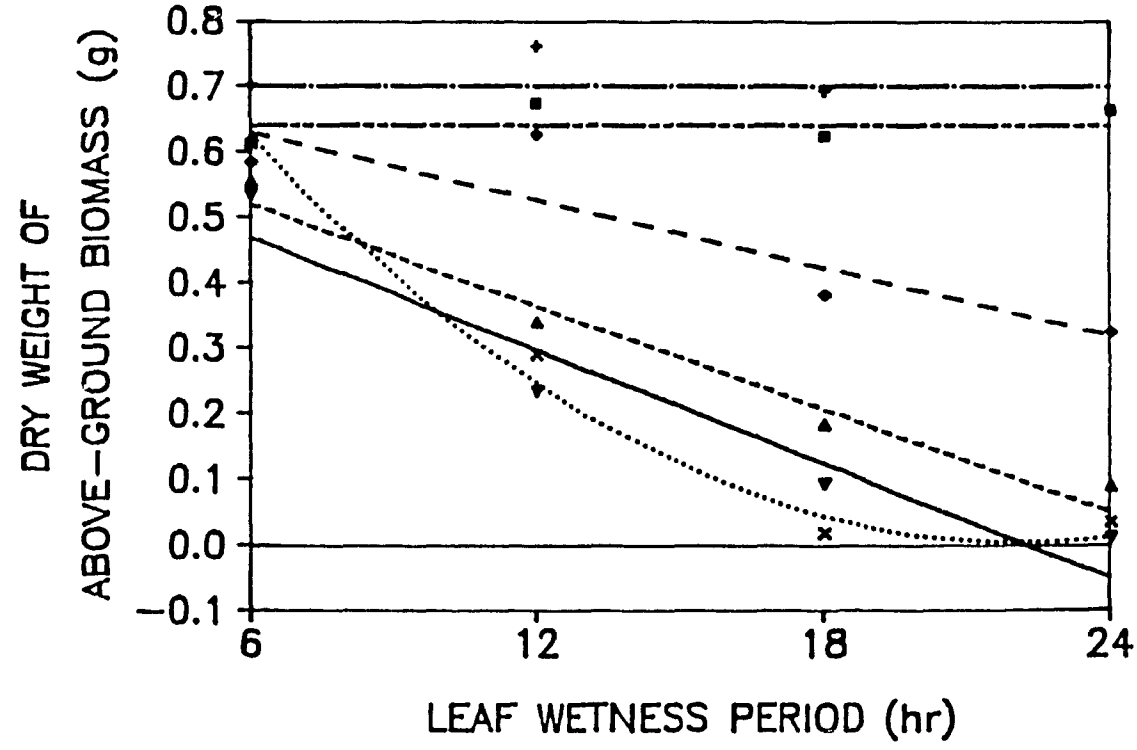
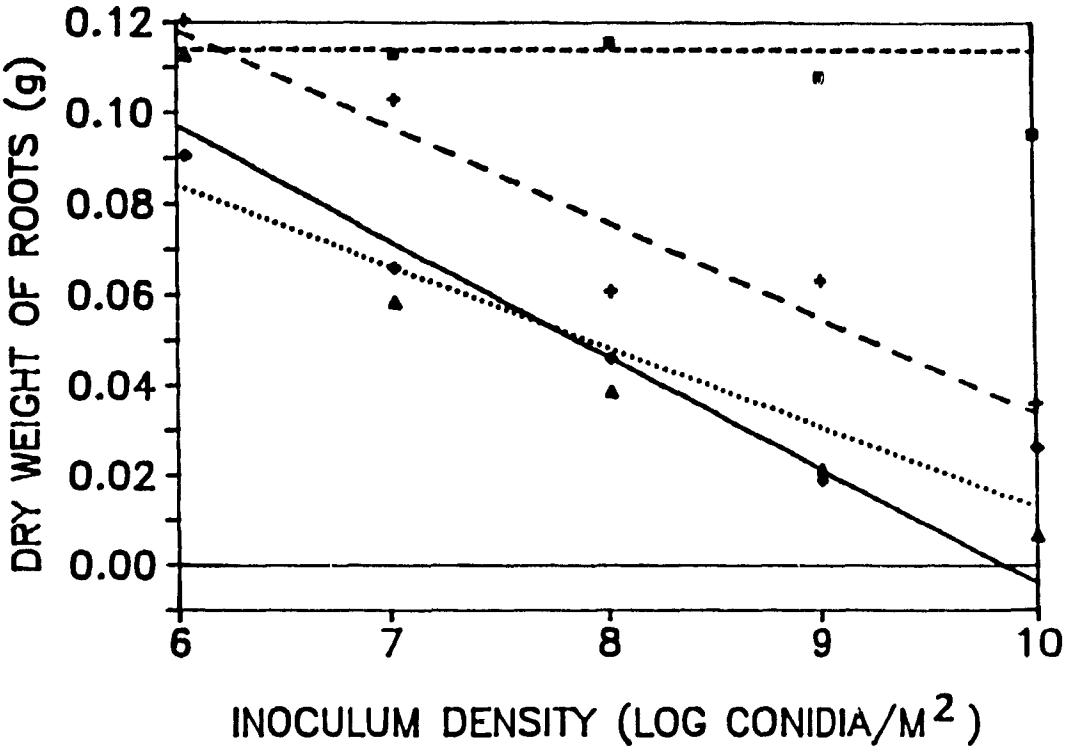

DRY WEIGHT OF
ABOVE-GROUND BIOMASS (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
6
12
18
24
LEAF WETNESS PERIOD (hr)

**Figure 3.** Continued

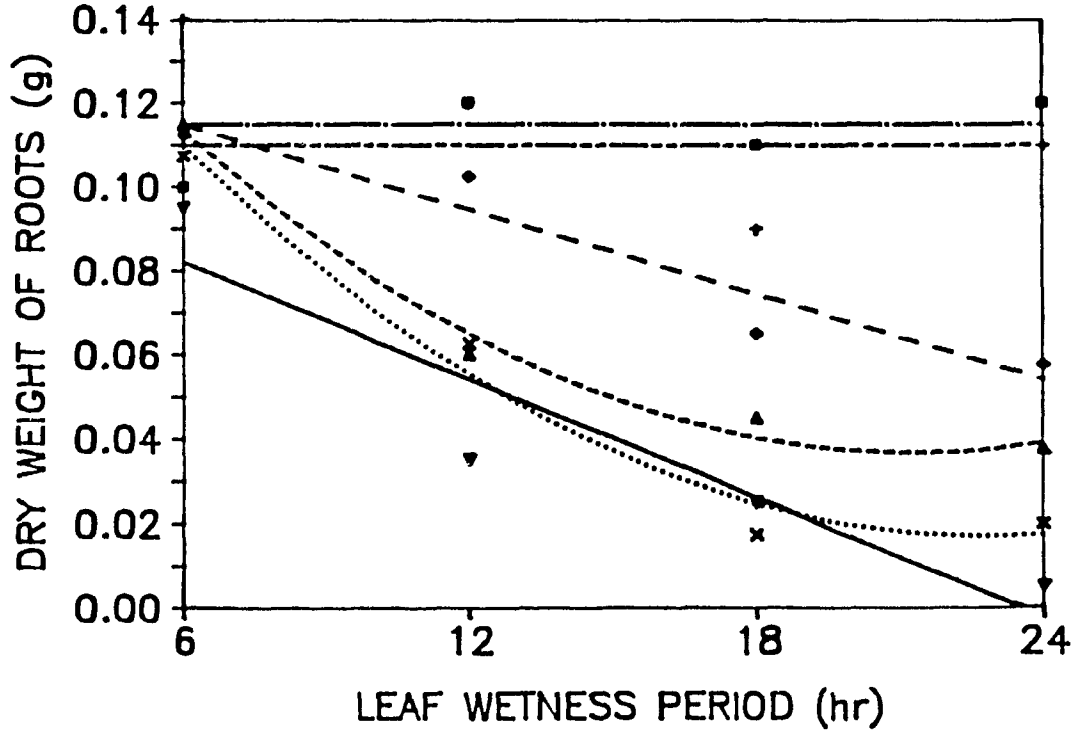
C) Dry weight of roots versus inoculum density at 4 levels of leaf wetness duration. There was no significant effect of 6 hr leaf wetness period (------ ) over all levels of inoculum density. Regression equation for 12 hr leaf wetness period (— — —): $Y = 0.24 + 0.02x$, for 18 hr ( ····· ): $Y = 0.19 - 0.02x$, and for 24 hr ( ——— ): $Y = 0.25 - 0.03x$ where Y = g of roots and x = log of inoculum density. F-statistic for the model of each level of leaf wetness was highly significant (P=0.0001).

D) Dry weight of roots versus leaf wetness duration at 6 levels of inoculum density. There was no significant effect of control 0 conidia/$m^2$ ( ——— ) and inoculum density of $10^6$ conidia/$m^2$ (——·——) over all levels of leaf wetness period. Regression equation for inoculum density of $10^7$ conidia/$m^2$ ( — — — ): $Y = 0.14 - 0.003x$, for $10^8$ conidia/$m^2$ ( ------ ): $Y = 0.19 - 0.01x + 0.0003x^2$, for $10^9$ conidia/$m^2$ ( ····· ): $Y = 0.19 - 0.02x + 0.0003x^2$, and for $10^{10}$ conidia/$m^2$ ( ——— ): $Y = 0.11 - 0.005x$ where Y = g of roots and x = hr of leaf wetness. F-statistic for the model of each level of inoculum density was highly significant ($P<0.001$).

C)


0.12
0.10
0.08
0.06
0.04
0.02
0.00
DRY WEIGHT OF ROOTS (g)
6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M 2 )

D)


0.14
0.12
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DRY WEIGHT OF ROOTS (g)
6
12
18
24
LEAF WETNESS PERIOD (hr)

after inoculation enhanced disease development on plants inoculated with $10^7$ conidia/$m^2$ or more. The effect of 18 and 24 hr leaf wetness periods on disease severity and mortality of field bindweed was not significantly different (Table 1) and the reduction in dry weight of above-ground biomass and roots was quite similar (Fig. 3). There were no significant effects of 6 hr leaf wetness duration or inoculum density of $10^6$ conidia/$m^2$ for any dependant variables measured.

**Cumulative wet periods interrupted by dry periods.** A continuous wet period (CWP, 18 hr) reduced significantly ($\alpha$=0.05) dry weight of above-ground biomass compared to interrupted wet periods (IWP, 6 + 6; 6 + 6 + 6) (Table 2). No significant differences were detected between means of dry weight of roots for the IWP and 18 hr CWP treatments, although values for IWP were much larger. Inoculated seedlings subjected to the IWP treatment developed much less severe disease symptoms than seedlings treated with 18 hr CWP. A CWP of 18 hr was significantly different from 6 hr except for disease severity and dry weight of roots when the ambient relative humidity (RH) during the dry period was 100%. IWP were not significantly different from a single 6 hr wet period treatment for all dependant variables measured. In general, a wet period of only 6 hr was too short and did not provide sufficient moisture for extensive disease development.

High air relative humidity (100%) during the dry period slightly enhanced the disease. Significant differences in dry

Table 2. Effect of cumulative short wet periods interrupted by dry periods on development of *P. convolvulus* disease on field bindweed. [u]

| wet/dry regime (hr) | Disease rating [v] [w] | | Mortality [x] (%) | | Dry weight of above-ground biomass (g) [y] | | Dry weight of roots (g) [y] | |
|---|---|---|---|---|---|---|---|---|
| | Ambient relative humidity during dry period (%) [z] | | | | | | | |
| | 80 | 100 | 80 | 100 | 80 | 100 | 80 | 100 |
| 0 | 0.0 a | 1.0 a* | 0 | 0 | 1.14 a | 0.72 a** | 0.33 a | 0.14 a*** |
| 6 | 0.6 a | 1.4 ab* | 0 | 0 | 1.04 ab | 0.34 b*** | 0.25 ab | 0.07 ab*** |
| 6 + 6 | 1.0 ab | 1.2 ab | 0 | 0 | 0.71 ab | 0.54 ab | 0.17 bc | 0.08 ab* |
| 6 + 6 + 6 | 1.1 ab | 1.7 ab | 0 | 0 | 0.61 b | 0.37 b | 0.15 bc | 0.07 ab* |
| 18 | 4.0 b | 4.0 b | 22.2 | 33.3 | 0.03 c | 0.05 c | 0.03 c | 0.01 b |

[u] Experiments were not combined because variances were not homogenous for parametric data. Similar trends, however, were observed for both experiments. Results from one experiment are presented.

[v] Ratings: 0 = no visible symptoms; 1 = $<$ 25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$75% necrosis.

[w] Means followed by the same letter in a column are not significantly different at the 0.15 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

[x] No significant difference between treatments at $\alpha$=0.05, according to the F-test

[y] Means followed by same letter in a column are not significantly different at $\alpha$=0.05, according to Tukey's W test.

[z] For each dependant parametric variable, mean values between 80 and 100% RH for each wet/dry regime differ significantly ($\alpha$=0.05*, $\alpha$=0.01**, $\alpha$=0.001***) according to the F-test on simple effects of RH. For disease ratings, mean values between 80 and 100% RH differ significantly at $\alpha$=0.05*, according to the Median test.

weight of roots were observed for the control, 6 hr CWP and IWP treatments. Reduction in dry weight of above-ground biomass and increase in disease severity between 80% and 100% RH was only significant for the control and 6 hr CWP. There was no effect of RH during the dry period following an 18 hr CWP.

**Effect of inoculum density and additive.** None of the additives tested enhanced disease development on Convolvulus arvensis. Dry weight of above-ground biomass of seedlings inoculated with a conidial suspension mixed with gelatin or SORBO TM and subjected to a short 12 hr leaf wetness period was only slightly reduced, as shown by the high Y intercept of their regression lines (Fig. 4). The steep slopes of the regression lines of gelatin and SORBO TM, however, indicate that their adverse effect on disease rapidly disappeared as the leaf wetness period increased. The same phenomenon was observed with the disease severity ratings (Table 3).

For the control, mortality of the weed increased as the leaf wetness period was extended (Fig. 5). The rate of increase was lower for the additive treatments. Mortality for gelatin and SORBO TM treatments was significantly lower than the control. An increased leaf wetness period had no significant effect on the highest concentration treatment of gelatin and SORBO TM, which resulted in the lowest percent mortality of the weed. BOND TM had no significant effect and was comparable to the control for all variables measured.

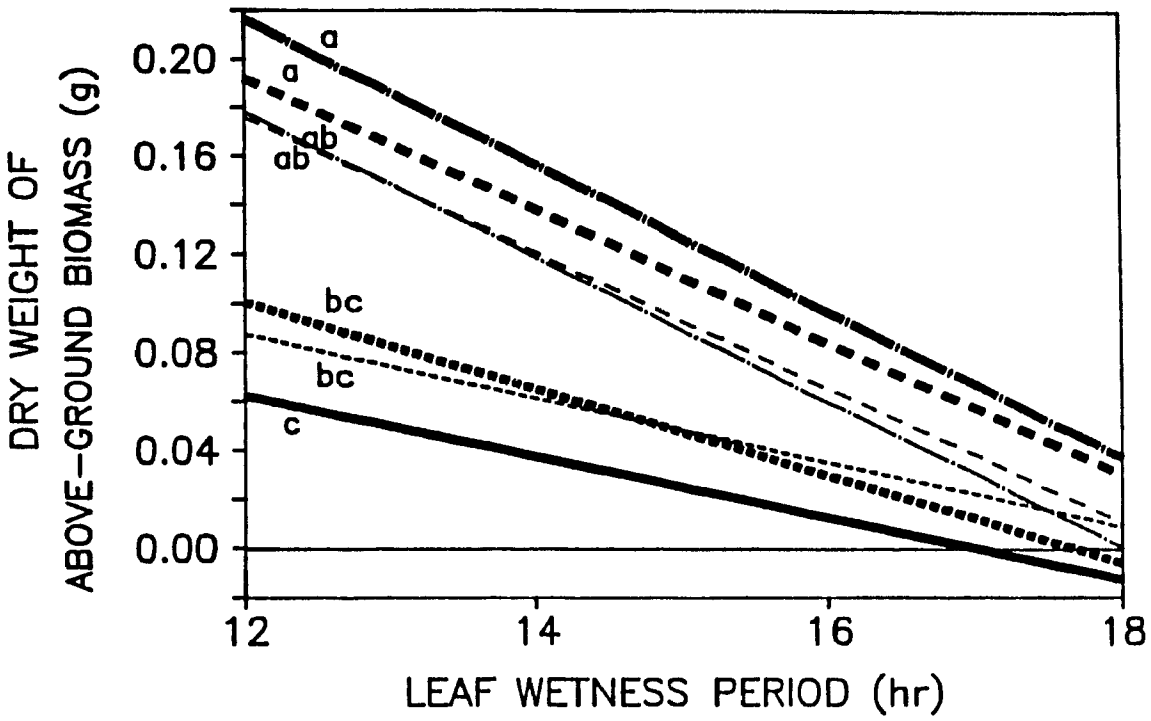


**Figure 4.** Effect of leaf wetness duration and additive on dry weight of above-ground biomass of field bindweed. Experiments were not combined because variances were not homogenous. Similar trends, however, were observed for both experiments. Results from one experiment are presented. The relationship between leaf wetness period and each additive is represented with a regression line. Since there was no significant (P=0.0906) interaction between leaf wetness period and additive, comparison among additives was performed on the mean values averaged over all levels of leaf wetness period. Additive treatments (lines) associated with the same letter have means that are not significantly different at $\alpha$=0.05, according to Tukey's W test. The various treatments are represented as follows: control (water) ——— (Y = 0.21 - 0.01x); gelatin 1% – – – (Y = 0.51 - 0.03x) and 2% – – – (Y = 0.52 - 0.03x); SORBO TM 20% ——— (Y = 0.53 - 0.03x) and 30% ——— (Y = 0.58 - 0.03x); BOND TM 0.74 L/ha ------- (Y = 0.31 - 0.02x) and 1.48 L/ha ••••••• (Y = 0.25 - 0.01x). In the regression equations Y = g of above-ground biomass and x = hr of leaf wetness.

**Table 3.** Effect of leaf wetness duration and additive on disease severity on field bindweed. [x]

| | | Leaf wetness period (hr) | | | |
|---|---|---|---|---|---|
| | | 12 | 14 | 16 | 18 |
| **Additive** | **Rate** | **Disease rating y** | | | |
| control | (water) | 2.5 ab/e [z] | 3.8 a/ef | 4.0 a/f | 4.0 a/f |
| BOND TM | 0.74 L/ha | 2.2 ab/e | 2.8 ab/ef | 4.0 a/f | 3.9 a/f |
| BOND TM | 1.48 L/ha | 2.7 a/e | 3.5 ab/ef | 3.9 a/f | 3.9 a/f |
| Gelatin | 1% | 1.5 b/e | 3.0 ab/ef | 3.8 a/f | 3.7 a/f |
| Gelatin | 2% | 1.5 b/e | 3.1 ab/ef | 3.6 a/f | 3.7 a/f |
| SORBO TM | 20% | 1.6 ab/e | 3.0 ab/ef | 3.5 a/f | 3.9 a/f |
| SORBO TM | 30% | 1.8 ab/e | 2.2 b/ef | 3.6 a/f | 3.3 a/f |

[x] Experiments were not combined and results from one experiment are presented.

[y] Ratings: 0 = no visible symptoms; 1 = <25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = >75% necrosis.

