ASCOCHYTA HYALOSPORA: A POTENTIAL MYCOHERBICIDE FOR CONTROL OF *CHENOPODIUM ALBUM*.

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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January, 1988

ABSTRACT

Lamb's-quarters (Chenopodium album L.) is a widespread weed of many crops such as corn, soybeans, sugar beets, and cereals. Normally, the weed is readily controlled using cultural and chemical methods, although triazine herbicide use in corn is not effective where triazine resistant populations of lamb's-quarters have developed. A pathogen, Ascochyta hyalospora (Cooke & Ellis) Boerema et al., was isolated from diseased lamb's-quarters plants collected during a weed disease survey at Ste-Anne-de-Bellevue, Quebec. In controlled environment studies the pathogen was restricted to plants in the family Chenopodiaceae, particularly the genus Chenopodium. Seedlings at the cotyledon to the 4-leaf stage of growth sustained more damage than older plants when sprayed at a rate of 1×10^8 conidia/m^2. Disease developed over a broad range of wetness periods (6 to 24 hours) and temperatures (12 to 30C). Most disease developed at temperatures of 18C to 24C with long periods of moisture (18 and 24 hours) resulting in more disease. Periods of continuous moisture produced more disease than equivalent periods of interrupted moisture. Conidia of Ascochyta hyalospora were able to infect lamb's-quarters when applied in a mixture containing atrazine at field rate (2.2 kg/ha). Ascochyta hyalospora would appear to have potential as a biological herbicide for control of lamb's-quarters, particularly triazine-resistant lamb's-quarters, but more testing is needed to determine efficacy under field conditions.

RESUME

Le chénopode blanc (Chenopodium album L.) est une mauvaise herbe répandue dans les cultures de maïs, de soya, de betteraves sucrières et de céréales. Normalement, cette mauvaise herbe est facilement enrayée grâce aux méthodes culturales et chimiques. Faisant exception à la règle, l'herbicide triazine, utilisé dans la culture du maïs, est inefficace sur les populations de chénopode blanc ayant développé de la résistance contre son action. Un pathogène, Ascochyta hyalospora (Cooke & Ellis) Boerema et al., a été isolé de plants de chénopode blanc malades, cueillis à Ste-Anne-de-Bellevue, Québec. Dans le cadre d'études en environnement contrôlé, le pathogène était restreint aux plantes appartenant à la famille Chénopodiacées, plus particulièrement au genre Chenopodium. De jeunes plants au stade de croissance de cotylédons jusqu'au stade de quatre feuilles ont subi plus de dommages que des plants plus âgés lorsqu'un taux de 1 X 10^8 conidies/m^2 a été appliqué. La maladie s'est developée sous une vaste gamme de périodes d'humidité (6 à 24 heures) et de températures (12 à 30C). Cependant, des températures de 18C à 24C associées avec de longues périodes de rosée (18 et 24 heures) ont résulté en plus de maladie. Des périodes de rosée continue ont également entraîné un meilleur développement de la maladie que des périodes équivalentes de rosée interrompue. Les conidies de Ascochyta hyalospora ont infecté le chénopode blanc lorsque appliquées en mélange avec l'herbicide atrazine au taux d'application recommendé (2.2 kg/ha). Ascochyta hyalospora, semble démontrer un potentiel comme agent de lutte

biologique contre le chénopode blanc, particulièrement contre les populations résistantes à l'herbicide triazine, mais de plus amples recherches sont nécessaires afin de déterminer son efficacité en champ.

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. A.K. Watson and Dr. L.A. Wymore for their valuable guidance and advice on my research, and for reviewing my thesis. I would also like to thank them and Dr. R. Releeder for serving on my committee. Grateful thanks are due to Dr. A.K. Watson for his financial assistance of myself and my project. Appreciation is extended to Dr. M. Fanous and F. El-Zayadi for their suggestions on experiment design and statistical analysis. A special note of thanks is extended to H. Cohen-Rimmer and A. Virly for their friendly assistance and advice on photography. I would also like to thank M. Pikarnegar and G. Rimmer for their technical assistance during the research. Special acknowledgement is due to L. Morin and H. Gadoury for the French translation of the abstract. Finally, I would like to express my happiness and satisfaction in having met and worked with the graduate students and technicians, past and present, of the Weed Biology Section and Room R2005.

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I GENERAL INTRODUCTION

1.1 Biological Control of Weeds

1.1.1 Inoculative and inundative biological control

Biological weed control can be defined as the deliberate use of insects, fungi, nematodes, bacteria, or viruses to suppress weeds below an economically damaging level (Andres, 1982). Generally, biological weed control can be divided into two primary methods, the inoculative tactic and the inundative tactic.

The inoculative tactic, sometimes called the classical approach, is the older method and it remains the most frequently used type of biological weed control. This method requires that a control agent, obtained from the weed's centre of origin, be released at a limited number of sites, where a population establishes itself on the weed over a period of time. As the population increases, the control agent spreads out from the original release sites. If the control agent is successful, the weed population will be suppressed to a subeconomic level. Once the control agent has been successfully established, further releases are usually not necessary. The likelihood of successful biological control is increased if the weed is not closely related to an economic crop species.

The inoculative tactic has been used exclusively for introduced weeds that have become major problems in undisturbed areas such as rangelands and waterways. These introduced weeds have become major problems because they have escaped their natural enemies that previously controlled them in their native range. In the past, biological control was attempted when conventional weed control techniques were not economical or

could not effectively control the weed. Most of the inoculative control agents have been insects but plant pathogens have also been used (Andres, 1982; Andres et al., 1976; Charudattan, 1985; Schroeder, 1983; Templeton, 1982; Wapshere, 1982).

The inoculative method has been used to control skeletonweed (Chondrilla juncea L.), an introduced weed in Australia that became a serious problem in fallow wheatfields. The rust pathogen, Puccinia chondrillina Bubak & Syd., was collected in southern Europe and released at several Australian sites in 1971. The pathogen was very successful against one of the three forms of the weed (Hasan, 1980; Schroeder, 1983). A European rust fungus, Phragmidium violaceum (Schulz) Winter, was released to control Rubus spp. in Chile. As a result of the introduced pathogen, large reductions occurred in the population of R. constrictus Lef. & M. The pathogen, however, caused less damage to the population of R. ulmifolius Schott (Hasan, 1980).

The second method of biological weed control is the inundative or bioherbicide tactic. This method requires a mass release of the control agent (usually a fungal plant pathogen) over a target area where the weed is present. The inundative release of the agent permits an immediate, explosive epidemic to develop. In this manner, satisfactory control of the weed can be achieved within a relatively short time. The uses of the presently available bioherbicides are similar to those of chemical herbicides. Bioherbicides generally are meant to be applied annually in annual or high-value perennial cropping situations. The control agents used in this method are indigenous pathogens that are endemic on the weed. They are cultured and mass produced on artificial (non-living) substrate.

With this type of production, the pathogen can be released in large doses at the best time to take advantage of the weed growth stage and optimum environmental conditions (Templeton, 1982 ; Templeton & Smith, 1977).

During the 1970's, advances were made in the field of inundative biological control. In particular, two bioherbicides -- COLLEGO® and DEVINE® -- were developed and were made available commercially in the 1980's. COLLEGO®, a formulation of Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. aeschynomene (C.g.a.), is recommended for the control of Aeschynomene virginica (L.) B.S.P. (northern jointvetch) in rice and soybeans in the southern United States. Between 90 and 100% of northern jointvetch can be controlled when the pathogen is applied post-emergence at the mid-to-late vegetative stage of plant growth (Smith, 1986). DEVINE®, another commercially available bioherbicide, is composed of spores of Phytophthora palmivora (E.J. Butler) E.J. Butler and is recommended for the control of stranglervine in citrus groves. For COLLEGO® and especially for DEVINE®, the areas upon which they are likely to be applied are small in size. Both bioherbicides, however, have been judged satisfactory by farmers and, in the case of COLLEGO®, most farmers who used it the first year it was available, have continued to use it in the second year (Bowers, 1986).

1.1.2 Developing biological herbicides

There are several reasons for developing biological herbicides. Principal among these are the increasing problems associated with the development of new chemical herbicides.

Chemical herbicide companies are confronted with the prospect of developing more specific chemicals (narrow spectrum) for smaller market uses. At the same time, development costs have increased greatly in recent years. Bioherbicides will generally be quite specific and there are claims that the development costs will be less than is the case with chemical herbicides (Bowers, 1982; Templeton, 1986). Environmental contamination due to chemical herbicide drift and water runoff is also a major concern (McWhorter and Chandler, 1982).

Development of bioherbicides can be divided into three stages. The first stage is the discovery stage where a potential weed pathogen is found through literature reviews and field surveys. The second stage consists of evaluating the pathogen for suitability of development by testing host range and the effects of various factors on efficacy (effects on disease development/weed control by factors such as temperature, moisture, plant age, adjuvants, pesticides, and inoculum concentration). The third stage, deployment, consists of small- and large-scale field tests using formulations of mass-produced propagules (TeBeest, 1985; Templeton, 1982).

Fungi, and imperfect fungi in particular, are assumed to hold the most promise for development as bioherbicides. There are several reasons for this assumption. Imperfect fungi are quite common as pathogens on plants. They can usually be artificially cultured and mass produced by liquid or solid substrate "fermentation" (Templeton, 1982). In addition fungi are easier to identify than are bacteria and viruses, and can infect the plant directly --- unlike bacteria and viruses which normally require wounds or natural openings in the plant for

infection to occur (Hasan, 1980). Finally, fungal propagules are often the easiest to store and have a relatively long shelf-life. The term mycoherbicide is often used to refer to bioherbicides that consist of fungal plant pathogens (Bowers, 1982; Quimby & Walker, 1982).

Daniel et al. (1973) listed three requirements for a mycoherbicide. First, the biocontrol agent must be specific to the weed and be safe to use on crops. Secondly, there should be abundant production of long-lasting, viable propagules in artificial culture, and thirdly the propagules of the control agent should be capable of rapidly infecting and reducing the weed population.

Damage to the weed caused by disease in nature should not be used as an indication of the pathogen's potential as a biocontrol agent. Although the disease is endemic and constrained by some aspect of the pathogen-host-environment interaction, these constraints could perhaps be overcome technically when the pathogen is used as a bioherbicide (Scheepens & Van Zon, 1981). For example, Templeton et al. (1979) have suggested that poor propagule production and poor dispersal are major constraints on C.g.a., keeping the disease at a low endemic level. These constraints are overcome by mass production of C.g.a. conidia and inundative release of the propagules at the most favourable stage of weed growth.

Most weed control researchers who have worked with bioherbicides view them as part of an integrated pest management or weed management program. The bioherbicides would work in conjunction with cultural and chemical control methods (Smith,

1982; Watson, 1986). The use of the bioherbicide may eliminate or reduce the use of certain herbicides or may be added to complement an existing but small number of herbicides used for control of a problem weed. The two existing commercial bioherbicides can be used in conjunction with chemical herbicides. COLLEGOR can be successfully tank-mixed and applied with at least two herbicides, acifluorfen (5-[2-chloro-4-(trifluoromethyl) phenoxyl]-2-nitrobenzoic acid) and bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one2,2-dioxode) (Smith, 1986; TeBeest & Templeton, 1985). DEVINER and chemical herbicides must be applied in separate sequential applications, due to reduced spore germination in the presence of herbicides (Smith, 1982).

1.2 Objectives of Research Project

The objectives of this research project were similar in nature to the first two phases of bioherbicide development (See Section 1.1.2). The first objective was to conduct a disease survey of several problem weeds. When diseased plants were found, the diseased tissue was collected and the plant pathogen isolated. The best weed-pathogen pathosystem was selected for further study. The second objective was to evaluate the pathogen according to several criteria considered important for developing the pathogen as a mycoherbicide. The studies were conducted in controlled environment conditions. The following characteristics of the pathosystem were examined: 1) host range of pathogen, 2) response of plant at different stages of development to different pathogen inoculum concentrations, 3) disease development and subsequent plant damage as a response to different wetness period temperatures and durations, and 4)

compatibility with other pathogens and chemical herbicides.

II WEED - PATHOGEN SURVEY

2.1 Survey and Collection of Weed Pathogens

2.1.1 Introduction

The initial stage in the development of bioherbicides is the "discovery" of the weed pathogen, with the first step being a review of the literature (Templeton & Trujillo, 1981). Some of the major references include the Sylloge Fungorum (Saccardo, 1882), Index of Fungi -Petraks List (Petrak, 1920-39), the Index of Fungi (Anonymous, 1940 - present), the Index of Plant Diseases in the United States (Anonymous, 1960), An Annotated Index of Plant Diseases in Canada (Conners, 1967), and Compendium of Plant Disease and Decay Fungi in Canada (Ginns, 1986). Although the literature record may not be complete, the literature review may suggest possible pathogens to search for on specific target weeds.

Two procedures for finding pathogens are field surveys and chance discoveries (TeBeest, 1985). In the southern U.S., there have been field surveys on an ongoing basis in several states. In South Carolina, areas containing natural weed infestations and research plots have been surveyed for the presence of pathogens (Ridings et al., 1985); in Arkansas, weed nurseries have been set up in different areas for collection of weed pathogens (TeBeest, 1985). Mortensen (1984), in western Canada, surveyed heavily infested areas of leafy spurge (Euphorbia esula-virgata complex) for the presence of diseased

plants. The sites were located over a wide area extending from Manitoba to British Columbia.

There are several advantages to collecting widely-distributed pathogens. They are likely to be effective in causing disease over a broad range of environmental conditions and a wide distribution would present less of a problem in using a bioherbicide over a large region, since there would be less danger of introducing a pathogen that was not previously present. Collecting fungi from a large geographic area might also provide a diverse genetic base on which to develop a bioherbicide[1]. A survey over a broad geographic area would also reveal those pathogens having a narrow, restricted distribution which might indicate limitations such as environmental restrictions on disease development, or poor dispersal mechanisms and might also indicate a need for more careful host range testing[1].

Field collecting of weed pathogens is most successful when disease has reached its climax, which for many diseases occurs along with maturation and senescence of the weed (Templeton & Trujillo, 1981). Periodic visits to the same survey site throughout a growing season might provide indication of natural constraints that could be hindering disease development in the field. Knowledge of these constraints could be helpful in developing formulations and application procedures of the bioherbicide (TeBeest, 1985; Templeton & TeBeest, 1979; Templeton & Trujillo, 1981).

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[1] Committee Report, Regional Project S-136. 1978-1983. Biological control of weeds with fungal plant pathogens. U.S. Department of Agriculture and State Agricultural Experiment Station Southern Regional Research Project S-136.

The objective of this part of the research project was to survey weed species on the Macdonald College campus of McGill University. Attempts were made to isolate causal organisms of diseases from Taraxacum officinale Weber, Plantago major L., Convolvulus arvensis L., and Chenopodium album L., as well as any other diseased weed that was collected. Periodic surveys were undertaken at two sites for the presence of two pathogens that had been previously collected from those areas.

2.1.2 Methods

Survey sites were located on the Macdonald College campus of McGill University and the Emile A. Lods Research Centre at Ste-Anne-de-Bellevue, Quebec. There were three main survey sites. The first survey site consisted of campus roadsides and lawns. Plantago major (broad-leaved plantain) and Taraxacum officinale (dandelion) were collected from these areas. The second collection site was a small, low-lying area of the Macdonald Campus front lawn. The shaded area, regularly cut with a lawn mower, contained a small patch of Convolvulus arvensis (field bindweed). The third collection site was a research field at the Lods Research Centre. The field was approximately one hectare and had a loam to light sandy loam soil type. The soil was tilled annually, and thus supported the growth of many species of annual agrestals such as Echinochloa crusgalli (L.) Beauv. (barnyard grass), Setaria glauca (L.) Beauv. (yellow foxtail), Setaria viridis (L.) Beauv. (green foxtail), Chenopodium album (lamb's-quarters), Amaranthus retroflexus L. (redroot pigweed), and Polygonum convolvulus L. (wild buckwheat). Surveys for diseased weeds from these three sites were conducted during the autumn of 1984 and the 1985

growing season. Specimens (whole plants or leaves) of diseased weeds were preserved for use as herbarium mounts.

Two methods were used to isolate fungi from the collected weeds. The first method involved surface disinfestation of the infected plant part and plating it on an agar medium. Plant parts containing lesions of the suspected disease were cut up into pieces no larger than 1 cm^2. The plant pieces were placed in 70% alcohol for at least 30 seconds followed by immersion in 2% sodium hypochlorite for 30 to 60 seconds. The plant pieces were then rinsed twice with sterile distilled water, dried on filter paper, and placed on an agar medium (usually acidified potato dextrose agar [PDA] in Petri dishes with or without an antibiotic such as Novobiocin®). The second method of isolation involved washing the leaves in running tap water for at least two hours, and then placing the washed leaves on moist filter paper in Petri plates. The plates were incubated for several days. Any fungal growth or fructification, resulting from either of the above two methods, was examined and the fungus was transferred to obtain a pure culture.

Pathogenicity testing involved spraying the plants with a mixture of water and conidia. The amount of inoculum varied according to the amount of sporulation of each isolate. The plants were placed in a moist environment for at least 20 hours at room temperature (20 to 24C). After the wetness period, the plants were kept in the greenhouse or in the growth chamber and observed for the development of disease symptoms. Isolates which failed to produce sufficient inoculum and/or did not

appear pathogenic or only weakly virulent were dropped from further consideration as possible candidate pathogens for bioherbicide development.

Two continuous surveys (a site surveyed periodically throughout a growing season) were conducted during the 1985 and 1986 growing seasons. The first continuous survey was conducted on the Macdonald College front lawn site. Diseased field bindweed, from which a Colletotrichum had been previously isolated (Ormeno, 1986), was collected every three to four weeks and tested for the presence of the Colletotrichum organism. The second continuous survey was conducted at the Lods Research Centre site. Lamb's-quarters plants were surveyed during the 1986 growing season and the first part of the 1987 season, for the presence of lesions, particularly those lesions caused by Ascochyta hyalospora (Cooke & Ellis) Boerema et al. Ascochyta hyalospora had been isolated from leaf lesions collected at the same site in the autumn of 1985.

2.1.3 Results

During the autumn of 1984 and the 1985 growing season, diseased dandelion, plantain, barnyard grass, yellow foxtail, green foxtail, field bindweed, and lamb's-quarters were collected. Fungi were successfully isolated from all the diseased weeds.

The results of the isolation and testing procedures of Koch's postulates can be divided into three categories. The first category of isolates (from the weeds dandelion, plantain, green foxtail, and yellow foxtail) did not sporulate or develop any fructifications. Consequently, no identification could be made, and no pathogenicity testing could be attempted using

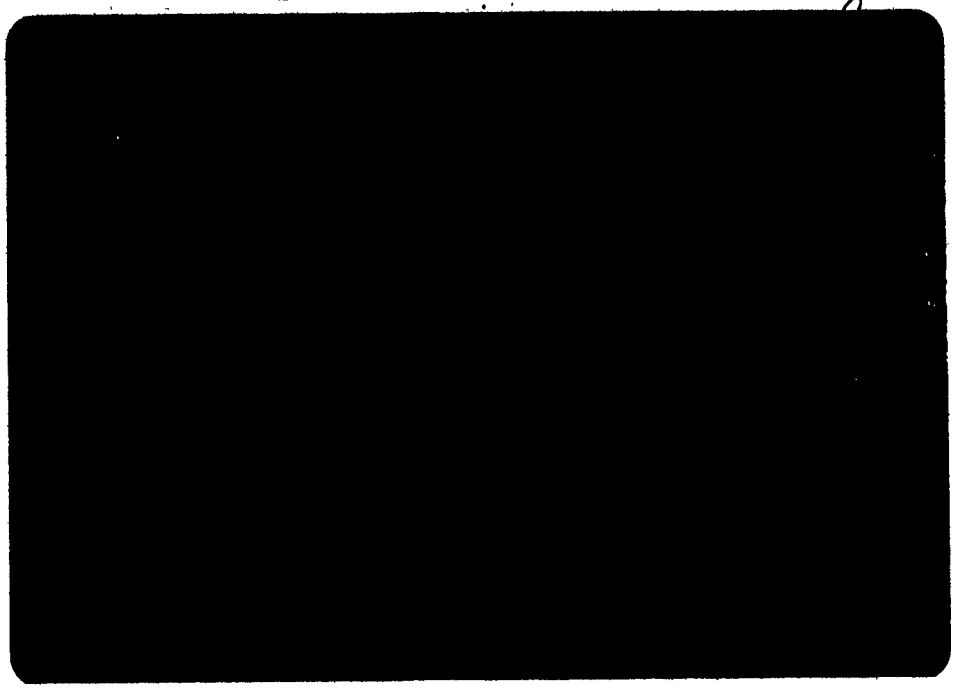
inoculum of the isolates.

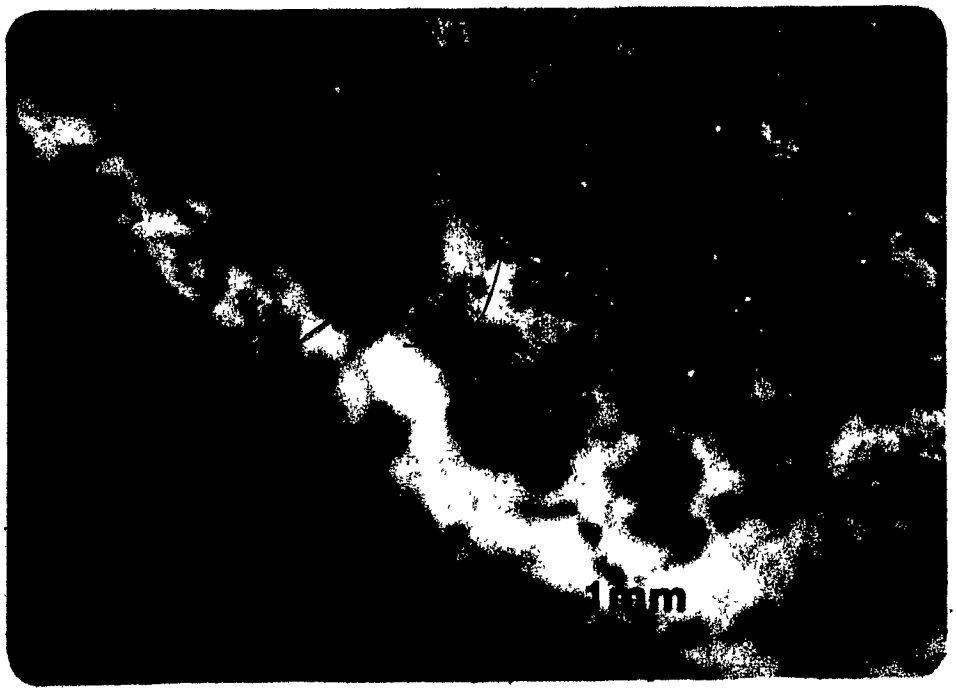
The second category applies to the results obtained with Colletotrichum on field bindweed (initially isolated and tested by Ormeno, 1986). Inoculum production was very poor, but there was enough to carry out some limited pathogenicity testing. Although Colletotrichum was re-isolated from some inoculated plants showing disease symptoms, conclusions on its infectivity were difficult to establish since the control plants also displayed symptoms of infection (Colletotrichum, however, was not re-isolated from any control plants).

The third category applies to isolates from barnyard grass and lamb's-quarters. Curvularia sp. was isolated from barnyard grass. The fungus produced adequate amounts of inoculum on PDA plates. Pathogenicity tests showed that spraying of barnyard grass plants resulted in the formation of necrotic lesions on the leaf tips and the leaf sheaths but the virulence of the fungus was low. Curvularia was re-isolated from surface sterilized necrotic lesions. Ascochyta hyalospora and Phoma sp. were isolated from diseased leaves of lamb's-quarters (See Figures 1 & 2). Lamb's-quarters leaves with lesions caused by Peronospora sp. and Cercospora sp. were also collected, but no isolation was attempted. Pathogenicity tests, using inoculum produced on torula yeast agar (TYA) [torula yeast, 15 g; potassium phosphate monobasic, 1 g; magnesium sulfate, 0.5 g; agar, 20 g; water, 1 L] indicated that Ascochyta hyalospora was more virulent than Phoma sp. Ascochyta hyalospora caused more mortality on plants at the 2-leaf stage of growth and also caused more defoliation on plants sprayed at

Figure 1. Leaf lesion, caused by Ascochyta hyalospora, on Chenopodium album (lamb's-quarters).

Figure 2. Close-up view of leaf lesion in Figure 1, showing pycnidia on necrotic tissue. Leaf was placed in moist chamber for 1/2 hour before photographing the lesion.




1μm

a mature stage of growth (beginning of flowering) (See also Section 4.2 and Section 4.6).

The continuous survey of field bindweed at the campus site during the summer of 1985, provided evidence of the presence of a Colletotrichum-induced disease in early July. The fungus was isolated from leaf lesions during four subsequent collection dates, including the last survey date at the end of September.

Results from the continuous surveys of lamb's-quarters during the summer of 1986 at the Lods Research Station site indicated that Ascochyta was present in mid-summer (July) and remained present until the survey ended in the autumn (end of September). Phoma pycnidia and conidia were prevalent on the leaves at the first survey date (July 4) in 1986, and remained for the remainder of the growing season. Phoma pycnidia and conidia were also more numerous on leaves that were surveyed (beginning in early June) during the 1987 growing season.

2.1.4 Discussion

Based on the above results, research work with all but one of the isolates was discontinued. Work with the fungi isolated from dandelion, plantain, green foxtail, and yellow foxtail was not pursued further due to the absence of sporulation in culture. Curvularia sp. was dropped from further consideration due to its weak virulence on barnyard grass. Testing with Colletotrichum sp. on field bindweed was discontinued due to the difficulty in obtaining inoculum and the "mixed" results in the pathogenicity tests. Further work with Phoma sp. on lamb's-quarters was postponed because of its weaker virulence on the plant in comparison with Ascochyta hyalospora.