[z] Means followed by the same letter in a column (a, b, c) or in a row (e, f) are not significantly different at the 0.30 and 0.15 experiment-wise error rate respectively, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

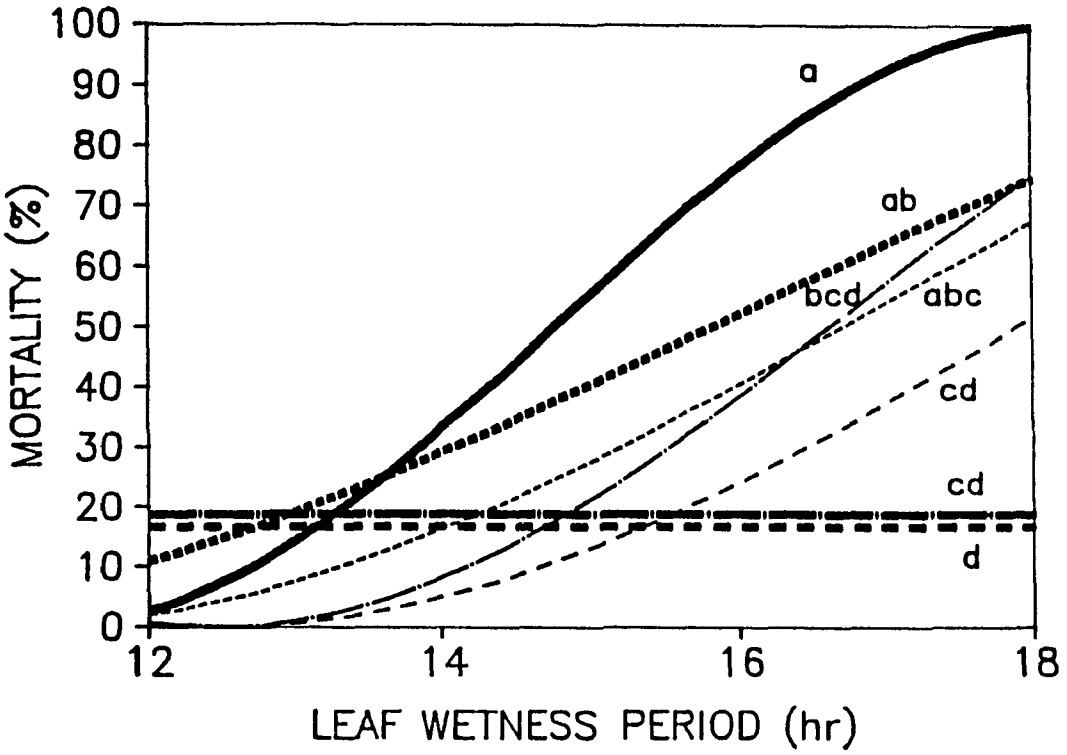


Figure 5. Effect of leaf wetness duration and additi e on mortality of field bindweed. Experiments were not combined because a significant difference (exp * leaf wetness; P=0.0140) was detected. Similar trends, however, were observed for both experiments. Results from one experiment are presented. The relationship between leaf wetness period and each additive is represented with a regression line. Regression parameters were estimated following arcsin transformation. Effects of gelatin 2% and SORBO $^{TM}$ 30% were not significant (P=0.2080 and P=0.0533, respectively) over all levels of leaf wetness period. Since there was no significant (P=0.1379) interaction between leaf wetness period and additive, comparison among additives was performed on the mean values averaged over all levels of leaf wetness period. Additive treatments (lines) associated with the same letters have means that are not significantly different at =0.05, according to Tukey's W test. The various treatments are represented as follows: control (water) ——— ($\arcsin\sqrt{Y}$ = -147.35 + 12.9x); gelatin 1% – – – ($\arcsin\sqrt{Y}$ = -102.62 + 8.26x) and 2% – · – · ; SORBO $^{TM}$ 20% ——— ($\arcsin\sqrt{Y}$ = -135.16 + 10.84x) and 30% ——·—· ; BOND $^{TM}$ 0.74 l/ha ------- ($\arcsin\sqrt{Y}$ = -85.81 + 7.84x) and 1.48 l/ha ••••••• ($\arcsin\sqrt{Y}$ = -61.68 + 6.75x). In the regression equations Y = percent mortality and x = hr of leaf wetness.

# DISCUSSION

The extent of disease caused by P. convolvulus on field bindweed was dependent on free moisture. The greater severity of disease following long leaf wetness periods observed under controlled environments may possibly result from increased conidia germination and penetration. An optimum leaf wetness period of 18 hr incited severe necrosis and blighting of inoculated plants. This requirement for moisture during infection is relatively long and may pose a constraint in field applications.

Primary inoculum to initiate disease is easily manipulated in the bioherbicide strategy of biological control of field bindweed. This study suggests that a high inoculum density ($10^9$ conidia/$m^2$) should be used to achieve adequate levels of infection under sub-optimal free moisture conditions.

Mortality of the seedlings increased with extended leaf wetness periods and increasing inoculum density. The level of mortality under identical environmental conditions, however, often varied between experiments. This phenomenon may be related to the difference in vigor of seedlings between each seedlot of field bindweed used or to slight differences in the leaf-stage of the plants at the time of inoculation.

Preliminary experiments indicated that the presence of excessive free water on the leaves (in dew cabinets) inhibited pycnidia formation and resulted in rapid decomposition of the leaves. Rotem et al. (1978) stated that insufficient aeration

on leaf surfaces covered with a thick film of water may be responsible for low sporulation of some fungal species. This problem was solved by covering the plants with small plastic bags which provided less free water on foliage. This moist environment favored sporulation of the fungus on infected plants. P. convolvulus required at least 48 hrs under moist conditions to produce pycnidia and conidia (secondary inoculum) on diseased leaves. The potential for secondary cycles of P. convolvulus to occur under natural conditions may depend on infected plant parts located near the humid soil. Insects and rain splash dispersal of inoculum from sporulating lesions could possibly spread the pathogen over a dense weed population. This secondary spread phenomenon has been observed between contiguous field plots sprayed with Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. aeschynomene, a bioherbicide to control northern jointvetch (TeBeest & Templeton, 1985), with Alternaria macrospora Zimm. a potential bioherbicide to control spurred anoda (Walker, 1981), and with Colletotrichum coccodes (Wallr.) Hughes on velvetleaf (L. Wymore, personal communication).

Interrupted short wetting periods were less effective in inciting disease symptoms on Convolvulus arvensis compared to a continuous long period of leaf wetness after plant inoculation. These findings differ from other pathogens such as Stemphylium botryosum Wallr. f.sp. lycopersici Rotem, Cohen & Wahl (Bashi & Rotem, 1974) and Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldwell (Damicone et al., 1987). A dry period

after a short wet period may have adversely affected germinated P. convolvulus conidia. High relative humidity during the dry period, however, appears to prevent excessive desiccation and allows some conidia to resume growth when rewet. Bashi & Rotem (1974) indicated that slow penetration rates, low survivability of germinating spores in dryness, or defense reactions of the host initiated during the first wet period which interfere with penetration during the following wet period may be responsible for limited infection during interrupted wetting periods of some fungal pathogens.

The definite requirement for free moisture of P. convolvulus represents a possible obstacle for this fungus to become a reliable bioherbicide over various types of environmental conditions. This constraint could be overcome with spray additives that provide protection against desiccation to germinating conidia , have spreader-sticker qualities or favor rapid germination and penetration, thus shortening the length of the continuous leaf wetness period necessary for development of the disease epidemic.

In the present study, SORBO TM was observed to slightly inhibit disease incidence following various periods of continuous leaf wetness periods. This phenomenon may have resulted from the stimulation of saprophytic growth on leaf surface in the presence of nutrients, to the detriment of rapid formation of appressoria and penetration pegs by P. convolvulus. The addition of SORBO TM to the spore suspension of Colletotrichum coccodes is reported to increase survival and

efficacy of this potential bioherbicide to control velvetleaf when the dew period is not provided immediately after inoculation (Wymore & Watson, 1986). On the other hand, sorbitol and other sugars are reported to reduce the severity of wilt symptoms of infected tomato plants by Fusarium oxysporum f. sp. lycopersici (Sacc.) Snyd. & Hans., probably as a result of repression of the pathogenic hydrolase (Patil & Dimond, 1967). These findings suggest that the effect of additive is possibly specific and will likely differ with each plant disease complex.

Gelatin, a heterogenous mixture of water soluble proteins, also adversely affected disease progress on field bindweed at concentrations of 1% and 2%. No apparent reduction in disease expression, however, was observed with the concentration of 0.1 % gelatin used as a standard sticker additive in all other experiments. Some amino acids have been reported as inducers of resistance against disease on host plants (Ahl, 1984). Further investigations are required to determine if gelatin and SORBO TM have a direct effect on the pathogen, the host, or the saprophytic microorganisms living on the leaf surfaces.

The synthetic latex, BOND TM, demonstrated no significant effects on P. convolvulus disease. Higher concentrations of this product still need to be evaluated. Some agricultural adjuvants are recognized to alter the outermost layer of cuticle of Vitis vinifera L. berries, which become less resistant to Botrytis cinera Pers. infection (Marois et al., 1987). The possible effects of adjuvants on the plant cuticle should be evaluated in

an attempt to formulate bioherbicides.

P. convolvulus, like several other foliar pathogens investigated as bioherbicides, required a minimum wetting period for infection to occur. Emphasis on the formulation of fungal propagules is necessary to make these products less dependant on environmental variations. Understanding of the physiological and morphological characteristics of the infection processes and the effectsof various humectants, surfactants, and adjuvants on the host-pathogen interactions could help in developing efficient formulations for mycoherbicides (Van Dyke & Trigiano, 1987). The use of host-specific phytotoxins isolated from biocontrol agents of weeds is another possibility to bypass the absolute requirement for moisture by living pathogenic propagules (Duke & Lydon, 1987). A major focus of research with fungal pathogens, like P. convolvulus, with great potential as an alternative method of weed control will be directed toward providing a stable efficient product which will promote acceptable weed control over a wide range of environmental conditions.

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## CONNECTING TEXT

Along with the epidemiological studies of a potential bioherbicide, it is essential to evaluate the level of control that can be achieved on the weed before planning large-scale field experiments. The use of very young field bindweed plants to determine the optimum conditions for maximum infection by Phomopsis convolvulus was appropriate. Efficacy testing of the fungus, however, had to be performed on plants of various ages to evaluate their respective sensitivity. Since field bindweed is a perennial, it was important to estimate the pressure exerted by the fungus on established plants originating from root sections.

The conditions in growth cabinet were optimum for plant growth, and did not necessarily correspond to the reality of the natural environment. Greenhouse experiments were therefore carried out to test the efficacy of Phomopsis convolvulus in term of weed control since it more accurately reflected the field conditions (Wymore et al., 1988).

## LITERATURE CITED

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## IV. EFFICACY OF *PHOMOPSIS CONVOLVULUS* FOR THE CONTROL OF FIELD BINDWEED.

### INTRODUCTION

Field bindweed (*Convolvulus arvensis* L. # CONAR), a member of the Convolvulaceae, is classified as a noxious weed in most agricultural regions of all Canadian provinces except Prince Edward Island and Newfoundland (Weaver & Riley, 1982). Infestations are encountered in most states of the United States, particularly in west and midwest regions (Phillips, 1978). It is most troublesome as a weed in cereals, beans, corn, and orchards. Its aggressiveness is primarily due to its extensive root system giving rise to adventitious shoots at a distance from the parent plant (Best, 1963). Intensive use of herbicides in combination with cultivation and crop rotation is the most effective program to control this perennial weed (Wiese & Rea 1959, Derscheid *et al.* 1970). Success of weed control strategies depends on environmental conditions, soil moisture, and field bindweed biotype, growth stage, morphology, and biological response (Wiese & Rea 1962, Meyer 1978, DeGennaro & Weller 1984). A new potential control method for field bindweed, involving the use of a fungus, *Phomopsis convolvulus* Ormeno has been suggested by Ormeno-Nunez *et al.* (1988). This foliar

pathogen possesses several characteristics common to other fungi used as bioherbicides (Templeton & TeBeest, 1979).

Efficacy testing of a potential bioherbicide is conducted soon after the development of an inoculum production method and after some knowledge on the epidemiology of the disease is known (TeBeest, 1985). The main objective of this research was to evaluate the level of control obtained with various densities of P. convolvulus conidia on different ages of field bindweed plants.

## MATERIALS AND METHODS

**Inoculum production.** A single-conidium isolate of P. convolvulus was grown on potato carrot agar slants in small vials and maintained under mineral oil at 4°C (Tuite, 1969). Conidial suspensions were obtained by flushing the surface of sporulating cultures growing on half strength PDA plates (Ormeno-Nunez et al., 1988). One ml of this dense suspension (approx. $10^8$ conidia/ml) was used to seed 10 g of pearl barley grains previously moistened with 20 ml of water in a 250 ml Erlenmeyer flask and autoclaved for 17 min. (100 kPa and 120°C). The flasks were incubated for 2 wk at room temperature (approximately 21°C) with 12-14 hr of fluorescent light. Fifty ml of deionized water was added to each flask to harvest conidia. Flasks were shaken on a rotary shaker at 250 rpm for 5-10 min. to suspend most conidia and the contents of each flask were poured through a soil sieve (250 um, Fisher Scientific)

lined with two layers of cheesecloth. Inoculum density in the filtrate was determined with the aid of a haemocytometer and adjusted to the desired density with water.

**Plant production.** Field bindweed seedlings were produced using a commercial sample of seeds (Valley Seed Co., Fresno, CA). Imbibed seeds (washed under warm running tap water for 2-3 hr and soaked in deionized water for 24 hr) were germinated on a moist filter papers in a Petri dishes at 24°C for 24-36 hr in the dark. Four germinated seeds were sown in potting medium (Pro-Mix BX, Premier Brands Inc., New Rochelle, NY) in 10 cm plastic pots and thinned to three seedlings per pot after emergence. Seedlings were grown in a growth cabinet (see conditions below) until the desired age before application of the fungus.

Plants from a single field bindweed population were collected on Macdonald campus in Ste-Anne-de-Bellevue, Quebec. They were maintained through vegetative propagation of their roots under greenhouse conditions (Ormeno-Nunez *et al.*, 1988). Established field bindweed plants were propagated from 10 cm root sections planted in potting medium at a depth of 3-4 cm in 15 cm plastic pots. The plants were grown for 2 months, aerial parts were cut at soil level, and 2 wk old regrowth was inoculated with the fungus.

Until time of inoculation, seedlings and established plants were grown in controlled environment chambers (Conviron, Model E-15, Controlled Environments, Winnipeg, Man.) adjusted to 23/18°C

day/night temperature, 15 hr photoperiod, and 250 $\mu Em^{-2}s^{-1}$. Plants were fertilized weekly with a water-soluble fertilizer (20-20-20, 1 g/L of water).

**General inoculation procedure.** Field bindweed plants were inoculated with various densities of conidia suspended in 0.1% (w/v) gelatin solution, using a spray chamber (Research Instrument Manufacturing Co. Ltd., Guelph, Ont.) with a full cone nozzle (TG 0.7), approx. 200 kPa air pressure, a speed of 0.85 kph, and a spray volume of 500 L/ha. Plants were placed in a dew chamber (Percival, Model E-54UDL, Boone, Iowa) at 20°C in the dark for 18 hr and subsequently transferred to a growth cabinet at the original conditions or to a greenhouse mist frame where moisture was maintained on the foliage for a 12 hr period each night (19:00 to 7:00). The greenhouse temperature ranged from 15 to 25°C, the photoperiod was approximately 14 hr and the light intensity at noon ranged from 150 to 250 $\mu Em^{-2}s^{-1}$. This additional nightly moisture simulated field conditions more closely than the low humidity atmosphere of growth cabinets.

**Assessment of weed control.** Mortality was evaluated for each individual plant and results for seedlings were pooled and averaged for each pot. Completely necrotic seedlings with a damaged and discolored hypocotyl and no potential for any regrowth were considered dead. Established field bindweed plants were classified dead when aerial parts were completely necrotic and when plants did not regrow 3 wk after diseased foliage was

removed. Dry weight of above-ground biomass or regrowth of plants at any growth stage was determined by cutting the aerial parts at the soil level and drying living tissue in paper bags for 4-5 days at 60°C. Root biomass was determined by soaking the root masses in water for few minutes, washing them with running water, and drying them (as above). Dry weight of diseased foliage, regrowth, and roots were pooled and averaged, respectively, for each pot containing three seedlings.

**Effect of inoculum density on young seedlings.** Field bindweed seedlings were inoculated with 0, $10^6$, $10^7$, $10^8$, $10^9$, and 1010 conidia/$m^2$, placed in a dew cabinet, and subsequently incubated under the greenhouse mist frame for 2 wk until assessmentsof mortality, dry weight of above-ground biomass, and dry weight of roots.

**Effect of inoculum density on established plants.** Field bindweed established plants were inoculated with 0, $10^6$, $10^7$, $10^8$, and $10^9$ conidia/$m^2$, placed in a dew cabinet, and transferred to the greenhouse mist frame. After 2 wk incubation, dry weight of above-ground biomass was assessed. Mortality and dry weight of regrowth and roots were evaluated 5 wk after inoculation.

**Effect of inoculum density and age of seedlings.** Field bindweed seedlings of various ages (1 wk old = cotyledon stage, 2 wk old = 3-5 leaf stage, 3 wk old = axillary shoots emerging, and 4 wk

old = numerous shoots and older leaves senescing) were inoculated with 0, $10^7$, $10^8$, and $10^9$ conidia/$m^2$. Plants were incubated in a dew cabinet and subsequently transferred to the greenhouse mist frame. Dry weight of above-ground biomass was assessed 4 wk after inoculation. Plants were allowed to regrow for 3 wk when mortality and dry weight of regrowth and roots were evaluated.

**Effect of number of applications of various inoculum densities on well-established seedlings.** Four wk old seedlings were inoculated with 0, $10^7$, $10^8$, and $10^9$ conidia/$m^2$, placed in a dew cabinet and subsequently incubated in the growth chamber at the original conditions. During this incubation period, infected plants and uninoculated plants produced new shoots from axillary or root buds. Two wk after the initial treatment, the same conidial densities were applied a second time (under similar conditions) to half of the plants. Weed control was assessed by determining the dry weight of above-ground biomass 4 wk after the first inoculation and the percent mortality and dry weight of regrowth and roots 7 wk after the first treatment.

**Data analysis.** All experiments were performed twice. A completely randomized design with 4 or 6 replicates per treatment was used for all experiments except for the experiment testing the effect of inoculum density on established plants. In this experiment a randomized complete block design with 3 blocks was used. Blocking was performed to remove variation due to

differences in the amount of foliage at time of inoculation. Mortality data were transformed with the arcsin transformation prior to analysis (Steel & Torrie, 1980). Factorial experiments were analyzed with a factorial analysis of variance (ANOVA) considering the effect of each factor individually and their interaction. Polynomial regression analysis was performed on all significant ($\alpha$=0.05) dependant variables. Results for the two trials of each factorial experiment were not pooled because homogeneity of variances was not detected using Barlett's test (Steel & Torrie, 1980). A similar trend, however, was observed between trials of these experiments.

## RESULTS

**Effect of inoculum density on young seedlings.** Field bindweed seedlings at the 3-5 leaf stage were severely diseased and effectively killed (70% mortality) by an inoculum density of 109 conidia/$m^2$ (Fig. 1, 2). This density reduced dry weight of above-ground biomass and roots by almost 100%. Lower densities induced less disease. Aerial plant parts and root biomass of seedlings inoculated with $10^7$ conidia/$m^2$ were reduced by 40% and 53%, respectively. A similar negative relationship was observed between levels of inoculum density and dry weight of above-ground biomass or roots. A high inoculum density (1010 conidia/$m^2$) was difficult to apply with a full cone nozzle because of the presence of the thick conidial matrix and

**Figure 1.** Effect of Phomopsis convolvulus inoculum density on 3-5 leaf stage seedlings of field bindweed, one week after inoculation. From left to right: uninoculated plants (control) and plants inoculated with $10^6$, $10^7$, $10^8$, and $10^9$ conidia/$m^2$.