The best pathosystem for further work appeared to be the Ascochyta hyalospora - Chenopodium album system. This decision was based on two reasons: the pathogenicity and virulence of Ascochyta on lamb's-quarters, and an adequate amount of Ascochyta inoculum that could be produced in culture.

Templeton & Trujillo's assertion (1981) that chances of finding disease are greatest at the mature or senescent stage of plant growth was confirmed by the results of the continuous survey for Ascochyta. The largest number of pathogens on lamb's-quarters was found in the autumn (Ascochyta, Phoma sp., and Cercospora sp.). Ascochyta and Phoma sp., however, were also found throughout the summer on both mature and immature plants.

No conclusions about the distribution of Ascochyta hyalospora through time can be made since only one complete season of surveys was conducted. The pattern observed in the continuous survey, where Phoma was more prevalent than Ascochyta, may have been due to climatic factors, or it may have been due to the apparently more active dispersal of Phoma conidia in water. When pieces of leaves with lesions were placed in water and observed under a microscope, it was noticed that Phoma conidia appeared to disperse much more readily in the water than did Ascochyta conidia. This observation might indicate that dispersal of Ascochyta conidia in the field is more limited than dispersal of Phoma conidia, resulting in more widespread disease caused by the Phoma pathogen (See Ascochyta - Phoma comparison in Section 4.6). Templeton et al. (1979) suggested that poor dispersal mechanisms for conidia of

Colletotrichum gloeosporioides f. sp. aeschynomene are part of the reason for the disease of northern jointvetch remaining at an endemic level. Further studies are required to evaluate the distribution of Ascochyta hyalospora - induced disease through time.

2.2 Biology and Control of Chenopodium album L.

2.2.1 Distribution and extent of weed problem

Lamb's-quarters (Chenopodium album L.) is an annual weed species with a world-wide distribution, ranging from lat 70° N to lat 50° S (Holm et al., 1977). According to Coquillat (1951), the weed is one of the five most widely distributed plants. It is a successful colonizer of disturbed soil and is a serious weed problem in many different crops including sugar beets, corn, soybeans, and cereal crops (Bassett & Crompton, 1978; Holm et al., 1977).

The primary detrimental aspect associated with lamb's-quarters infestations is reduced crop yields, particularly if the infestation is heavy. Minor problems caused by lamb's-quarters include crop seed contamination, livestock poisoning if large quantities are consumed, and human allergenic reactions to the pollen (Holm et al., 1977; Wodehouse, 1971).

2.2.2 Description

The morphological features of the plant are quite variable. The plant is an erect annual herb that grows up to 2.5 metres in height. The ridged, branching stems often have reddish parallel stripes arranged length-wise. The simple, alternate leaves have shapes that range from ovate-lanceolate to rhombic-lanceolate. The leaves can have up to 10 shallow lobes but these are not distinct. The leaf surface is glabrous with

mealy, farinose texture. No stipules are present. The inflorescence is a spike pannicle and has green perfect flowers with five sepals and no petals. The sepals nearly cover the fruit (utricle) at maturity. The seeds are usually shiny black, lens-shaped, and about 2 mm in diameter (Bassett & Crompton, 1978; Holm et al., 1977).

2.2.3 Lamb's-quarters: the weed

Several characteristics, such as abundant seed production, seed dormancy, and competitive ability, contribute to the success of lamb's-quarters as a crop weed. Reports on seed yield vary widely. Stevens (1932) reported that an average-sized plant produced up to 72,000 seeds, whereas Holm et al. (1977) stated that large plants have produced up to 500,000 seeds. Ervio (1971) reported a maximum of 42,000 seeds per plant, with an average of 8,300 seeds per plant over three years. This variability likely depends on the conditions under which the plants are growing (Bassett & Crompton, 1978). Several studies have shown that the seeds of lamb's-quarters are capable of long dormancy in the soil due to physiological and external (soil burial) factors (Williams & Harper, 1965). Lewis (1973) reported up to 23% viability of lamb's-quarters seeds after 20 years of soil burial. Lamb's-quarters seed germination also exhibits wide variability in response to various environmental stimuli (Williams & Harper, 1965). Other advantageous characteristics cited for lamb's-quarters include phenotypic plasticity which enables the plant to grow rapidly to positions where it can effectively trap light, and aggressive competition for nutrients with rapid growth in soils with high

nitrogen content (Glauninger & Holzner, 1982; Williams, 1964).

Thick, uniform patches of the weed commonly develop in an infested field since the plant has no special method of dispersal other than the shedding of seeds around the mother plant. If not controlled, the weed can quickly become troublesome and competitive in the crop (Bassett & Crompton, 1978; Holm et al., 1977). In one experiment, lamb's-quarters at densities up to 277 plants/m^2, reduced grain corn yield up to 58% (Sibuga & Bandeen, 1980).

2.2.4 Control of lamb's-quarters

Normally, lamb's-quarters is readily controlled through cultural and chemical methods. For example, preplant incorporated, pre-emergence, and post-emergence applications of atrazine generally provide excellent control of lamb's-quarters in corn. Shallow mechanical cultivation is also recommended to help control small seedlings (Ontario Ministry of Agriculture and Food [OMAF], 1986).

There are problems, however, with some of the control methods. Apart from the general concern with chemical pesticide contamination of the environment, there is a more specific concern with triazine resistant lamb's-quarters plants. These resistant plants have become a problem in North America and western Europe. In western Europe, triazine resistance has also been reported in the related species, C. polyspermum L. and C. ficifolium Smith (Bandeen et al., 1982; Gressel et al., 1982).

When this resistance appears, other control measures are necessary such as alternative herbicides, greater use of crop rotation, and mechanical cultivation (Parochetti, 1980). These other control measures, however, are not always possible

or not always desirable. With increased use of reduced tillage techniques, less reliance is placed on mechanical cultivation and crop rotation. Therefore, increased rates or increased numbers of applications of chemical herbicides are often necessary for weed control in reduced tillage systems.

2.3 The Genus Ascochyta Lib.

2.3.1 Taxonomy and description

The genus Ascochyta Lib. is a member of the Fungi Imperfecti, a miscellaneous grouping of anamorphic fungi. The taxon, Fungi Imperfecti (Deuteromycotina), is often treated as a Subdivision, although this is not strictly correct (Hawksworth et al., 1983). Classification above the rank of genus remains disputed. Sutton (1980) provided the following description of the Genus Ascochyta Lib.:

> Mycelium immersed, branched, septate, hyaline to pale brown. Conidiomata pycnidial, amphigenous, separate, globose, brown, immersed, unilocular, thin-walled, of textura angularis. Ostiole central, circular, slightly papillate. Conidiophores absent. Conidiogenous cells enteroblastic, phialidic, determinate, discrete, doliiform to lageniform, hyaline, smooth, formed from the inner cells of the pycnidial wall. Conidia hyaline, medianly 1 septate, continuous or constricted, thin-walled, smooth, ±guttulate, cylindrical to irregular.

This description conflicts with the description by Boerema & Bollen (1975) who describe conidiogenesis as annellidic. They also allowed for a much broader conidial description by describing the conidia as having two, occasionally three, but rarely four cells.

Several teleomorphs have been associated with Ascochyta species. Pleospora Rabenh. ex Ces. & de Not. has been associated with Ascochyta-induced diseases of Chenopodium

(Menzies, 1966; van der Aa & van Kesteren, 1979; Webster & Lucas, 1959). For example, Pleospora calvescens (Fr. ex Desm.) Tul. has been identified as the teleomorphic stage of Ascochyta caulina (P. Karst.) v.d. Aa & v. Kest. (Boerema et al., 1987). Mycosphaerella Johanson has been identified as the teleomorphic stage of Ascochyta pinodes Jones (Martens et al., 1984) as well as Ascochyta chrysanthemi F. Stevens, although the teleomorphic stage of the latter has recently been reclassified as Didymella Sacc. ex Sacc. (Walker & Baker, 1983). Punithalingam (1979) has suggested that Didymosphaeria Fuckel is also a teleomorphic stage of Ascochyta.

The genus Ascochyta Lib. was first described in 1830, with Ascochyta pisi Lib. as the type species. There are now over 600 formally described species in the genus. Most of these species are plant pathogens with restricted host ranges (Punithalingam, 1979).

2.3.2 Diseases incited by Ascochyta spp.

Ascochyta incites diseases on many different crops worldwide, including A. rabiei (Pass.) Labrousse on chickpea (Cicer arietinum L.), A. oryzae Catt. on rice (Oryza sativa L.), A. tritici Hori & Enjoji on wheat (Triticum aestivum L.), A. avenae (Petr.) Sprague & Johnson on oats (Avena sativa L.), A. sorghi Sacc. on sorghum (Sorghum vulgare Pers.), A. gossypii Woron. on cotton (Gossypium hirsutum L.), A. caulicola Laub. on clover (Trifolium L.), A. fabae Speg. on broad bean (Vicia faba L.), A. lentis Vassil. on lentil (Lens culinaris Medic.), A. adzamethica Schoschiaschvili on peanuts (Arachis hypogaea L.), and A. pinodes Jones on pea (Pisum sativum L.). Blight and foot rot of peas is one of the most serious Ascochyta-induced

diseases in Canada (Martens et al.,1984 ; Nene, 1982 ; Ou, 1972 ; Rossman et al., 1987).

There are several Ascochyta pathogens that incite diseases within the family Chenopodiaceae, including A. betae Prill. et. Del. and A. chochrjakovii Meln. on beet (Beta vulgaris L.), A. spinaciae Bond.-Mont. and A. spinaciicola Meln. on spinach (Spinacia oleracea L.), A. haloxyli (Syd.)Jacz. on the genus Haloxylon Bunge, and A. boni-henrici Ranoj. on Atriplex sp., Spinacia oleracea and several Chenopodium species (C. ambrosioides L., C. bonus-henricus L., C. foliosum Aschers, and C. polyspermum L.) (Melnik, 1977). A. caulina(P. Karst.) v.d. Aa & v.d. Kest. is a Eurasian pathogen that incites disease on Chenopodium and Atriplex species. A. hyalospora (Cooke & Ellis) Boerema et al., occurring in North and South America, incites diseases on several species of Chenopodiaceae. The species is very similar to A. caulina (van der Aa & van Kesteren, 1979).

2.3.3 Potential Ascochyta mycoherbicides

At least two Ascochyta weed pathogens have been investigated for use as biocontrol agents. Scheepens and Van Zon (1981) have examined the possibility of using A. caulina for control of Chenopodium and Atriplex weed species in Europe. Studies of the potential of Ascochyta spp. as biocontrols of bracken fern have been conducted in California and Great Britain (Burge & Irvine, 1985; Webb and Lindow, 1987). In addition, a toxin of the Ascochyta pathogen, which causes systemic necrotic spots on bracken fern, has been examined for potential use as a herbicide (Lindow, 1985).

III BIOLOGY OF *ASCOCHYTA HYALOSPORA*

3.1 Introduction

The fungal plant pathogen isolated from diseased lamb's-quarters at Ste-Anne-de-Bellevue was identified as *Ascochyta hyalospora* (Cooke & Ellis) Boerema *et al.* by Dr. E. Punithalingam of the Commonwealth Mycological Institute (Herb. IMI #302770). The name, a new combination proposed by Boerema *et al.* (1977), replaces three synonyms previously used: *Diplodia hyalospora* Cooke & Ell. (Cooke & Ellis, 1878), *Diplodina ellisii* Sacc. (Saccardo, 1884), and *Phleospora chenopodii* Ell. & Kell. (Ellis & Kellerman, 1888). The conidia of *Diplodia hyalospora*, from stems of *Chenopodium* sp. collected in New Jersey, had dimensions of 20-26 X 9 µm with one to two septa. The conidia were constricted at the septa (Cooke & Ellis, 1878). The conidia of *Phleospora chenopodii*, from leaves of *Chenopodium album* collected in Kansas, had dimensions of 20-35(mostly 20-25) X 8-11 µm and were described as 3-septate. Constrictions were also present at the septa (Ellis & Kellerman, 1888). Boerema *et al.* (1977) reported that conidia from colonies grown on oatmeal agar had an ellipsoidal or cylindrical shape with dimensions of 20-30 X 8-12 µm. The conidia had one or sometimes two septa. Conidia from leaves of *Chenopodium album* often had two or three septa. *Ascochyta hyalospora* colonies grown on oatmeal agar consisted of a dark mycelial mat with scattered pycnidia measuring approximately 175-250 µm in diameter. Short dark hyphae were commonly observed growing around the opening of the pycnidia.

There appears to be no information in the literature specifically relating to *Ascochyta hyalospora* or its synonyms

concerning the cardinal temperature points for growth on media. Nor does there appear to be any information on the infection process and mode of penetration of the fungus. Previous reports indicate that Ascochyta pisi Lib. and Ascochyta rabiei (Pass.) Labrousse usually penetrate directly through the cuticle (Brewer & MacNeill, 1953; Heath & Wood, 1969; Pandey et al., 1987). Heath & Wood (1969) observed that a small proportion of penetrations by A. pisi occurred through stomata. A majority of the penetrating germ tubes invaded through epidermal cells (especially guard cells), while a lower proportion of germ tubes penetrated between cells. Pandey et al. (1987), working with A. rabiei on chickpea stems, observed that penetration did not occur through stomata but occurred directly through the cuticle. Hyphae penetrated through the cell junctions of the epidermis and through the junction of the subsidiary cells and guard cells of the stomatal complex.

The first objective of this section was to compare the morphological and cultural characteristics of Ascochyta hyalospora collected from the Ste-Anne-de-Bellevue site with descriptions presented in the literature. The second objective was to determine the effect of different media and temperatures on colony growth. The third objective was to observe the first few hours of the infection process on the leaf surface, using time-series photography.

3.2 Methods

Description of Ascochyta hyalospora from Ste-Anne-de-Bellevue site

Lamb's-quarters plants at the 4- to 6-leaf growth stage were inoculated with Ascochyta hyalospora conidia at a rate of

1 X 10^8 conidia/m^2. After disease had developed on the plants, diseased stems and leaves were excised and placed on moist filter paper in petri plates. The pycnidia or conidia that developed on the plant parts were placed in a water droplet on a glass slide and examined with a Reichert Diavar microscope under 125 and 500 X magnification. The dimensions of the pycnidia and conidia were measured using an eyepiece micrometer. Ten pycnidia were selected from the stem and 10 from the leaves. Twenty-five conidia originating from the stem and 25 from the leaves were selected for measurement.

Ascochyta hyalospora cultures were grown on oatmeal agar [30 g oatmeal boiled in 1 L water for one hour, then filtered; 20 g agar added to remaining liquid; autoclaved] and malt agar [Difco brand] for eight days at 27C. Characteristics and colour of the cultures were described using a mycological colour chart (Rayner, 1970).

Effect of agar medium and temperature on colony growth

Three tests were set up whereby Ascochyta hyalospora was grown on several media at different temperatures. In the first test, the fungus was grown on three media: potato dextrose agar (PDA) [Difco brand], Czapek-Dox agar (CDA) [sodium nitrate, 3.0 g; dipotassium phosphate, 1.0 g; magnesium sulfate, 0.5 g; potassium chloride, 0.5 g; ferrous sulfate, 0.01 g; agar, 20.0 g, distilled water, 1 L], and oatmeal agar (OA). Three day/night temperature regimes were used (16/10C, 22/16C, and 28/22C) with a 14 hr light/10 hr dark photoperiod regime (light intensity 135 to 185 microeinsteins m^{-2} sec^{-1}). A #2 cork borer was used to cut out 6-mm diameter plugs of the fungus (grown on

1/2PDA:1/2TYA at 24/22C light/dark temperature), which were transferred and inverted onto the centre of a Petri plate of one of the three media. Four plates of each medium were placed in growth cabinets at each temperature regime. Two perpendicular radial measurements were made eight days after the start of the experiment.

The materials and methods for the second test were the same except for the addition of another medium, torula yeast agar (TYA) [torula yeast, 15 g; potassium phosphate monobasic, 1 g; magnesium sulfate, 0.5 g; agar, 20 g; water, 1 L]. In the third test, the procedures were similar to those of the first two tests with colony growth being evaluated on PDA, CDA, TYA, and 1/2PDA:1/2TYA [one half strength PDA plus half weight of the ingredients listed for TYA in 1 L water] media kept at constant temperature (5C, 13C, 23C, and 33C) in the dark. Treatment combinations were replicated three times in the test.

Infection process on the leaf surface

An experiment was set up to observe the germination, germ tube growth, and appressorium formation of Ascochyta hyalospora conidia on the leaf surface of lamb's-quarters. Leaves of lamb's-quarters plants at the 8-leaf stage of growth were sprayed to wetness using a spray suspension of 1.6 X 10^6 conidia/ml. The suspension also contained 20% (v/v) sorbo (ATKEMIX INC., Brantford, Ont.). After inoculation, the plants were placed in a dark dew chamber at 22C for time periods of 3.5, 6.5, 17.5, 24, 30, and 42 hours. Leaves were excised and cut into sections of approximately 1-cm^2. The sections were fixed in Farmer's solution (two parts absolute ethanol: one part glacial acetic acid) for 24 hours, then placed in lactophenol

(equal parts of distilled water, glycerin, lactic acid, and melted phenol) for 24 hours, and then transferred to 0.1% acid fuchsin in lactophenol (1 part of 1 g acid fuchsin in 99 ml of 95% ethanol: 9 parts lactophenol) for approximately five minutes to obtain adequate staining. Excess stain was removed by dipping the sections in lactophenol for approximately one minute. Sections were mounted on glass slides in 50% glycerin, surrounded with a ring of Permount® (Fisher Scientific Co), and covered with a glass cover slip. Stained material was examined with a Reichert Diavar microscope (125 X and 500 X magnification).

3.3 Results

Description of Ascochyta hyalospora from Ste-Anne-de-Bellevue site

All pycnidia that developed on leaves and on stems of lamb's-quarters were dark brown to black. The conidial matrix was dark brown and had a granular appearance under stereomicroscope magnification of 50X. The immersed to superficial pycnidia on the leaves were mainly glabrous, whereas the partly immersed pycnidia on the stem had many hair-like hyphae present. Pycnidia and conidia from the stem were (198-) 248(-327) µm in diameter and (15.0-)22.3(-27.5) µm X (7.5-) 8.8(-10.0) µm, respectively. Pycnidia and conidia from leaves were (168-)199 (-243) µm in diameter and (20.0-)24.5(-33.0) µm X (7.5-)8.5(-10.0) µm, respectively. Conidia from both plant structures commonly had one to three septa.

Ascochyta hyalospora colonies attained diameters of 39 - 40 mm on oatmeal agar and 27 - 29 mm on malt agar after eight days (Table 1). On oatmeal agar, the fungus appeared as a dark

mat of appressed mycelium with dark pycnidia scattered about the centre of the colony. The obverse side of the colony had an olivaceous colour while the reverse was greenish-glaucous to olivaceous in colour. The appearance on malt agar was similar.

TABLE 1. Description of Ascochyta hyalospora growth on oatmeal and malt agar.

	Oatmeal Agar	Malt Agar
CHARACTER		
COLOUR	a)obverse side-olivaceous b)reverse side-greenish glaucous in central zone, olivaceous around the edge.	a)obverse side-olivaceous b)reverse side-olivaceous
TEXTURE	outer edge of colony appressed, sodden. central zone of colony has cobwebby hyphal growth with dark pycnidia scattered throughout.	appressed growth, sodden much cobwebby aerial mycelium, with pycnidia scattered throughout both central and outer zones of colony.
SHAPE IN SECTION	flat	convex to flat
MARGIN	entire	uneven crenate
COLONY DIAMETER (8 DAYS)	39 - 40 mm	27 - 29 mm

Effect of agar medium and temperature on colony growth

Table 2 shows the overall treatment means for the three tests. In test #1, 22/16C produced the most growth on the three media, whereas in test #2 the most growth occurred at 28/22C for the same three media. Colonies grown on TYA in test #2 had a temperature optimum for growth at 22/16C. In test #3, the temperature optimum for colony growth on the four media tested was 23C. Low temperatures in the three tests (16/10C, 13C, and

TABLE 2. Effect of medium and temperature on the colony diameter (mm) of *Ascochyta hyalospora* after eight days growth.

Test #1 (means±s.e. of 4 replications).

Medium	Temperature Regime(C)*		
	16/10	22/16	28/22
PDA	17.25±4.05	23.63±3.07	23.00±2.27
Czapek-Dox agar	12.13±0.95	16.00±2.16	15.63±4.15
oatmeal agar	19.87±4.01	27.50±6.70	20.25±10.81

Test #2 (means±s.e. of 4 replications).

Medium	Temperature Regime(C)*		
	16/10	22/16	28/22
PDA	13.75±4.33	19.75±1.85	23.13±1.03
Czapek-Dox agar	13.00±1.78	15.25±2.02	18.25±2.40
oatmeal agar	21.88±6.25	25.50±10.61	27.37±5.20
torula yeast agar	27.87±3.09	33.38±4.23	26.50±9.16

Test #3 (means±s.e. of 3 replications).

Medium	Temperature Regime(C)**			
	5	13	23	33
torula yeast agar	6.00±0	18.17±2.36	37.00±1.50	8.50±0.87
PDA	6.00±0	11.67±1.04	22.00±0.87	11.67±1.26
1/2PDA:1/2TYA	6.00±0	16.00±1.80	30.50±2.78	8.67±0.76
Czapek-Dox agar	6.00±0	6.17±0.29	13.83±0.76	6.17±0.29

* light/dark temperature, 14-hour photoperiod
** continuous darkness

5C) produced the least amount of growth. A high temperature of 33C in test #3 was detrimental to growth. The effect of media on radial growth can be summed up by ranking them in the order of most to least radial growth (some exceptions present): TYA > Oatmeal > PDA > CDA.

Infection process on the leaf surface

Germinated conidia were observed on the leaf surface after 3.5 hours of leaf wetness at 22C (Figure 3). More than one germ tube was commonly produced from multiple celled conidia (Figures 3, 4, and 5). After a 6.5 hour incubation period, germ tube length increased (Figure 4). Swellings at the tips of the germ tubes were probably appressoria. No penetration of cuticle or epidermal cells by the appressoria was observed in any stained leaf section after any incubation period. Appressorium formation commonly occurred on or near anticlinal cell junctions (Figure 5). One incidence was observed of appressorium formation on a stomatal complex. In that case the appressorium formed over the junction of the guard cell with another cell. Forty-two hours after inoculation, infected areas were readily noticed when the leaf section was cleared and stained (Figure 6). Presumably this was the reaction of the plant cells to fungal infection, but there was no further investigation to confirm this conjecture.

3.4 Discussion

The pycnidium and conidium dimensions of the Ste-Anne-de-Bellevue fungus were similar to those reported in the literature for Ascochyta hyalospora. The cultural characteristics of the Ste-Anne-de-Bellevue fungus also resemble the description of a closely related fungus, Ascochyta caulina

Figure 3. Germinated conidia on leaf surface after 3 1/2 hour wetness period.

Figure 4. Continued growth of germ tubes (GT) after 6 1/2 hour wetness period. Note formation of swollen tips (S) on germ tubes.

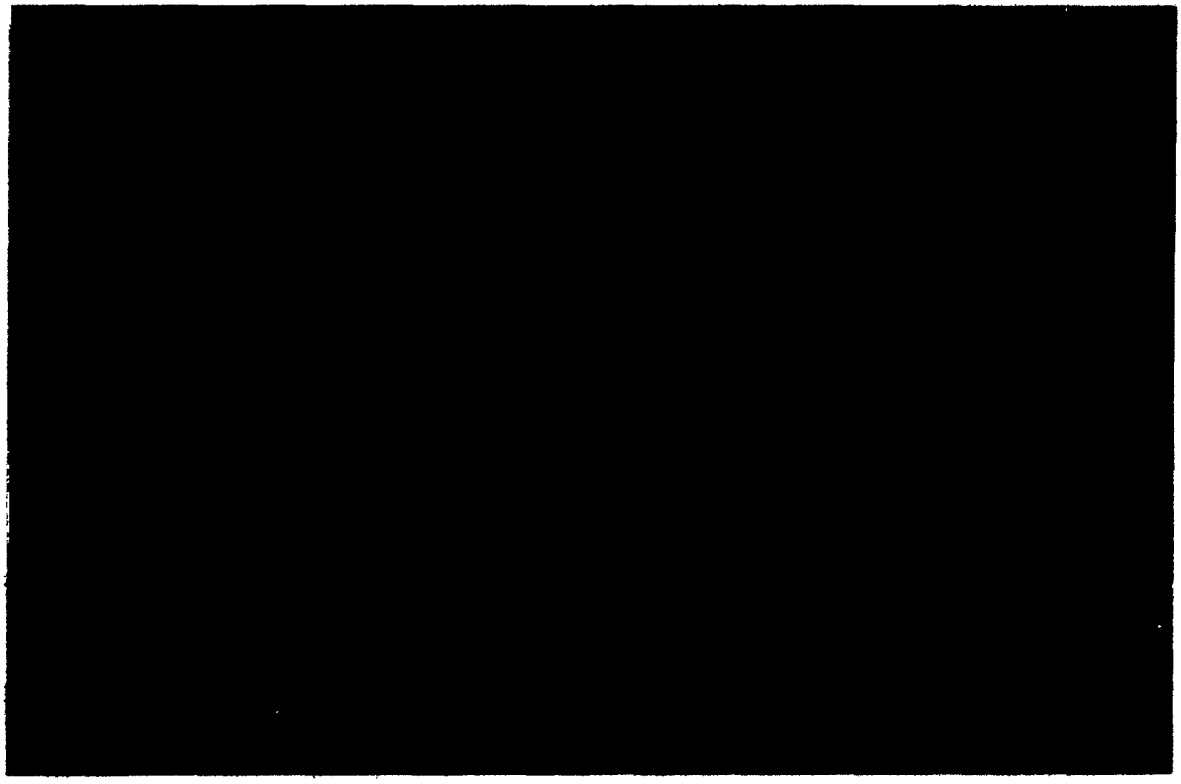


Figure 5. Conidia with two germ tubes after 24 hour wetness period. Note swollen tips (S) formed near or on anticlinal cell divisions (CD), but not stoma (ST).