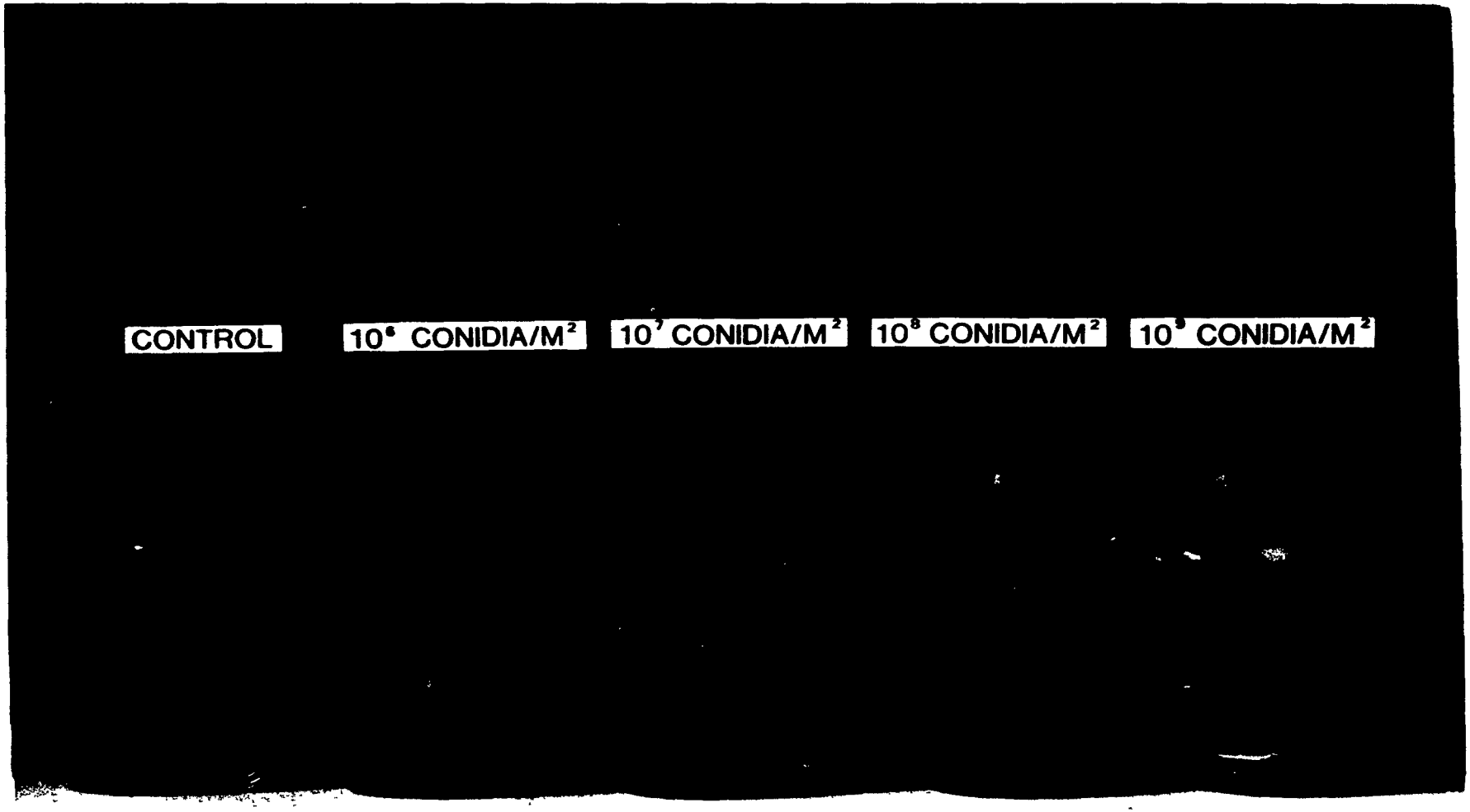

CONTROL
10⁶ CONIDIA/M²
10⁷ CONIDIA/M²
10⁸ CONIDIA/M²
10⁹ CONIDIA/M²

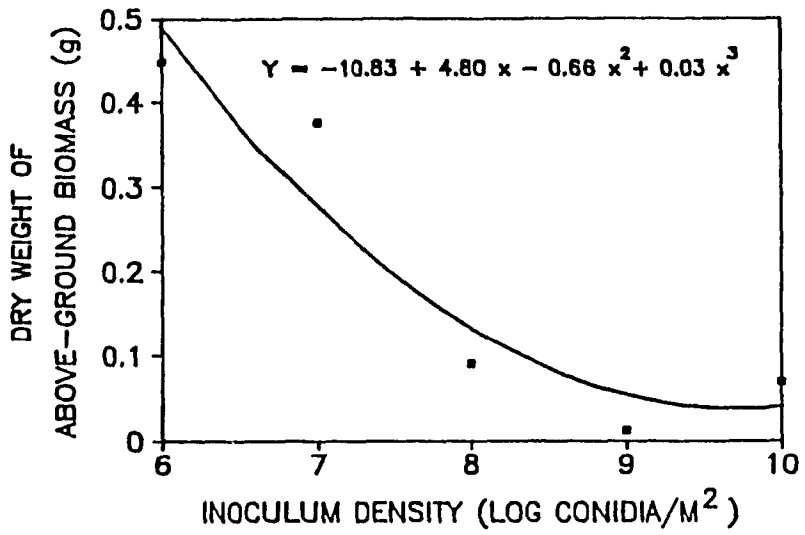
**Figure 2.** Effect of inoculum density on field bindweed seedlings (3-5 leaf stage) under the greenhouse environment. Results are from pooled experiments. Data points represent means of 8 replicates.

**A)** Dry weight of above-ground biomass versus inoculum density. Mean value for the control (0 conidia/$m^2$) was 0.61 g. In the regression equation Y = g of above-ground biomass and x = log of inoculum density. F-statistic for the model was highly significant (P=0.0001).

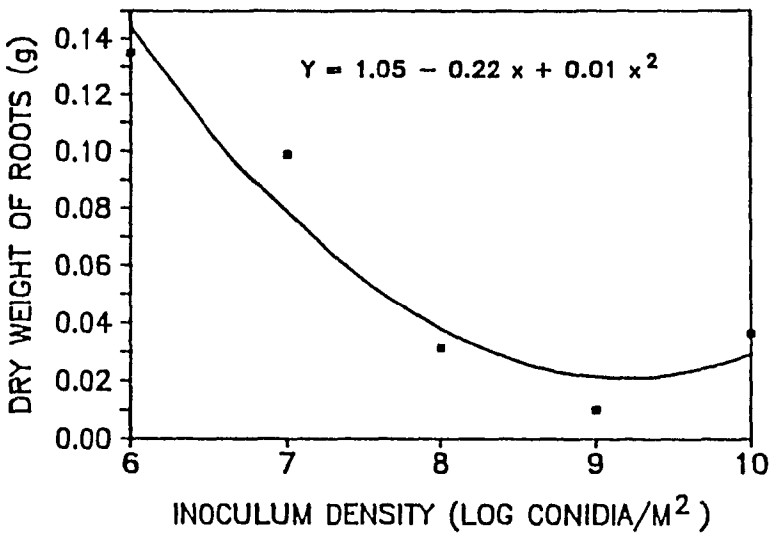
**B)** Dry weight of roots versus inoculum density. Mean value for the control (0 conidia/$m^2$) was 0.19 g. In the regression equation Y = g of roots and x = log of inoculum density. F-statistic for the model was highly significant (P=0.0001).

**C)** Percent mortality versus inoculum density. Inoculum densities of 0, $10^6$, and $10^7$ conidia/$m^2$ were not included in the analysis because no variance was detected. Mean values for these treatments were 0. In the regression equation Y = % mortality and x = log of inoculum density. F-statistic for the model was highly significant (P=0.0043).

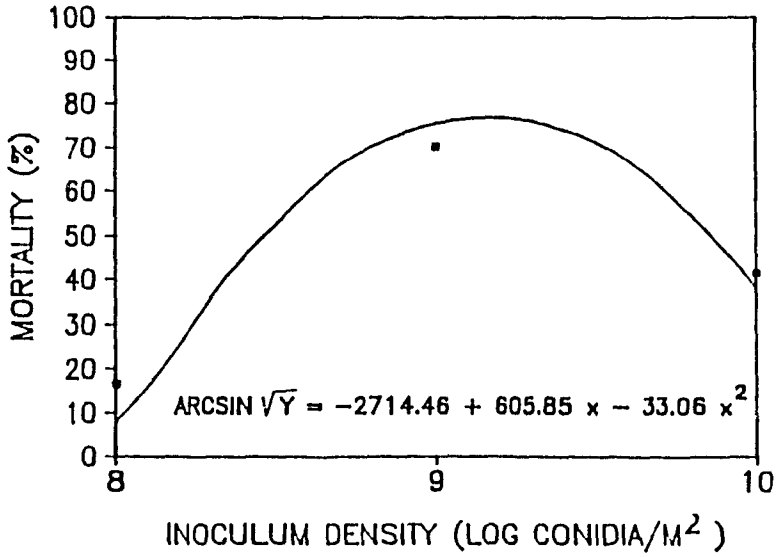
A)


DRY WEIGHT OF ABOVE-GROUND BIOMASS (g)
0.5
0.4
0.3
0.2
0.1
0
Y = −10.83 + 4.80 x − 0.66 x² + 0.03 x³
6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M²)

B)


DRY WEIGHT OF ROOTS (g)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Y = 1.05 − 0.22 x + 0.01 x²
6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M²)

C)


MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
ARCSIN √Y = −2714.46 + 605.85 x − 33.06 x²
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M²)

resulted in uneven coverage of the plants. Consequently, fewer plants (42%) died after inoculation with $10^{10}$ conidia/$m^2$.

**Effect of inoculum density on established plants.** Aerial parts of established field bindweed plants became heavily necrotic 1 wk after inoculation with $10^9$ conidia/$m^2$. This inoculum density reduced dry weight of above-ground biomass by 82%. (Fig. 3). Lower inoculum densities ($10^7$ and $10^8$ conidia/$m^2$) were slightly less effective and reduced foliage biomass by only 30% to 40%. The few disease lesions developing on plants inoculated with 106 conidia/$m^2$ did not reduce foliage biomass. Due to a large experimental error resulting from the high variation between experimental units (established plants), no significant ($P>0.1$) effect of inoculum density was detected on dry weight of roots, regrowth, and mortality. A decreasing trend in the dry weight of roots and regrowth, however, was observed as the inoculum density was increasing (Table 1). Additionaly, some plants inoculated with $10^9$ conidia/$m^2$ were killed after 2 wk incubation.

**Effect of inoculum density and seedling age.** Increasing inoculum density generally increased disease severity on all ages of seedlings although the extent of damage varied with plant age (Fig. 4). Field bindweed seedlings at the cotyledon stage (1 wk old) were severely injured and killed (95% mortality) with 108 conidia/$m^2$. A lower inoculum density ($10^7$ conidia/$m^2$) reduced the dry weight of above-ground biomass by 50%, but killed only

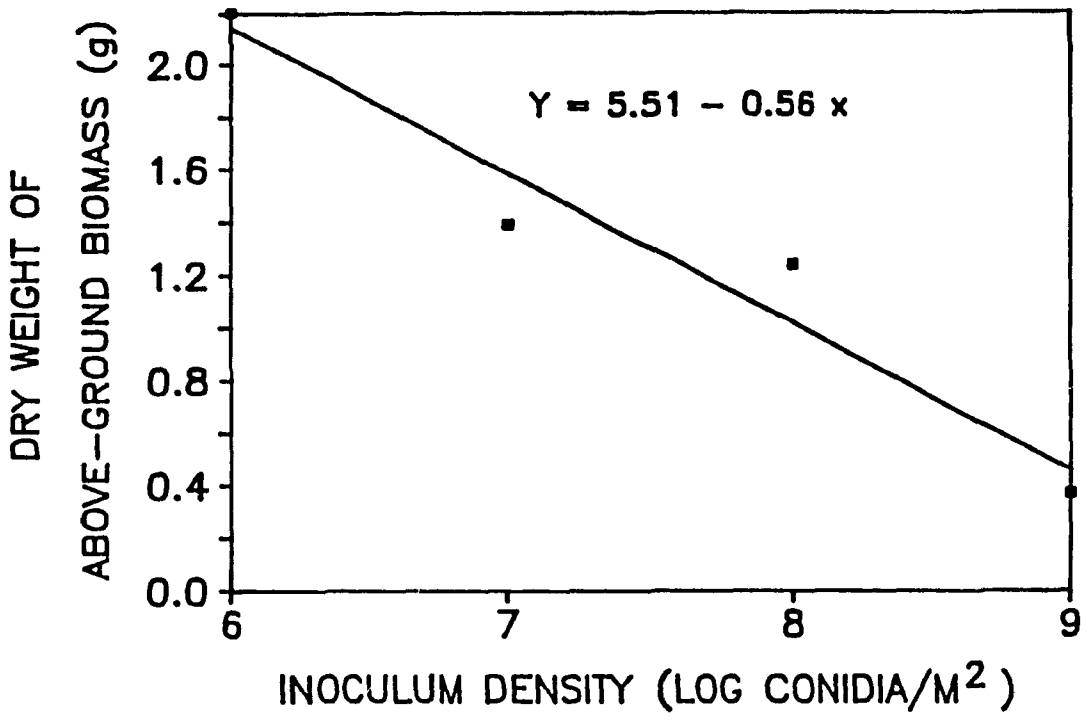



**Figure 3.** Effect of inoculum density on dry weight of above-ground biomass of established field bindweed plants under the greenhouse environment. Results are from pooled experiments. Data points represent means of 6 real replicates. Mean value for the control (0 conidia/$m^2$) was 2.0 g. In the regression equation, Y = g of above-ground biomass and x = log of inoculum density. F-statistic for the model was highly significant (P=0.0015).

**Table 1.** Effect of inoculum density on established field bindweed plants. [y]

| Inoculum density (log conidia/$m^2$) | Dry weight of regrowth (g) | Dry weight of roots (g) | Mortality (%) |
|---|---|---|---|
| uninoculated control | 0.55 [z] | 0.80 | 0.0 |
| 6 | 0.94 | 1.13 | 0.0 |
| 7 | 0.57 | 0.74 | 0.0 |
| 8 | 0.21 | 0.65 | 0.0 |
| 9 | 0.14 | 0.49 | 33.3 |

[y] Results are from pooled experiments.

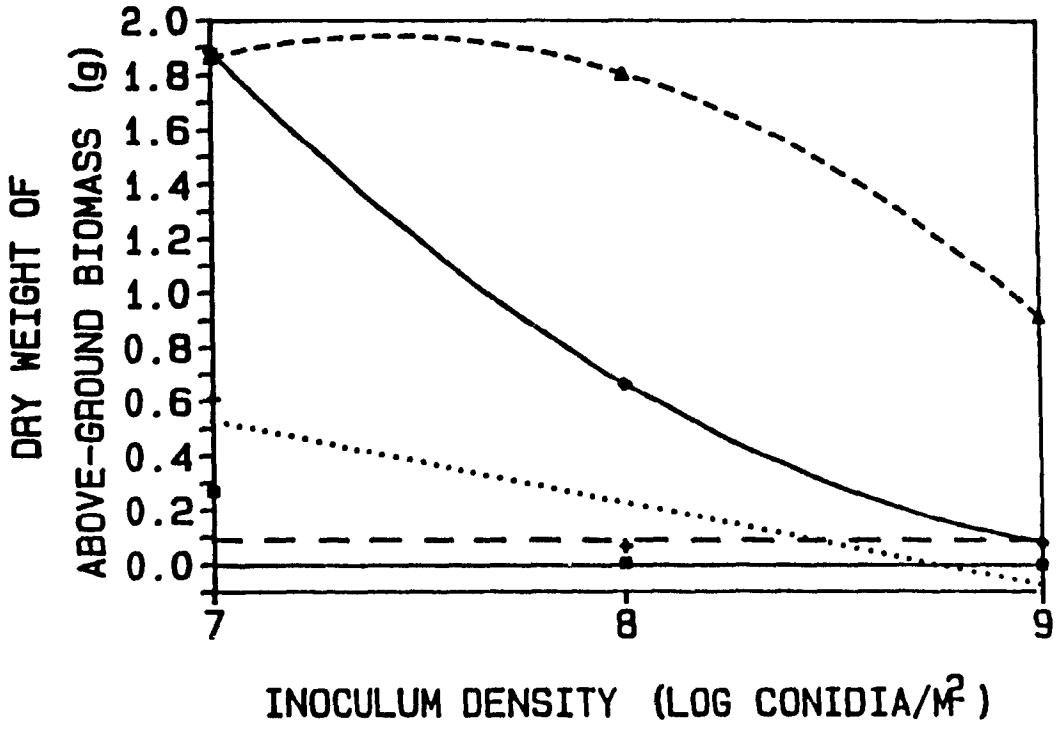
[z] Mean value of 6 real replicates.

**Figure 4.** Effect of inoculum density and age of field bindweed seedlings. Experiments were not combined because variances were not homogenous. Similar trends, however, were observed for both experiments and therefore results from one experiment are presented. Data points represent means of 6 replicates. Seedling ages are represented by the various line patterns: 1 wk old (cotyledon stage) — — ; 2 wk old (3-5 leaf stage) ····· ; 3 wk old (axillary shoot emerging) ——— ; 4 wk old (numerous shoots) - - - - .

A) Dry weight of above-ground biomass versus inoculum density at 4 levels of seedling age. There was no significant ($P=0.2153$) difference between inoculum densities applied on 1 wk old seedlings. Regression equation for 2 wk old seedlings: $Y = 2.65 - 0.30x$, for 3 wk old seedlings: $Y = 28.22 - 5.98x + 0.32x^2$, and for 4 wk old seedlings: $Y = -21.0 + 6.18x - 0.42x^2$, where Y = g of above-ground biomass and x = log of inoculum density. Mean values for the control (0 conidia/$m^2$) were 0.62 g, 1.30 g, 2.12 g and 2.81 g for 1, 2, 3, and 4 wk old seedlings, respectively.

B) Dry weight of roots versus inoculum density at 4 levels of seedling age. There was no significant ($P>0.05$) difference between inoculum densities applied on 1 and 2 wk old seedlings. Regression equation for 3 wk old seedlings: $Y = 8.30 - 1.90x + 0.11x^2$ and for 4 wk old seedlings: $Y = 11.54 - 2.56x + 0.14x^2$, where Y = g of roots and x = log of inoculum density. Mean values for the control (0 conidia/$m^2$) were 0.05 g, 0.19 g, 0.53 g, and 0.89 g for 1, 2, 3, and 4 wk old seedlings, respectively.

A)


DRY WEIGHT OF ABOVE-GROUND BIOMASS (g)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)

B)

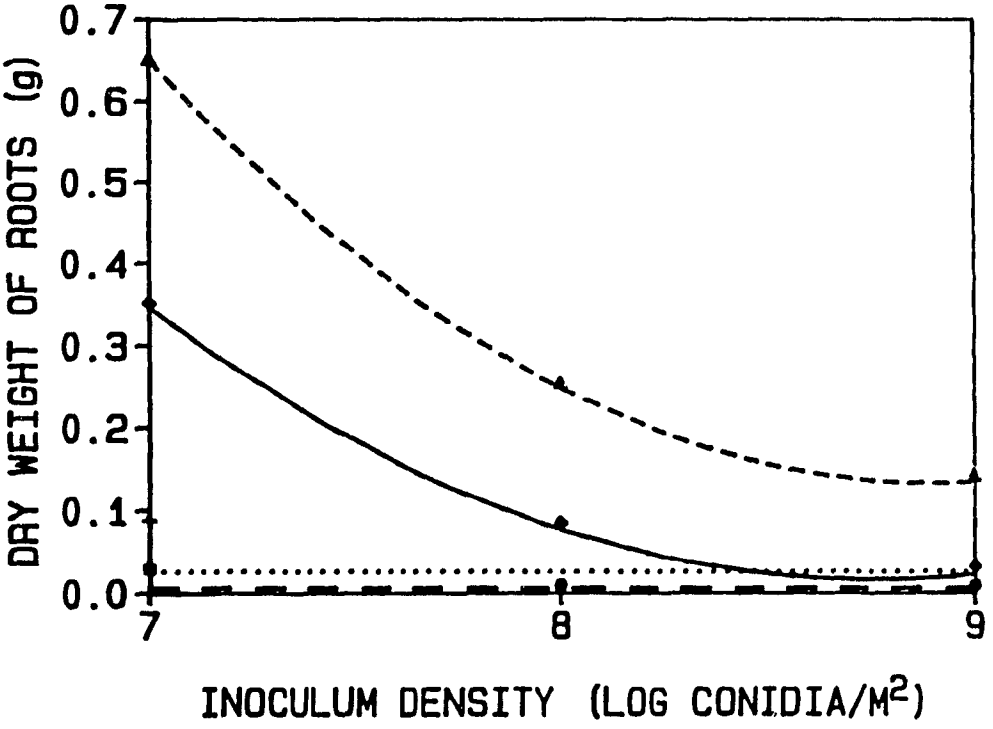
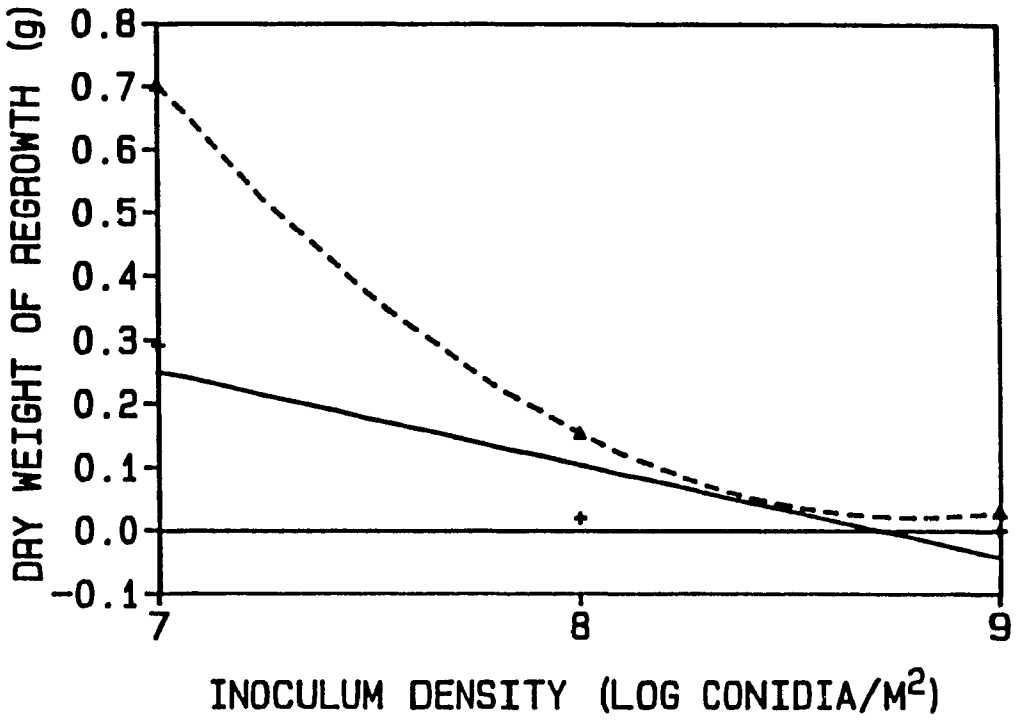

DRY WEIGHT OF ROOTS (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)

**Figure 4.** continued

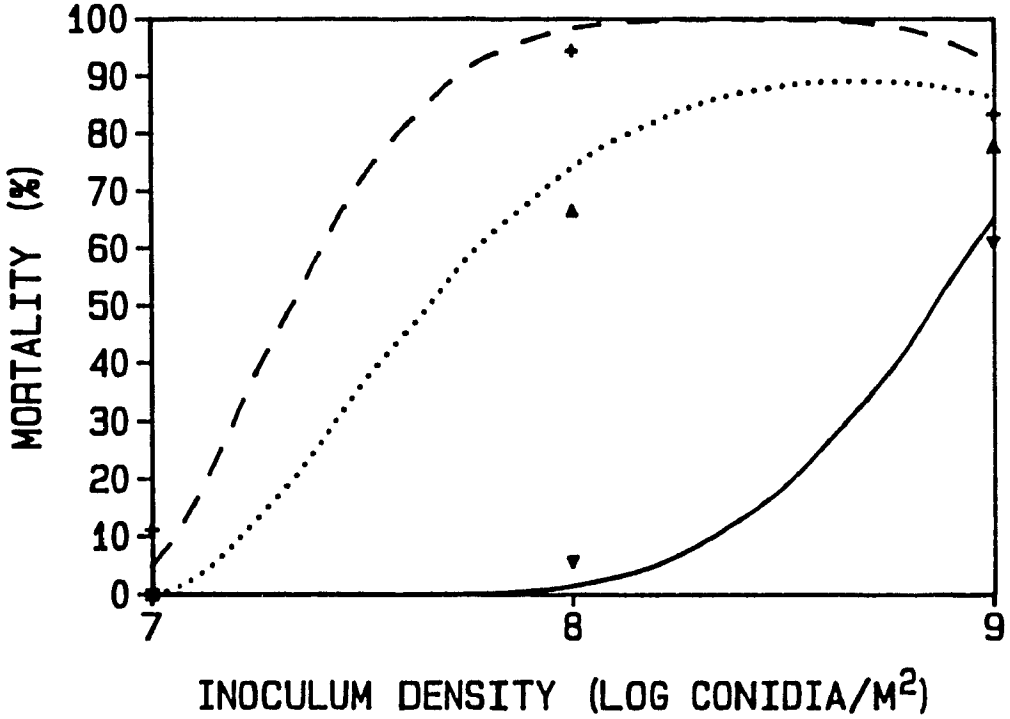
C) Dry weight of regrowth versus inoculum density at 2 levels of seedling age. One and two wk old seedlings inoculated with $10^7$, $10^8$, and $10^9$ conidia/$m^2$ did not regrow after infected foliage was cut. Uninoculated 2 wk old seedlings, however, regrew slightly (mean value was 0.13 g). Regression equation for 3 wk old seedlings: $Y = 1.28 - 0.15x$, and for 4 wk old seedlings: $Y = 16.40 - 3.72x + 0.21x^2$, where Y = g of regrowth and x = log of inoculum density. Mean values for the control (0 conidia/$m^2$) were 0.52 g and 0.85 g for 3 and 4 wk old seedlings, respectively.