Figure 6. Dark spots with a discrete outline have formed on leaf (DS) after 42 hour wetness period.

(Van der Aa & Van Kesteren, 1979), in addition to the description of Ascochyta hyalospora given by Boerema et al. (1977). These results confirm the CMI identification of the isolate as Ascochyta hyalospora.

The best temperature range for maximum radial growth on agar medium was 20 - 25C. Growth was greatly reduced at colder temperatures (e.g. 13C) and at warmer temperatures (e.g. 33C). A. hyalospora is mesophilic (Hawksworth et al., 1983) but additional studies are needed to determine cardinal temperatures.

The infection process was similar to that described for other Ascochyta pathogens mentioned in Section 3.1, particularly Ascochyta rabiei. The majority of the penetrations of A. hyalospora appeared to be directly through the cuticle. Invasion into the epidermis often appeared to occur through cell junctions (assuming that the swelling at tip of germ tube was an appressorium and that penetration occurred there). Development of Ascochyta hyalospora in this test appeared to progress more rapidly than that reported for Ascochyta rabiei on chickpea stems where conidia began to germinate 12 hours after inoculation. The rapidity of A. hyalospora germination is more similar to that of Colletotrichum gloeosporioides f. sp. aeschynomene (C.g.a), the pathogen used for control of northern jointvetch. The conidia of C.g.a. were observed to germinate and produce appressoria within five hours of inoculation. Although the precise time of penetration for A. hyalospora was not determined, there were changes in plant cells within 48 hours of inoculation, which would seem to indicate successful infection. It is not known whether appressorium formation

always took place before penetration and colonization of the plant. Heath & Wood (1969) reported that 62 % of the penetrating germ tubes of A. pisi developed appressoria. Based on the results of this section, it would seem that infection of Chenopodium album leaves by Ascochyta hyalospora can proceed rapidly under optimal conditions.

Preece (1976) has commented on the need to study pre-penetration stages on the leaf surface, with a special emphasis on studying where the stages are most likely to occur, the time of development, and the surrounding environment at each stage. The pathogen is probably most vulnerable to changes in the environment (temperature, moisture, fungicides) at this period in its development (Preece, 1976). Similarly, this stage of the disease cycle is also a vulnerable period for a mycoherbicide agent. Further studies should be carried out to examine the effect of external environmental factors (temperature, moisture, light, nutrients on leaf surface, etc.) on the pre-penetration stages of Ascochyta hyalospora.

IV EVALUATION OF THE POTENTIAL OF ASCOCHYTA HYALOSPORA AS A MYCOHERBICIDE FOR CHENOPODIUM ALBUM

4.1 General Materials and Methods

4.1.1 Isolation of Ascochyta hyalospora and use of subcultures

The Ascochyta hyalospora isolate (CH-1) was originally obtained from diseased leaf tissue. Pieces of diseased leaf tissue were immersed in 70% ethanol for 30 seconds, transferred to 2% sodium hypochlorite for approximately 60 seconds, and rinsed twice with sterile distilled water. After drying on filter paper, the leaf pieces were placed on PDA medium. For

storage, the isolated strain was grown on PDA in a glass storage vial. When the colony had grown to cover the agar surface, enough sterile mineral oil was added to immerse the culture surface in oil. The storage vials were placed in a refrigerator at 4C.

Two subcultures, CH-1b and CH-1c (both subcultures of CH-1), were used in the studies along with CH-1. The subculture CH-1b, was begun through single conidium isolation of CH-1 conidia. CH-1c was a mycelial transfer from the advancing margin of an Ascochyta colony growing from a piece of stem tissue infected with CH-1. The isolate CH-1 was used in Section 4.2 (Tests #1 & #2), Section 4.4 (in vivo tests), parts of Section 4.5, and in part of Section 4.6 (Ascochyta-Phoma Comparison Test). Subculture CH-1b was used in parts of Section 4.5. Subculture CH-1c was used in Section 4.2 (Test #3), Section 4.3, Section 4.4 (in vitro test), parts of Section 4.5, and parts of Section 4.6. There was no indication that the subcultures were pysiologically or morphologically different from CH-1.

4.1.2 Preparation of test plants

Two methods were used to prepare plants for the experiments: the filter paper method and the soil method. In the filter paper method, seeds of lamb's-quarters were germinated by placing them on moistened filter paper in Petri plates. The plates were placed in an incubator (Hotpack Model 525-2, Hotpack Canada Ltd, Waterloo, Ontario) with a 12-hour photoperiod and 24/22C day/night temperature. After seven to 10 days, one or two seedlings at the cotyledon stage were transplanted to Pro-Mix (ProMix BX, Premier Brands, Inc., New

York, N.Y.) in 7.5-cm pots. One day after transplanting, 30 ml of 10-52-10 fertilizer solution (1.25 g/L) were applied to the plants. The soil method was an improvement over the filter paper method, since it produced more robust seedlings. In this method, seeds were sown in a 12.5-cm pot and covered with a thin layer of Pro-Mix. Seven seedlings at the cotyledon or early 2-leaf stage were transferred to a 12.5-cm pot to be used for experimental purposes. One day after transplanting into Pro-Mix, 80 ml of 10-52-10 fertilizer solution (1.25 g/L) was added to each pot. Once the plants became established, they were thinned to five plants per pot.

All plants were maintained in growth cabinets (Sherer Controlled Environment Lab., Model CEL 37-14, Sherer-Gilett Co., Marshall, Michigan; Model EY 15 and Model E15, Controlled Environments Ltd., Winnipeg, Manitoba) with a 14-hr photoperiod and 22/16C day/night temperature. The average light intensity at the bottom of the growth cabinets ranged from 134 to 210 microeinsteins m^{-2} sec^{-1}.

4.1.3 Production and preparation of inoculum

For each experiment, "starter" cultures were begun by placing a small piece of mycelium from the stock (storage) culture onto the centre of a Petri plate. The medium used for the starter culture was either torula yeast agar (TYA) or a mixture of 1/2 strength TYA and 1/2 strength PDA. One to three week old starter cultures were then used to start additional plates upon which inoculum for experiments was produced. A mixture of pycnidia and conidia from the starter culture was transferred with a needle to the inoculum production plate. To facilitate better coverage of the plate, separate colonies were

begun at three locations on the plate. The production plates were incubated for two to three weeks in an incubator (12 hr photoperiod with 24/22C day/night temperature).

Conidia were harvested by scraping, with a spatula, the fungal growth from the surface of the inoculum production plates. The mycelium, pycnidia, and conidia were transferred to a beaker of water and the mixture was stirred on a magnetic stirrer for 15 to 30 minutes. The stirred material was filtered through several layers of cheesecloth. When a large number of plates were harvested, a number 60 soil sieve (250 um diameter) was used in addition to cheesecloth. Conidia were washed twice by centrifugation (once at 5000 rpm and a second time at 3000 rpm) and resuspended in distilled water. Inoculum density was determined with the aid of a hemacytometer and adjusted to the desired level by adding distilled water.

4.1.4 Inoculation techniques

Plants were sprayed using a DeVilbiss atomizer. Application rates were calculated on the basis of number of conidia per unit area of soil surface with a spray volume of 500L/ha. Two methods of spraying were used, depending on the experiment. For some experiments, plants were placed on a tray and moved beneath a spray nozzle at a rate required to give the proper spray volume (laboratory bench inoculation method). For other experiments, plants in pots were placed on a turntable (approximately 33 rpm) and the proper spray volume was applied by adjusting the height of the nozzle (turntable method). Unless otherwise indicated, a standard rate of 1 X 10^8 conidia/m^2 was applied to the plants.

After inoculation, the plants were placed in one of two dew chambers (Model E-54UDL and Model DC 20 , Percival Manufacturing Company, Boone, Iowa). Unless otherwise noted, the standard wetness period conditions were 20 hours of wetness in a dark dew chamber at 22C. After the leaf wetness period, the plants were kept in the growth cabinets and under the conditions previously described in Section 4.1.2.

4.1.5 Response measurements

Depending on the experiment, three plant responses to the treatments were measured: mortality, height, and dry weight. In addition, percent leaf area affected by disease was rated in some experiments. Mortality was expressed as a percentage, and was recorded at the time of disease rating and at harvest. Height was measured as the vertical distance from the soil surface to the growing point and was recorded at the time of plant harvesting. The above-ground portion of the plants were harvested for dry weight measurements. After harvesting, the plants were placed in a 70C oven for at least three days, and then weighed.

At the time of disease rating, the four oldest leaves (the four lowest leaves) of the sprayed plants were rated according to the Barratt-Horsfall rating scale (Horsfall & Barratt, 1945). Either one of two methods was used for analyzing the Barratt-Horsfall grades. Analysis in the first method (See Section 4.6) involved analysis of variance for ratings recorded on a single date. Before analysis the grades were converted to mid-point percentages and one average percent leaf disease value was calculated for each pot (experimental unit). These averages were transformed using the angular

transformation.

The second method, area-under-the-disease-progress-curve (AUDPC) procedure, required ratings at several dates (at least three). The Barratt-Horsfall grades were converted to mid-point percentages and an average percent leaf disease at each rating date was calculated for each experimental unit. The following formula was used to obtain an AUDPC value for each experimental unit:

$$\sum_{i=1}^{n-1} [(Y_{i+1}+Y_i)/2][X_{i+1}-X_i],$$

where Y_i is the % disease at the ith rating date, X_i is the time (days) after inoculation of the ith rating date, and n is the total number of rating dates (Shaner & Finney, 1977).

AUDPC, the integral over time of percent diseased foliage, is thought to be a good measurement since it indicates the amount of disease present over time and not just at a single rating date. AUDPC measurements are probably more closely related to height and dry weight than are single-date disease measurements, since duration of healthy leaf area is related to the amount of photosynthate that is produced (Waggoner, 1986; Watson, 1947).

4.2 Effect of Inoculum Dose and Plant Age on Disease Development and Subsequent Plant Damage

4.2.1 Introduction

Two important aspects in determining whether a pathogen will make a good mycoherbicide are the spray rate and the stage of weed growth at time of inoculation. It is commonly observed in mycoherbicide research that increasing inoculum dose promotes weed damage, whereas increased weed development stage at time of inoculation hinders disease development on the weed. Scheepens

and van Zon (1981) found that young lamb's-quarters plants were susceptible to Ascochyta caulina whereas older plants were more resistant. Boyette et al. (1979), working with Colletotrichum gloeosporioides f. sp. jussiaeae on Jussiaea decurrens (Walt.) DC (winged water primrose), found that doses of 5 X 10^6, 1 X 10^7, and 2 X 10^7 spores/ml produced weed mortalities of 67, 87, and 100%, respectively. Scheepens (1987), working with Cochliobolus lunatus Nelson & Haasis on Echinochloa crusgalli (L.) Beauv., provided an example of decreasing percentage necrosis as an inoculum dose of 0.4 X 10^6 spores/ml was applied to 22-, 30-, and 47-day old plants. Webb and Lindow (1987) found that there was decreasing disease development on Pteridium aquilinum (L.) Kuhn (bracken fern) when Ascochyta pteridis (Bres.) Sacc. was applied to fronds of increasing age. In order to obtain an equivalent 50% disease incidence, the inoculum concentration had to be higher as the age of the fronds increased.

While the previous examples illustrate plants having increased resistance to pathogens with increasing age, this situation is not necessarily typical. Populer (1978) has proposed four patterns of susceptibility to pathogens based on age of plant or plant part. One pattern (Type IB) is illustrated by the previous examples where there is decreasing susceptibility with increasing age. Other patterns are: early juvenile resistance followed by a period of susceptibility that endures until the plant enters a more resistant phase during the adult-growth stage (Type IA), increasing susceptibility with increasing age (Type II), and juvenile susceptibility followed by some type of resistance with susceptibility reappearing again

at a later mature stage (Type III). These resistance patterns may be applicable for age of plant, age of plant part, or both. There is limited information to indicate the type of pattern that would describe the Ascochyta disease of lamb's-quarters. Boerema et al. (1977) isolated Ascochyta hyalospora from contaminated seed and seedlings of Chenopodium quinoa Willd., the South American crop plant that is closely related to C. album. There is no indication in their paper that epidemics occur on mature crop plants. At Ste-Anne-de-Bellevue, Ascochyta hyalospora was often obtained from lamb's-quarters plants growing in late summer or early fall. Lesions could be found on mature leaves. It is not known, however, when the leaves were first infected.

It is important to determine the oldest stage of lamb's-quarters development that can be effectively controlled by Ascochyta hyalospora. For effective use in crops such as soybeans and corn, effective weed control should be possible from an early seedling stage of lamb's-quarters to a more mature stage of weed development. Corn can be used to illustrate this point. Corn exhibits slow early development, and therefore can be affected by severe competition from lamb's-quarters that may germinate early under the normally cool temperature conditions of spring (Glauninger & Holzner, 1982). Therefore, from a competitive standpoint the field should be free from weeds for several weeks. One set of recommendations for corn (Upfold & Morris, 1987) indicates that the six to eight weeks following corn emergence is the most crucial time period for weed control. Weed competition at this time can be harmful. During this six

to eight week period lamb's-quarters plants with a wide range of sizes could develop in the field.

In addition to efficacy over a wide range of weed growth stages, the pathogen should be effective with a spray rate as low as possible to reduce costs. Currently, 1.9 X 10^7 conidia/m^2 is the recommended rate for COLLEGO®, the commercially available bioherbicide used to control northern jointvetch in rice. This rate is applied in July or August when the weed is at the mid-to-late stages of vegetative growth and is emerging above the rice crop canopy (Smith, 1986; TeBeest & Templeton, 1985).

The objective of this section was to determine the effect of increasing inoculum and increasing weed maturity on disease development and on plant mortality, height, and dry weight. The experiments were carried out as growth chamber studies only.

4.2.2 Methods

Test #1

The response of plants at three growth stages to five application rates of Ascochyta hyalospora inoculum were observed in an experiment set up as a randomized complete block design (RCBD). The experimental unit, replicated 10 times, consisted of one plant in a 7.5-cm pot. The plants were prepared by the filter paper germination method (See Section 4.2.2).

The preparation of test plants was coordinated to provide three growth stages (cotyledon, 2- to 4-leaf, and >8-leaf) at the time of inoculation. Inoculum of A. hyalospora was applied at five rates: 0 conidia/m^2, 1X10^5 conidia/m^2, 1X10^6 conidia/m^2, 1X10^7 conidia/m^2, and 1X10^8 conidia/m^2. Plants were

inoculated using the laboratory bench inoculation method, and subjected to the standard wetness period (See Section 4.1.4). Height, dry weight, and mortality data were recorded 22 days after spraying.

Test #2

This test was similar to Test #1, except that plants were inoculated at 3, 13, and 23 days. The three transplant ages corresponded approximately to the cotyledon, 4- to 6-leaf stage, and the 8- to 10-leaf stage of growth.

Disease ratings were begun two days after inoculation and continued every other day for 12 days to give six rating dates. Using the Barratt-Horsfall scale, ratings were recorded for the four oldest leaves of the 13- and 23-day old transplants. The 3-day old plants had to be excluded, since no leaves were present for rating. Height, dry weight, and mortality data were recorded 22 days after spraying.

Test #3

In the third experiment, the response of plants, at three transplant ages, to eight rates of Ascochyta hyalospora inoculum was studied in an experiment set up as an RCBD. The experimental unit, replicated six times, consisted of five plants in a 12.5-cm pot. The plants were prepared by germinating the seed in soil (Section 4.2.2). At the time of spraying, the three transplant ages were 5 (2-leaf), 10 (4- to 6-leaf), and 15 (6- to 8-leaf) days. The application rates were 0, $1.6X10^7$, $3.1X10^7$, $6.3X10^7$, $1.3X10^8$, $2.5X10^8$, $5X10^8$, and $1X10^9$ conidia/m^2. Plants were inoculated using the turntable method (Section 4.2.4). Fifteen days after inoculation, mortality data

were recorded. Height, dry weight, and mortality data were recorded 15 days after spraying.

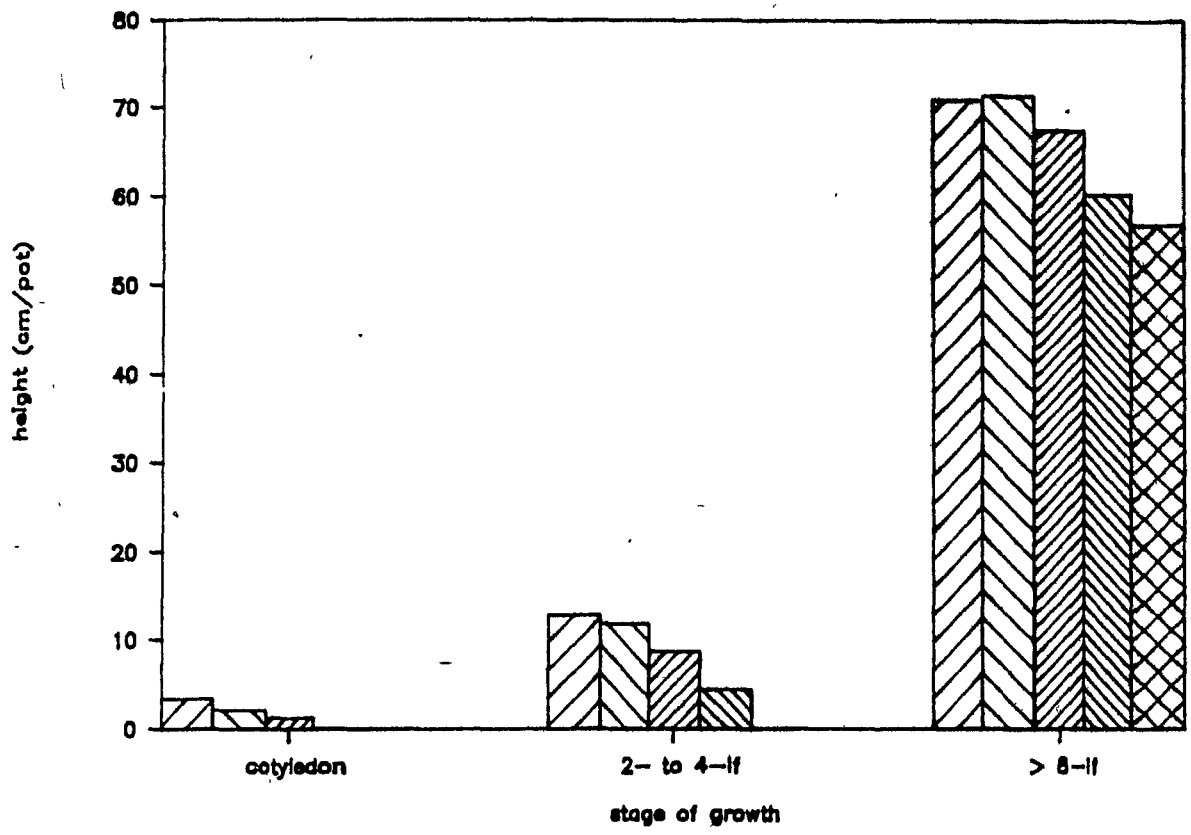
4.2.3 Results

The means of dry weight and height from test #1 and #2 are presented in Figures 7 to 10. For each growth stage in both experiments, height and dry weight generally decrease with the application of higher rates of conidia. There was, however, a noticeable difference in the rate of height and dry weight decrease among the growth stages. At the oldest growth stages, the rate of decrease in height and dry weight, with increasing inoculum from zero to 1 X 10^8 conidia/m^2, was much lower than at the middle and youngest growth stages. For example, in Figure 10, 23-day old transplants suffered a 56.9 % decrease when dry weight for treatments of 0 and 1 X 10^8 conidia/m^2 are compared. Over the same rates, 13-day old transplants suffered a 98.9 % decrease in dry weight and 3-day old transplants suffered a decrease of essentially 100 % (no plant tissue could be found for measurement).

Analysis of log AUDPC values (Table 3) for 13- and 23-day old transplants at the four levels of spray rate in test #2 (see means in Table 4), showed interaction to be present ($P = 0.0361$). The AUDPC values were log transformed to maintain homogeneity of variance. Fitted orthogonal polynomials (Table 5) for 13- and 23-day old transplants are shown in Figure 11. This figure shows that at the middle spray rates there was greater disease development on the leaves of the 23-day old transplants compared to the 13- day old transplants. At low and high spray rates, disease development was approximately equal.

Figure 7. Effect of spray rate and growth stage on height of lamb's-quarters (test #1).

Figure 8. Effect of spray rate and growth stage on dry weight of lamb's-quarters (test #1).


height (cm/pot)
80
70
60
50
40
30
20
10
0
cotyledon
2– to 4–lf
> 8–lf
stage of growth

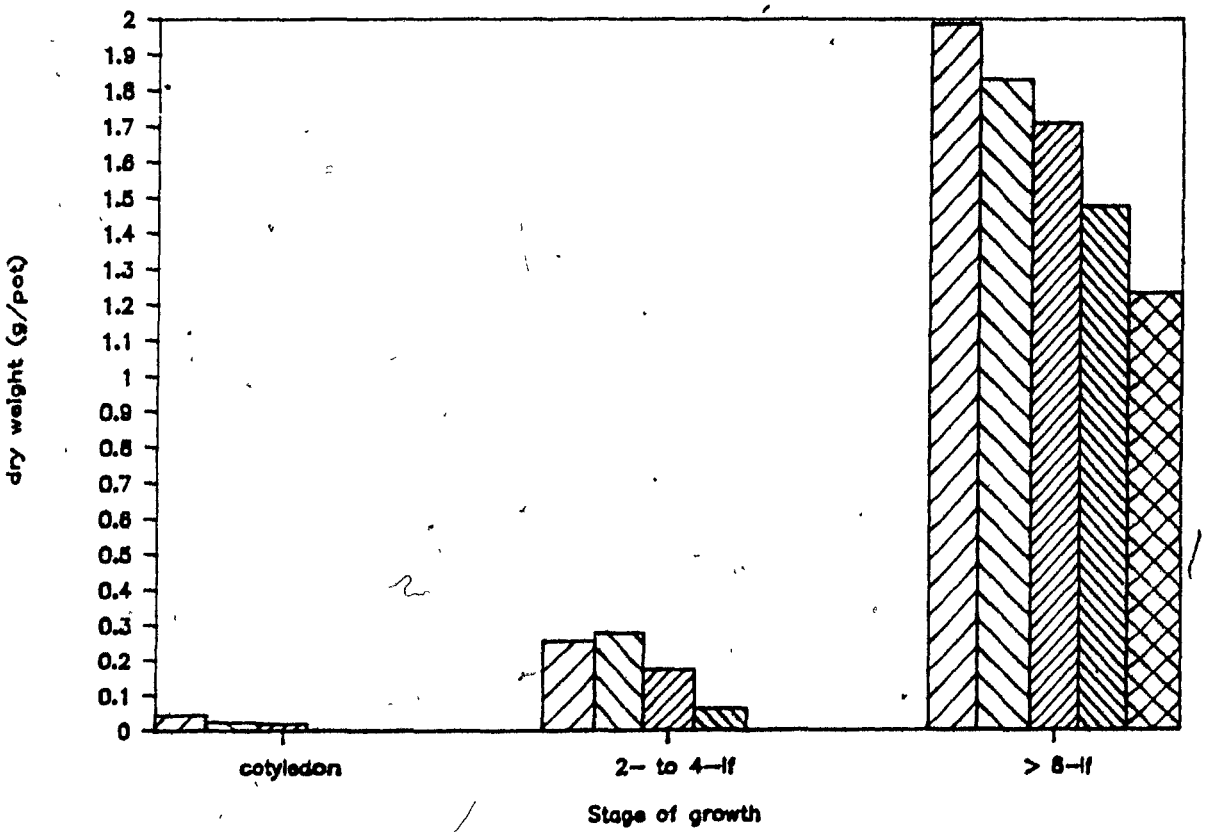
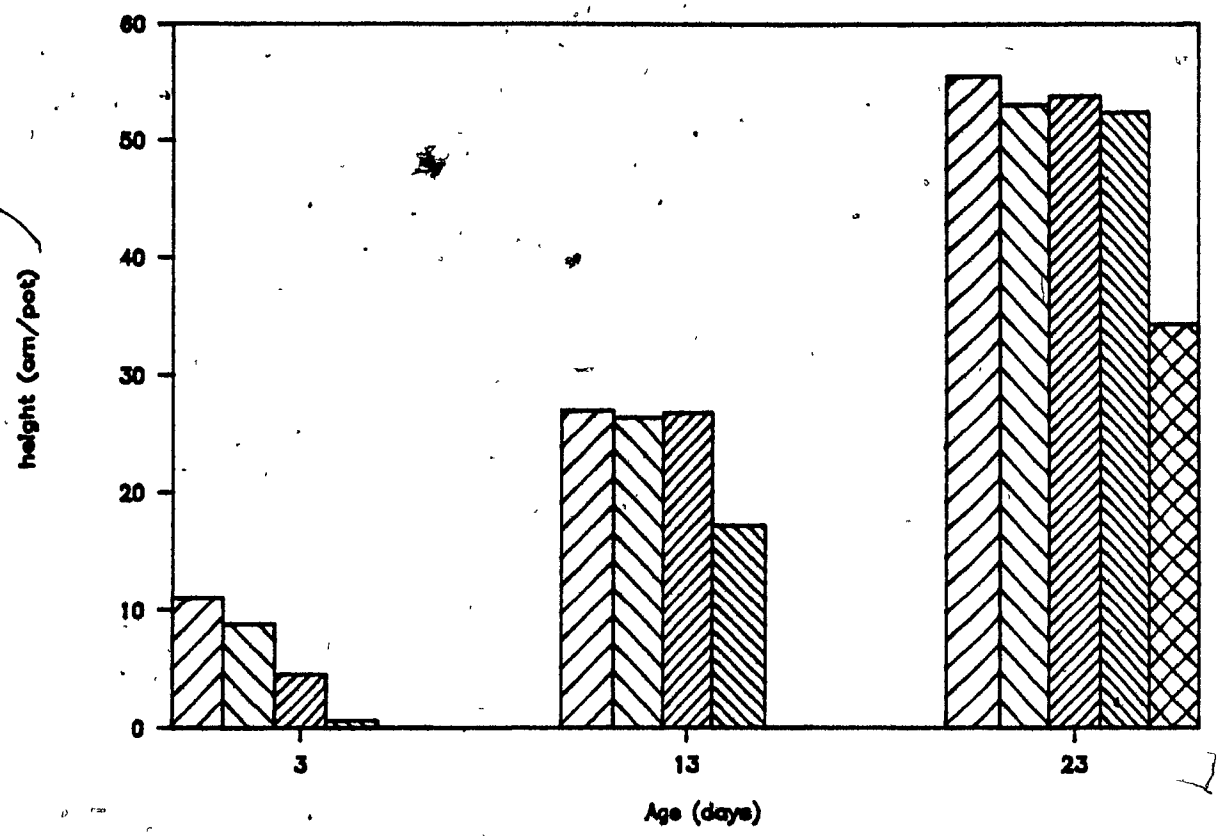

dry weight (g/pot)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
cotyledon
2– to 4–lf
> 8–lf
Stage of growth

Figure 9. Effect of spray rate and plant age on height of lamb's-quarters (test #2).

Figure 10. Effect of spray rate and plant age on dry weight of lamb's-quarters (test #2).


height (cm/pot)
60
50
40
30
20
10
0
3
13
23
Age (days)

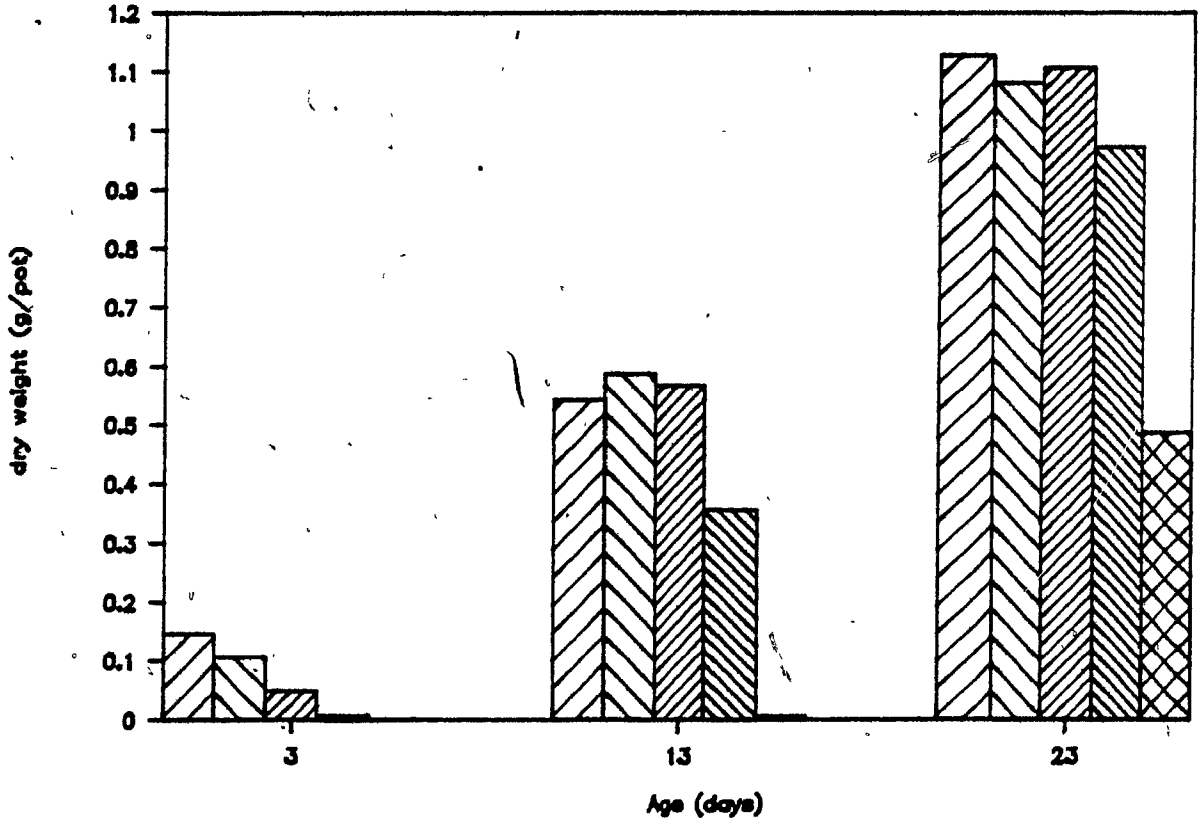

dry weight (g/pot)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3
13
23
Age (days)

TABLE 3. Analysis of variance on the effects of plant age and spray rate on disease development on lamb's-quarters in test #2.

Source*	df	Mean Square	Pr > F
R	9	0.1906	0.1626
A	1	1.4193	0.0013
D	3	15.0555	0.0001
A * D	3	0.3806	0.0361
Error	63	0.1259	

* R=block, A=age, D=spray rate

TABLE 4. Effect of plant age and spray rate on disease development (log AUDPC/pot) in test #2. *

Age (days)	Spray Rate (log conidia/m^2)			
	5	6	7	8
13	0.92	2.05	2.61	3.00
23	1.14	2.69	2.85	2.97

standard error = ±0.11

* means of ten replicates

Because of the different procedures (five plants/pot and 2-1f growth stage) used in test #3, the dry weight and height (few or no zero values) could be analyzed for the presence of trends. Height data for rates of 5×10^8 and 1×10^9 conidia/m^2 were not used in the analysis since many zeros were present due to mortality at these rates. In addition, the height and dry weight were log transformed to maintain homogeneity of variance (Tables 6 & 7). Interaction (Table 8) between spray rate and age was found to be present for both height and dry weight responses ($P = 0.0001$ for both responses). Fitted orthogonal polynomials (Tables 9 & 10) for the three age groups are shown in Figures 12 & 13. No significant trend was

TABLE 5. Orthogonal polynomial contrasts and fitted regression equations for disease development (log AUDPC/pot) in test #2.

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
D IN A					
D in A1	3	8.1483	0.0001	Log AUDPC = - 9.8425 + 3.0816*D	0.91
D linear	1	23.0061	0.0001	- 0.1849*D^2	
D quadratic	1	1.3670	0.0016		
D cubic	1	0.0717	0.4532		
D in A2	3	7.2877	0.0001	Log AUDPC = -73.3258 + 32.6149*D	0.75
D linear	1	15.8943	0.0001	- 4.6432*D^2 + 0.2198*D^3	
D quadratic	1	5.0993	0.0001		
D cubic	1	0.8696	0.0108		
ERROR	63	0.1259			

* D=spray rate, A1=13-day old plants, A2=23-day old plants

TABLE 6. Effect of plant age and spray rate on height (log cm/pot) of lamb's-quarters in test #3.*

Age (days)	Spray Rate(log conidia/m^2) 7.2	7.5	7.8	8.1	8.4
5	1.63	1.53	1.47	1.17	0.53
10	1.95	1.93	1.93	1.86	1.73
15	2.16	2.18	2.10	2.04	1.94

standard error = ±0.06

* means of six replicates

TABLE 7. Effect of plant age and spray rate on dry weight (log g/pot) of lamb's-quarters in test #3.*

Age (days)	Spray Rate(log conidia/m^2) 7.2	7.5	7.8	8.1	8.4	8.7	9.0
5	0.21	0.18	0.15	0.09	0.03	0.01	0.01
10	0.52	0.48	0.50	0.43	0.37	0.19	0.07
15	0.69	0.66	0.61	0.57	0.48	0.42	0.38

standard error = ±0.025

* means of six replicates

TABLE 8. Analysis of variance on the effects of plant age and spray rate on height and dry weight of lamb's-quarters in test #3.

Source*	Height df	Mean Square	Pr > F	Dry Weight df	Mean Square	Pr > F
R	5	0.1210	0.0004	5	0.0159	0.0013
A	2	5.4420	0.0001	2	2.1569	0.0001
D	4	0.7943	0.0001	6	0.2734	0.0001
A * D	8	0.2487	0.0001	12	0.0188	0.0001
Error	70	0.0232		100	0.0037	

* R=block, A=age, D=spray rate

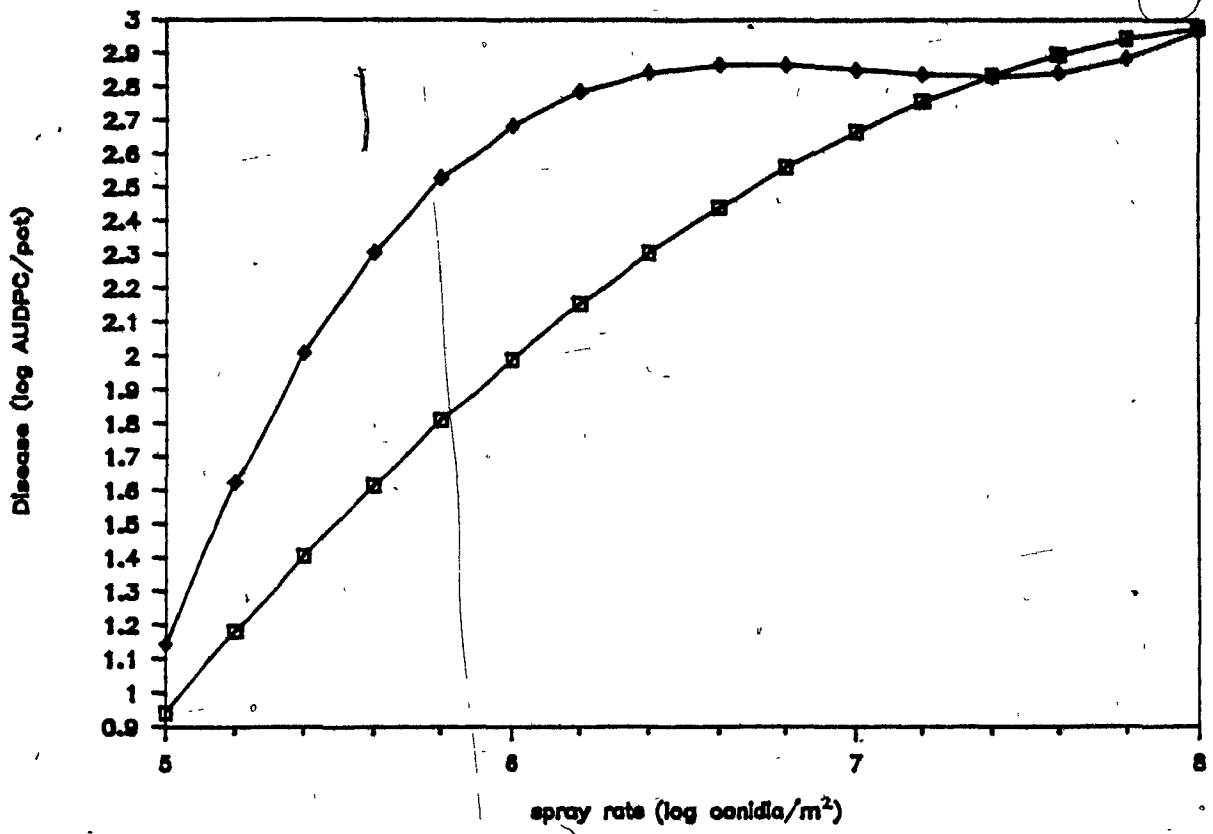
TABLE 9. Orthogonal polynomial contrasts and fitted regression equations for height (log cm/pot) in test #3.

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
D IN A					
D in A5	4	1.1835	0.0001	log ht = -54.8346 + 15.2835*D	0.71
D linear	1	3.9115	0.0001	$- 1.0342*D^2$	
D quadratic	1	0.7278	0.0001		
D cubic	1	0.0921	0.0504		
D quartic	1	0.0495	0.7438		
D in A10	4	0.0495	0.0863		
D in A15	4	0.5885	0.0479	log ht = 3.611 - 0.1958*D	0.59
D linear	1	0.2071	0.0039		
D quadratic	1	0.0233	0.3206		
D cubic	1	0.0027	0.7347		
D quartic	1	0.0024	0.7497		
ERROR	70	0.0232			

* D=spray rate, A5=5-day old plants, A10=10-day old plants, A15=15-day old plants

Figure 11. Effect of spray rate and plant age on disease development (test #2).

Figure 12. Effect of spray rate and plant age on height of lamb's-quarters (test #3).


Disease (log AUDPC/pot)
3
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
5
6
7
8
spray rate (log conidia/m²)

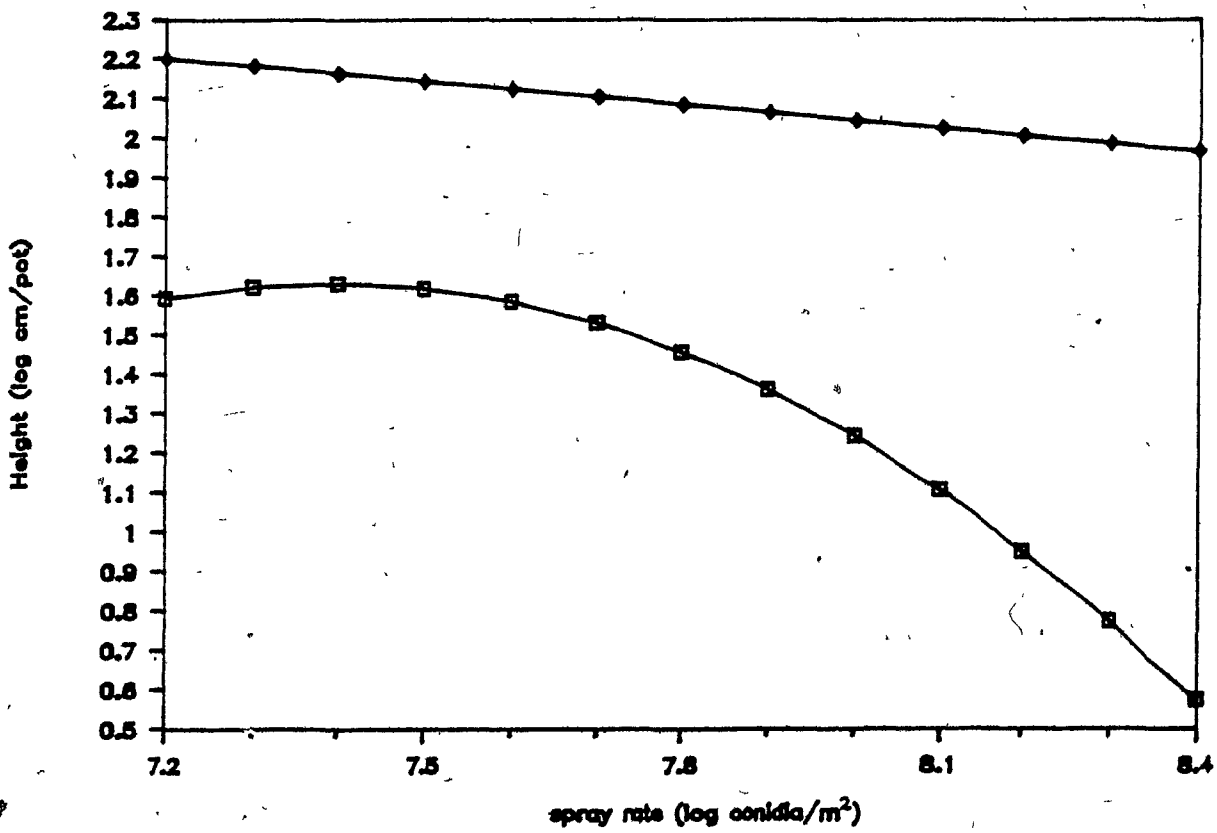

Height (log cm/pot)
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
7.2
7.5
7.8
8.1
8.4
spray rate (log conidia/m²)

TABLE 10. Orthogonal polynomial contrasts and fitted regression equations for dry weight (log g/pot) in test #3.

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
D IN A					
D in A5	4	0.0647	0.0001	log dry wt = -45.45333314	0.78
D linear	1	0.0454	0.0007	+ 17.47012997*D - 2.20825405*D^2	
D quadratic	1	0.1268	0.0001	+ 0.09204047*D^3	
D cubic	1	0.0779	0.0001		
D quartic	1	0.0088	0.1246		
D in A10	4	0.2634	0.0001	log dry wt = 9.651854297	0.78
D linear	1	0.0000038	0.9744	- 4.429154089*D + 0.699739476*D^2	
D quadratic	1	0.8475	0.0001	- 0.036232855*D^3	
D cubic	1	0.1070	0.0001		
D quartic	1	0.0990	0.0001		
D in A15	4	0.1267	0.0001	log dry wt = -34.03406422	0.85
D linear	1	0.0343	0.0029	+ 13.04186948*D - 1.61090675*D^2	
D quadratic	1	0.3283	0.0001	+ 0.06518502*D^3	
D cubic	1	0.1101	0.0001		
D quartic	1	0.0340	0.0030		
ERROR	100	0.0037			

* D=spray rate, A5=5-day old plants, A10=10-day old plants, A15=15-day old plants

found in the height response of 10-day old transplants whereas a decreasing linear trend was found for 15-day old transplants and a decreasing quadratic trend was found for 5-day old transplants. Analysis of dry weight trends included the responses at spray rates of 5 X 10^8 and 1 X 10^9 conidia/m^2. The fitted equations of dry weight response for all three age groups include cubic terms but the predicted responses plotted in Figure 13 do not show this in all cases. There was a decelerating rate of dry weight decrease for 5- and 15-day old transplants as the spray rate was increased from approximately 1.3 X 10^8 to 1 X 10^9 conidia/m^2. On the other hand, the dry weight response of the 10 day old transplants displayed an accelerating rate of decrease as the spray rate was increased to 1 X 10^9 conidia/m^2.

The mortality patterns were similar for all three experiments, although the spray rate needed to obtain equivalent mortality varied from one experiment to another. In test #1 (Figure 14), 100% mortality for cotyledon and 2- to 4-leaf stages occurred at spray rates of 1 X 10^7 conidia/m^2 and 1 X 10^8 conidia/m^2, respectively. No mortality was recorded for plants having more than eight leaves at the time of inoculation. Although the pattern was similar in test #2 (Figure 15), higher spray rates were generally needed to obtain mortality similar to that obtained with lower rates in test #1. For test #3 (Figure 16), minimum rates needed to obtain at least some mortality were 1 X 10^7 conidia/m^2 for the 2-leaf stage, and 1 X 10^8 conidia/m^2 for the 4-leaf and 6- to 8-leaf stages. The highest mortalities in the three age groups occurred at the highest rate. At that spray rate, mortalities of 96.7 %, 66.7 %, and 13.3 % occurred

Figure 13. Effect of spray rate and plant age on dry weight of lamb's-quarters (test #3).

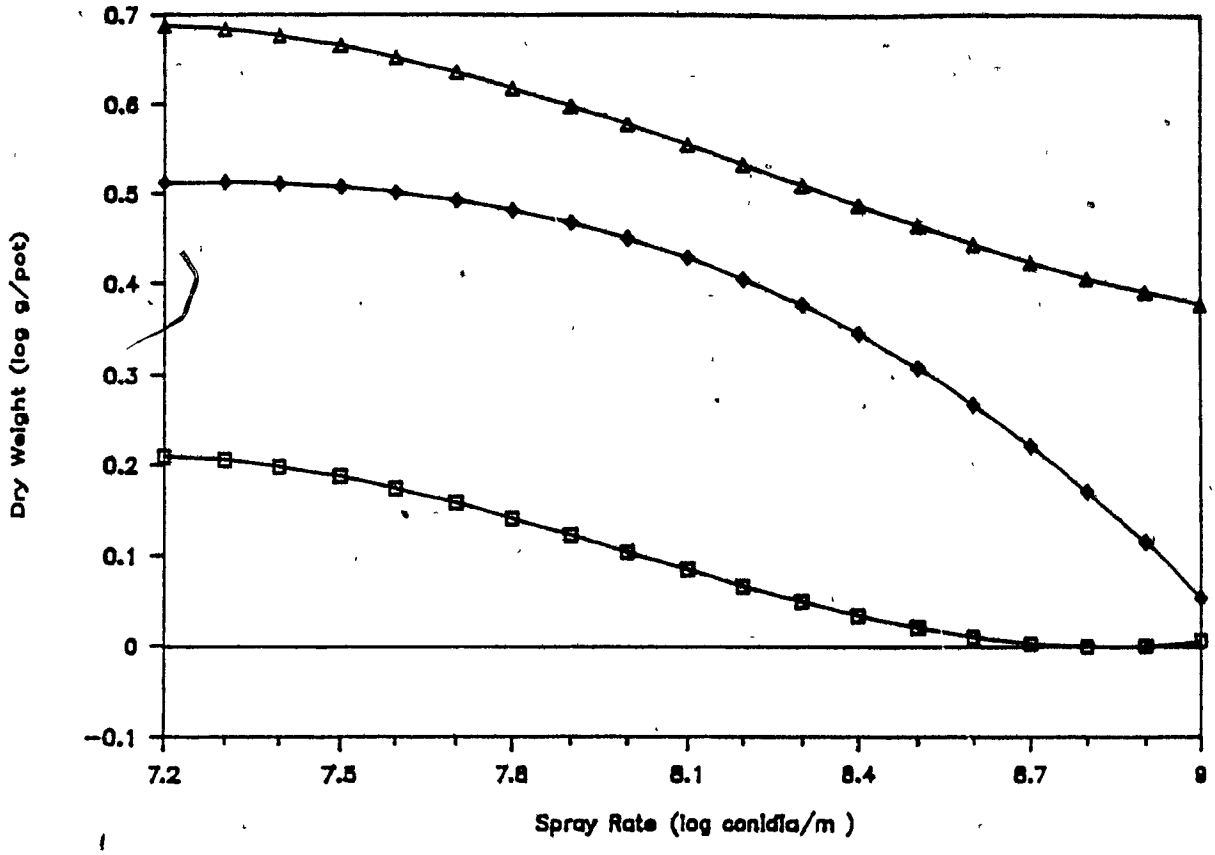
Age

□ 5 days ◇ 10 days △ 15 days

Figure 14. Effect of spray rate and growth stage on mortality of lamb's-quarters (test #1). Note: Zero rate on horizontal axis was not log transformed.

Growth Stage

□ cotyledon ◇ 2- to 4-leaf △ >8-leaf


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
Dry Weight (log g/pot)
7.2
7.5
7.8
8.1
8.4
8.7
9
Spray Rate (log conidia/m)

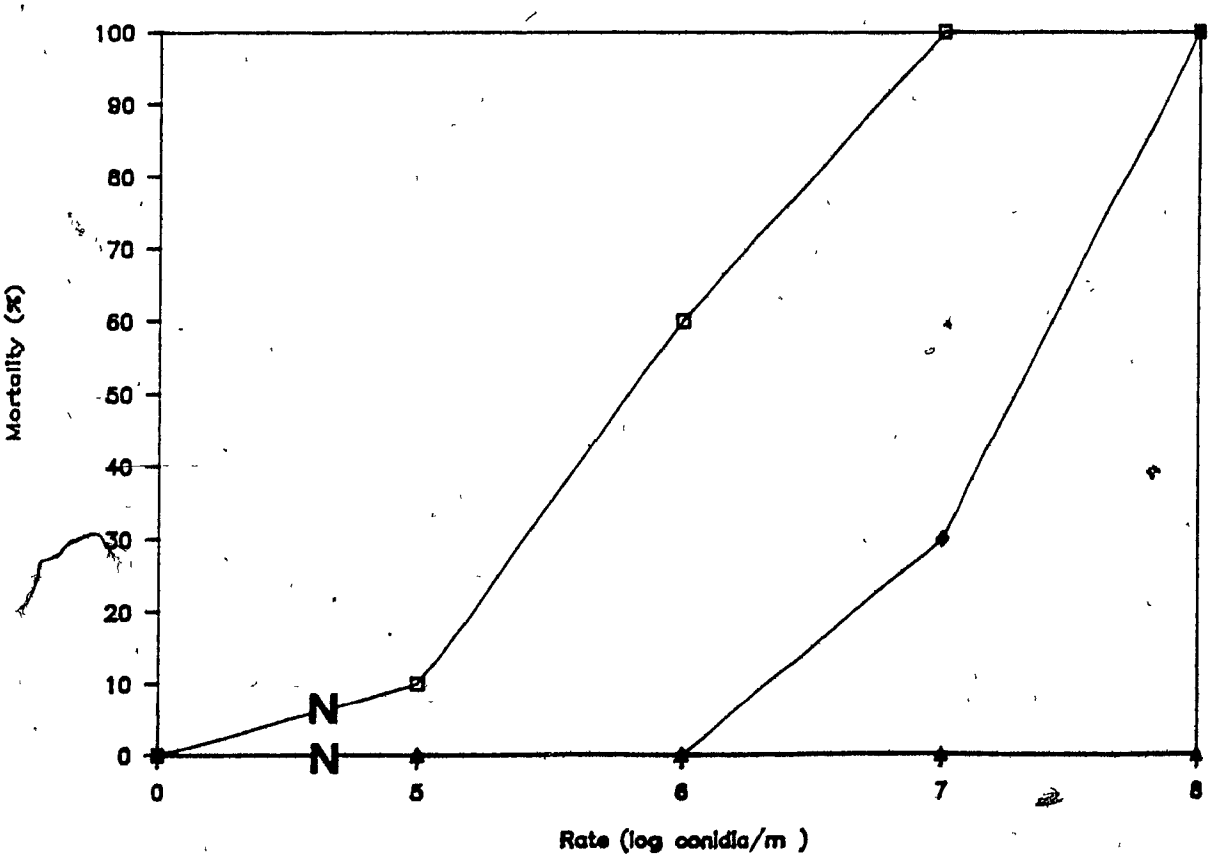

100
90
80
70
60
50
40
30
20
10
0
Mortality (%)
N
N
0
5
6
7
8
Rate (log conidia/m)

Figure 15. Effect of spray rate and plant age on mortality of lamb's-quarters (test #2). Note: Zero rate on horizontal axis was not log transformed.