D) Percent mortality versus inoculum density at 3 levels of seedling age. There was no significant (P=1.0) effect of inoculum density on 4 wk old seedlings and none of the densities were effective in killing these seedlings. Regression equation for 1 wk old seedlings: $\arcsin\sqrt{Y} = -2683.27 + 660.97x - 39.40x^2$, for 2 wk old seedlings: $\arcsin\sqrt{Y} = -1774.78 + 425.21x - 24.49x^2$, and for 3 wk old seedlings: $\arcsin\sqrt{Y} = 1111.16 - 302.10x + 20.51x^2$, where Y = % mortality and x = log of inoculum density. No plants were killed with the control treatment (0 conidia/$m^2$).

C)


DRY WEIGHT OF REGROWTH (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)

D)


MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)

few plants (approximately 10% mortality).

Growth of 3-5 leaf stage seedlings (2 wk old) was suppressed with $10^8$ conidia/$m^2$ (67% mortality and 95% reduction in foliage). Young seedlings (1 and 2 wk old) did not have a well-developed root system at time of inoculation and therefore no plants regrew after infected foliage was removed. After 4 wk incubation, disease caused by all inoculum densities had developed extensively and may have affected the roots of these seedlings since no significant (P 0.1) differences in root biomass was detected between the levels of inoculum density. This result differs from that obtained in the experiment where only 3-5 leaf stage seedlings were inoculated (Fig. 2B). The shorter incubation period (2 wk) after inoculation, in the methodology of this experiment, may have accounted for less severe effects of the disease on field bindweed roots.

The rate of decrease in dry weight of above-ground biomass ,as the inoculum density increased, was more rapid for 3 wk old seedlings than for older plants (Fig. 4A). An inoculum density of $10^9$ conidia/$m^2$ reduced foliage biomass of 3 and 4 wk old seedlings by 96% and 67%, respectively. For these ages, the decrease in dry weight of roots followed a similar pattern over the levels of inoculum density (Fig. 4B). Regrowth potential of 4 wk old seedlings was much higher than 3 wk old seedlings, as indicated by the Y intercept of their regression curve and line (Fig. 4C). Inoculum densities of $10^8$ and $10^9$ conidia/$m^2$ severely suppressed the amount of regrowth produced by well-established seedlings (3 and 4 wk old). None of the older seedlings (4 wk

old), however, were completely killed since a few shoots regrew from the roots. Sixty percent of 3 wk old seedlings were killed when plants were inoculated with $10^9$ conidia/$m^2$ (Fig. 4D).

**Effect of number of applications of various inoculum densities on well-established seedlings.** The negative relationship observed between inoculum density and dry weight of above-ground biomass was similar for plants inoculated once or twice (Fig. 5). Two inoculations with P. convolvulus significantly (P=0.0217) reduced above-ground biomass in comparison to one inoculation. The superiority of two inoculations treatment, however, was much lower than expected. New shoots produced during the incubation period between the first and second inoculation, developed much less severe disease symptoms than did the older plant tissue inoculated the first time. Based on the results obtained with inoculation of regrowth from established field bindweed plants (Fig. 3), young shoots should have been severily affected or possibly killed with the high inoculum densities. There was no significant (P>0.2) effect of inoculum density and number of inoculations on dry weight of roots and regrowth. No plants were killed in any of the treatments in this experiment.

## DISCUSSION

Application of high levels of inoculum at particularly susceptible growth stages of the weed may compensate for possible constraints preventing a disease epidemic such as sub-

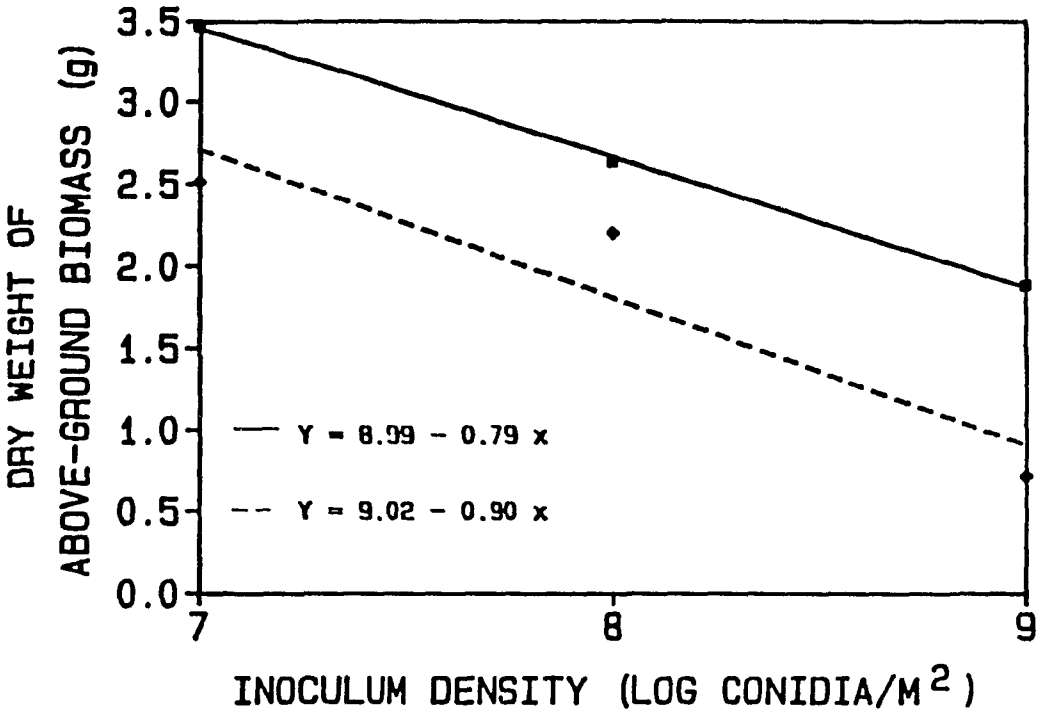



**Figure 5.** Effect of number of applications of various inoculum densities on dry weight of above-ground biomass of field bindweed seedlings (4 wk old) under a controlled environment. Experiments were not combined because variances were not homogenous. Similar trends, however, were observed for both experiments and therefore results from one experiment are presented. Data points represent means of 4 replicates. The solid line ( ——— ) represents one inoculation and the dashed line ( - - - - ) represents two inoculations. Mean values for the control (0 conidia/$m^2$) were 3.86 and 3.10 g for one and two inoculation treatments, respectively. In the regression equations Y = g of above-ground biomass and x = log of inoculum density. F-statistic for both models was significant ($P<0.05$).

optimal environmental conditions, low pathogen virulence, or host resistance (Templeton & TeBeest, 1979). The field bindweed foliar disease caused by Phomopsis convolvulus was dependant on the amount of initial inoculum. Under optimum conditions, the effectiveness of various inoculum densities in killing the weed was related to the plant growth stage. Field bindweed tissue of any age was susceptible to the fungus. Many plants completely collapsed and died when inoculated with a high inoculum density ($10^9$ conidia/$m^2$) at a young growth stage. Older plants inoculated with the same conidial density were severely blighted and stunted, regrew only slightly, and their root biomass development was suppressed.

The effect of the disease on roots was more severe when infected foliage remained on the young seedlings for longer periods. This observation suggests that the fungus may invade weakened roots and affect the ability of old plants to regrow. This hypothesis, however, still needs to be investigated.

The regenerative ability of old seedlings and established plants represented a major obstacle in obtaining complete death of this perennial weed. Field bindweed plants have a well-developed root system and chemical herbicides also frequently fail to provide complete control. The vigorous regrowth, from the subterranean root buds, occurring after the foliage has been destroyed makes this weed difficult to eradicate completely with herbicides (Weaver & Riley, 1982). The potential advantage of mycoherbicides over chemicals, is the self perpetuating capacity

of the living agents and possible occurrence of secondary disease cycles during the same growing season.

In this research, the application of a specific number of fungal spores per surface area with a spray chamber resembling the conventional equipment used for field application aid in the interpretation of the results and possible comparison with future field testing. Most studies evaluating potential bioherbicide used inoculum "sprayed to runoff". This common phytopathological method is not appropriate for evaluating bioherbicides because the spray volumes that will be used in the field are usually much less (Walker & Riley, 1982).

The greenhouse environment was satisfactory to initiate the efficacy testing of P. convolvulus on field bindweed. The additional moisture provided at night simulated the dew period often occurring in the field and important for infection and disease development. The results, however, should be interpreted carefully since greenhouse conditions often make plants more succulent and possibly more susceptible to disease (TeBeest, 1985). High humidity and low light intensity, commonly encountered in greenhouses, is reported to stimulate the development of a thin cuticle which may slightly alter the overall plant defense mechanism against fungal invasion (Martin, 1964).

Ormeno-Nunez et al. (1988) suggested that two or more applications of P. convolvulus on aged field bindweed plants may be required to kill them. In the present study, a second

inoculation of the fungus on previously infected plants caused limited disease symptoms. The young healthy shoots which emerged after the first inoculation may have acquired some tolerance to P. convolvulus. This phenomenon resembles the induced systemic protection against pathogens extensively studied in tobacco, cucumber, and bean (De Wit, 1985). In cucumber plants, systemic protection against Colletotrichum lagenarium (Pass.) Ell. & Halst. is typically induced by inoculating the first true leaf with a conidia suspension when the second true leaf is not fully expanded. After a lag period of a few days, a challenge inoculation of the second leaf resultsin fewer and smaller necrotic lesions expanding less rapidly than those in unprotected leaves (Dean & Kuc, 1986). The natural enhancement of resistance that was observed in field bindweed shoots growing from axillary and root buds of infected plants may affect the efficiency of this fungal disease to control the weed over a long period. More research is required to determine the extent and persistance of this inducable systemic protection to P. convolvulus in field bindweed.

The fungus P. convolvulus requires extensive testing under field conditions prior to fully evaluate its bioherbicide potential for the control of field bindweed infestations. Adequate formulation of the product, combination with growth regulators (Wymore et al., 1987) or herbicides, and appropriate application methods (Khodayari et al., 1987) could possibly enhance its effectiveness and reduce the amount of inoculum

required to achieve acceptable control. Research on integration of mycoherbicides into weed and pest management programs is emphasized by Klerk *et al.* (1985) and should be considered with *P. convolvulus* in the near future.

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## V. GENERAL DISCUSSION AND CONCLUSIONS

Phomopsis convolvulus possesses the essential characteristics to become an effective bioherbicide to control field bindweed. It is host-specific, it can be easily produced in liquid and solid substrate culture, it causes rapid disease symptoms over a range of environmental conditions, and it can provide good control of field bindweed plants.

The success in producing large numbers of conidia on various crude agricultural products and in liquid media offers several alternatives to develop an effective large-scale spore production system and represents a great asset for future industrial production. The production of pycnidium-like structures and normal conidia in liquid fermentation is particularly intriguing and differs from all other organisms investigated as possible bioherbicide. This phenomenon represents a great opportunity to study the development of pycnidia.

Field bindweed seedlings inoculated with 108 conidia/m2 or more and incubated for at least 18 hr in a dew cabinet at 20°C were severely affected by Phomopsis convolvulus. In general, an increase of the leaf wetness period on plants inoculated with the same inoculum density resulted in greater disease expression. These results indicate that a primary constraint

restricting disease epidemics under natural conditions could be poor inoculum production and dissemination as with most other fungi evaluated and used as bioherbicides (Templeton et al., 1979). This obstacle, however, is easily eliminated by massive inoculation of the target weed. Sub-optimal moisture conditions also represent a possible major disease constraint. The development of genetically engineered organisms requiring less moisture to penetrate and infect plant tissue (Charudattan, 1985) or the application of host-specific toxins isolated from the pathogen (Duke & Lydon, 1987) provide plausible strategies to overcome the absolute requirement for free water on the plant surface. An adequate formulation of the biological product, however, could be developed at lower research cost and provide sufficient humidity to allow germination and penetration of the infective propagules.

Phomopsis convolvulus was effective in reducing growth and regeneration of field bindweed plants as well as causing death in certain cases. The effect of the fungus as a control agent was related to the age of the plants. Younger plants (cotyledon and 3-5 leaf stage) required a lower dosage of inoculum to achieve good control and high mortality. Older seedlings and established plants required an inoculum density of 109 conidia/$m^2$ to obtain a reduction in foliage, roots, and regrowth biomass. The indirect effect of this foliar disease on root biomass and regenerative ability of Convolvulus arvensis was important to estimate since this weed is a perennial. It would be interesting to determine if the fungus has some direct

pathogenic effects on the root biomass of plants having heavily diseased foliage. The acquired tolerance to Phomopsis convolvulus observed on healthy shoots emerging from previously infected field bindweed plant should be scrutinized to determine its impact on the efficacy of this potential bioherbicide. A similar phenomenon has never been reported for any other fungus investigated as a bioherbicide.

Phomopsis convolvulus is certainly a good candidate to be used as a microbial herbicide to suppress field bindweed populations. It represents an alternative control measure that could exert an additional stress on this noxious perennial weed. Integration of this promising biological herbicide with other chemical agricultural products will have to be considered in an attempt to plan an adequate weed control program for Convolvulus arvensis. Efficacy testing of this potential bioherbicide has been recently initiated in the field. Future research should include long term evaluation of the stress caused by this fungal pathogen on heavy infestations of field bindweed.

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# APPENDIX A

Results not presented in the text.

When the results of two trials of each experiment could not be pooled statistically, data of both trials were presented in the text. When the results were similar, however, only one set of data was presented. Results not presented in the text are presented in this appendix. The main differences between trials of each experiment were principally due to variation in vigor of field bindweed plants grown from various seedlots and possible slight changes of environmental conditions in growth cabinets or effects of uncontrolled parameters in the greenhouse environment. The genetic heterogeneity of this weed was often observed and may explain the slight differences in biological response of field bindweed to identical treatments. Similar general conclusions, however, were drawn from both trials of each experiment. The regression equations are presented in appendix C: Statistical analysis of parametric data.

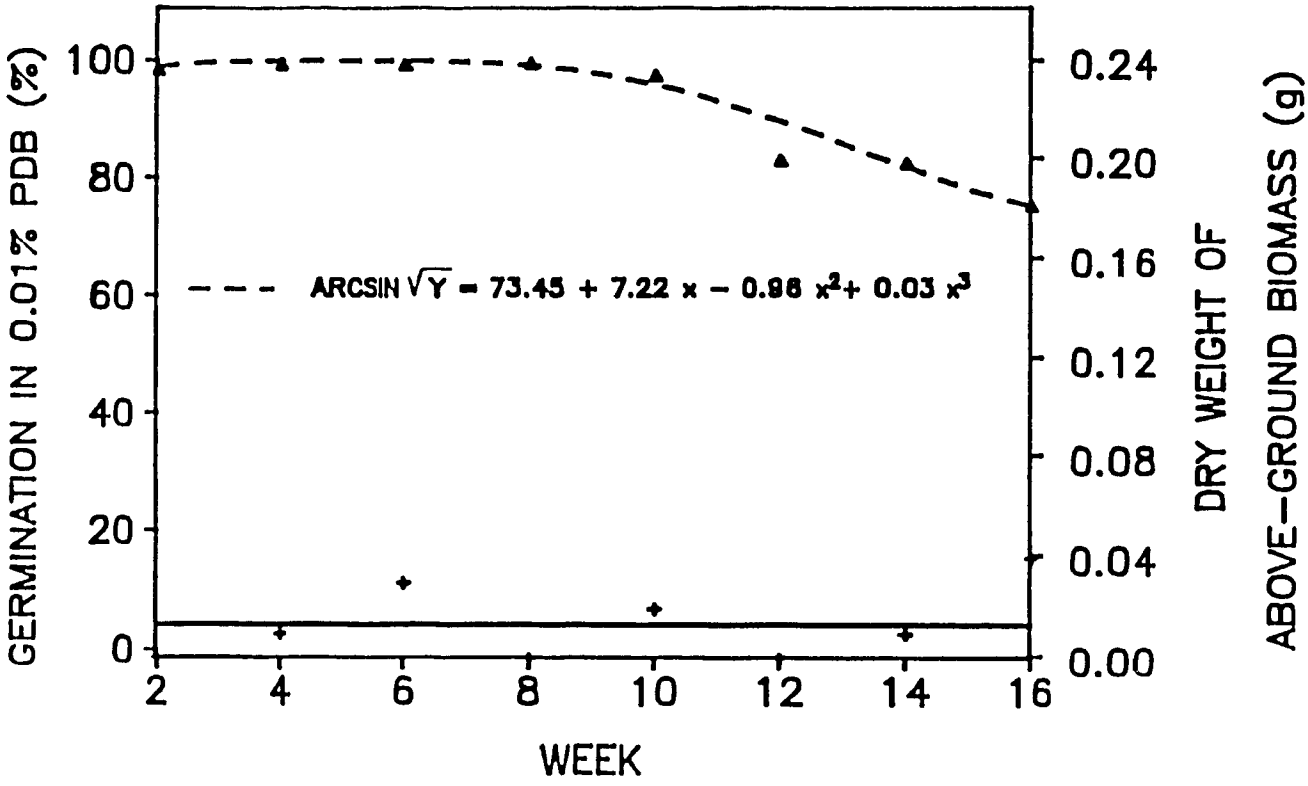



**Figure 1.** Viability and virulence of *P. convolvulus* conidia produced on pearl barley grains over time (experiment 2). The dashed line ( - - - ) represents germination in 0.01% PDB and the solid line (———) represents the dry weight of above-ground biomass of field bindweed. There was no significant (P=0.1600) difference in dry weight of above-ground biomass over time. Regression parameters for germination data were estimated following arcsin transformation. In the regression equation Y = percent germination and x = no. of weeks. F-statistic for the model was highly significant (P=0.0001). Data points represent means of 4 replicates (2 sample units/replicate) for both dependant variables.

**Table 1.** Pathogenicity of conidia produced on pearl barley grains over time.

| Duration of incubation (weeks) | Disease ratings x | |
|---|---|---|
| | Exp 1 | Exp 2 |
| 2 | 4.0 a y | 4.0 z |
| 4 | 4.0 a | 4.0 |
| 6 | 4.0 a | 4.0 |
| 8 | 4.0 a | 4.0 |
| 10 | 3.9 ab | 3.8 |
| 12 | 4.0 a | 4.0 |
| 14 | 3.5 ab | 4.0 |
| 16 | 2.8 b | 3.6 |

[x] Ratings: 0 = no visible symptoms; 1 = <25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = >75% necrosis.