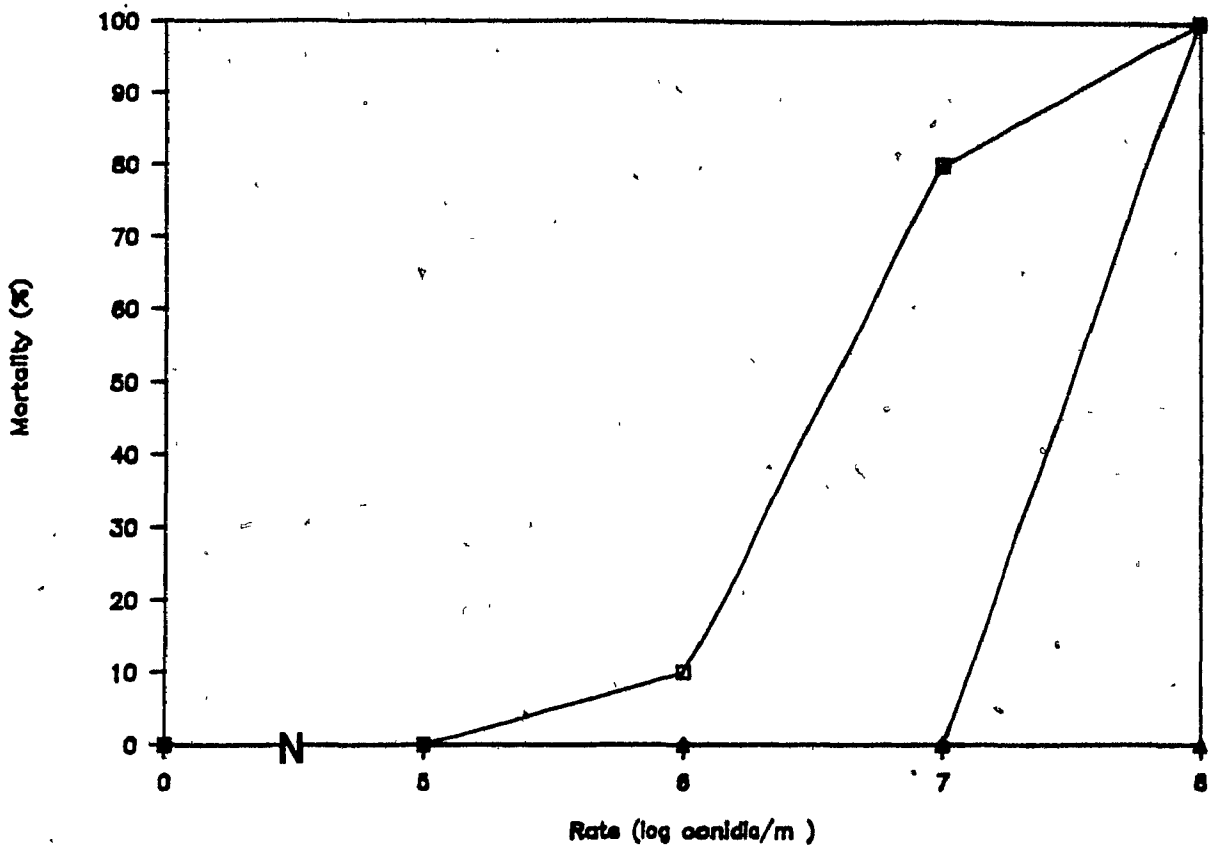
Age

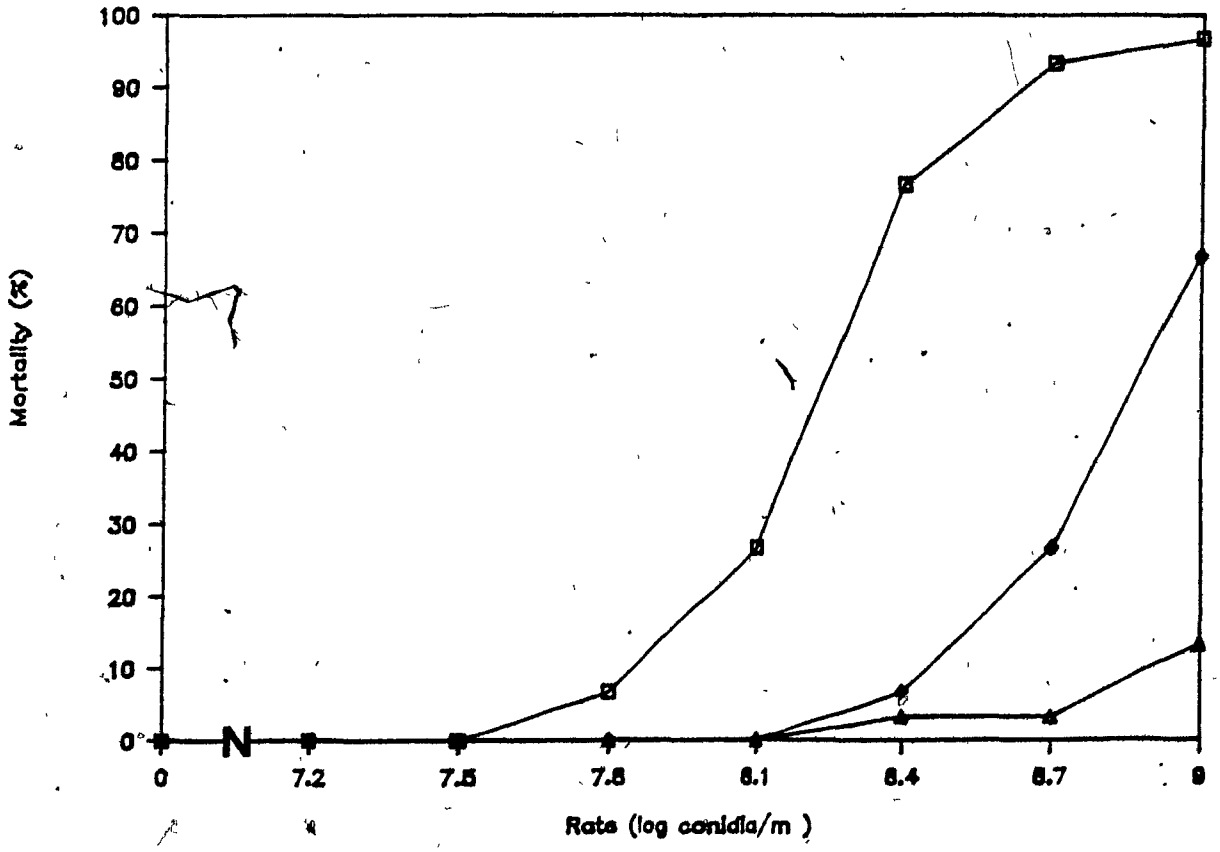
□ 3 days ◇ 13 days △ 23 days

Figure 16. Effect of spray rate and plant age on the mortality of lamb's-quarters (test #3). Note: Zero rate on horizontal axis was not log transformed.

Age

□ 5 days ◇ 10 days △ 15 days


100
90
80
70
60
50
40
30
20
10
0
Mortality (%)
N
0
5
6
7
8
Rate (log conidia/m)


100
90
80
70
60
50
40
30
20
10
0
Mortality (%)
N
0
7.2
7.5
7.8
8.1
8.4
8.7
9
Rate (log conidia/m)

in the 5-day old, 10-day old, and 15-day old age groups, respectively.

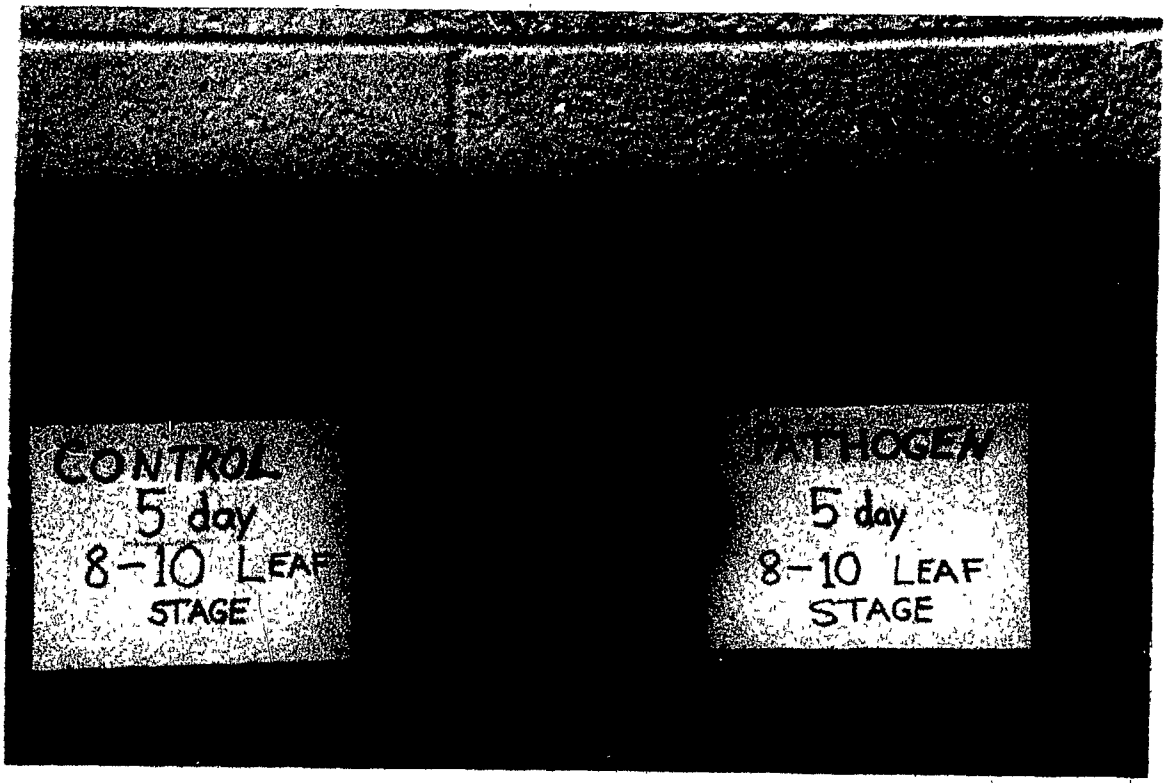
At younger growth stages (4- to 6-leaf stage or less), death of the plant often occurred within five days of inoculation when inoculated with a high spray rate (i.e. 1 X 10^8 or higher). At the time of death, pycnidia had usually developed on stems of the plant (Figure 17), even if the stem was not directly exposed to moisture. Heavy defoliation typically occurred on more mature plants when inoculated with a high spray rate and kept in a dew chamber for 20 hours (Figure 18). In addition to desiccation of the sprayed leaves, stem epinasty often occurred. No plants at this stage of growth were killed at spray rates of 1 X $10^8/m^2$.

4.2.4 Discussion

The results of the experiments reported in this section generally show results quite similar to the results of previous bioherbicide research. Increasing inoculum dose results in increased disease development, whereas increasing maturity of plant generally results in less disease development (Boyette et al., 1979; Scheepens, 1987; Webb & Lindow, 1987). There is a discrepancy in the results of the present studies, however. Increasing maturity resulted in less damage to the plant as measured by mortality, height and dry weight, but there was not, however, decreased disease development of leaves with advancing maturity. AUDPC measurements, which provide a direct measurement of disease development on leaves, showed more disease development on the oldest growth stages except at the highest spray rate where disease development appeared to be approximately equal. The remaining response measurements, which

Figure 17. Stem of dead plant inoculated at approximately 4-leaf growth stage. At time of death, pycnidia (P) appeared on stem.

Figure 18. Lamb's-quarters plants five days after inoculation with water or 1 X 10^8 conidia/m^2. At time of inoculation the plants had reached the 8- to 10-leaf stage.


CONTROL
5 day
8-10 LEAF
STAGE
PATHOGEN
5 day
8-10 LEAF
STAGE

provide indirect measurements of damage due to disease development on leaves as well as other plant parts and functions, showed the more typical response of decreasing susceptibility with increasing age of plant. In this case it would appear that measurement of disease development on leaves was not a good indicator of the effect of the pathogen on other responses such as mortality, height, and dry weight. The leaf tissue was equally (or even more) susceptible to infection at older ages, but the plant can tolerate infection at older ages.

The mortality, height, and dry weight responses provide an indication of the pathogen's potential for use as a mycoherbicide. Disease development on the stem correlated much better with the indirect measurements of mortality, height, and dry weight. Stem infection (Figure 11 of Section 4.3.3) was observed to be greater at the cotyledon to 4- to 6- leaf growth stage. AUDPC measurements were only collected and reported for test #2. More evidence is needed for a more firm conclusion as to how disease development on leaves is related to the age of the leaves and what effect this has on overall plant health.

Based on the results of the growth chamber experiments presented in this section and the report by Boerema et al. (1977) finding Ascochyta hyalospora a seed contaminant and cause of a seedling disease of Chenopodium quinoa, it would appear that the disease incited by inundative inoculation of Chenopodium album with A. hyalospora follows a Type IB age resistance pattern (Populer, 1978). The resistance pattern, illustrated by the mortality, height, and dry weight responses, would appear to be more related to disease development on the stem rather than disease development on the leaf. The stem

appeared to become less susceptible to infection with increased maturity.

The apparent difference in stem disease development between young plants and older plants bears further investigation to determine the basis for the difference. Is there some type of resistance mechanism present in the older stem that is not present in younger stems? Goodman *et al.* (1986) list several possible factors involved in resistance including pre- and post-invasion structural barriers (e.g. lignification, papilla formation), already existing antimicrobial substances (e.g. phenolic compounds), and postinfectional resistance (e.g. phytoalexins, hypersensitive reactions).

The minimum spray rate needed for good efficacy was 1 X 10^8 conidia/m^2. This is five times the recommended rate of approximately 1.9 X 10^7 conidia/m^2 for COLLEGO® (TeBeest & Templeton, 1985). Field testing would provide more realistic indications of the optimum *Ascochyta* spray rate, since plant vitality and method of spray application (particularly nozzle type) would be different.

The results of the age-rate experiments, if verified by field testing, would indicate that weed control can be obtained by spraying plants at the cotyledon to 6-leaf stage of growth. One of the keys in controlling weeds at older growth stages would appear to be in finding a way to increase disease development in older stems.

4.3 Effect of Wetness Period Temperature and Wetness Period Duration on Disease Development and Subsequent Plant Damage

4.3.1 Introduction

The most important environmental factors affecting plant disease are probably moisture, and to a lesser extent, temperature. Free moisture on the leaf surface is needed for the germination and penetration by infective propagules of most foliar plant pathogens (Rotem, 1978; Zadoks & Schein, 1979). In the field, the free moisture may be provided by several natural and man-made processes such as rain, dew, fog, and irrigation (Miller, 1969; Wallin, 1963). The duration of leaf surface moisture is more important than the amount that is present (Rotem, 1978; Van der Wal, 1978).

In bioherbicide research, determination of environmental requirements for disease development is important for establishing the bioherbicide's potential for weed control. These environmental requirements include wetness period (WP) temperature and WP duration. TeBeest et al. (1978) found that disease on northern jointvetch plants inoculated with Colletotrichum gloeosporioides f. sp. aeschynomene (C.g.a.) and kept at a WP (12 hours) temperature of 28C, developed more rapidly than for plants kept at higher (32C or 36C) and lower (20C or 24C) temperatures. In a separate experiment, they examined the effect of WP duration and found that at least 12 hours of moisture (at 28C) were required in order to obtain 100% infection. Less disease development and slower disease development occurred at shorter wetness periods.

The previous example illustrates the effect of temperature and wetness period, tested separately, on disease

development. Other experiments, however, have shown that an interaction often exists between WP temperature and WP duration. In these studies the minimum WP duration required for infection is dependent on the WP temperature. Hence, most experiments that are set up to study leaf surface wetness also must take into account the relationship or interaction between the duration and the temperature (Jones, 1986; Rotem et al., 1971). This was illustrated by Rotem et al. (1971) who worked with Phytophthora infestans (Mont.) De Bary on potato leaves. The maximum, optimum, and minimum levels of WP temperature, WP duration, and inoculum concentration were highly dependent on the combination of the levels of each individual factor. Several studies in bioherbicide research, with WP temperature and WP duration as factors, also showed this to be true (Boyette & Walker, 1985; Capo, 1979; Webb & Lindow, 1987).

The objective of this experiment was to evaluate the joint effect of wetness period temperature and wetness period duration on the infection of lamb's-quarters by Ascochyta hyalospora.

4.3.2 Methods

Lamb's-quarters plants were prepared by the soil germination method (See Section 4.1.2). Standard harvesting procedures (See Section 4.1.3) were used to collect the required amount of inoculum for spray application onto the plants. The two factors comprising the experiment were WP duration and WP temperature. Five WP durations (0 hr, 6 hr, 12 hr, 18 hr, and 24 hr) and four temperatures (12C, 18C, 24C, and 30C) were tested in all combinations for a 5 X 4 factorial. The

experiment was a randomized complete block design (RCBD) with the treatment combinations blocked in time. Each treatment combination was replicated four times. Since the experiment was blocked in time, plant growth and inoculum production were synchronized to obtain similar sets of experimental materials for each block. An experimental unit consisted of a 12.5-cm pot containing five plants at the 4-leaf stage of growth. The different temperatures were achieved by setting each of four growth cabinets to the required temperature. Temperature was adjusted and rotated among the cabinets for each new block of treatment combinations. Different WP durations were achieved by placing the recently sprayed pot in a humid plastic bag. The covered pots were then placed in the growth cabinets. Approximately every six hours, the plants that remained in the plastic bags were lightly misted with water. At the end of each wetness period duration, the pot of plants at each temperature was unbagged, but left in the growth cabinet with the other plants. The growth cabinets were kept dark for the entire 24-hour period. At the end of this 24-hour period the plants were removed from the growth cabinets and placed in a growth cabinet set at standard conditions (See Section 4.1.2).

Four, eight, and 12 days after inoculation, the area affected by disease on the four oldest leaves of each plant was scored using the Barrett-Horsfall rating system. Area-under-the-disease-progress-curve (AUDPC) values were calculated based on the scores from the three rating dates. Since no disease was present at 0 WP, the values were analyzed as a 4 X 4 factorial. Significant interaction between WP temperature and WP duration was examined further by using orthogonal polynomial regression

(PC-SAS GLM).

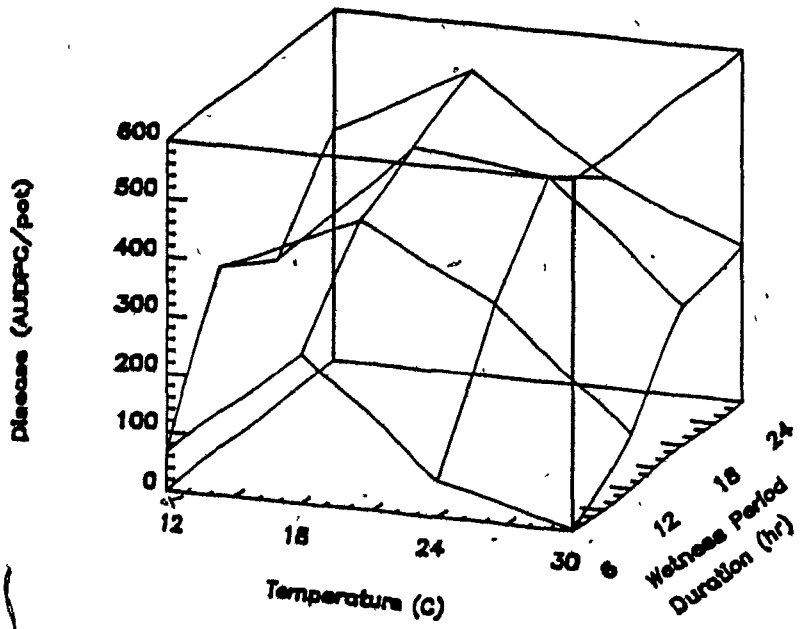
Each block was harvested separately two weeks after inoculation. The total height of plants in each experimental unit (pot) was measured, the plants harvested, dried in 70C ovens for several days, and then weighed. The procedure was repeated for each block. Analysis of the height (cm/pot) and dry weight (g/pot) means was similar to that of log AUDPC.

4.4.3 Results

A three-dimensional presentation of AUDPC means (disease development) in Figure 19, indicates that there is a convex-shaped response surface with the highest value occurring at 18C temperature and 24 hour duration. The lowest mean value was the treatment combination of 30C temperature and six hour duration. Due to the widely varying responses to the effect of the treatment combinations, the assumption of homogeneous variance was not met. Therefore, the data were transformed using the logarithmic transformation [log(y+1)]. The means of the disease development data (log AUDPC/pot) can be found in Table 11. The responses of dry weight and height were quite similar to each other (Figures 20 & 21). The three-dimensional presentation of the means shows concave relationships. At each temperature, the dry weight and height means generally decreased with increasing length of WP duration. The decrease in dry weight and height with increasing WP duration was greater at 18C and 24C than at 12C and 30C. Using a control of WP=0 hours averaged over all temperatures, the largest decrease in dry weight (60%) occurred after a 12 hour WP at 24C. The largest decrease in height was also 60% but this occurred after an 18

Figure 19. Effect of wetness period temperature and wetness period duration on disease development of lamb's-quarters (means of four replicates).

Figure 20. Effect of wetness period temperature and wetness period duration on dry weight of lamb's-quarters (means of four replicates).


Disease (AUDPC/pot)
600
500
400
300
200
100
0
12
18
24
30
Temperature (C)
6
12
18
24
Wetness Period Duration (hr)

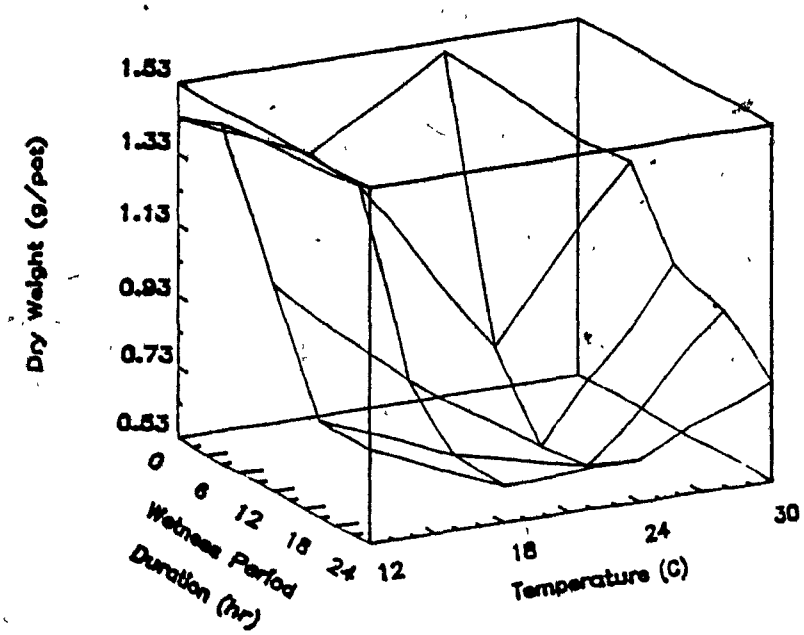
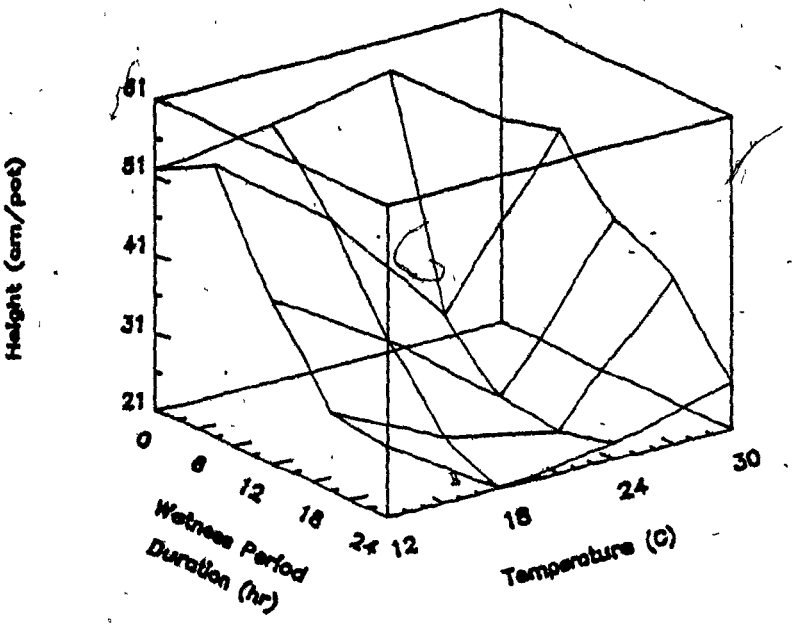

Dry Weight (g/pot)
1.53
1.33
1.13
0.93
0.73
0.53
0
6
12
18
24
Wetness Period Duration (hr)
12
18
24
30
Temperature (C)

Figure 21. Effect of wetness period temperature and wetness period duration on height of lamb's-quarters (means of four replicates).


Height (cm/pot)
61
51
41
31
21
0
6
12
18
24
Wetness Period
Duration (hr)
12
18
24
30
Temperature (C)

hour WP at 24C and after a 24 hour WP at 18C. The dry weight and height means are shown in Tables 12 & 13.

TABLE 11. Effect of wetness period temperature and wetness period duration on disease development (log AUDPC/pot) in lamb's-quarters inoculated with Ascochyta hyalospora.*

Wetness Period (hrs)	Temperature(C) 12	18	24	30
6	1.57	2.34	1.41	0.46
12	2.42	2.59	2.44	1.79
18	2.36	2.64	2.63	2.28
24	2.58	2.67	2.54	2.39

standard error = ±0.14

* means of four replicates

TABLE 12. Effect of wetness period temperature and wetness period duration on dry weight (g/pot) of lamb's-quarters inoculated with Ascochyta hyalospora.*

Wetness Period (hrs)	Temperature(C) 12	18	24	30
0	1.43	1.27	1.50	1.19
6	1.49	1.25	0.74	1.20
12	1.11	0.78	0.54	0.99
18	0.80	0.64	0.55	0.93
24	0.79	0.63	0.65	0.81

standard error = ±0.11

* means of four replicates

TABLE 13. Effect of wetness period temperature and wetness period duration on height (cm/pot) of lamb's-quarters inoculated with Ascochyta hyalospora.*

Wetness Period (hrs)	Temperature(C) 12	18	24	30
0	52	54	57	47
6	56	45	29	48
12	42	32	22	41
18	30	24	21	37
24	30	21	23	27

standard error = ±4

* means of four replicates

Interaction between WP temperature and WP duration was present (P = 0.0001) in the analysis of variance of disease development (Table 14). Using orthogonal polynomial contrasts, the response of disease development to WP temperature was investigated at each WP duration (T in WP, Table 15). Differences among means were found at six hours (P=0.0001) and 12 hours (P=0.0001), whereas no significant differences were found among the means across the temperature levels at 18 hours (P=0.1849) and 24 hours (P=0.5724). When the fitted equations were plotted, a quadratic trend was evident at six hours and to a lesser extent at 12 hours (Figure 22).

The disease development response can also be evaluated by considering the effect of WP duration at each WP temperature (WP in T, Table 15). Differences among the means were found at T=12C (P=0.0001), T=24C (P=0.0001), and T=30C (P=0.0001). There were no significant differences for T=18C (P=0.3349). All three temperatures (12C, 18C, and 30C) had a quadratic component

(Table 15, Figure 23). These response curves in Figure 23 show a large difference between 30C and the other temperatures after six hours of wetness. After 24 hours of wetness the differences are much less.

TABLE 14. Analysis of variance on the effects of wetness period temperature and wetness period duration on disease development in lamb's-quarters inoculated with Ascochyta hyalospora.

Source*	df	Mean Square	P > F
R	3	0.4581	0.0019
T	3	1.8787	0.0001
WP	3	4.1695	0.0001
T X WP	9	0.3951	0.0001
Error	45	0.0791	

* R=block, T=temperature, WP=duration

Analyses of the sources of variation for dry weight and height responses showed that, as with disease development, the T X WP interaction existed for both responses (P=0.0170 and P=0.0103, respectively)(Table 16). For dry weight, partitioning the SS for WP temperature at each WP duration (T in WP, Table 17)indicated that there were no significant differences between temperatures at WP=0 hours (P=0.2015), WP=18 hours (P=0.0970), and WP=24 (P=0.5697). There were, however, differences among the means at WP=6 hours (P=0.0002) and WP=12 hours (P=0.0039). A cubic trend was found to be present at WP=6 hours, whereas a quadratic trend was present at WP=12 hours (Figure 24).

Investigating the dry weight responses to WP duration at each temperature level (WP in T, Table 17), all temperature levels were found to have significant differences between WP durations (P=0.0001 for each temperature level). The level

TABLE 15. Orthogonal polynomial contrasts and fitted regression equations for disease development (log AUDPC/pot).

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
T IN WP					
T in WP6	3	2.3745	0.0001	Log AUDPC = - 11.817 + 2.055*T	0.71
T linear	1	3.6161	0.0001	$- 0.094*T^2 + 0.0013*T^3$	
T quadratic	1	2.9383	0.0001		
T cubic	1	0.5690	0.0102		
T in WP12	3	0.5034	0.0011	Log AUDPC = 0.749 + 0.207*T	0.59
T linear	1	0.8215	0.0024	$- 0.0057*T^2$	
T quadratic	1	0.6822	0.0052		
T cubic	1	0.0066	0.7737		
T in WP18	3	0.1328	0.1849		
T in WP24	3	0.0533	0.5724		

TABLE 15. (continued)

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
WP IN T					
WP in T12	3	0.8153	0.0001	Log AUDPC = 0.696 + 0.1816*WP	0.57
WP linear	1	1.7693	0.0001	$- 0.0044*WP^2$	
WP quadratic	1	0.4019	0.0291		
WP cubic	1	0.2747	0.0689		
WP in T18	3	0.0918	0.3349		
WP in T24	3	1.3036	0.0001	Log AUDPC = - 0.043 + 0.293*WP	0.73
WP linear	1	2.5890	0.0001	$- 0.0078*WP^2$	
WP quadratic	1	1.2532	0.0002		
WP cubic	1	0.0685	0.3568		
WP in T30	3	3.1440	0.0001	Log AUDPC = - 1.367 + 0.359*WP	0.85
WP linear	1	7.8970	0.0001	$- 0.00849*WP^2$	
WP quadratic	1	1.4947	0.0001		
WP cubic	1	0.0404	0.4785		
ERROR	45	0.0791			

* T=temperature, WP=duration, WP6=6 hr, WP12=12 hr, WP18= 18 hr, WP24=24 hr, T12=12C, T18=18C, T24=24C, T30=30C

T=24C exhibited a quadratic response while the remaining levels showed strong linear responses (Figure 25). There was a significant cubic trend at T=12C (P=0.0445) but a cubic term was not included in the regression equation since a large proportion of the SS belonged to the linear component (88 %). Only a small proportion of the SS (12%) could be attributed to the cubic term.

TABLE 16. Analysis of variance on the effects of wetness period temperature and wetness period duration on dry weight and height of lamb's-quarters inoculated with Ascochyta hyalospora.

Source*	df	Dry Weight Mean Square	P > F	Height Mean Square	P > F
R	3	1.2715	0.0001	945.9458	0.0001
T	3	0.3983	0.0002	540.2792	0.0001
WP	4	1.2596	0.0001	2098.1375	0.0001
T X WP	12	0.1178	0.0170	167.7375	0.0103
Error	57	0.0508		66.9985	

* R=block, T=temperature, WP=duration.

Height was quite similar to dry weight, in both the response to WP at each temperature (WP in T, Table 18) and the response to temperature at each WP duration (T in WP, Table 18). There was, however, one additional equation added to the response to temperature (WP=18 hours at P=0.0321)(T in WP, Table 18). The response at this level was quadratic in nature. The other levels had trends similar to those in dry weight (Figures 26 & 27).

Figure 22. Effect of wetness period temperature and wetness period duration on disease development with data arranged as temperature within duration (T in WP).

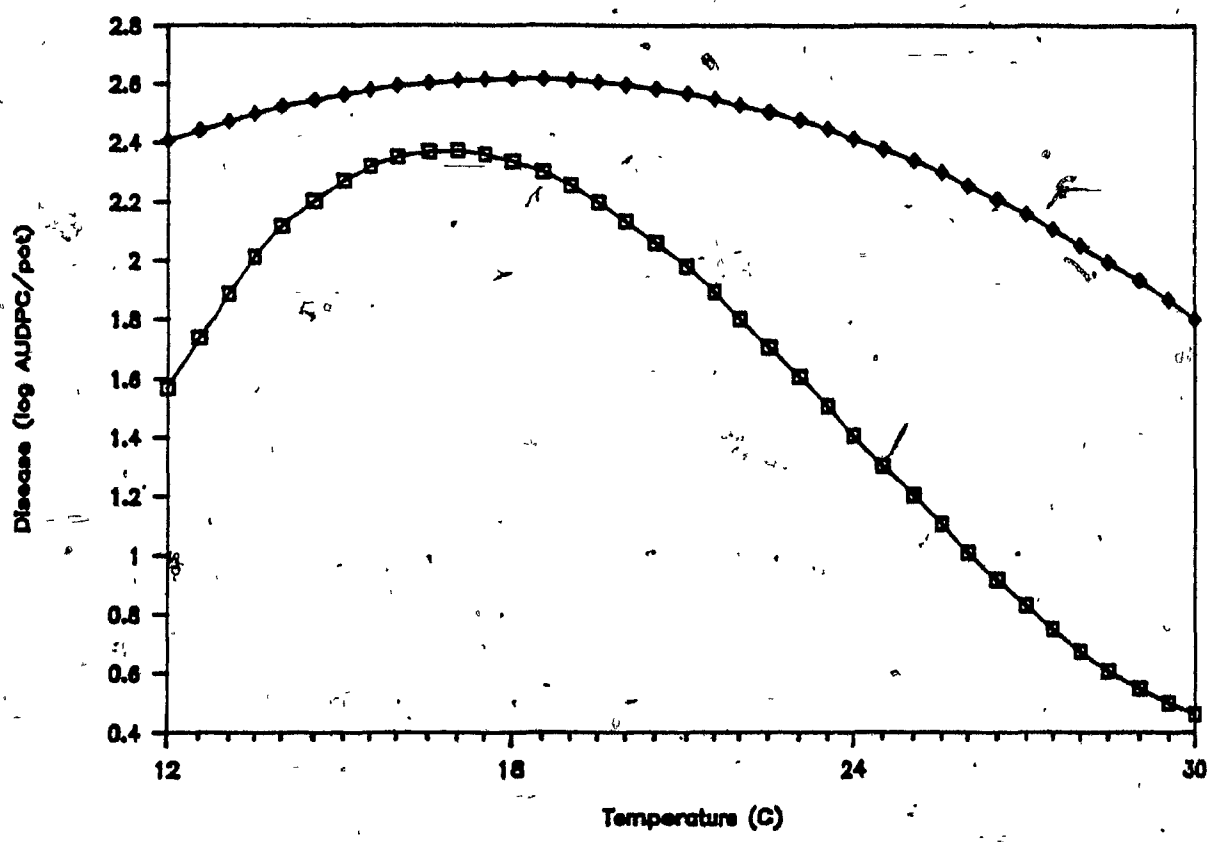
Wetness Period Duration

□ 6 hrs ◇ 12 hrs

Figure 23. Effect of wetness period temperature and wetness period duration on disease development with data arranged as duration within temperature (WP in T).

Temperature

□ 12C ◇ 24C △ 30C


Disease (log AUDPC/pot)
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
12
18
24
30
Temperature (C)

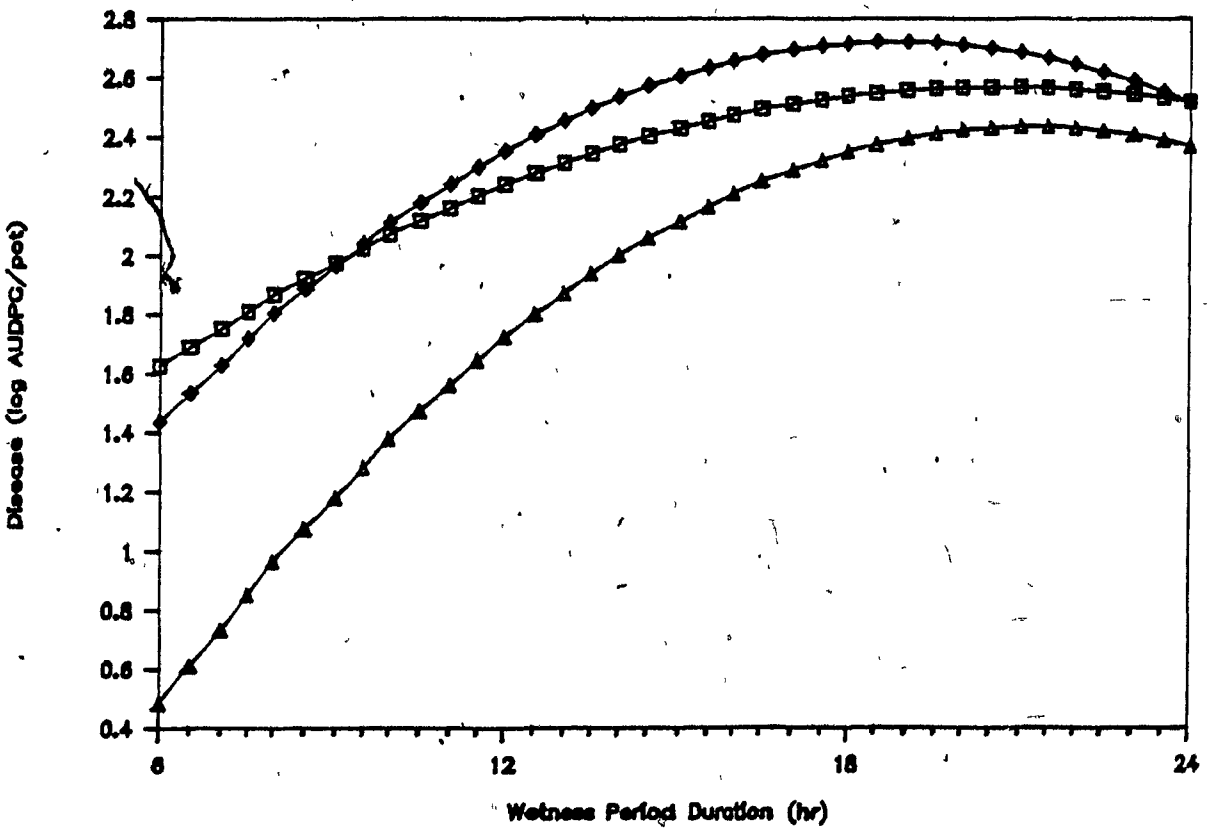

Disease (log AUDPC/pot)
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
6
12
18
24
Wetness Period Duration (hr)

TABLE 17. Orthogonal polynomial contrasts and fitted regression equations for dry weight (g/pot).

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
T IN WP					
T in WP0	3	0.0808	0.2015		
T in WP6	3	0.3900	0.0002	dry wt = -3.87775 + 0.9795833*T	0.47
T linear	1	0.3696	0.0091	$- 0.0559653*T^2 + 0.0009653*T^3$	
T quadratic	1	0.4872	0.0030		
T cubic	1	0.3130	0.0160		
T in WP12	3	0.2523	0.0039	dry wt = 3.21086 - 0.2379146*T	0.35
T linear	1	0.0706	0.2430	$+ 0.0054288*T^2$	
T quadratic	1	0.6111	0.0010		
T cubic	1	0.0751	0.2289		
T in WP18	3	0.1121	0.0970		
T in WP24	3	0.0344	0.5697		

TABLE 17. (continued)

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
WP IN T					
WP in T12	4	0.4405	0.0001	dry wt = 1.5159 - 0.032725*WP	0.50
WP linear	1	1.5421	0.0001		
WP quadratic	1	0.0009	0.8929		
WP cubic	1	0.2143	0.0445		
WP quartic	1	0.0046	0.7642		
WP in T18	4	0.4110	0.0001	dry wt = 1.2918 - 0.0313833*WP	0.55
WP linear	1	1.4183	0.0001		
WP quadratic	1	0.0347	0.4118		
WP cubic	1	0.1333	0.1106		
WP quartic	1	0.0577	0.2906		
WP in T24	4	0.6453	0.0001	dry wt = 1.4488 - 0.1233464*WP	0.59
WP linear	1	1.4236	0.0001	+ 0.00382937*WP^2	
WP quadratic	1	1.0643	0.0001		
WP cubic	1	0.0915	0.1847		
WP quartic	1	0.0020	0.8440		
WP in T30	4	0.6453	0.0001	dry wt = 1.23215 - 0.0172667*WP	0.13
WP linear	1	0.4293	0.0052		
WP quadratic	1	0.0039	0.7823		
WP cubic	1	0.0103	0.6535		
WP quartic	1	0.0208	0.5250		
ERROR	57	0.0508			

* T=temperature, WP=duration, WP0=0 hr, WP6=6 hr, WP12=12 hr, WP18=18 hr, WP24=24 hr T12=12C, T18=18C, T24=24C, T30=30C

Figure 24. Effect of wetness period temperature and wetness period duration on dry weight with data arranged as temperature within duration (T in WP).

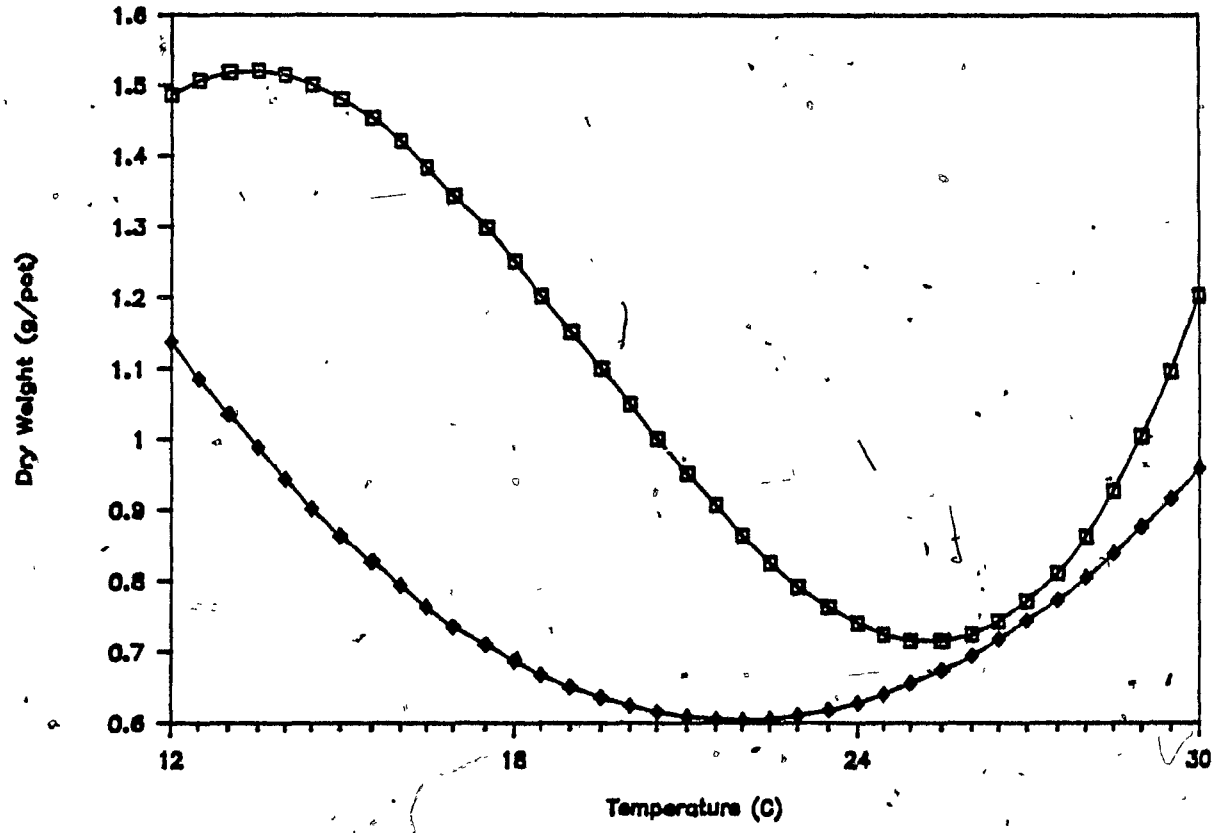
Wetness Period Duration

□ 6 hrs ◆ 12 hrs

Figure 25. Effect of wetness period temperature and wetness period duration on dry weight with data arranged as duration within temperature (WP in T).

Temperature

□ 12C ◆ 18C ▲ 24C ▼ 30C


Dry Weight (g/pot)
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
12
18
24
30
Temperature (C)

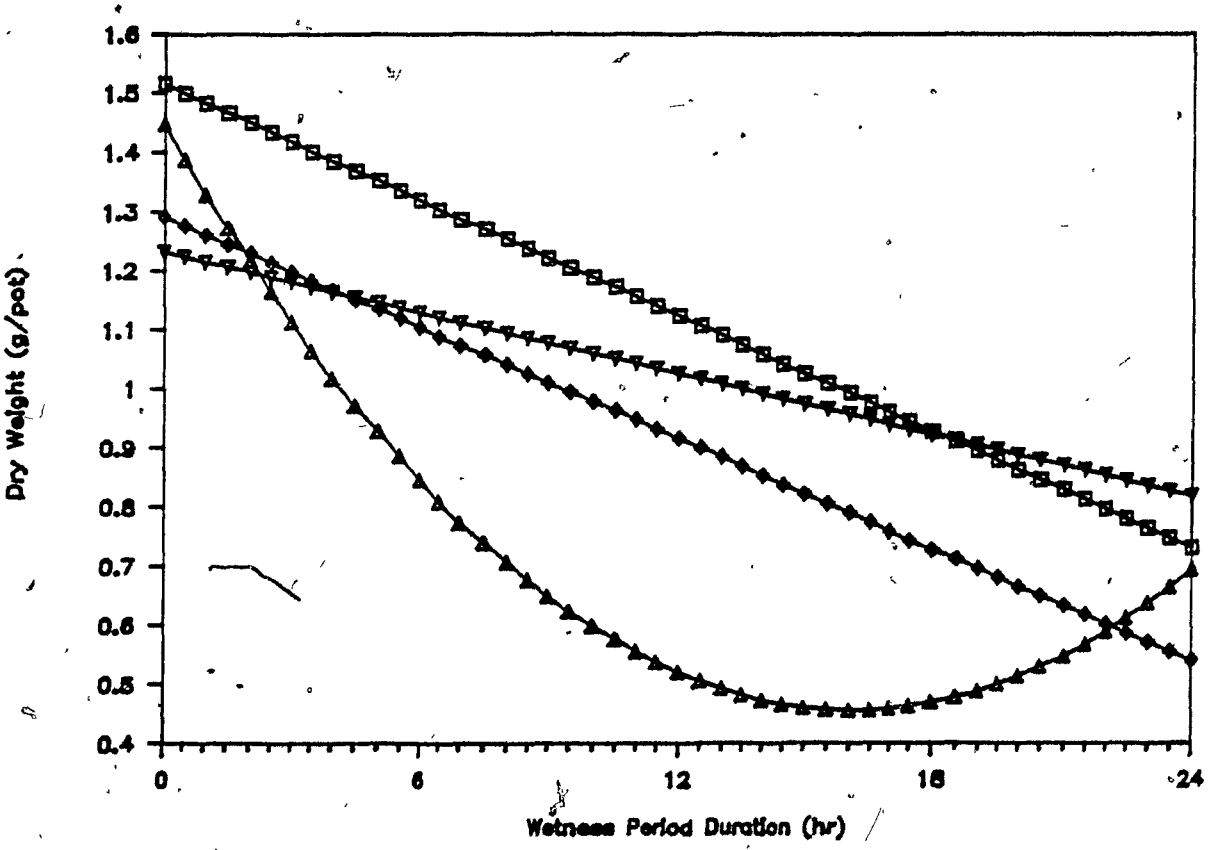

Dry Weight (g/pot)
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0
6
12
18
24
Wetness Period Duration (hr)

TABLE 18. Orthogonal polynomial contrasts and fitted regression equations for height (cm/pot).