[y] Means followed by the same letter are not significantly different at the 0.35 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks.

[z] No significant difference among treatments in the column at the 0.35 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

Table 2. Effect of leaf wetness duration and inoculum density on disease severity and mortality of field bindweed (experiment 2).

| Inoculum density (log conidia/$m^2$) | Leaf wetness duration (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 | 12 | 18 | 24 | 6 | 12 | 18 | 24 |
| | Disease rating [x] | | | | Mortality (%) [y] | | | |
| uninoculated control | 0.0 a/e [z] | 0.0 a/e | 0.0 a/e | 0.0 a/e | 0 | 0 | 0 | 0 |
| 6 | 0.0 a/e | 0.3 ab/e | 0.7 ab/ef | 1.0 ab/f | 0 | 0 | 0 | 0 |
| 7 | 0.3 ab/e | 1.0 abc/ef | 1.1 abc/f | 1.0 ab/ef | 0 | 0 | 0 | 0 |
| 8 | 0.8 b/e | 2.3 bc/ef | 2.5 bc/ef | 3.4 b/f | 0 | 0 | 8.3 | 16.7 |
| 9 | 1.1 b/e | 2.9 c/ef | 3.6 c/f | 3.9 b/f | 0 | 0 | 25.0 | 66.7 |
| 10 | 0.8 b/e | 2.9 c/ef | 3.3 c/ef | 3.7 b/f | 0 | 0 | 16.7 | 50.0 |

[x] Ratings: 0 = no visible symptoms; 1 = $<$25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$75% necrosis.

[y] For mortality data, inoculum densities 0, $10^6$, $10^7$ conidia/$m^2$ and leaf wetness durations 6 and 12 hr were not included in the analysis because no variance was detected over all levels of each factor for each treatment. No significant effect (P=0.1017) among the levels of inoculum density was observed and a significant (P=0.0413) difference was detected between 18 and 24 hr leaf wetness durations, according to the F-test.

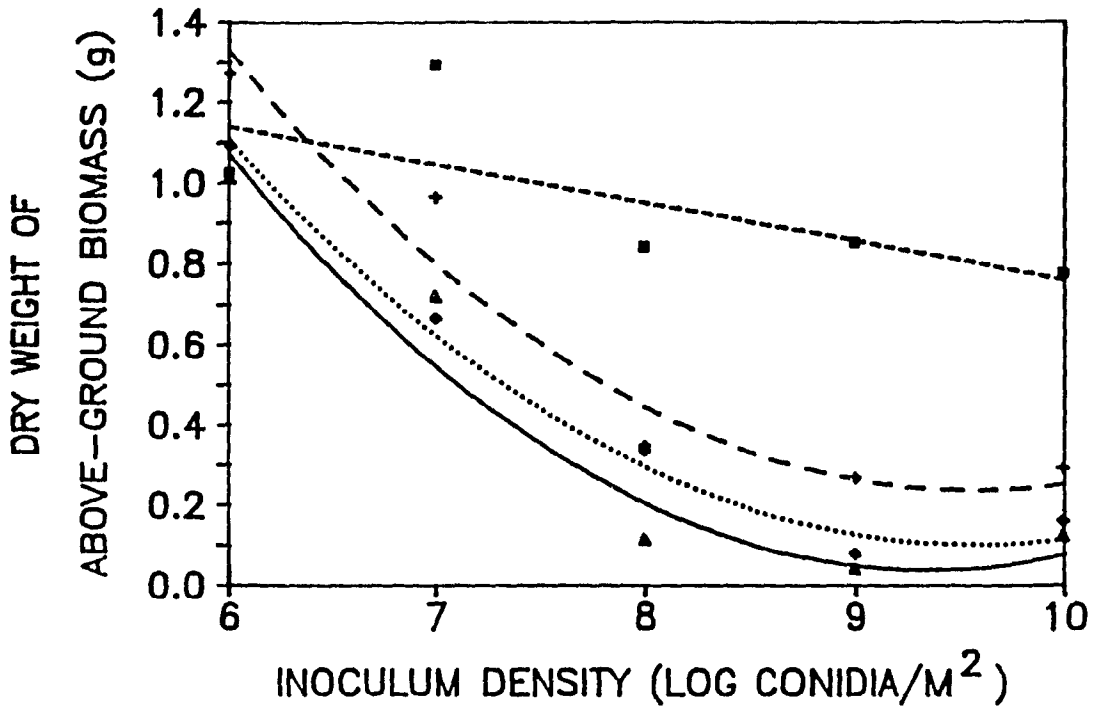
[z] Means followed by the same letter in a column (a,b) or in a row (e,f) are not significantly different at the 0.30 and 0.15 experiment-wise error rate respectively, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

**Figure 2.** Effect of leaf wetness duration and inoculum density on field bindweed (experiment 2). Data points represent means of 4 replicates.

A) Dry weight of above-ground biomass versus inoculum density at 4 levels of leaf wetness duration. Six hr leaf wetness period is represented by ------, 12 hr by — — — , 18 hr by ·····, and 24 hr by ———. F-statistic for the model of each level of leaf wetness period was highly significant (P=0.0001).

B) Dry weight of above-ground biomass versus leaf wetness duration at 6 levels of inoculum density. There was no significant effect of control 0 conidia/$m^2$ ( —·— ) and inoculum density of $10^6$ conidia/$m^2$ ( —·— ) over all levels of leaf wetness period. Inoculum density of $10^7$ conidia/$m^2$ is represented by— — —, $10^8$ conidia/$m^2$ by ·-·-·-·, $10^9$ conidia/$m^2$ by ····· , and $10^{10}$ conidia/$m^2$ by ———. F-statistic for the model of each level of inoculum density was highly significant ($P<0.005$).

A)


DRY WEIGHT OF
ABOVE-GROUND BIOMASS (g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M$^2$)

B)

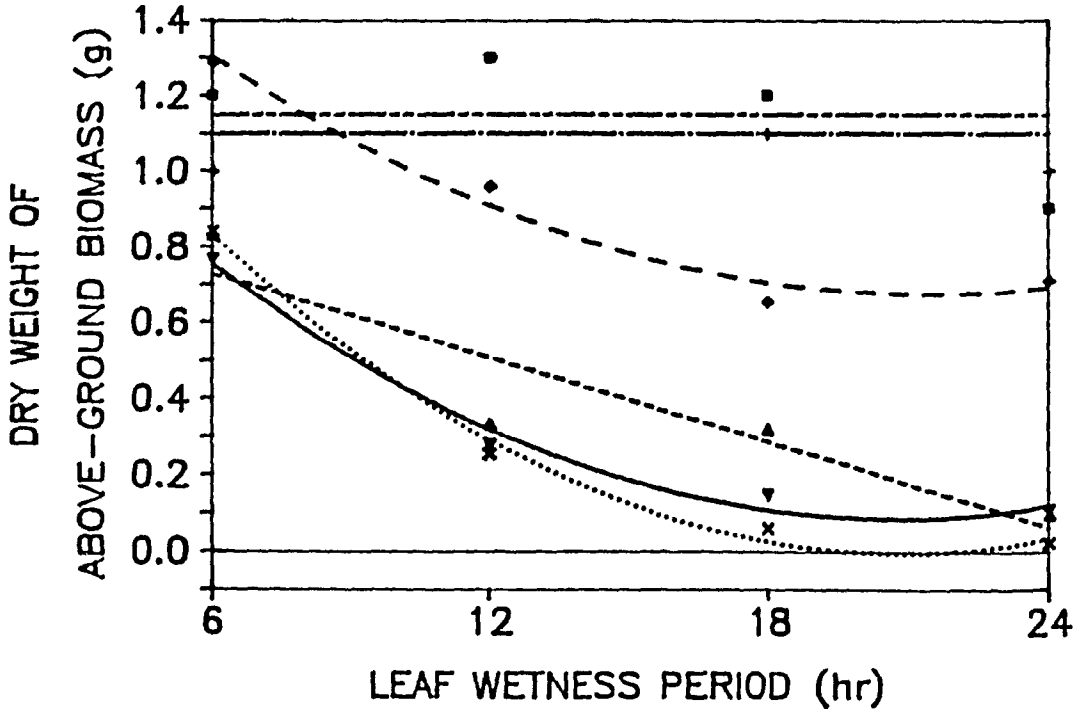
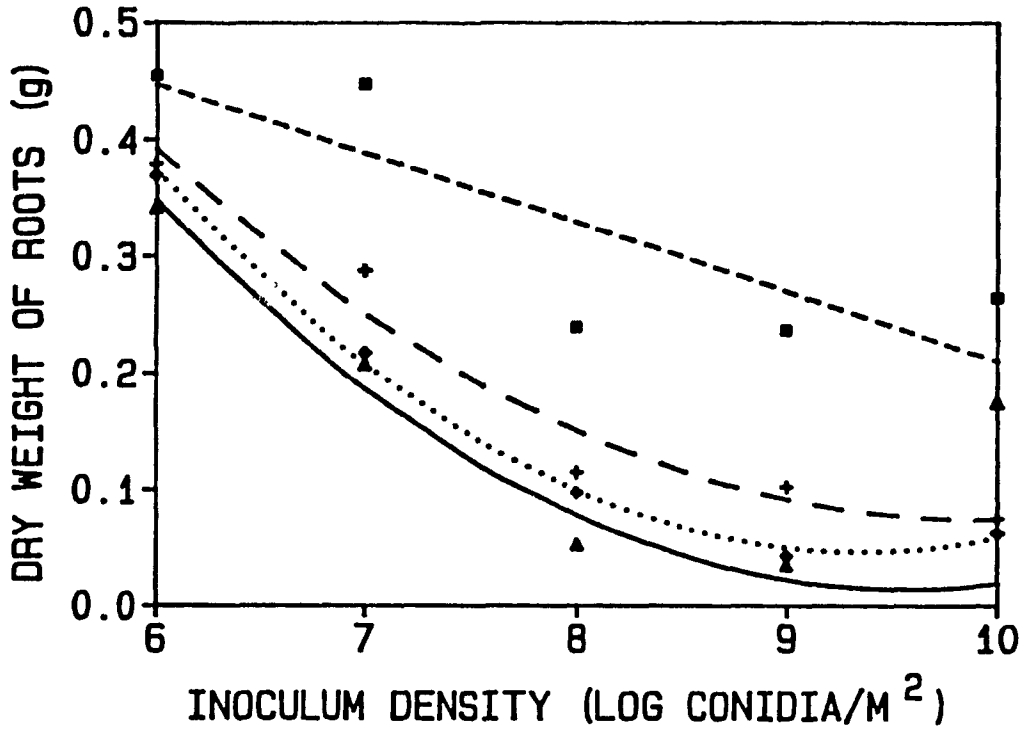

DRY WEIGHT OF
ABOVE-GROUND BIOMASS (g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6
12
18
24
LEAF WETNESS PERIOD (hr)

**Figure 2. Continued.**

C) Dry weight of roots versus inoculum density at 4 levels of leaf wetness duration. Six hr leaf wetness period is represented by ------, 12 hr by — — —, 18 hr by ·····, and 24 hr by ———. F-statistic for the model of each level of leaf wetness was highly significant ($P<0.001$).

D) Dry weight of roots versus leaf wetness duration at 6 levels of inoculum density. There was no significant effect of control 0 conidia/$m^2$ ( ——— ) and inoculum density of $10^6$ conidia/$m^2$ ( —·— ) over all levels of leaf wetness period. Inoculum density of $10^7$ conidia/$m^2$ is represented by — — —, $10^8$ conidia/$m^2$ by ------, $10^9$ conidia/$m^2$ by ·····, and $10^{10}$ conidia/$m^2$ by ———. F-statistic for the model of each level of inoculum density was highly significant ($P<0.001$).

C)


DRY WEIGHT OF ROOTS (g)
0.5
0.4
0.3
0.2
0.1
0.0
6
7
8
9
10
INOCULUM DENSITY (LOG CONIDIA/M 2)

D)

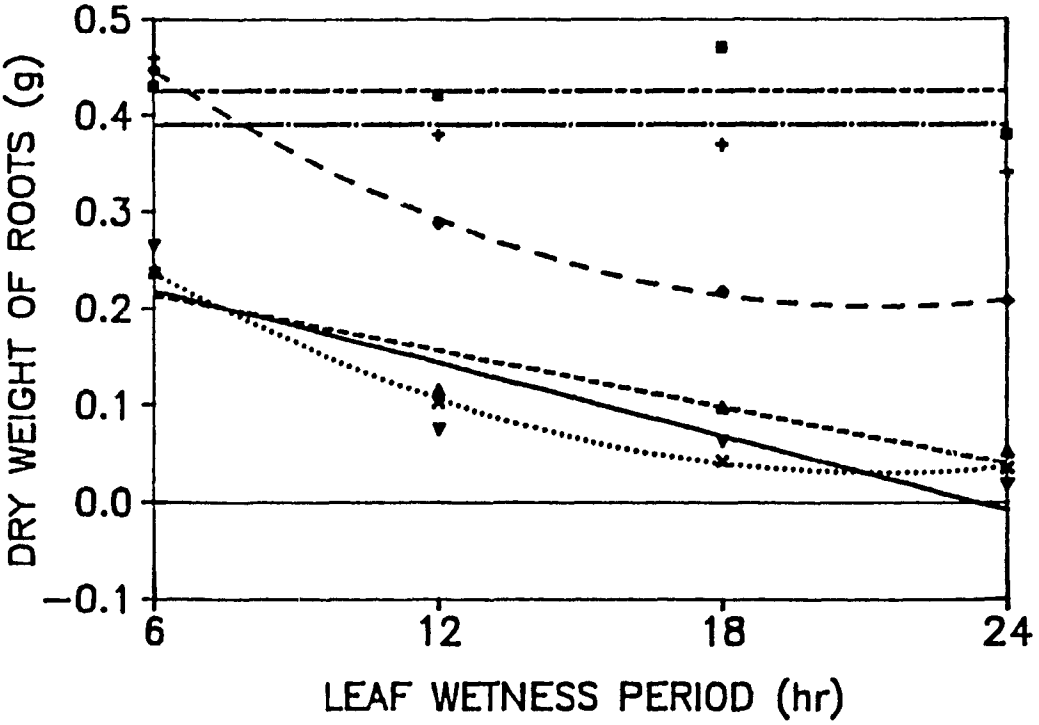

DRY WEIGHT OF ROOTS (g)
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
6
12
18
24
LEAF WETNESS PERIOD (hr)

Table 3. Effect of cumulative short wet periods interrupted by dry periods on development of P. convolvulus disease on field bindweed (experiment 2).

| wet/dry regime (hr) | Disease rating [w] [x] | | Mortality [y] (%) | | Dry weight of above-ground biomass (g) [y] | | Dry weight of roots (g) [y] | |
|---|---|---|---|---|---|---|---|---|
| | Ambient relative humidity during dry period (%) [z] | | | | | | | |
| | 80 | 100 | 80 | 100 | 80 | 100 | 80 | 100 |
| 0 | 0.0 a | 0.8 a* | 0 a | 0 a | 0.75 a | 0.54 a** | 0.22 a | 0.15 a*** |
| 6 | 0.8 ab | 1.7 ab* | 0 a | 0 a | 0.60 ab | 0.20 b*** | 0.14 b | 0.06 bc*** |
| 6 + 6 | 1.0 ab | 1.4 ab* | 0 a | 0 a | 0.35 b | 0.24 b | 0.10 b | 0.07 b |
| 6 + 6 + 6 | 1.2 ab | 2.2 ab* | 0 a | 0 a | 0.41 b | 0.19 bc** | 0.14 b | 0.05 bc*** |
| 18 | 4.0 b | 4.0 b | 77.8 b | 100.0 b | 0.03 c | 0.0 c | 0.0 c | 0.0 c |

[w] Ratings: 0 = no visible symptoms; 1 = <25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = >75% necrosis.

[x] Means followed by the same letter in a column are not significantly different at the 0.15 experiment wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

[y] Means followed by the same letter in the column are not significantly different at $\alpha$=0.05, according to the Tukey's test.

[z] For each dependant parametric variable, mean values between 80 and 100% RH for each wet/dry regime differ significantly ($\alpha$=0.05*, $\alpha$=0.01**, $\alpha$=0.001***) according to the F-test on simple effects of RH. For disease ratings, mean values between 80 and 100% RH differ significantly at $\alpha$=0.05*, according to the Median test.

Table 4. Effect of leaf wetness duration and additive on disease severity and dry weight of above-ground biomass of field bindweed (experiment 2).

| | | Leaf wetness period (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | 14 | 16 | 18 | 12 | 14 | 16 | 18 |
| Additive | Rate | Disease rating [w] [x] | | | | Dry weight of above-ground biomass (g) | | | |
| control | (water) | 3.5 e [y] | 3.6 e | 3.7 e | 3.9 e | 0.08 a [z] | 0.08 ab | 0.04 ab | 0.01 a |
| BOND [TM] | 0.74 L/ha | 2.7 e | 3.1 e | 3.6 e | 3.7 e | 0.12 a | 0.13 ab | 0.07 ab | 0.03 a |
| BOND [TM] | 1.48 L/ha | 3.1 e | 3.7 e | 4.0 e | 3.8 e | 0.06 a | 0.03 b | 0.02 b | 0.03 a |
| Gelatin | 1% | 2.0 e | 3.1 ef | 3.4 ef | 4.0 f | 0.18 a | 0.12 ab | 0.07 ab | 0.01 a |
| Gelatin | 2% | 2.6 e | 3.3 e | 3.1 e | 3.7 e | 0.17 a | 0.10 ab | 0.07 ab | 0.04 a |
| SORBO [TM] | 20% | 1.8 e | 3.1 ef | 3.2 ef | 3.9 f | 0.28 a | 0.13 ab | 0.12 a | 0.01 a |
| SORBO [TM] | 30% | 1.6 e | 2.5 ef | 3.4 fg | 3.9 g | 0.25 a | 0.24 a | 0.05 ab | 0.01 a |

[w] Ratings: 0 = no visible symptoms; 1 = $<$25% necrosis; 2 = 25%-50% necrosis; 3 = 51%-75% necrosis; 4 = $>$ 75% necrosis.

[x] No significant difference between additive treatments at each level of leaf wetness period at the 0.30 experiment-wise error rate according to the Kruskal-Wallis one-way analysis of variance by ranks.

[y] Means followed by the same letter in a row (e, f, g) are not significantly different at the 0.15 experiment-wise error rate, according to the Kruskal-Wallis one-way analysis of variance by ranks followed by a multiple-comparison procedure.

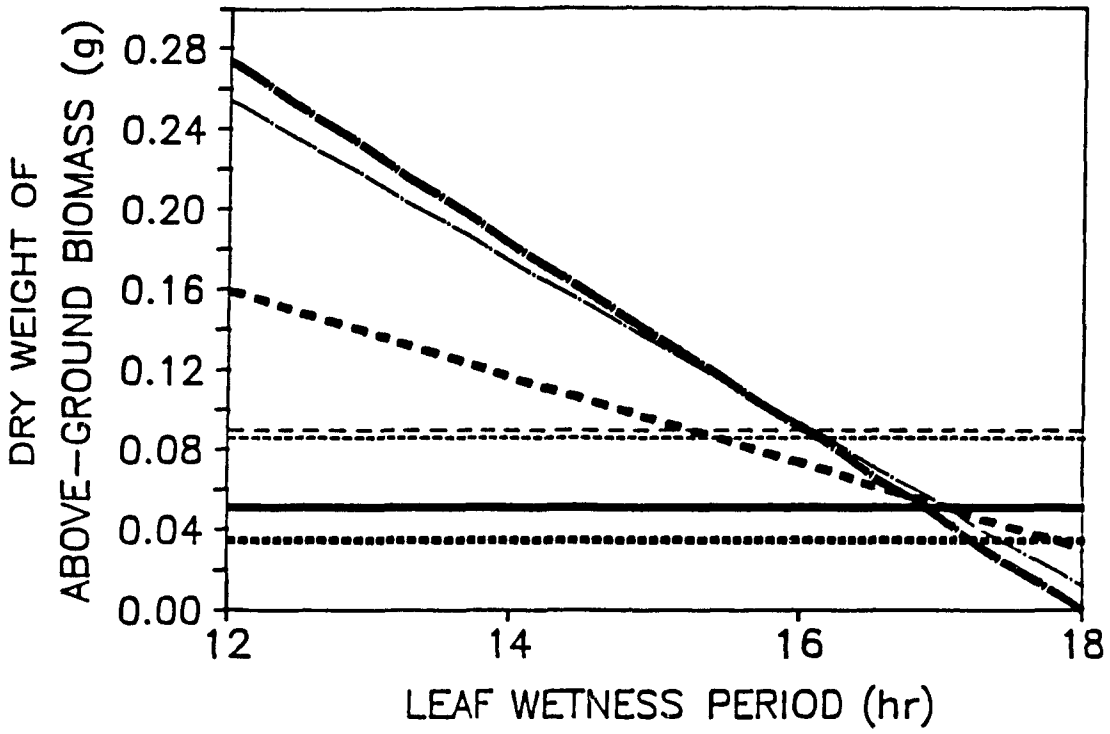
[z] Means followed by the same letter in a column are not significantly different at $\alpha$=0.05, according to the Tukey's W test.