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
T IN WP					
T in WP0	3	74.7500	0.3503		
T in WP6	3	529.0833	0.0002	ht = - 107.75 + 30.9375*T	0.55
T linear	1	304.2000	0.0374	$- 1.8298611*T^2 + 0.0324074*T^3$	
T quadratic	1	930.2500	0.0004		
T cubic	1	352.8000	0.0255		
T in WP12	3	335.0000	0.0038	ht = 115.45 - 8.366667*T	0.36
T linear	1	28.8000	0.5147	$+ 0.1944444*T^2$	
T quadratic	1	784.0000	0.0012		
T cubic	1	192.2000	0.0958		
T in WP18	3	210.5000	0.0321	ht = 85.475 - 6.4333333*T	0.27
T linear	1	54.4500	0.3711	$+ 0.1597222*T^2$	
T quadratic	1	529.0000	0.0068		
T cubic	1	48.0500	0.4006		
T in WP24	3	61.8958	0.4352		

TABLE 18. (continued)

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
WP IN T					
WP in T12	4	572.5000	0.0001	ht = 55.90 - 1.1583333*WP	0.48
WP linear	1	1932.1000	0.0001		
WP quadratic	1	8.6429	0.7208		
WP cubic	1	336.4000	0.0290		
WP quartic	1	12.8571	0.6630		
WP in T18	4	796.7000	0.0001	ht = 52.90 - 1.4583333*WP	0.71
WP linear	1	3062.5000	0.0001		
WP quadratic	1	82.5714	0.2716		
WP cubic	1	40.0000	0.4429		
WP quartic	1	1.7286	0.8730		
WP in T24	4	938.4500	0.0001	ht = 55.2428 - 4.4809524*WP	0.64
WP linear	1	2310.4000	0.0001	+ 0.1339286*WP^2	
WP quadratic	1	1301.7857	0.0001		
WP cubic	1	129.6000	0.1697		
WP quartic	1	12.0143	0.6736		
WP in T30	4	938.4500	0.0001	ht = 50.35 - 0.85*WP	0.34
WP linear	1	1040.4000	0.0002		
WP quadratic	1	103.1429	0.2198		
WP cubic	1	4.2250	0.8026		
WP quartic	1	27.0321	0.5278		
ERROR	57	66.9985			

* T=temperature, WP=duration, WP0=0 hr, WP6=6 hr, WP12=12 hr, WP18=18 hr, WP24=24 hr
T12=12C, T18=18C, T24=24C, T30=30C

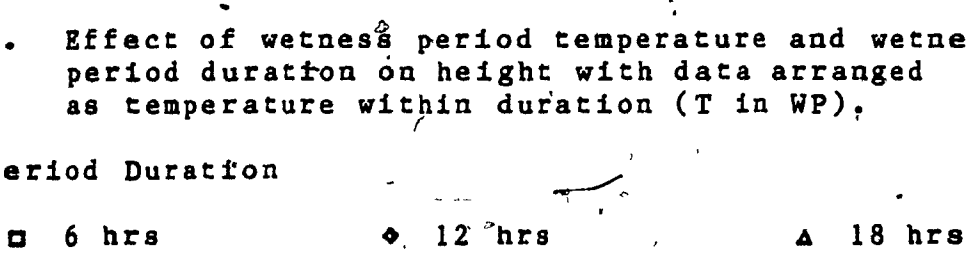
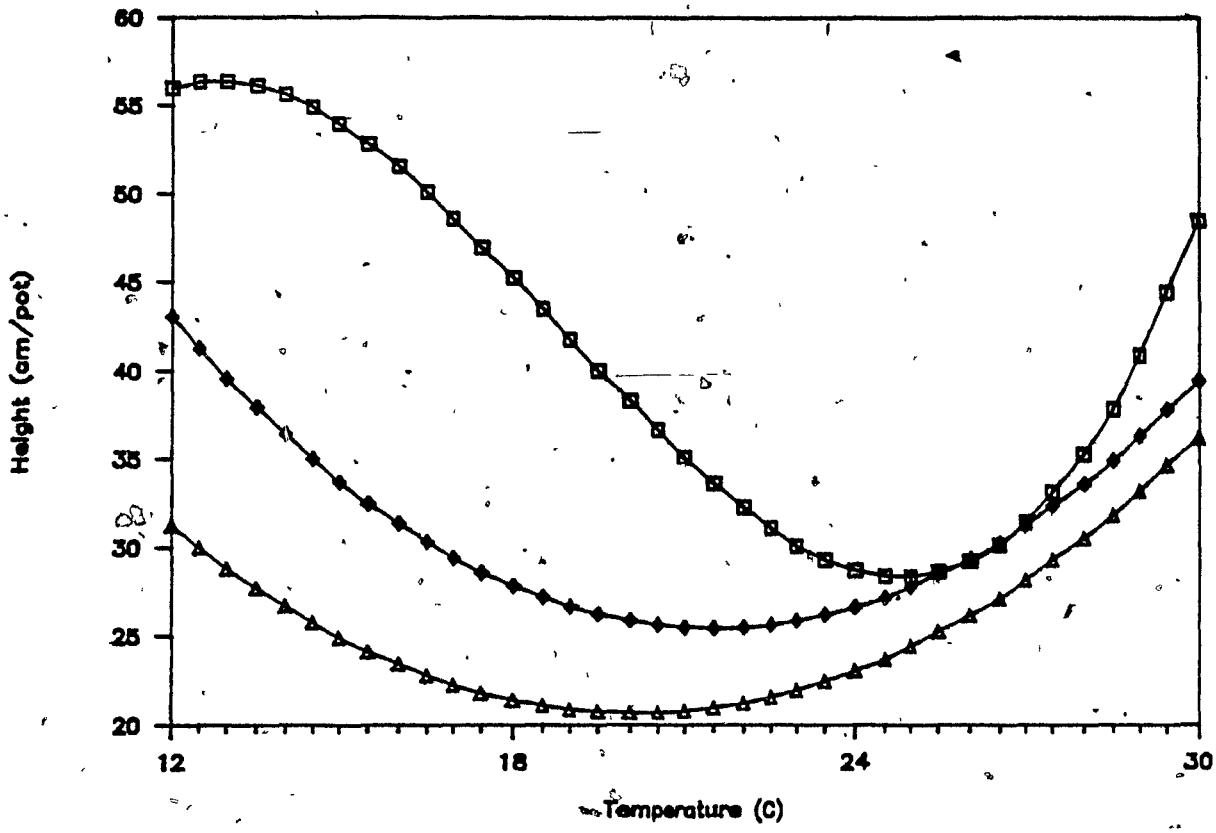
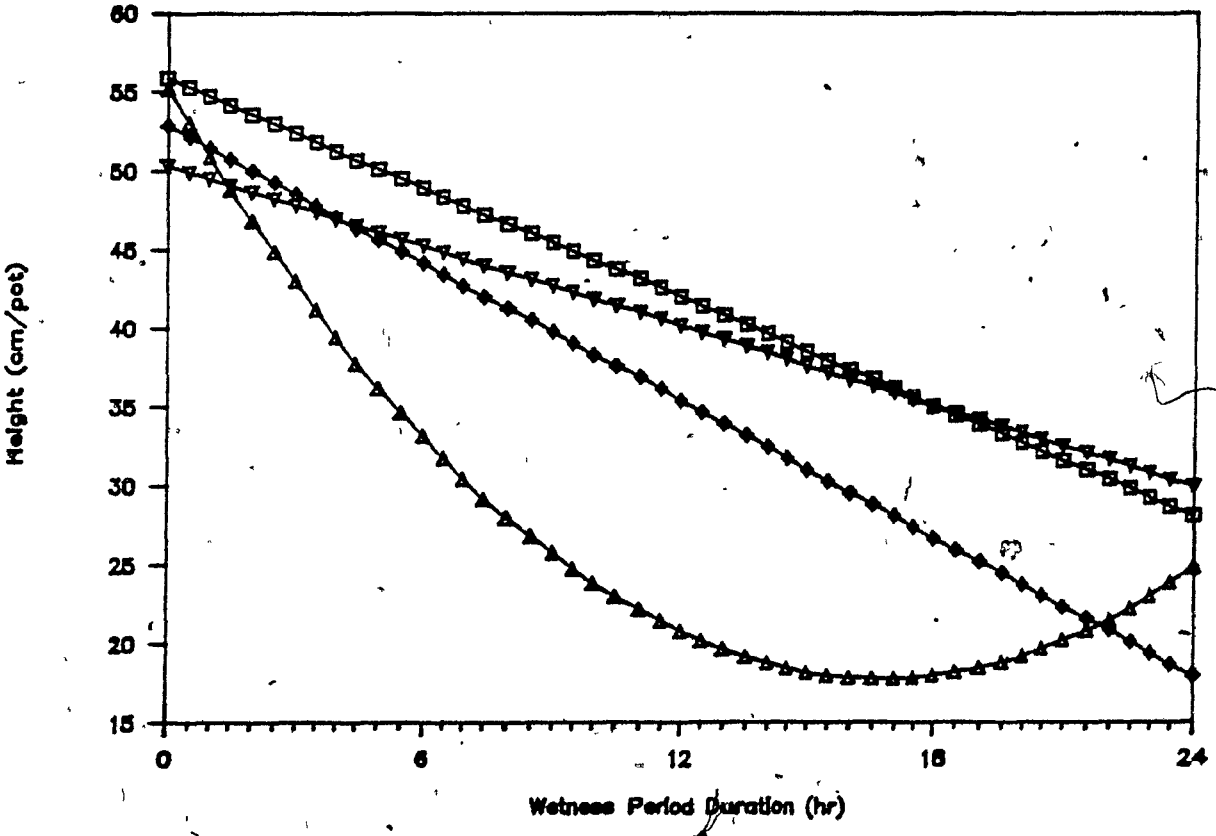


Figure 26. Effect of wetness period temperature and wetness period duration on height with data arranged as temperature within duration (T in WP).

Figure 27. Effect of wetness period temperature and wetness period duration on height with data arranged as duration within temperature (WP in T).


Height (cm/pot)
60
55
50
45
40
35
30
25
20
12
18
24
30
Temperature (C)


Height (cm/pot)
60
55
50
45
40
35
30
25
20
15
0
6
12
18
24
Wetness Period Duration (hr)

4.3.4 Discussion

Similar to other host-pathogen studies (Section 4.3.1), there was interaction between WP temperature and WP duration in this experiment. The WP duration needed to obtain a certain level of infection was dependent on temperature. The minimum WP duration for infection to occur could be obtained only with an optimum temperature. Therefore, longer periods of wetness were required when temperatures were higher or lower than the optimum temperature. In general, the longer the WP duration, the greater the amount of disease and the greater the decrease in height and dry weight of lamb's-quarters plants. The rate at which this occurred depended on the temperature. There did, however, appear to be a maximum amount of disease. There were no large increases in disease beyond this maximum amount, even with wetness periods of longer duration. For example, the maximum log AUDPC for 18C appeared to have been reached after only a six hour wetness period. In addition, the quadratic trend of the other three temperatures indicated a slowing down or levelling off of increase in log AUDPC, particularly after 18 hours of leaf wetness.

It is important to note that at the wetness period of 0 hours there were no significant differences among the means of dry weight and height. This indicates the importance of a wetness period of a minimum length in order for infection to occur. The result at WP=0 hours also serves as a control, indicating that the brief time (24 hours) spent at different WP temperatures in the dark growth cabinets, by itself did not affect the plant height and dry weight.

The effect of temperature on disease development,

indicated by all three response variables (log AUDPC, dry weight, and height), was most apparent after a wetness period of six hours. At this point, there was a quadratic response which could indicate a faster rate of infection at optimum temperatures (18C for log AUDPC and 24C for height and dry weight). At wetness periods of longer duration, the response flattened and became more horizontally linear in character, until 18 hours (24 hours for height) where there were no significant differences among the means at the four temperatures. This indicated that infection levels at other suboptimal temperatures eventually became more equal to those at the optimum temperature when the wetness period was long enough.

The apparent increase in height and dry weight from 18 to 24 hours WP at 24C was unexpected. This phenomenon, however, has been noted before in other experiments. Rotem et al. (1971), suggested that decreased infection levels at longer durations of leaf wetness might be due to poor gas exchange during an extended period of incubation in a plastic bag. They found the phenomenon at all temperatures tested. In the experiment reported here, the phenomenon was only present at 24C for the fitted response curves of dry weight and height. The fitted response curves for the other temperatures were still decreasing at the 24 hour WP. The increase of the response variables at 24 hours WP and 24C may also be an artefact due to the large amount of variability in the data.

It is difficult, if not impossible, to relate the performance (disease development, dry weight and height decrease) of the pathogen in this experiment with its possible

performance in the field as a bioherbicide. This is due, in part, to the fact that there are likely to be more than just temperature and wetness period factors interacting in the field. Taking this into account, it can be stated that the optimum field temperatures would probably lie between 18C - 24C, and that the duration of the minimum wetness period required in the field would probably lie between 12 - 18 hours.

4.4 Effect of Interrupted Wetness Periods on Disease Development and Subsequent Plant Damage

4.4.1 Introduction

In mycoherbicide research, wetness periods (WP) of short duration can be a constraint on disease development. Andersen and Lindow (1984), working with Alternaria sp. on Carduus pycnocephalus L. (Italian thistle), found that 100% mortality occurred after a 24 hour wetness period if an adequate amount of inoculum was applied. A four hour WP was needed for infection to occur. They recognized that most dew-forming conditions in the field were not long enough for optimum infection, even though some infection would likely occur. The WP requirements for another potential mycoherbicide, Fusarium lateritium Nees ex Fr. on Abutilon theophrasti Medic. (velvetleaf) and Sida spinosa L. (prickly sida), were restrictive. Boyette and Walker (1985), however, concluded that these restrictions would not be too stringent for the typical summer weather of the southeastern U.S. TeBeest et al. (1978), working with Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. aeschynomene on Aeschynomene virginica (L.) B.S.P. (northern jointvetch), found that disease development was rapid at the optimum temperature. They suggested, however, that immediate

penetration might not be necessary since the appressoria formed by Colletotrichum would be able to remain dormant under adverse conditions.

The standard WP duration used in the present studies on Ascochyta hyalospora (20 hours) is longer than the WP most likely to occur in the field. Based on collected field evidence, WP's are rarely as long as 20 hours, although shorter wetness periods occur frequently. A study of dew on wheat in England indicated that water is present at least some time during most 24-hour periods. Night-time dew periods ranged from four to 14 hours (Burrage, 1972). In Quebec, during a six week period in June and July of 1987, WP's were recorded in 33 of the 42 24-hr periods. The WP's, averaging approximately six hours in duration, ranged from 5 minutes to 31 hours (Armour & Wymore, 1987). Kolbe (1985) reported that at the Hofchen Experimental Station in West Germany, the average number of nights with dew in June and July of the years 1966 -1983 were 11 and 12, respectively.

Wetness periods are not always continuous but are interrupted by dry periods ranging from a few minutes to a day or more (Jones, 1986). These dry periods may increase the mortality of spores that have not completed the germination process, but on the other hand the spores might survive the dry period if the previous wet period was too short for the germination process to advance into a sensitive stage. Thus the effect of each wetness period can be cumulative. According to Jones (1986), the extent to which each additional WP increases the development of disease may depend on the many factors affecting the survival of the spores.

Bashi and Rotem (1974) have observed that plant pathogens are adapted to incite disease under varying wet period conditions. Conidia of Stemphylium botryosum f. sp. lycopersici (R., G. & W.), under continuous wet period (CWP) or interrupted wet period (IWP) conditions, infected tomatoes equally well. Alternaria porri f. sp. solani Neerg. was able to infect potato leaves under IWP with short first wetness periods (4 hrs) separated by long dry periods (20 hrs) but did much better under CWP conditions. Phytophthora infestans (Mont.) De Bary infected poorly under IWP conditions where the first wetness period was short (4 hrs) but did much better when the first wetness period was longer (6 hrs) or continuous. Uromyces phaseoli (Reb.) Wint. was affected similarly except it needed an even longer first wetness period (8 hrs) to achieve some infection.

Other studies with several plant pathogens have also recorded varying reactions to the effect of CWP and IWP conditions on disease development. Studies with Coccomyces hiemalis Higgins on Prunus cerasus L. 'Montmorency' (sourcherry) (Eisensmith et al., 1982) and Glomerella cingulata (Stonem.) Spauld & Schrenk var. orbiculare S.F. Jenkins & Winstead on Cucumis sativus L. 'Green Prolific' (cucumber) indicate that IWP conditions reduce the amount of infection compared to a CWP with an equivalent number of wet hours. Studies with Stagonospora maculata (G.) Sprague on Dactylis glomerata L. (orchardgrass) (Graham and Sprague, 1953) and Venturia inaequalis (Cke) Wint. on Malus sylvestris Mill. (apple) (Schwabe, 1980) appeared to show approximately the same amount of infection whether the wetness period was interrupted or continuous. Moore (1964)

found that long dry periods of 24 hours or more reduced leaf infection caused by Venturia inaequalis.

The effect of IWP on the conidia of several fungi has been shown in two in vitro studies. When conidia of Botrytis cinerea Pers. ex Fr., Cercospora musae Zinn., or Monilinia fructicola (Wint.) Honey were germinated on moist dialyzing membrane and then exposed to dry periods of eight to 12 hours, 60 to 90 % of the conidia survived (Good and Zathureczky, 1967). In a similar experiment, Grindle and Good (1961) found that higher relative humidities and temperatures during the dry period contributed to increased mortality of conidia of Monilinia fructicola.

The objectives of the tests in this section were to observe and measure the effects of interrupted wetness periods on Ascochyta hyalospora conidia on dialyzing membranes and on leaves of whole plants.

4.4.2 Methods

In vitro dialyzing membrane experiment

Dialyzing membrane was cut into 1-cm squares and placed onto water agar (WA) in plates. In order to re-photograph the same location, two perpendicular lines were marked with a needle onto the surface of each membrane square. Twenty-five µl of conidial suspension were placed on the surface of each membrane square. The petri plates containing the squares were then placed in darkness at approximately 23C for varying periods of time, depending on the treatment. If the treatment required a dry period, the squares were transferred for 14 hours to a glass desiccator containing $CaSO_4$ (Drierite). During the course of the dry period, the glass desiccator was kept in the dark at

room temperature. When the dry period ended, the squares were then transferred back to the petri plates. At the end of each moisture period, photographs were taken of pre-marked locations on the membrane squares. There were three treatments: 1) 2 hours on water agar (WA) --- photographed (PHOTO) --- 14 hours in desiccator --- 8 hours on WA --- PHOTO --- 14 hours in desiccator --- 2 hours on WA plus 12 hours on lamb's-quarters agar (LQA) --- PHOTO, 2) 10 hours on WA --- PHOTO --- 14 hours in desiccator --- 2 hours on WA plus 12 hours on LQA --- PHOTO, and 3) 12 hours on WA plus 8 hours on LQA --- PHOTO. A minimum of four squares were used per treatment.

In vivo plant inoculation experiment

An experiment (randomized complete block design) was set up to measure the effect of an IWP on area-under-the-disease-progress-curve (AUDPC), height, and dry weight of lamb's-quarters plants. Four levels of wetness period conditions and two levels of inoculation were tested. The levels of the wetness period factor consisted of 0 hours wetness period, 10 hours continuous wetness period (CWP), 10 hours wetness period --- 14 hours dry period --- 10 hours wetness period (interrupted wetness period or IWP), and 20 hour continuous wetness period (CWP). The wetness periods were timed to coincide with the 10 hours of darkness during the usual 14 hr day/10 hr night under which the plants were grown. The dry cycle of the IWP treatment was obtained by placing the plants in the growth cabinet. Therefore, the first three levels were exposed to the same light/dark cycle. The plants in the 20 hr CWP level were kept in darkness for the full 20 hours. Dew

cabinet and growth cabinet temperatures were kept at standard conditions (See Section 4.1). The two levels of the inoculation factor were a control level in which the plants were sprayed only with distilled water, and an inoculum level where a suspension of conidia in distilled water was sprayed at a rate of 1 X 10^8 conidia/m^2. The experiment was a 4 X 2 factorial with ten replications of the treatment combinations. For the first test, plants at the 4- to 6-leaf growth stage were used. The experiment was repeated once with plants at the 6-leaf stage.

Disease development and the response of the plants to the disease were recorded by calculating the area-under-the-disease-progress-curve (AUDPC) for those inoculated plants exposed to a wetness period, by measuring the height of the plants, and by determining the above-ground dry weight. AUDPC values for the first experiment were calculated from the Barratt-Horsfall ratings that were recorded two, four, six, eight, 10, and 12 days after inoculation. Ratings for the second experiment were recorded two, four, and 10 days after inoculation. Analysis of variance was carried out on log AUDPC values (AUDPC values were log transformed to stabilize the variance), and the three treatment means of each experiment were compared using an LSD test.

Height measurements, for both experiments were measured 22 days after spraying. The plants were then cut at the soil surface, dried for several days in a 70C oven, and weighed. Analyses of variance were carried out on the height and dry weight measurements. Means within inoculated and uninoculated treatment combinations were compared using Tukey's test. Paired

comparisons of inoculated and uninoculated treatments at each wetness period level were made using the LSD test.

4.4.3 Results

Time-course photographs for two of the three treatments of the in vitro test are shown in Figure 28 (Treatment #1) and Figure 29 (Treatment #2). Figure 28a shows several germinated conidia. Figure 28b (after a dry period of 14 hours and an additional wet period of eight hours) shows that the germ tubes present after two hours have grown further and many more conidia have germinated. After a second 14 hour dry period and an additional 14 hour wet period, the germ tubes resumed growth (Figure 28c). Germination following an initial 10-hr WP is shown in Figure 29a. After a 14 hour dry period, the germ tubes resumed growth and more conidia germinated during an additional 14 hour wet period (Figure 29b).

The infection level for the two in vivo tests was lower than expected. The differences between some of the treatments were not as large as might have been expected based on previous work with Ascochyta hyalospora. The AUDPC measurements did show, however, differences between the treatments (Table 19 and Figure 30). In Test #1, 20 hours CWP produced a higher log AUDPC measurement than 10 hours CWP and 20 hours IWP. The log AUDPC measurements for the latter two treatments were not significantly different at $LSD_{.05}$. The results were different for Test #2, where additional hours of moisture led to increased amounts of log AUDPC so that 10 hrs CWP < 20 hours IWP < 20 hours CWP. According to the LSD test, 20 hours IWP was not significantly different from 20 hours CWP.

Figure 28

a. Treatment #1. Photographed after 2 1/2 hours on water agar.

b. Treatment #1. The same location as in Figure 28a but interrupted by a 14-hr dry period followed by an 8-hour moisture period.

c. Treatment #1. Same location subjected to a further 14-hour dry period followed by a 14-hour moisture period.

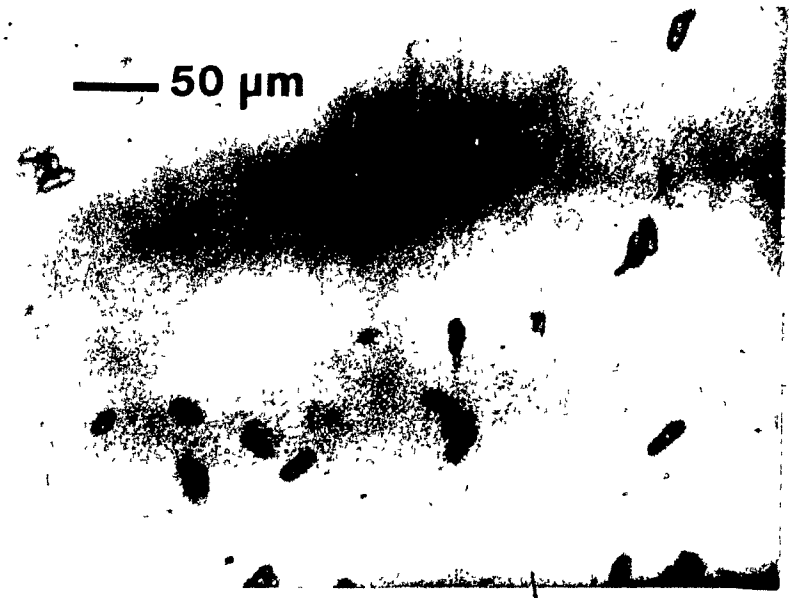

50 µm

Figure 29

a. Treatment #2. Photographed after 10 hours on water agar.

b. Treatment #2. The same location as in Figure 29a but interrupted by a 14-hour dry period followed by a 14-hour moisture period.

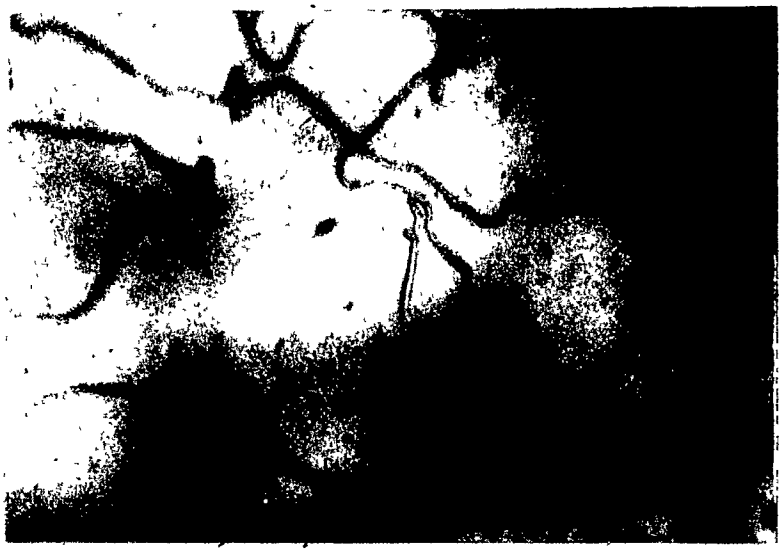


TABLE 19. Analysis of variance on the effects of wetness period regime on disease development in lamb's-quarters inoculated with Ascochyta hyalospora.

Source*	df	Mean Square	P > F
Test #1			
R	9	0.1910	0.0059
TRT	2	2.3815	0.0001
ERROR	18	0.0476	
Test #2			
R	9	0.1295	0.0879
TRT	2	0.8762	0.0002
ERROR	18	0.0620	

* R=block, WP=wetness period condition

The results of the analyses of variance for the height and dry weight measurements of tests #1 and #2 are shown in Table 20. Interaction between the two factors was significant at the 0.05 level except for the height measurement in test #1. Mean comparisons are shown in Tables 21 to 24. Overall, there were no significant differences among the height and dry weight measurements of the uninoculated treatments but there was a decreasing trend as cumulative wetness period increased. Among inoculated treatments, height and dry weight show a decreasing trend as the length of wetness period is increased. The differences between 10 hrs CWP and 20 hrs IWP are not as great as the differences between 20 hr's IWP and 20 hrs CWP. This is most clearly demonstrated by Tukey's test on dry weight and height in test #1 (Table 21 and 23). The paired comparisons of the uninoculated treatments with the inoculated treatments showed no significant differences except in the case of 20 hrs CWP (Tables 21, 22, & 23).

Figure 30. Effect of wetness period conditions on disease development in lamb's-quarters inoculated with Ascochyta hyalospora in test #1 and #2.

Wetness Period Conditions

10 hrs CWP 20 hrs IWP 20 hrs CWP

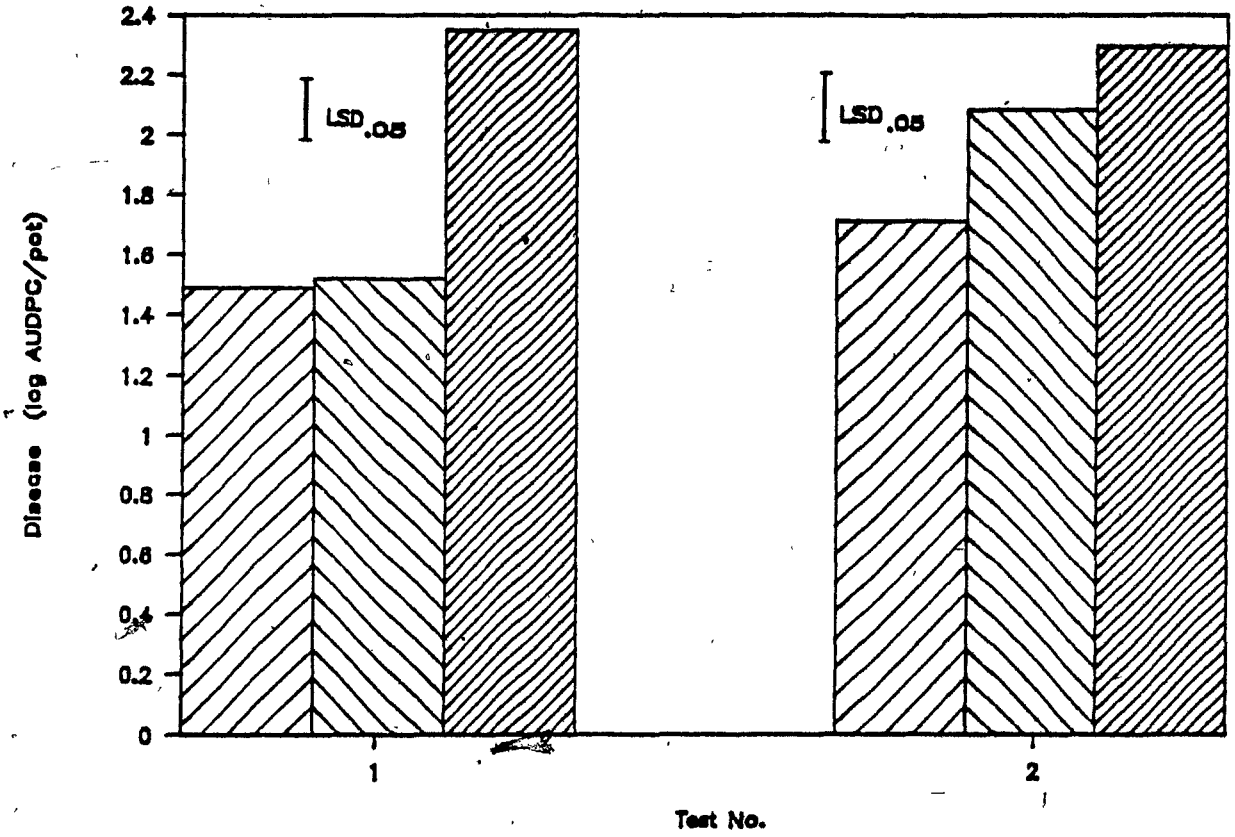

2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LSD.05
LSD.05
Disease (log AUDPC/pot)
1
2
Test No.