**Figure 3.** Effect of leaf wetness duration and additive on field bindweed (experiment 2). The relationship between leaf wetness period and each additive is represented with a regression line when appropriate. The various treatments are represented as follows: control (water) ——; gelatin 1% - - - and 2% - - -; SORBO TM 20% —— and 30% ——; BOND TM 0.74 L/ha ..... and 1.48 L/ha ..... .

A) Dry weight of above-ground biomass versus leaf wetness period.

B) Percent mortality versus leaf wetness period. Since there was no significant (P=0.1726) interaction between leaf wetness period and additive, comparison among additives was performed on the mean values averaged over all levels of leaf wetness period. Additive treatments (lines) associated with the same letter have means that are not significantly different at $\alpha$=0.05, according to Tukey's W test.

A)


DRY WEIGHT OF ABOVE-GROUND BIOMASS (g)
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
12
14
16
18
LEAF WETNESS PERIOD (hr)

B)

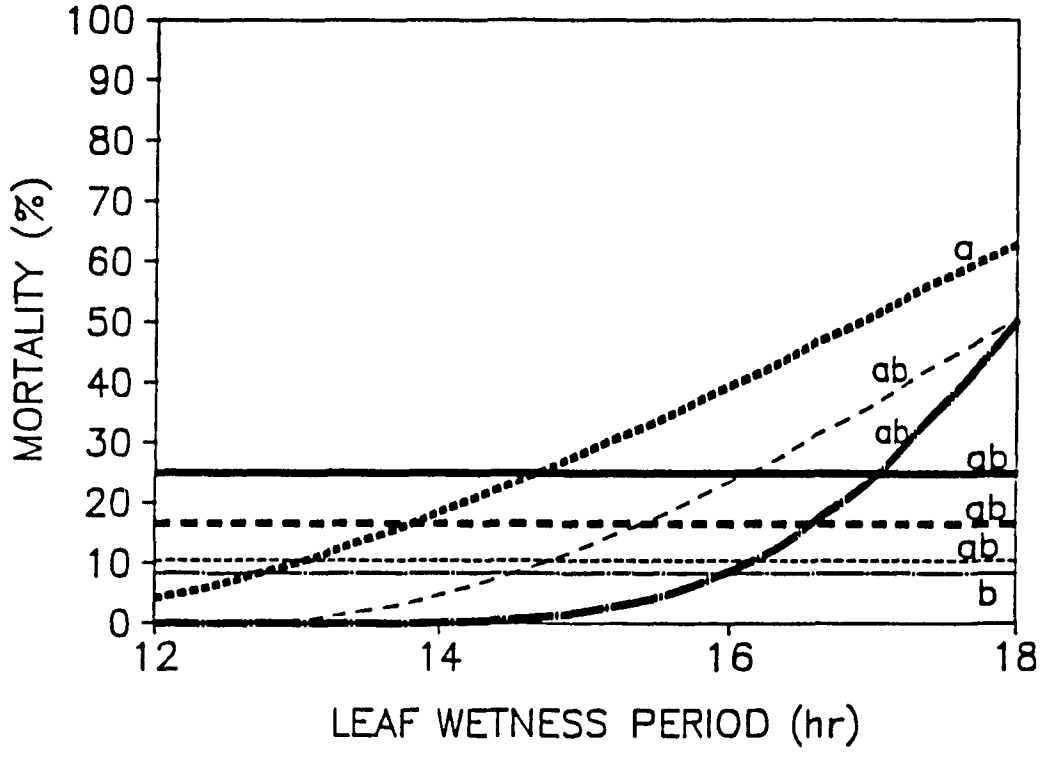

MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
12
14
16
18
LEAF WETNESS PERIOD (hr)
a
ab
ab
ab
ab
ab
b

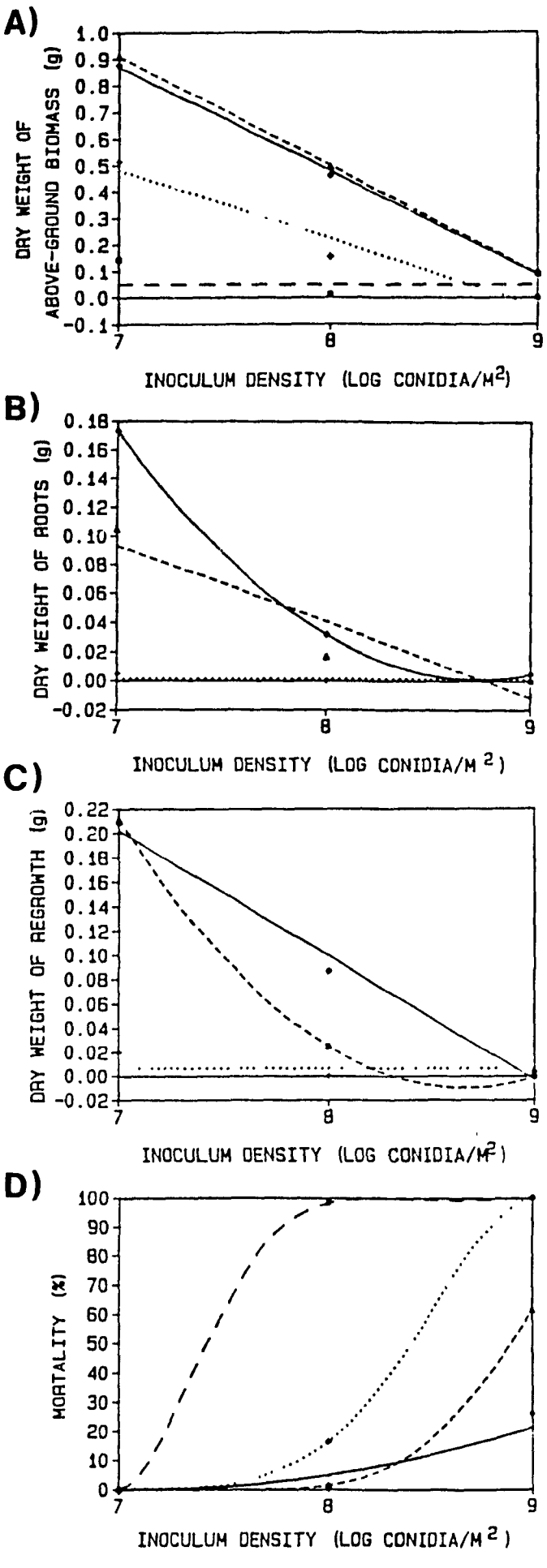
**Figure 4.** Effect of inoculum density and age of field bindweed seedlings under the greenhouse environment (experiment 2). Data points represents means of 6 replicates. Seedling ages are represented by the various line patterns: 1 wk old (cotyledon stage) — — ; 2 wk old (3-5 leaf stage) ····· ; 3 wk old (axillary shoot emerging) ——— ; 4 wk old (numerous shoots) - - - - .

**A)** Dry weight of above-ground biomass versus inoculum density at 4 levels of seedling age. There was no significant ($P=0.3126$) difference between inoculum densities applied on 1 wk old seedlings.

**B)** Dry weight of roots versus inoculum density at 3 levels of seedling age. No values for the dry weight of roots were recorded for 1 wk old seedlings inoculated with the various inoculum densities. There was no significant ($P>0.05$) difference between inoculum densities applied on 2 wk old seedlings.

**C)** Dry weight of regrowth versus inoculum density at 3 levels of seedling age. One wk old seedlings inoculated with the various inoculum densities did not regrow after infected foliage was cut. There was no significant ($P>0.05$) difference between inoculum densities applied on 2 wk old seedlings.

**D)** Percent mortality versus inoculum density at 4 levels of seedling age.


A)
DRY WEIGHT OF ABOVE-GROUND BIOMASS (g)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)
B)
DRY WEIGHT OF ROOTS (g)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M 2)
C)
DRY WEIGHT OF REGROWTH (g)
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M2)
D)
MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
7
8
9
INOCULUM DENSITY (LOG CONIDIA/M 2)

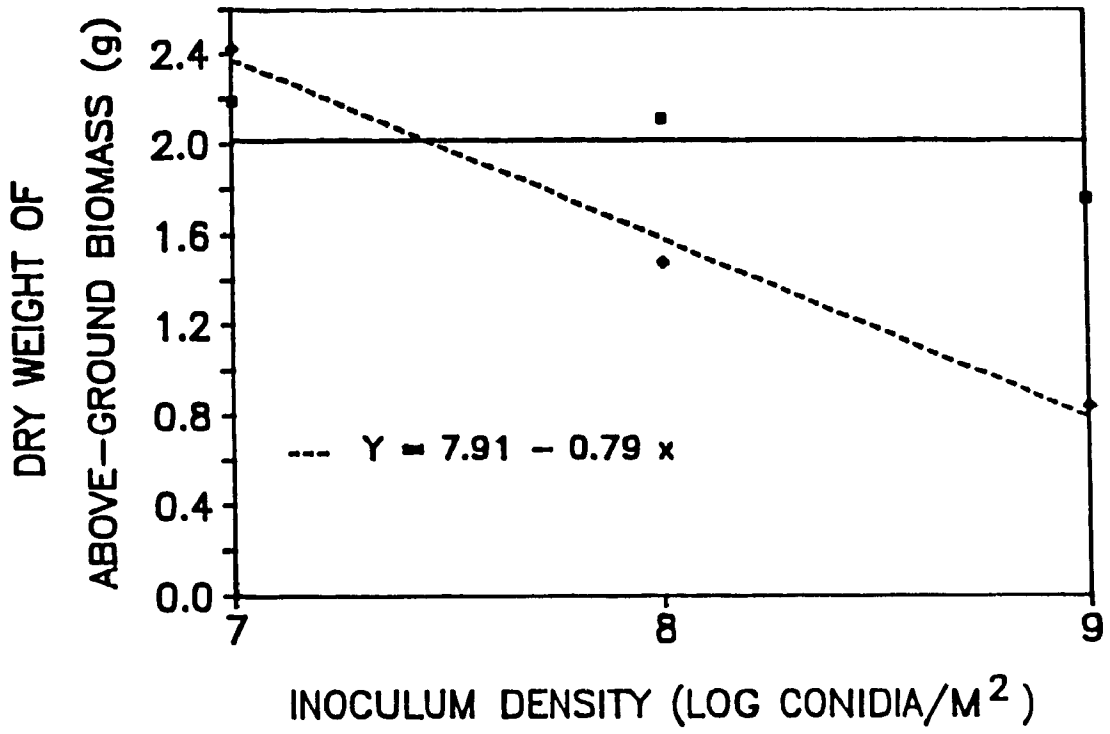



**Figure 5.** Effect of number of applications of various inoculum densities on dry weight of above-ground biomass of field bindweed seedlings (4 wk old) under controlled environment (experiment 2). Data points represent means of 4 replicates. The solid line ( ——— ) represents one inoculation and the dashed line ( - - - - ) represents two inoculations. There was no significant (P=0.2627) effect of inoculum density when only one inoculation was performed. In the regression equation associated with two inoculations treatment Y = g of above-ground biomass and x = log of inoculum density. F-statistic for the model was highly significant (P=0.0001).

# APPENDIX B

## Statistical analysis of non-parametric data.

Non-parametric statistical tests were selected to analyze the disease ratings. The Kruskal-Wallis one-way analysis of variance by ranks or the Median test were used when appropriate. In this section, one example of the statistical results obtained with each test are presented. The experiment evaluating the effect of cumulative short wet periods interrupted by dry periods on the disease development was chosen because it provided exemples of both tests.

**Example 1. Kruskal-Wallis one-way analysis of variance by ranks.**

Kruskal-Wallis one-way analysis of variance of all wet/dry regimes (dew) when the relative humidity during the dry period was 80%.

N P A R 1 W A Y P R O C E D U R E

Wilcoxon Scores (Rank Sums) for Variable Disease Rating Rank Classified by Variable DEW

| DEW | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|---|---|---|---|---|---|
| 0 | 3 | 6.0 | 24.0 | 6.74642763 | 2.0 |
| 6 | 3 | 15.0 | 24.0 | 6.74642763 | 5.0 |
| 6 + 6 | 3 | 27.0 | 24.0 | 6.74642763 | 9.0 |
| 6 + 6 + 6 | 3 | 30.0 | 24.0 | 6.74642763 | 10.0 |
| 18 | 3 | 42.0 | 24.0 | 6.74642763 | 14.0 |

Average Scores were used for Ties

Kruskal-Wallis Test (Chi-Square Approximation)

CHISQ= 13.605 DF= 4 Prob >CHISQ= 0.0087 **

** significant at the 0.01 level.

**Example 2. Median test.**

Median test to detect differences between the levels of relative humidity during the dry period when the wet/dry regime was 6 hr.

N P A R 1 W A Y  P R O C E D U R E

Median Scores (Number of Points above Median)
for Variable Disease Rating Rank
Classified by Variable HUM

| HUM | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|---|---|---|---|---|---|
| 80 | 3 | 0.0 | 1.50000000 | 0.670820393 | 0.0 |
| 100 | 3 | 3.0 | 1.50000000 | 0.670820393 | 1.0 |

Average Scores were used for Ties

Median 1-Way Analysis (Chi-Square Approximation)
CHISQ= 5.0000 DF= 1 Prob > CHISQ= 0.0253 *

* significant at the 0.05 level.

# APPENDIX C

## Statistical analysis of parametric data

**Analysis 1.** Production of conidia on various solid media.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ media.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ media.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Percent germination in water * MS | Pr > F | Percent germination in PDB ^ MS | Pr > F | df | No. of conidia produced # MS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|
| 1 | media | 5 | 738.946962 | 0.0001 | 0.00004976 | 0.2583 | 5 | 0.50844256 | 0.0020 |
| | error | 30 | 96.288293 | | 0.00004742 | | 12 | 0.06713204 | |
| 2 | media | 5 | 1191.495529 | 0.0001 | 0.00007705 | 0.0709 | 5 | 0.37120593 | 0.0001 |
| | error | 30 | 74.206655 | | 0.00003367 | | 12 | 0.02489564 | |
| pooled | media | 5 | 1792.5440579 | 0.0001 | 0.00010240 | 0.0246 | 5 | 0.82759888 | 0.0001 |
| | error | 60 | 104.8088936 | | 0.00003689 | | 30 | 0.04559306 | |

*. Arcsin transformation.
^. Square-root transformation.
#. Log (Y+1) transformation.

**Analysis 2.** Production of conidia on two quantities of pearl barley grains moistened with three different volumes of water.

Statistical model: $Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ijk}$ is the observation associated with the $jk^{th}$ BW combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of B.
$\beta_k$ is the effect due to the $k^{th}$ level of W.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ BW combination.
$E_{ijk}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation * | No. of conidia produced ᵛ df | MS | Pr > F |
|---|---|---|---|---|
| 1 | B | 1 | 351795902 | 0.3571 |
| | W | 2 | 3427066611 | 0.0048 |
| | B * W | 2 | 5925059011 | 0.0006 |
| | error | 11 | 380774731 | |
| 2 | B | 1 | 6200707 | 0.7446 |
| | W | 2 | 3930782631 | 0.0001 |
| | B * W | 2 | 1461511423 | 0.0001 |
| | error | 12 | 55769181 | |

ᵛ Square-root transformation.
* B = quantity of pearl barley grains, W = volume of water.

**Analysis 3.** Production of conidia on pearl barley grains harvested once and twice.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ harvest.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ harvest.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | Percent germination in PDB * df | MS | Pr > F | No. of conidia produced * df | MS | Pr > F |
|---|---|---|---|---|---|---|---|
| 1 | harvest | 1 | 0.00012955 | 0.1558 | 1 | 8062020947 | 0.0001 |
| | error | 14 | 0.00005756 | | 6 | 79801482 | |
| 2 | harvest | 1 | 0.00000000 | 0.9961 | 1 | 4577792056 | 0.0001 |
| | error | 14 | 0.00003444 | | 6 | 40193791 | |
| pooled | harvest | 1 | 0.00006444 | 0.2415 | 1 | 12394958389 | 0.0001 |
| | error | 30 | 0.00004514 | | 14 | 79358449 | |

* Square-root transformation.

**Analysis 4.** Viability and virulence of conidia produced on pearl barley grains over time.

Statistical model: $Y_{ij} = \mu + T_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ week.
$\mu$ is the overall mean.
$T_j$ is the effect due to the $j^{th}$ week.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Percent germination in PDB ¤ MS | Pr > F | Dry weight of above-ground biomass MS | Pr > F |
|---|---|---|---|---|---|---|
| 1 | week | 7 | 793.2711604 | 0.0001 | 0.01665860 | 0.0001 |
| | error | 54 | 35.8489418 | | 0.00284414 | |
| 2 | week | 7 | 1027.496698 | 0.0001 | 0.00136267 | 0.1600 |
| | error | 54 | 28.6761601 | | 0.00086019 | |

| Experiment | Dependant variable | Regression equation ¤ | $R^2$ ¥ | Pr ¤ |
|---|---|---|---|---|
| 1 | percent germination in PDB ¤ | $Y = 75.21141699 + 6.87629282\ X - 1.00956496\ X^2 + 0.03349209\ X^3$ | 0.686964 | 0.0001 |
| | dry weight of above-ground biomass | $Y = 0.0370840868 - 0.0153764504\ X + 0.0013140917\ X^2$ | 0.409653 | 0.0001 |
| 2 | percent germination in PDB ¤ | $Y = 73.44699524 + 7.22090241\ X - 0.95896927\ X^2 + 0.02847168\ X^3$ | 0.774984 | 0.0001 |

¤ Arcsin transformation.
¤ Y is the dependant variable and X is the number of weeks.
¥ Coefficient of determination
¤ Level of significance

**Analysis 5.** Effect of quantity of conidia used to inoculate pearl barley grains on the final production of conidia.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ seed inoculum density.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ seed inoculum density.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | No. of conidia produced * MS | Pr > F |
|---|---|---|---|---|
| 1 | seed inoculum density | 3 | 2108701828.7 | 0.0005 |
| | error | 8 | 111187865.5 | |
| 2 | seed inoculum density | 3 | 3583387094 | 0.0001 |
| | error | 8 | 114961719 | |

| Experiment | Dependant variable | Regression equation * | $R^2$ ~ | Pr ° |
|---|---|---|---|---|
| pooled | no. of conidia produced * | $Y = -440273.427 + 141140.2063\ X - 9182.0534\ X^2$ | 0.832886 | 0.0001 |

* Square-root transformation.
* Y is the dependant variable and X is the seed inoculum density.
~ Coefficient of determination.
° Level of significance.

**Analysis 6.** Production of conidia in various liquid media seeded with different types of seed inoculum.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ combination.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ combination.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | No. of conidia produced ˇ MS | Pr > F |
|---|---|---|---|---|
| 1 | combinations * | 4 | 170544179.6 | 0.0218 |
| | error | 10 | 36460882.5 | |
| 2 | combinations | 4 | 276382778 | 0.0005 |
| | error | 10 | 20391348 | |
| pooled | combinations | 4 | 371938431.6 | 0.0001 |
| | error | 25 | 34831535.8 | |

ˇ Square-root transformation.
* Combinations of liquid medium and type of seed inoculum.

**Analysis 7.** Effect of modifications of the Richard's (V-8) liquid medium on final production of conidia.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ recipe.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ recipe.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | No. of conidia produced * MS | Pr > F |
|---|---|---|---|---|
| 1 | recipes | 2 | 8631284.13 | 0.0001 |
| | error | 9 | 77432.88 | |
| 2 | recipes | 2 | 6268317.41 | 0.0001 |
| | error | 9 | 64361.20 | |
| pooled | recipes | 2 | 14797627.07 | 0.0001 |
| | error | 21 | 76378.46 | |

* Square-root transformation.

**Analysis 8.** Effect of quantity of conidia used to inoculate 100 ml of Richard's (V-8) liquid medium.

Statistical model: $Y_{i,j} = \mu + T_j + E_{i,j}$

Definitions: $Y_{i,j}$ is the observation associated with the $j^{th}$ seed inoculum density.
$\mu$ is the overall mean.
$T_j$ is the effect due to the $j^{th}$ seed inoculum density.
$E_{i,j}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | No. of conidia produced * df | MS | Pr > F |
|---|---|---|---|---|
| 1 | seed inoculum density | 3 | 53189923.57 | 0.0196 |
| | error | 8 | 8943435.20 | |
| 2 | seed inoculum density | 3 | 76627790.74 | 0.0086 |
| | error | 8 | 9561757.87 | |

| Experiment | Dependant variable | Regression equation * | $R^2$ ▾ | Pr ▪ |
|---|---|---|---|---|
| pooled | no. of conidia produced * | Y = 40598.65039 - 3252.57148 X | 0.549792 | 0.0001 |

* Square-root transformation.
* Y is the dependant variable and X is the seed inoculum density.
▾ Coefficient of determination.
▪ Level of significance.

**Analysis 9.** Effect of low-temperature (−10°C) storage on conidia.

Statistical model: $Y_{i,j} = \mu + T_j + E_{i,j}$

Definitions: $Y_{i,j}$ is the observation associated with the $j^{th}$ day.
$\mu$ is the overall mean.
$T_j$ is the effect due to the $j^{th}$ day.
$E_{i,j}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Percent germination in PDB * MS | Pr > F | Dry weight of above-ground biomass MS | Pr > F |
|---|---|---|---|---|---|---|
| 1 | days | 1 | 28532.39098 | 0.0001 | 1.15281667 | 0.0001 |
| | error | 14 | 10.82324 | | 0.008155 | |
| 2 | days | 1 | 28198.45309 | 0.0001 | 0.20475625 | 0.0002 |
| | error | 14 | 0.35728 | | 0.00785625 | |

* Arcsin transformation.

**Analysis 10.** Comparison of methods to produce conidia.

Statistical model: $Y_{ij} = \mu + T_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ method.
$\mu$ is the overall mean.
$T_j$ is the effect due to the $j^{th}$ method.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Percent germination in PDB * MS | Pr > F | Dry weight of above-ground biomass MS | Pr > F | df | No. of conidia produced * MS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|
| 1 | methods | 2 | 0.00034297 | 0.0180 | 0.00051667 | 0.7607 | 2 | 2829705211.7 | 0.0003 |
| | error | 15 | 0.00006456 | | 0.00185444 | | 6 | 64863114.6 | |
| 2 | methods | 2 | 0.00030678 | 0.1820 | 0.01068889 | 0.0773 | 2 | 3540139387 | 0.0005 |
| | error | 15 | 0.00016037 | | 0.00350222 | | 6 | 154731211 | |
| pooled | methods | 2 | 0.00064924 | 0.0048 | 0.00641944 | 0.2002 | 2 | 8143963294 | 0.0001 |
| | error | 33 | 0.00010284 | | 0.00379975 | | 15 | 156824978 | |

* Square-root transformation.

**Analysis 11.** Effect of short-term cold (4°C) storage on conidia.

Statistical model: $Y_{i,j} = \mu + \tau_j + E_{i,j}$

Definitions: $Y_{i,j}$ is the observation associated with the $j^{th}$ day.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ day.
$E_{i,j}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | Percent germination in PDB ⁼ df | MS | Pr > F | Dry weight of above-ground biomass df | MS | Pr > F |
|---|---|---|---|---|---|---|---|
| 1 | days | 5 | 2344.320549 | 0.0001 | 5 | 0.00327750 | 0.4774 |
| | error | 42 | 9.6275 | | 18 | 0.00347639 | |
| 2 | days | 5 | 8449.020320 | 0.0001 | 5 | 0.09125 | 0.0001 |
| | error | 42 | 17.779344 | | 18 | 0.00493056 | |

| Experiment | Dependant variable | Regression equation ⁼ | $R^2$ ᵛ | Pr ⁿ |
|---|---|---|---|---|
| 1 | percent germination in PDB ⁼ | $Y = 89.41761262 - 14.36290163\ X + 8.55179492\ X^2 - 1.31328743\ X^3$ | 0.961120 | 0.0001 |
| 2 | percent germination in PDB ⁼ | $Y = 87.37681494 - 31.50354834\ X + 37.06662239\ X^2 - 14.83707952\ X^3 + 1.61884194\ X^4$ | 0.978246 | 0.0001 |

⁼ Arcsin transformation.
⁼ Y is the dependant variable and x is the number of days.
ᵛ Coefficient of determination
ⁿ Level of significance

**Analysis 12.** Effect of low-temperature (−70°C) storage on conidia.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ month.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ month.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Percent germination in PDB ~ MS | Pr > F | Dry weight of above-ground biomass MS | Pr > F |
|---|---|---|---|---|---|---|
| 1 | months | 8 | 842.625860 | 0.0001 | 0.00043460 | 0.1154 |
| | error | 63 | 21.703114 | | 0.00025533 | |
| 2 | months | 8 | 2392.97077 | 0.0001 | | |
| | error | 63 | 43.01651 | | | |

| Experiment | Dependant variable | Regression equation * | $R^2$ ▾ | Pr ▪ |
|---|---|---|---|---|
| 1 | percent germination in PDB ~ | $Y = 84.98745663 - 3.05583409\ X + 1.50201504\ X^2 - 0.19806526\ X^3$ | 0.780106 | 0.0001 |
| 2 | percent germination in PDB ~ | $Y = 86.65342030 - 20.73758058\ X - 15.9171214\ X^2 + 3.6249235\ X^3$ | 0.785573 | 0.0001 |

~ Arcsin transformation.
* Y is the dependant variable and X is the number of months.
▾ Coefficient of determination
▪ Level of significance

**Analysis 13.** Effect of moist conditions on sporulation of Phomopsis convolvulus on infected leaves.

Statistical model: $Y_{ij} = \mu + \tau_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ day.
$\mu$ is the overall mean.
$\tau_j$ is the effect due to the $j^{th}$ day.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | No. of conidia produced * df | MS | Pr > F |
|---|---|---|---|---|
| 1 | days | 5 | 864612.614 | 0.0003 |
| | error | 18 | 100664.324 | |
| 2 | days | 5 | 334300.824 | 0.0001 |
| | error | 17 | 30734.719 | |

| Experiment | Dependant variable | Regression equation * | $R^2$ ▾ | Pr ▪ |
|---|---|---|---|---|
| 1 | no. of conidia produced * | $Y = 6.4677568 + 224.6562185\ X$ | 0.575863 | 0.0001 |
| 2 | no. of conidia produced * | $Y = -2744.578232 + 2331.380733\ X - 485.475347\ X^2 + 32.847788\ X^3$ | 0.734059 | 0.0001 |

* Square-root transformation.
* Y is the dependant variable and X is the number of days.
▾ Coefficient of determination
▪ Level of significance

**Analysis 14.** Effect of leaf wetness and inoculum density on disease expression.

Statistical model: $Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ijk}$ is the observation associated with the $jk^{th}$ LI combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of L.
$\beta_k$ is the effect due to the $k^{th}$ level of I.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ LI combination.
$E_{ijk}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation ~ | df | Dry weight of above-ground biomass MS | Pr > F | Dry weight of roots MS | Pr > F | df | Percent mortality ˇ MS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|
| 1 | L | 3 | 0.60287458 | 0.0001 | 0.01986833 | 0.0001 | 1 | 1254.503328 | 0.0708 |
| | I | 4 | 0.67916562 | 0.0001 | 0.01416250 | 0.0001 | 2 | 8665.061452 | 0.0001 |
| | L * I | 12 | 0.05055896 | 0.0002 | 0.00072667 | 0.1699 | 2 | 666.543189 | 0.1699 |
| | error | 60 | 0.01268125 | | 0.00050167 | | 18 | 340.229434 | |
| 2 | L | 3 | 1.25942458 | 0.0001 | 0.1547 | 0.0001 | 1 | 4469.611486 | 0.0413 |
| | I | 4 | 2.19155750 | 0.0001 | 0.26639687 | 0.0001 | 2 | 2407.730483 | 0.1017 |
| | L * I | 12 | 0.11586 | 0.0001 | 0.00315104 | 0.8114 | 2 | 329.737807 | 0.7051 |
| | error | 60 | 0.02598375 | | 0.00503 | | 18 | 925.415725 | |

ˇ Arcsin transformation. For this variable analysis was only performed on densities $10^{8}$, $10^{9}$, $10^{10}$ conidia/$m^2$ and leaf wetness period of 18 and 24 hr.
~ L = leaf wetness period, I = inoculum density.

## Analysis 14. continued

Experiment 1:

| Dependant variable | Contrast | df | MS | Pr > F | Regression equation ˣ | R² ⁼ |
|---|---|---|---|---|---|---|
| dry weight of above-ground biomass | model | 19 | 0.27010493 | 0.0001** | | |
| | error | 60 | 0.01268125 | | | |
| | L in I10⁴ | 3 | 0.00811667 | 0.5922 | | |
| | L in I10⁷ | 3 | 0.04569333 | 0.0184* | Y = 0.7325 - 0.01725 X | 0.438864 |
| | L linear in I10⁷ | 1 | 0.214245 | 0.0001** | | |
| | L quadratic in I10⁷ | 1 | 0.01 | 0.3781 | | |
| | L cubic in I10⁷ | 1 | 0.04608 | 0.0614 | | |
| | L in I10⁸ | 3 | 0.16880625 | 0.0001** | Y = 0.67625 - 0.026125 X | 0.814952 |
| | L linear in I10⁸ | 1 | 0.49141125 | 0.0001** | | |
| | L quadratic in I10⁸ | 1 | 0.01500625 | 0.2810 | | |
| | L cubic in I10⁸ | 1 | 0.00000125 | 0.9921 | | |
| | L in I10⁹ | 3 | 0.32077292 | 0.0001** | Y = 1.175625 - 0.106354167 X + 0.002413194 X² | 0.890448 |
| | L linear in I10⁹ | 1 | 0.83028125 | 0.0001** | | |
| | L quadratic in I10⁹ | 1 | 0.12075625 | 0.0031** | | |
| | L cubic in I10⁹ | 1 | 0.01128125 | 0.3494 | | |
| | L in I10¹⁰ | 3 | 0.21750625 | 0.0001** | Y = 0.6425 - 0.028875 X | 0.829969 |
| | L linear in I10¹⁰ | 1 | 0.60031125 | 0.0001** | | |
| | L quadratic in I10¹⁰ | 1 | 0.04950625 | 0.0528 | | |
| | L cubic in I10¹⁰ | 1 | 0.00210125 | 0.6854 | | |
| | I in L6 | 4 | 0.0172825 | 0.2576 | | |
| | I in L12 | 4 | 0.2150575 | 0.0001** | Y = 1.5635 - 0.14 X | 0.731715 |
| | I linear in L12 | 1 | 0.784 | 0.0001** | | |
| | I quadratic in L12 | 1 | 0.04345714 | 0.0691 | | |
| | I cubic in L12 | 1 | 0.009 | 0.4029 | | |
| | I quartic in L12 | 1 | 0.02377286 | 0.1760 | | |
| | I in L18 | 4 | 0.3025575 | 0.0001** | Y = 5.156857143 - 1.093035714 X + 0.058392857 X² | 0.861322 |
| | I linear in L18 | 1 | 1.0080625 | 0.0001** | | |
| | I quadratic in L18 | 1 | 0.19094464 | 0.0003** | | |
| | I cubic in L18 | 1 | 0.00625 | 0.4854 | | |
| | I quartic in L18 | 1 | 0.00497286 | 0.5336 | | |
| | I in L24 | 4 | 0.295945 | 0.0001** | Y = 4.967 - 1.05825 X + 0.05625 X² | 0.881853 |
| | I linear in L24 | 1 | 1.0017225 | 0.0001** | | |
| | I quadratic in L24 | 1 | 0.1771875 | 0.0001** | | |
| | I cubic in L24 | 1 | 0.001444 | 0.7573 | | |
| | I quartic in L24 | 1 | 0.00343 | 0.6049 | | |

ˣ Y is the dependant variable and X is the leaf wetness period or the inoculum density.
⁼ Coefficient of determination.
* Significant at the 0.05 level.
** Significant at the 0.01 level.

## Analysis 14. continued

Experiment 2:

| Dependant variable | Contrast | df | MS | Pr > F | Regression equation ˣ | $R^2$ ⁼ |
|---|---|---|---|---|---|---|
| dry weight of above-ground biomass | model | 19 | 0.73341178 | 0.0001** | | |
| | error | 60 | 0.02598375 | | | |
| | L in $I10^{6}$ | 3 | 0.05745625 | 0.0960 | | |
| | L in $I10^{7}$ | 3 | 0.13364917 | 0.0031** | $Y = 1.895625 - 0.1146875\ X + 0.002690972\ X^2$ | 0.720993 |
| | L linear in $I10^{7}$ | 1 | 0.83028125 | 0.0001** | | |
| | L quadratic in $I10^{7}$ | 1 | 0.15015625 | 0.0193* | | |
| | L cubic in $I10^{7}$ | 1 | 0.02278125 | 0.3528 | | |
| | L in $I10^{8}$ | 3 | 0.38592292 | 0.0001** | $Y = 0.95 - 0.0369583333\ X$ | 0.709162 |
| | L linear in $I10^{8}$ | 1 | 0.98346125 | 0.0001** | | |
| | L quadratic in $I10^{8}$ | 1 | 0.07700625 | 0.0903 | | |
| | L cubic in $I10^{8}$ | 1 | 0.09730125 | 0.0577 | | |
| | L in $I10^{9}$ | 3 | 0.57472292 | 0.0001** | $Y = 1.648125 - 0.1593125\ X + 0.003836806\ X^2$ | 0.878251 |
| | L linear in $I10^{9}$ | 1 | 1.40715125 | 0.0001** | | |
| | L quadratic in $I10^{9}$ | 1 | 0.30525625 | 0.0011** | | |
| | L cubic in $I10^{9}$ | 1 | 0.01176125 | 0.5037 | | |
| | L in $I10^{10}$ | 3 | 0.37035625 | 0.0001** | $Y = 1.418125 - 0.129479167\ X + 0.003142361\ X^2$ | 0.734269 |
| | L linear in $I10^{10}$ | 1 | 0.89253125 | 0.0001** | | |
| | L quadratic in $I10^{10}$ | 1 | 0.20475625 | 0.0067** | | |
| | L cubic in $I10^{10}$ | 1 | 0.01378125 | 0.4693 | | |
| | I in L6 | 4 | 0.1774755 | 0.0001** | $Y = 1.71 - 0.095\ X$ | 0.301134 |
| | I linear in L6 | 1 | 0.361 | 0.0004** | | |
| | I quadratic in L6 | 1 | 0.01382857 | 0.4685 | | |
| | I cubic in L6 | 1 | 0.1625625 | 0.0151* | | |
| | I quartic in L6 | 1 | 0.17250893 | 0.0125* | | |
| | I in L12 | 4 | 0.86815 | 0.0001** | $Y = 8.146214286 - 1.657071429\ X + 0.086785714\ X^2$ | 0.821280 |
| | I linear in L12 | 1 | 2.88369 | 0.0001** | | |
| | I quadratic in L12 | 1 | 0.42177857 | 0.0002** | | |
| | I cubic in L12 | 1 | 0.07056 | 0.1046 | | |
| | I quartic in L12 | 1 | 0.09657143 | 0.0580 | | |
| | I in L18 | 4 | 0.7093925 | 0.0001** | $Y = 7.361785714 - 1.519178571\ X + 0.079464286\ X^2$ | 0.914978 |
| | I linear in L18 | 1 | 2.4552025 | 0.0001** | | |
| | I quadratic in L18 | 1 | 0.35361607 | 0.0005** | | |
| | I cubic in L18 | 1 | 0.0235225 | 0.3452 | | |
| | I quartic in L18 | 1 | 0.00522893 | 0.6553 | | |
| | I in L24 | 4 | 0.78412 | 0.0001** | $Y = 8.147214286 - 1.737321429\ X + 0.093035714\ X^2$ | 0.864881 |
| | I linear in L24 | 1 | 2.4750625 | 0.0001** | | |
| | I quadratic in L24 | 1 | 0.4847 | 0.0001** | | |
| | I cubic in L24 | 1 | 0.09025 | 0.0673 | | |
| | I quartic in L24 | 1 | 0.08 | 0.0731 | | |

ˣ Y is the dependant variable and X is the leaf wetness period or the inoculum density.
⁼ Coefficient of determination.
* Significant at the 0.05 level.
** Significant at the 0.01 level.

## Analysis 14. continued

| Experiment | Dependant variable | L | I | Regression equation ˣ | $R^2$ ˢ | Pr ˢ |
|---|---|---|---|---|---|---|
| 1 | dry weight of roots | 12 | - | Y = 0.244 - 0.021 X | 0.692308 | 0.0001 |
| | | 18 | - | Y = 0.1905 - 0.01775 X | 0.705825 | 0.0001 |
| | | 24 | - | Y = 0.2485 - 0.02525 X | 0.684539 | 0.0001 |
| | | - | $10^7$ | Y = 0.135 - 0.003375 X | 0.569779 | 0.0007 |
| | | - | $10^8$ | Y = 0.185625 - 0.0140208333 X + 0.0003298611 $X^2$ | 0.874275 | 0.0001 |
| | | - | $10^7$ | Y = 0.188125 - 0.0150208333 X + 0.0003298611 $X^2$ | 0.838524 | 0.0001 |
| | | - | $10^{10}$ | Y = 0.11 - 0.0046666667 X | 0.607752 | 0.0004 |
| 2 | dry weight of roots | 6 | - | Y = 0.801 - 0.059 X | 0.462622 | 0.0010 |
| | | 12 | - | Y = 2.112285714 - 0.410928571 X + 0.020714286 $X^2$ | 0.779563 | 0.0001 |
| | | 18 | - | Y = 2.605714286 - 0.547571429 X + 0.029285714 $X^2$ | 0.875187 | 0.0001 |
| | | 24 | - | Y = 2.438642857 - 0.507964286 X + 0.026607143 $X^2$ | 0.758588 | 0.0001 |
| | | - | $10^7$ | Y = 0.675 - 0.0444166667 X + 0.0010416667 $X^2$ | 0.807675 | 0.0001 |
| | | - | $10^8$ | Y = 0.27125 - 0.0096666667 X | 0.585171 | 0.0006 |
| | | - | $10^7$ | Y = 0.430625 - 0.0376875 X + 0.0008854167 $X^2$ | 0.848412 | 0.0001 |
| | | - | $10^{10}$ | Y = 0.29375 - 0.0125833333 X | 0.528780 | 0.0014 |

ˢ Level of significance.
ˣ Y is the dependant variable and X is the leaf wetness period or the inoculum density.
ˢ Coefficient of determination.

**Analysis 15.** Effect of cumulative short wet periods interrupted by dry periods on disease expression.

Statistical model: $Y_{ij} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ij}$ is the observation associated with the $jk^{th}$ HR combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of H.
$\beta_k$ is the effect due to the $k^{th}$ level of R.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ HR combination.
$E_{ij}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation * | df | Dry weight of above-ground biomass | | Dry weight of roots | | Percent mortality ˅ | |
|---|---|---|---|---|---|---|---|---|
| | | | MS | Pr > F | MS | Pr > F | MS | Pr > F |
| 1 | H | 1 | 0.67800333 | 0.0001 | 0.09408000 | 0.0001 | 37.6843207 | 0.6393 |
| | R | 4 | 0.649875 | 0.0001 | 0.03612833 | 0.0001 | 652.3400248 | 0.0165 |
| | H * R | 4 | 0.111995 | 0.0045 | 0.00813833 | 0.0142 | 37.6843207 | 0.9203 |
| | error | 20 | 0.02117333 | | 0.002 | | 166.3541962 | |
| 2 | H | 1 | 0.27265333 | 0.0001 | 0.02133333 | 0.0001 | 94.111267 | 0.3293 |
| | R | 4 | 0.32342833 | 0.0001 | 0.02490333 | 0.0001 | 7282.576757 | 0.0001 |
| | H * R | 4 | 0.033645 | 0.0077 | 0.00205 | 0.0063 | 94.111267 | 0.4307 |
| | error | 20 | 0.00716 | | 0.00041667 | | 94.111267 | |

˅ Arcsin transformation.
* H = relative air humidity during the dry period, R = 3-day wet/dry regime.

**Analysis 16.** Effect of leaf wetness and additive on disease expression.

Statistical model: $Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ijk}$ is the observation associated with the $jk^{th}$ DL combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of D.
$\beta_k$ is the effect due to the $k^{th}$ level of L.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ DL combination.
$E_{ijk}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation ˇ | df | Percent mortality ˜ MS | Pr > F | Dry weight of above-ground biomass MS | Pr > F |
|---|---|---|---|---|---|---|
| 1 | D | 6 | 1960.409088 | 0.0008 | 0.02261607 | 0.0001 |
| | L | 3 | 12491.202216 | 0.0001 | 0.10279643 | 0.0001 |
| | D * L | 18 | 652.330502 | 0.1379 | 0.00311448 | 0.0906 |
| | error | 84 | 455.251867 | | 0.00199821 | |
| 2 | D | 6 | 908.034646 | 0.0406 | 0.02284732 | 0.0001 |
| | L | 3 | 4636.230193 | 0.0001 | 0.11028899 | 0.0001 |
| | D * L | 18 | 534.264627 | 0.1726 | 0.00850010 | 0.0208 |
| | error | 84 | 392.123742 | | 0.00432530 | |

ˇ D = additive, L = leaf wetness period.
˜ Arcsin transformation.

**Analysis 16.** continued.

| Experiment | Dependant variable | D | Regression equation ˅ | R² ˢ | Pr ˢ |
|---|---|---|---|---|---|
| 1 | percent mortality ˜ | control(water) | Y = -147.3536022 + 12.9048728 X | 0.687992 | 0.0001 |
| | | gelatin 1% | Y = -102.6243474 + 8.2555523 X | 0.449901 | 0.0045 |
| | | sorbo 20% | Y = -135.158763 + 10.8448052 X | 0.649057 | 0.0002 |
| | | bond 0.74 L/ha | Y = -85.81267274 + 7.83526041 X | 0.378021 | 0.0113 |
| | | bond 1.48 L/ha | Y = -61.68409453 + 6.7507799 X | 0.444685 | 0.0048 |
| | dry weight of above-ground biomass | control(water) | Y = 0.2125 - 0.0125 X | 0.443262 | 0.0049 |
| | | gelatin 1% | Y = 0.5075 - 0.027625 X | 0.677265 | 0.0001 |
| | | gelatin 2% | Y = 0.51625 - 0.027 X | 0.465165 | 0.0036 |
| | | sorbo 20% | Y = 0.53375 - 0.029625 X | 0.857346 | 0.0001 |
| | | sorbo 30% | Y = 0.575 - 0.029875 X | 0.570554 | 0.0007 |
| | | bond 0.74 L/ha | Y = 0.31375 - 0.01775 X | 0.576773 | 0.0006 |
| | | bond 1.48 L/ha | Y= 0.245 - 0.013125 X | 0.364162 | 0.0133 |
| 2 | percent mortality ˜ | gelatin 1% | Y = -101.9493304 + 8.1880506 X | 0.393026 | 0.0093 |
| | | sorbo 30% | Y = 271.7602652 - 42.466682 X + 1.6604716 X² | 0.767609 | 0.0001 |
| | | bond 1.48 L/ha | Y = -69.13154818 + 6.76383491 X | 0.382160 | 0.0107 |

˜ Arcsin transformation.
ˢ Level of significance.
˅ Y is the dependant variable and X is the leaf wetness period.
² Coefficient of determination.

**Analysis 16.** continued.

Experiment 2:

| Dependant variable | Contrast | df | MS | Pr > F | Regression equation ▾ | $R^2$ ▪ |
|---|---|---|---|---|---|---|
| dry weight of above-ground biomass | model | 27 | 0.02299825 | 0.0001** | | |
| | error | 84 | 0.00432530 | | | |
| | L in D control | 3 | 0.004575 | 0.3716 | | |
| | L in D gelatin 1% | 3 | 0.00880771 | 0.1150 | | |
| | L in D gelatin 2% | 3 | 0.01308333 | 0.0341* | Y = 0.4175 - 0.0215 X | 0.539067 |
| | L linear in D gelatin 2% | 1 | 0.03698 | 0.0044** | | |
| | L quadratic in D gelatin 2% | 1 | 0.0.002025 | 0.4957 | | |
| | L cubic in D gelatin 2% | 1 | 0.000245 | 0.8125 | | |
| | L in D sorbo 20% | 3 | 0.04807292 | 0.0001** | Y = 0.73875 - 0.040375 X | 0.633853 |
| | L linear in D sorbo 20% | 1 | 0.13041125 | 0.0001** | | |
| | L quadratic in D sorbo 20% | 1 | 0.00105625 | 0.6225 | | |
| | L cubic in D sorbo 20% | 1 | 0.01275125 | 0.0897 | | |
| | L in D sorbo 30% | 3 | 0.06320625 | 0.0001** | Y = 0.82125 - 0.045625 X | 0.573171 |
| | L linear in D sorbo 30% | 1 | 0.16653125 | 0.0001** | | |
| | L quadratic in D sorbo 30% | 1 | 0.00030625 | 0.7908 | | |
| | L cubic in D sorbo 30% | 1 | 0.02278125 | 0.0242* | | |
| | L in D bond 0.74 L/ha | 3 | 0.00875625 | 0.1167 | | |
| | L in D bond 1.48 L/ha | 3 | 0.00092292 | 0.8869 | | |

▾ Y is the dependant variable and X is the leaf wetness period.
▪ Coefficient of determination.
* Significant at the 0.05 level.
** Significant at the 0.01 level.

**Analysis 17.** Effect of inoculum density on field bindweed seedlings (3-5 leaf stage).

Statistical model: $Y_{ij} = \mu + T_j + E_{ij}$

Definitions: $Y_{ij}$ is the observation associated with the $j^{th}$ density.
$\mu$ is the overall mean.
$T_j$ is the effect due to the $j^{th}$ density.
$E_{ij}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | Dry weight of above-ground biomass df | MS | Pr > F | Dry weight of roots df | MS | Pr > F | Percent mortality ~ df | MS | Pr > F |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | density | 4 | 0.21225 | 0.0001 | 4 | 0.01483 | 0.0004 | 2 | 1036.289698 | 0.1641 |
| | error | 15 | 0.00564 | | 15 | 0.00149167 | | 9 | 466.000672 | |
| 2 | density | 4 | 0.1143125 | 0.0001 | 4 | 0.0081875 | 0.0009 | 2 | 3269.514405 | 0.0090 |
| | error | 15 | 0.005875 | | 15 | 0.00097 | | 9 | 392.618246 | |

| Experiment | Dependant variable | Regression equation * | $R^2$ ▾ | Pr ▪ |
|---|---|---|---|---|
| pooled | dry weight of above-ground biomass | $Y = -10.83446429 + 4.79846726\ X - 0.65928571\ X^2 + 0.02885417\ X^3$ | 0.805638 | 0.0001 |
| | dry weight of roots | $Y = 1.049642857 - 0.224339286\ X + 0.012232143\ X^2$ | 0.627848 | 0.0001 |
| | percent mortality ~ | $Y = -2714.463010 + 605.851112\ X^{MS} - 33.058642\ X^2$ | 0.405166 | 0.0043 |

~ Arcsin transformation. For this variable analysis was only performed on densities $10^8$, $10^7$, and $10^{10}$ conidia/$m^2$.
* Y is the dependant variable and X is the density of inoculum.
▾ Coefficient of determination
▪ Level of significance

**Analysis 18.** Effect of inoculum density on established field bindweed plants.

Statistical model: $Y_{i,j} = \mu + \rho_i + \tau_j + E_{i,j}$

Definitions: $Y_{i,j}$ is the observation associated with the $j^{th}$ density and the $i^{th}$ block.
$\mu$ is the overall mean.
$\rho_i$ is the effect due to the $i^{th}$ block.
$\tau_j$ is the effect due to the $j^{th}$ density.
$E_{i,j}$ is the random error associated with the $ij^{th}$ experimental unit.

| Experiment | Source of variation | df | Dry weight of above-ground biomass | | Dry weight of roots | | Dry weight of regrowth | | Percent mortality * | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | MS | Pr > F | MS | Pr > F | MS | Pr > F | MS | Pr > F |
| 1 | block | 2 | 0.54355833 | 0.0573 | 0.13563333 | 0.0828 | 0.18810833 | 0.1101 | 591.758606 | 0.4219 |
| | density | 3 | 1.208475 | 0.0082 | 0.10443056 | 0.1176 | 0.18374444 | 0.1058 | 2367.034425 | 0.0701 |
| | error | 6 | 0.113725 | | 0.03492222 | | 0.05771944 | | 591.758606 | |
| 2 | block | 2 | 2.395675 | 0.0406 | 2.15585833 | 0.0300 | 1.384275 | 0.0360 | | |
| | density | 3 | 2.45196389 | 0.0324 | 0.39640833 | 0.3799 | 0.74261111 | 0.1012 | | |
| | error | 6 | 0.41836389 | | 0.32415833 | | 0.22738611 | | | |

| Experiment | Dependant variable | Regression equation = | $R^2$ v | Pr * |
|---|---|---|---|---|
| pooled | dry weight of above-ground biomass | Y = 5.514166667 - 0.56200000 X | 0.372613 | 0.0015 |

= Arcsin transformation.
= Y is the dependant variable and X is the density of inoculum.
v Coefficient of determination
* Level of significance

**Analysis 19.** Effect of inoculum density and age of field bindweed seedlings.

Statistical model: $Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ijk}$ is the observation associated with the $jk^{th}$ AI combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of A.
$\beta_k$ is the effect due to the $k^{th}$ level of I.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ AI combination.
$E_{ijk}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation ⁼ | df | Dry weight of above-ground biomass | | Dry weight of roots | | Dry weight of regrowth | | Percent mortality ⁼ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | MS | Pr > F | MS | Pr > F | MS | Pr > F | MS | Pr > F |
| 1 | A | 3 | 7.86518704 | 0.0001 | 0.43332593 | 0.0001 | 0.34888519 | 0.0001 | 10572.76865 | 0.0001 |
| | I | 2 | 4.99118472 | 0.0001 | 0.37592917 | 0.0001 | 0.40488472 | 0.0001 | 13093.68618 | 0.0001 |
| | A * I | 6 | 0.87519954 | 0.0001 | 0.08578843 | 0.0001 | 0.17533102 | 0.0001 | 2805.24409 | 0.0001 |
| | error | 60 | 0.09031333 | | 0.00742389 | | 0.01857278 | | 404.27224 | |
| 2 | A | 3 | 0.83233519 | 0.0001 | 0.02039444 | 0.0001 | 0.4439398 | 0.0001 | 7129.52341 | 0.0001 |
| | I | 2 | 1.93468889 | 0.0001 | 0.03364306 | 0.0001 | 0.08012639 | 0.0001 | 23939.19674 | 0.0001 |
| | A * I | 6 | 0.14918519 | 0.0006 | 0.01154306 | 0.0001 | 0.02160231 | 0.0004 | 2755.50806 | 0.0001 |
| | error | 60 | 0.03205444 | | 0.00144667 | | 0.00442528 | | 125.67785 | |

⁼ A = age of plants, I = inoculum density.
· Arcsin transformation.
ˇ Y is the dependant variable and X is the inoculum density.
* Coefficient of determination.
⁼ Significant at the 0.05 level.
⁼⁼ Significant at the 0.01 level.

## Analysis 19. continued.

Experiment 1:

| Dependant variable | Contrast | df | MS | Pr > F | Regression equation ¥ | R² = |
|---|---|---|---|---|---|---|
| dry weight of above-ground biomass | model | 11 | 3.52992071 | 0.0001** | | |
| | error | 60 | 0.09031333 | | | |
| | I in A1 | 2 | 0.14228889 | 0.2153 | | |
| | I in A2 | 2 | 0.66343889 | 0.0014** | Y = 2.647777778 - 0.3025 X | 0.628365 |
| | I linear in A2 | 1 | 1.098075 | 0.0009** | | |
| | I quadratic in A2 | 1 | 0.22880278 | 0.1167 | | |
| | I in A3 | 2 | 5.10671667 | 0.0001** | Y = 28.22 - 5.98416667 X + 0.3175 X² | 0.870291 |
| | I linear in A3 | 1 | 9.81020833 | 0.0001** | | |
| | I quadratic in A3 | 1 | 0.403225 | 0.0388* | | |
| | I in A4 | 2 | 1.70433889 | 0.0001** | Y = -21.0 + 6.1775 X - 0.41583333 X² | 0.497407 |
| | I linear in A4 | 1 | 2.71700833 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.69166944 | 0.0075** | | |
| dry weight of roots | model | 11 | 0.23332424 | 0.0001** | | |
| | error | 60 | 0.00742389 | | | |
| | I in A1 | 2 | 0.0008 | 0.8980 | | |
| | I in A2 | 2 | 0.0128 | 0.1870 | | |
| | I in A3 | 2 | 0.18028889 | 0.0001** | Y = 8.303333333 - 1.895 X + 0.108333333 X² | 0.717457 |
| | I linear in A3 | 1 | 0.31363333 | 0.0001** | | |
| | I quadratic in A3 | 1 | 0.04694444 | 0.0146* | | |
| | I in A4 | 2 | 0.43940556 | 0.0001** | Y = 11.535 - 2.56416667 X + 0.14416667 X² | 0.748406 |
| | I linear in A4 | 1 | 0.795675 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.08313611 | 0.0014** | | |
| dry weight of regrowth | model | 11 | 0.26440101 | 0.0001** | | |
| | error | 60 | 0.01857278 | | | |
| | I in A1 | 2 | 0.00005 | 0.9973 | | |
| | I in A2 | 2 | 0.0 | 1.0000 | | |
| | I in A3 | 2 | 0.16115556 | 0.0005** | Y = 1.277777778 - 0.146666667 X | 0.388729 |
| | I linear in A3 | 1 | 0.25813333 | 0.0004** | | |
| | I quadratic in A3 | 1 | 0.06417778 | 0.0679 | | |
| | I in A4 | 2 | 0.76967222 | 0.0001** | Y = 16.395 - 3.72333333 X + 0.21166667 X² | 0.666029 |
| | I linear in A4 | 1 | 1.36013333 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.17921111 | 0.0029** | | |
| percent mortality ¤ | model | 11 | 6794.28571 | 0.0001** | | |
| | error | 60 | 404.27224 | | | |
| | I in A1 | 2 | 8703.07652 | 0.0001** | Y = -2683.272032 + 660.966383 X - 39.401308 X² | 0.666184 |
| | I linear in A1 | 1 | 11196.30083 | 0.0001** | | |
| | I quadratic in A1 | 1 | 6209.85221 | 0.0002** | | |
| | I in A2 | 2 | 7871.96910 | 0.0001** | Y = -1774.779086 + 425.212303 X - 24.491556 X² | 0.553408 |
| | I linear in A2 | 1 | 13244.59291 | 0.0001** | | |
| | I quadratic in A2 | 1 | 2499.34529 | 0.0178* | | |
| | I in A3 | 2 | 4954.37283 | 0.0001** | Y = 1111.157561 - 302.100756 X + 20.513643 X² | 0.777193 |
| | I linear in A3 | 1 | 8165.54745 | 0.0001** | | |
| | I quadratic in A3 | 1 | 1683.29822 | 0.0457* | | |
| | I in A4 | 2 | 0.0 | 1.0000 | | |

**Analysis 19.** continued.

Experiment 2:

| Dependant variable | Contrast | df | MS | Pr > F | Regression equation ▾ | $R^2$ = |
|---|---|---|---|---|---|---|
| dry weight of above-ground biomass | model | 11 | 0.66013586 | 0.0001** | | |
| | error | 60 | 0.03205944 | | | |
| | I in A1 | 2 | 0.03801667 | 0.3126 | | |
| | I in A2 | 2 | 0.42177222 | 0.0001** | Y = 2.290555556 - 0.258333333 X | 0.726691 |
| | I linear in A2 | 1 | 0.80083333 | 0.0001** | | |
| | I quadratic in A2 | 1 | 0.04271111 | 0.2530 | | |
| | I in A3 | 2 | 0.91783889 | 0.0001** | Y = 3.605555556 - 0.390833333 X | 0.800378 |
| | I linear in A3 | 1 | 1.83300833 | 0.0001** | | |
| | I quadratic in A3 | 1 | 0.00266944 | 0.7739 | | |
| | I in A4 | 2 | 1.00461667 | 0.0001** | Y = 3.773333333 - 0.409166667 X | 0.626445 |
| | I linear in A4 | 1 | 2.00900833 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.000225 | 0.9335 | | |
| dry weight of roots | model | 11 | 0.01797525 | 0.0001** | | |
| | error | 60 | 0.00144667 | | | |
| | I in A1 | 2 | 0.0 | 1.0000 | | |
| | I in A2 | 2 | 0.00005 | 0.9660 | | |
| | I in A3 | 2 | 0.04911667 | 0.0001** | Y = 4.385 - 1.004166667 X + 0.0575 $X^2$ | 0.630509 |
| | I linear in A3 | 1 | 0.08500833 | 0.0001** | | |
| | I quadratic in A3 | 1 | 0.013225 | 0.0037** | | |
| | I in A4 | 2 | 0.01910556 | 0.0001** | Y = 0.4605555556 - 0.0525 X | 0.495918 |
| | I linear in A4 | 1 | 0.033075 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.00513611 | 0.0644 | | |
| dry weight of regrowth | model | 11 | 0.03845896 | 0.0001** | | |
| | error | 60 | 0.00442528 | | | |
| | I in A1 | 2 | 0.00008889 | 0.9801 | | |
| | I in A2 | 2 | 0.0008 | 0.8351 | | |
| | I in A3 | 2 | 0.06377222 | 0.0001** | Y = 0.9194444444 - 0.1025 X | 0.508173 |
| | I linear in A3 | 1 | 0.126075 | 0.0001** | | |
| | I quadratic in A3 | 1 | 0.00146944 | 0.5666 | | |
| | I in A4 | 2 | 0.08027222 | 0.0001** | Y = 6.045 - 1.399166667 X + 0.080833333 $X^2$ | 0.539504 |
| | I linear in A4 | 1 | 0.13440833 | 0.0001** | | |
| | I quadratic in A4 | 1 | 0.02613611 | 0.0181* | | |
| percent mortality * | model | 11 | 7800.0011 | 0.0001** | | |
| | error | 60 | 125.67785 | | | |
| | I in A1 | 2 | 14144.49174 | 0.0001** | Y = -2681.879498 + 647.784488 X - 37.775329 $X^2$ | 0.967770 |
| | I linear in A1 | 1 | 22581.08150 | 0.0001** | | |
| | I quadratic in A1 | 1 | 5707.90198 | 0.0001 | | |
| | I in A2 | 2 | 12169.49037 | 0.0001** | Y = 1018.676474 - 292.039251 X + 20.963654 $X^2$ | 0.941679 |
| | I linear in A2 | 1 | 22581.08150 | 0.0001** | | |
| | I quadratic in A2 | 1 | 1757.89924 | 0.0004** | | |
| | I in A3 | 2 | 1411.35664 | 0.0001** | Y = -102.5493455 + 14.4597402 X | 0.377037 |
| | I linear in A3 | 1 | 2819.00906 | 0.0001** | | |
| | I quadratic in A3 | 1 | 3.70422 | 0.1194 | | |
| | I in A4 | 2 | 4480.38216 | 0.0001** | Y = 1045.303397 - 284.461247 X + 19.337676 $X^2$ | 0.876776 |
| | I linear in A4 | 1 | 7464.98117 | 0.0001** | | |
| | I quadratic in A4 | 1 | 1495.78284 | 0.0010** | | |

**Analysis 20.** Effect of number of applications of various inoculum densities on disease expression.

Statistical model: $Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + E_{ijk}$

Definitions: $Y_{ijk}$ is the observation associated with the $jk^{th}$ NI combination.
$\mu$ is the overall mean.
$\alpha_j$ is the effect due to the $j^{th}$ level of N.
$\beta_k$ is the effect due to the $k^{th}$ level of I.
$(\alpha\beta)_{jk}$ is the interaction associated with the $jk^{th}$ NI combination.
$E_{ijk}$ is the random error associated with the $ijk^{th}$ experimental unit.

| Experiment | Source of variation [w] | df | Dry weight of above-ground biomass | | Dry weight of roots | | Dry weight of regrowth | |
|---|---|---|---|---|---|---|---|---|
| | | | MS | Pr > F | MS | Pr > F | MS | Pr > F |
| 1 | N | 1 | 4.36053750 | 0.0217 | 0.0294 | 0.3084 | 0.00010417 | 0.8730 |
| | I | 2 | 5.93077917 | 0.0024 | 0.03578750 | 0.2874 | 0.00408750 | 0.3766 |
| | N * I | 2 | 0.28253750 | 0.6704 | 0.01958750 | 0.4947 | 0.00137917 | 0.7107 |
| | error | 18 | 0.69085694 | | 0.02675833 | | 0.00396250 | |
| 2 | N | 1 | 1.1484375 | 0.0122 | 0.00201667 | 0.6216 | 0.00010417 | 0.9411 |
| | I | 2 | 2.03538750 | 0.0002 | 0.00635417 | 0.4670 | 0.03048750 | 0.2212 |
| | N * I | 2 | 0.71396250 | 0.0211 | 0.00112917 | 0.8693 | 0.00040417 | 0.9785 |
| | error | 18 | 0.14816250 | | 0.00799722 | | 0.01855972 | |

[w] N = number of inoculations, I = inoculum density.

**Analysis 20.** continued.

| Experiment | Dependant variable | N | Regression equation ˅ | $R^2$ ▪ | Pr ▪ |
|---|---|---|---|---|---|
| 1 | dry weight of above-ground biomass | 1 | Y = 8.991666667 - 0.79125 X | 0.563806 | 0.0049 |
| | | 2 | Y = 9.019166667 - 0.90125 X | 0.406672 | 0.0257 |

| Experiment | Dependant variable | Contrast | df | MS | Pr > F | Regression equation ˅ | $R^2$ ▪ |
|---|---|---|---|---|---|---|---|
| 2 | dry weight of above-ground biomass | model | 5 | 1.3294275 | 0.0002** | | |
| | | error | 18 | 0.1481625 | | | |
| | | I in N1 | 2 | 0.213525 | 0.2627 | | |
| | | I in N2 | 2 | 2.535825 | 0.0001** | Y = 7.9125 - 0.79125 X | 0.737751 |
| | | I linear in N2 | 1 | 5.0086125 | 0.0001** | | |
| | | I quadratic in N2 | 1 | 0.0630375 | 0.5225 | | |

▪ Level of significance.
˅ Y is the dependant variable and X is the inoculum density.
▪ Coefficient of determination.