TABLE 20. Analysis of variance on the effects of inoculation state (inoculated or not inoculated) and wetness period regime on dry weight and height of lamb's-quarters.

Test #1

Source*	df	Dry Weight Mean Square	P > F	Height Mean Square	P > F
R	9	0.0488	0.0035	208.4458	0.0008
S	1	0.1804	0.0012	738.1125	0.0005
W	3	0.1558	0.0001	644.6792	0.0001
S*W	3	0.0495	0.0298	75.3792	0.2642
ERROR	63	0.0155		55.5569	

Test #2

Source*	df	Dry Weight Mean Square	P > F	Height Mean Square	P > F
R	9	0.0847	0.0001	570.8458	0.0001
S	1	0.0405	0.0576	165.3125	0.1342
W	3	0.1514	0.0001	831.0458	0.0001
S*W	3	0.0589	0.0022	252.1458	0.0202
ERROR	63	0.0108		71.8014	

* R=block, S=inoculation state, WP=wetness period condition

TABLE 21. Effect of wetness period condition (continuous or interrupted) and inoculated state on the dry weight (g/pot) of lamb's-quarters in test #1.

Wetness Period Condition[a]	Plant Dry Weight (g/pot)		
	Uninoculated[b]	Inoculated[c]	Difference[d]
0 hours wet	0.755	0.784 a	-0.029 ns
10 hours wet	0.774	0.681 a	0.093 ns
10 hours wet - 14 hours dry - 10 hours wet	0.768	0.666 a	0.102 ns
20 hours wet	0.675	0.460 b	0.215 **

[a] Average of 10 replications. [b] No significant differences among means according to Tukey's Test at the 5% level. [c] Mean separation within columns by Tukey's Test at 5% level.
[d] Difference between uninoculated and inoculated.
** = significant at 1% level, ns = not significant.

TABLE 22. Effect of wetness period condition (continuous or interrupted) and inoculated state on the dry weight (g/pot) of lamb's-quarters in test #2.

Wetness Period Condition[a]	Plant Dry Weight (g/pot)		
	Uninoculated[b]	Inoculated[c]	Difference[d]
0 hours wet	0.751	0.852 a	-0.101 *
10 hours wet	0.779	0.747 ab	0.032 ns
10 hours wet - 14 hours dry - 10 hours wet	0.753	0.655 bc	0.098 *
20 hours wet	0.677	0.526 c	0.151 **

[a] Average of 10 replications. [b] No significant differences among means according to Tukey's Test at the 5% level. [c] Mean separation within columns by Tukey's Test at 5% level.
[d] Difference between uninoculated and inoculated.
** = significant at 1% level, * = significant at 5% level, ns = not significant.

TABLE 23. Effect of wetness period condition (continuous or interrupted) and inoculated state on the height (cm/pot) of lamb's-quarters in test #1.

Wetness Period Condition[a]	Plant Height (cm/pot)		
	Uninoculated[b]	Inoculated[c]	Difference[d]
0 hours wet	49.9	47.4 a	2.5 ns
10 hours wet	47.5	42.3 a	5.2 ns
10 hours wet - 14 hours dry - 10 hours wet	46.1	41.1 a	5.0 ns
20 hours wet	41.0	29.4 b	11.6 **

[a] Average of 10 replications. [b] No significant differences among means according to Tukey's Test at 5% level. [c] Mean separation within columns by Tukey's Test at 5% level. [d] Difference between uninoculated and inoculated. ** = significant at 1% level, ns = not significant.

TABLE 24. Effect of wetness period condition (continuous or interrupted) and inoculated state on the height (cm/pot) of lamb's-quarters in test #2.

Wetness Period Condition[a]	Plant Height (cm/pot)		
	Uninoculated[b]	Inoculated[c]	Difference[d]
0 hours wet	56.8	63.5 a	-6.7 ns
10 hours wet	55.4	53.3 ab	2.1 ns
10 hours wet - 14 hours dry - 10 hours wet	55.5	45.8 b	9.7 *
20 hours wet	48.0	41.6 b	6.4 ns

[a] Average of 10 replications. [b] No significant differences among means according to Tukey's Test at 5% level. [c] Mean separation within columns by Tukey's Test at 5% level. [d] Difference between uninoculated and inoculated. * = significant at 5% level, ns = not significant.

4.4.4 Discussion

The *in vitro* dialyzing membrane experiment showed that *Ascochyta* conidia can germinate after exposure to IWP conditions, and can also resume germ tube growth after this

exposure. Based on qualitative observations, IWP conditions would appear to have little effect on conidium mortality or conidium survivability. This experiment, however, did not test the ability of the conidia to infect and penetrate the plant after exposure to a dry period.

The results of the in vivo plant inoculation experiment indicated that IWP conditions do reduce infection by Ascochyta hyalospora conidia. The tests showed differences between 20 hrs CWP and 20 hrs IWP that are much more apparent than the differences between 20 hrs IWP and 10 hrs CWP.

The apparent differences in the results of the in vitro experiment and the in vivo experiment might be due to factors other than moisture conditions. As Jones (1986) has explained, it is difficult to determine how much each wet or dry period affects the amount of infection since there is poor understanding of the other factors which might be involved. For example, the apparent differences between the in vivo and in vitro experiments might be due to the fact that one experiment was carried out on dialyzing membrane whereas the other experiment was carried out on plant surfaces. Factors on the leaf surface such as nutrients, saprophytic microflora, physical features (periclinal and anticlinal cell walls), and pollen grains have been implicated as having an effect on the pre-penetration stages of a pathogen (Chou, 1970; Blakeman, 1980; Fokkema, 1976; Preece, 1976; Preece et al., 1967). Another factor involved might be light. There was no light present during the wet and dry periods of the in vitro experiment, whereas the 14-hr dry periods of the in vivo experiment occurred

in the presence of growth chamber lighting. Previous studies have implicated light as having an effect on infection (Leach & Anderson, 1982; Rotem et al., 1985; Visser et al., 1961). Future experiments examining the effect of IWP conditions on infection should be designed to examine the possible effects of other factors such as light and infection surface.

4.5 Host Range

4.5.1 Introduction

The determination of the host range for a potential biocontrol agent is an important component of biological weed control research with some authors claiming it to be the most important component (Schroeder, 1983; Leonard, 1982). This is probably true in the classical method of biological weed control, where the usual requirement for exotic control agents is strict host specialization. Unlike the endemic pathogens used in the bioherbicide method, exotic agents have not been exposed to many species of plants in the area of introduction. Therefore, extensive host range studies are required to determine whether or not an introduced biocontrol agent will attack plants other than the target host in the areas of introduction. In the bioherbicide method, strict host specialization may not be as essential. Endemic pathogens have presumably co-evolved with indigenous or naturalized plant species in the region. There is little danger of the control agent becoming a pathogen of a previously unreported host, since it has not already done so in nature (Leonard, 1982; Wapshere, 1982).

These differences between the two types of biological control approaches would suggest that a slightly different

emphasis should be used in the host range testing of bioherbicides. Watson (1985) has suggested that host range testing of bioherbicides is comparable to crop safety testing for chemical herbicides. Strict host specialization is not required, since bioherbicides can be restricted to those crops where the response to the pathogen is not in doubt.

Zherbele (1971) has stated that most Ascochyta species have restricted host ranges. Some species are specific to one host but can also be found on other members of the same genus. Ascochyta hyalospora and its three synonyms (Diplodia hyalospora, Diplodina ellisii, and Phleospora chenopodii) have been observed on several Chenopodiaceae species. Seymour (1929) presented a list of at least six Chenopodiaceae species from North America on which Ascochyta hyalospora or its synonyms were found. Among these were Chenopodium album, C. capitatum (L.) Ascherson, C. hybridum L., C. rubrum L., Spinacia oleracea L., and Atriplex patula L. Boerema et al. (1977) found that Ascochyta hyalospora, isolated from seeds of Chenopodium quinoa Willd. (from Bolivia), caused stem necroses and leaf spots on mature plants of C. quinoa and C. album. Conners (1967) listed the fungus as occurring on Chenopodium album, C. capitatum, and C. gigantospermum in Manitoba and Saskatchewan. Ellis & Kellerman (1888) found the fungus on leaves of Chenopodium album from Kansas. Cooke & Ellis (1878) found the fungus on stems of an unidentified Chenopodium species.

The objective of this section was to conduct host range testing of Ascochyta hyalospora under controlled environment conditions in the growth chamber and the greenhouse.

Test plants were selected primarily from the family Chenopodiaceae, although some species in other families were also tested.

4.5.2 Methods

The selection of plant species for the host range tests was done in accordance with the centrifugal phylogenetic system of Wapshere (1974) although in a much reduced form. Plants that were tested included selected species in the genus Chenopodium, economic species in the family Chenopodiaceae, and common unrelated crop species.

Seeds of each test species were sown in prepared soil mix (Pro-Mix) in 12.5-cm diameter pots and thinned to three plants per pot after emergence. In some cases, the three plants were transplanted into 12.5-cm pots. One pot of the test species was sprayed with the fungus while a second pot containing the same species was sprayed with water. In all but one test, a lamb's-quarters plant was included to check for the efficacy of the inoculum. Standard inoculum concentrations and spray volumes were used (Section 4.1.4) for inoculation. After inoculation all plants were subjected to a period of leaf wetness ranging from 20 to 24 hours at a temperature ranging from 20 to 24C. The plants were then placed in either a growth chamber (See Section 4.1.2) or the greenhouse. Seven to 14 days after inoculation, plants were scored susceptible (S), resistant (R), or immune (I) to A. hyalospora. A plant was scored susceptible when large spreading lesions developed. While small spots developed on resistant plants, they did not expand beyond their initial limits. Immune plants exhibited no symptoms. In most cases where a susceptible response was observed, two

additional tests were performed to confirm infection by the pathogen. A second inoculation test was carried out and an attempt was made to re-isolate the pathogen from surface disinfested tissue collected from diseased plants.

4.5.3 Results

Most species belonging to the genus Chenopodium showed a susceptible reaction (Table 25). The symptoms included chlorotic spots, expanding necrotic lesions, and defoliation. Pycnidium formation was rare except in cases where the infected tissue was touching the moist soil surface. Ascochyta hyalospora was re-isolated in most cases. Chenopodium ficifolium Sm., C. hybridum L., and C. sandwicheum and C. album were most susceptible. The species, C. bonus-henricus L., C. botrys L., and C. rubrum L., were immune in most of the tests. C. glaucum, however, was susceptible in one test but exhibited no symptoms in two other tests.

Many economic species in the family Chenopodiaceae exhibited a resistant response (Table 26). Three days after inoculation in Test # 2, red spots began to form on some varieties of beets. Within two weeks of inoculation, all varieties of beets tested displayed spotting to some extent. The red spots did not expand but were still present one month after inoculation. No pycnidia developed on these spots. Although no quantitative measurements were taken, visual comparisons with the control revealed no differences in the size of the plants. One month after inoculation, Ascochyta hyalospora could be re-isolated from surface disinfested red spots on the leaves of selected plants. When excised leaves

were placed on moist filter paper, pycnidia eventually developed within the tissue. Many more red spots formed on the leaves of plants sprayed at a later stage of growth (Test # 2 of Table 26) than on leaves of plants sprayed while still young (Test # 1 of Table 26).

Brown, necrotic-like flecks formed on the leaves of many spinach (Spinacea oleracea L.) and swiss chard (Beta vulgaris L. var cicla) varieties. The flecks were not numerous and did not expand. Ascochyta hyalospora was re-isolated from surface sterilized tissue and pycnidia formed on excised leaves incubated on moist filter paper. An immune response was observed on the Swiss Chard varieties "Dorat" and "Common Green" (Table 26). The one variety of sugar beets (Klein Wanzleben) that was tested did not display symptoms (Table 26). An immune response (Test # 3, Table 26) was recorded for "Hybrid Melodie" spinach, but the variety was found to be resistant in the second test, since pycnidia developed on fallen senescent leaves. Plants in Test # 3 & # 4 were incubated in the greenhouse after inoculation and leaf wetness period. With one exception, no reactions to the fungus were observed in these two tests. The unrelated crop plants listed in Table 27 did not show any reaction to inoculation with Ascochyta hyalospora. They were, therefore, labelled immune.

TABLE 25. Response to *Ascochyta hyalospora* of species in the genus *Chenopodium*.

Name	TEST # 1[1] Growth Stage[2]	Response[3]	Re-isolation[4]	TEST # 2 Growth Stage	Response	Re-isolation
Chenopodium murale L.	>8	I	-	>6	S	K*
C. bonus-henricus L.	>8	I	-	-	-	-
C. opulifolium Scrad.	-	-	-	4-6	S	K*
C. ficifolium Sm.	>8	S	K*	2-4	S	K*
C. hybridum L.	>8	I	-	2-4	S	K*
C. urbicum L.	-	-	-	4	S	K*
C. glaucum L.	-	-	-	4	I	-
C. polyspermum L.	-	-	-	4	S	K*
C. foliosum Aschers.	>8	I	-	2-4	S	K*
C. amaranticolor Coste & Reynier	-	-	-	4	S	K*
C. rubrum L.	-	-	-	4-6	I	-
C. foetidum Schrad.	>8	I	-	2-4	S	K*
C. album L.	?	S	-	4-6	S	K*
C. polyspermum L. var *spicatum* Mog.	>8	S	-	-	-	-
C. sandwicheum Mog.	>8	S	K*	-	-	-

TABLE 25. (continued)

Name	TEST # 3[1] Growth Stage[2]	Response[3]	Re-isolation[4]	TEST # 4 Growth Stage	Response	Re-isolation
C. glaucum L.	>8	I	-	mature	S	-
C. amaranticolor Coste & Reynier	>8	S	K*	mature	S	-
C. album L.	>8	S	K*	mature	S	-
C. quinoa Willd.	>8	S	K*	mature	S	-
C. capitatum (L.) Aschers	>8	S	K*	mature	S	-
C. botrys L.	>8	I	-	mature	I	-

[1] Test Conditions

	Test #1	Test #2	Test #3	Test #4
Subculture	CH-1c	CH-1c	CH-1b	CH-1b
Wetness Period (WP) Duration	20 hours in humid plastic bags	20 hours in dew chamber	20 hours in dew chamber	20 hours in dew chamber
WP air temperature	approx. 20C	22C	22C	22C
Post WP location	greenhouse	growth chamber	growth chamber	growth chamber

[2] Growth stage/number of leaves at time of inoculation.

[3] Response of inoculated plant. S = susceptible, I = immune, R = resistant.

[4] Re-isolation of affected tissue. K = re-isolation attempted, * Ascochyta hyalospora successfully re-isolated, - no testing.

TABLE 26. Response to *Ascochyta hyalospora* of several economic species in the family Chenopodiaceae.

Name	TEST # 1[1] Growth Stage[2]	Response[3]	Re-isolation[4]	TEST # 2 Growth Stage	Response	Re-isolation
Swiss Chard - *Beta vulgaris* L. (Cicla group)						
"Common Green"	2	I	-	>4	I	-
"Silver Giant"	2	R	-	>4	I	-
"Burgundy Crimson"	2	R	K	>4	R	-
Spinach - *Spinacia oleracea* Mill.						
"America"	2	R	-	>4	R	K*
"Long Standing Bloomsdale"	2	R	K*	>4	R	K*
"Tyee Hybrid"	2	R	-	>4	R	K*
Beets - *Beta vulgaris* L.						
"Red Ace Hybrid"	cot	R	-	>4	R	K*
"Garnet"	cot	R	-	-	-	-
"Detroit Dark Red"	cot	R	-	>4	R	-

TABLE 26. (continued)

Name	TEST # 1[1] Growth Stage[2]	Response[3]	Re-isolation[4]	TEST # 2 Growth Stage	Response	Re-isolation
"Ruby Queen"	cot	R	K*	>4	R	K*
"Burpee's Golden"	2	R	-	>4	R	-
"Tendersweet Cylindra"	cot-2	R	-	>4	R	K*
"Early Wonder"	cot	R	K*	>4	R	K*
"Badger Baby"	2	R	K*	-	-	-
"Lutz Green Leaf"	cot	R	-	>4	R	-
"Spinach Beet"	2	R	-	>4	R	-

Name	TEST # 3 Growth Stage	Response	Reisol-ation	TEST # 4 Growth Stage	Response	Reisol-ation
Swiss Chard - *Beta vulgaris* L. (Cicla group)						
"Dorat"	2-4	I	-	mature	I	-
Spinach - *Spinacia oleracea* Mill.						
"Hybrid Melodie"	4	I	-	mature	S	-

TABLE 26. (continued)

Name	TEST # 3[1] Growth Stage[2]	Response[3]	Re-isolation[4]	TEST # 4 Growth Stage	Response	Re-isolation
Sugar Beet - Beta vulgaris L. var rapa						
"Klein Wanzleben"	2-6	I	-	mature	I	-
Kochia						
"Childsii"	small	I	-	mature	I	-

[1] Test Conditions

	Test #1	Test #2	Test #3	Test #4
Subculture	CH-1c	CH-1c	CH-1b	CH-1b
Wetness Period (WP) Duration	20 hours in dew chamber	20 hours in dew chamber	20 hours in dew chamber	20 hours in dew chamber
WP air temperature	22C	22C	22C	22C
Post WP location	growth chamber	growth chamber	greenhouse	greenhouse

[2] Growth stage/number of leaves at time of inoculation.

[3] Response of inoculated plant. S = susceptible, I = immune, R = resistant.

[4] Re-isolation of affected tissue. K = re-isolation attempted, * Ascochyta hyalospora successfully re-isolated, - no testing.

TABLE 27. Response to *Ascochyta hyalospora* of several unrelated economic species.

Name	TEST # 1[1] Growth Stage[2]	Response[3]	TEST # 2 Growth Stage	Response	TEST # 3 Growth Stage	Response
Soybean - *Glycine max*						
"Maple Arrow"	cot.	I	-	-	2-4	I
Sweetcorn - *Zea mays*						
"Early Sunglow"	?	I	2-3	I	5	I
Spring Wheat - *Triticum aestivum*						
"Concorde"	shoot (stage I)	I	2-3	I	-	-
Oats - *Avena sativa*						
"Scott"	shoot (stage I)	I	2	I	4-6	I
Peas - *Pisum sativum*						
"Green Arrow"	-	-	2-3	I	5	I

[1] Test Conditions

	Test #1	Test #2	Test #3
Subculture	CH-1	CH-1	CH-1b
Wetness Period (WP) Duration	21 hours in dew chamber	24 hours in dew chamber	20 hours in dew chamber
WP air temperature	23C	24C	22C
Post WP location	growth chamber	greenhouse	greenhouse

[2] Growth stage/number of leaves at time of inoculation.

[3] Response of inoculated plant. S = susceptible, I = immune, R = resistant.

4.5.4 Discussion

The results of the host range tests presented here showed that Ascochyta hyalospora was not limited to one host species. The most severe effects, however, were limited to the genus Chenopodium. Some species were more heavily damaged than C. album including C. hybridum, C. sandwicheum, and C. ficifolium. It should be noted that C. ficifolium is a triazine resistant weed in western Europe (Gressel et al., 1982). It has also escaped into some areas of Quebec and the eastern seaboard of the United States (Bouchard et al., 1978). Perhaps there is some potential use of A. hyalospora as a bioherbicide for control of C. ficifolium, although at the present time the weed is not a problem in North America.

The resistant spotting observed on beets, spinach, and swiss chard could preclude the use of Ascochyta hyalospora as a bioherbicide in these crops. The immune response of sugar beets observed in the greenhouse tests is suspect, since the swiss chard and spinach in the greenhouse tests displayed immune responses (with one slight exception), but in the growth chamber tests the majority of the swiss chard and spinach varieties displayed brown, necrotic-like flecking. It is possible that sugar beet may have responded in the same manner if it had been used in the growth chamber tests.

Based on the limited testing that has been completed, there is no evidence to dispute the claim of Zherbele (1971) that most Ascochyta species have restricted host ranges. More species, however, need to be tested in the family Chenopodiaceae. In particular, closely related genera such as Atriplex, should be tested since synonyms of Ascochyta

hyalospora have been listed as pathogens (Seymour, 1929). The host range tests should include field testing as well as growth chamber and greenhouse tests, since Watson (1985) has noted numerous examples from the literature where host ranges were extended in growth chamber and greenhouse conditions.

4.6 Integration of Ascochyta hyalospora with Other Weed Control Techniques

4.6.1 Introduction

Many researchers have recognized that mycoherbicides need to be included in integrated pest management programs due to factors such as narrow host specificity and environmental and host constraints (Charudattan, 1985; Quimby & Walker, 1982; Smith, 1986; Wymore et al., 1987). This integration may demand compatibility with the various components in the program such as chemical pesticides, other biopesticides, and the type and timing of cultivation practices (Charudattan, 1985; Smith, 1986).

The bioherbicide may be combined with a herbicide or chemical to enhance the activity of both components. Scheepens (1987) found that there was positive interaction between Cochliobolus lunatus Nelson & Haasis and a low rate of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triaine) on the control of Echinochloa crus-galli (L.) Beauv. Mixtures of thiadiazuron, a plant growth regulator, and Colletotrichum coccodes (Wallr.) Hughes acted synergistically to raise the mortality level of Abutilon theophrasti Medic. (Wymore et al., 1987).

COLLEGO®, the commercially registered mycoherbicide for control of northern jointvetch, has use restrictions with

chemical pesticides. The technical label for COLLEGO® states that the mycoherbicide is not compatible with fungicides, insecticides, and some herbicides such as 2,4-D (2,4-dichloro phenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Tests have shown, however, that insecticides such as malathion (0,0-dimethyl phosphorodithioate of diethyl mercaptosuccinate) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate), along with herbicides such as acifluorfen and bentazon, do not decrease disease development (Smith, 1986).

Similarly, two or more fungi may be mixed to provide control of two or more weeds. In field experiments, joint application of Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. aeschynomene (COLLEGO®) and Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. jussiaeae controlled Aeschynomene virginica (L.) B.S.P. (northern jointvetch) and Jussiaea decurrens (Walt.) D.C. (winged water primrose) (Boyette et al., 1979).

The objective of the research reported in this section was to determine if Ascochyta hyalospora would grow and cause infection in the presence of herbicides. In addition, the efficacy on lamb's-quarters of Ascochyta alone was compared to its efficacy when combined with Phoma sp., a pathogen commonly found on lamb's-quarters in the Ste-Anne-de-Bellevue area.

4.6.2 Methods

Experiment #1 Ascochyta - Phoma combination experiment

The separate and combined effects of Ascochyta hyalospora and Phoma sp. on lamb's-quarters were studied in an RCBD experiment. Each experimental unit consisted of a 12.5-cm

pot containing five plants that had been prepared using the soil germination method (Section 4.2.2). When the plants reached the 4-leaf stage of growth, five replications of the experimental unit were inoculated with the treatments. The treatments were: control (water only), Ascochyta hyalospora (1×10^8 conidia/m^2), Ascochyta hyalospora + Phoma sp. (5×10^7 conidia/m^2 + 5×10^7 conidia/m^2), and Phoma sp. (1×10^8 conidia/m^2). Conidia production and harvesting for both pathogens followed the standard procedures as outlined previously (Section 4.1.3). The plants were inoculated using the turntable sprayer and placed under standard wetness period conditions (Section 4.1.4). After the wetness period the plants were placed in a growth chamber.

Ten days after inoculation, the five blocks of treated plants were rated for disease development on the four bottom leaves of each plant. The grades were converted to mid-point percentages and averaged to obtain one value for each experimental unit. This value was transformed using the arcsin square root transformation. Analysis of variance procedures were used to obtain main effect sum of squares. Tukey's Studentized Range Test was used to compare the treatment means.

Two weeks after inoculation, the height of the first four blocks (the fifth block was used in a destructive sampling test) of plants was measured. The plants were harvested, placed in a 70C oven for at least three days, and then weighed to obtain dry weight measurements. Analysis of variance and Tukey's test were used to detect differences among the means.

Experiment #2 Effect of herbicides on Ascochyta hyalospora growth

The effect of herbicide concentrations on the radial growth of Ascochyta colonies was measured in a replicated experiment. The Petri plates that were used were set up in a completely randomized design (CRD) with four replicates. Three herbicides -- DPX-F6025 (2-(([(4-chloro-6-methoxypyrimidine-2-yl)amino carbonyl]amino sulfonyl))benzoic acid, ethyl ester), AC 263 499 (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), and atrazine -- were mixed with PDA to form a logarithmic series (0, 1, 10, 100, and 1000 ppm) of herbicide concentrations. An additional concentration of 10000 ppm was added for atrazine and AC 263 499, but not for DPX-F6025 since there was not enough material.

An agar plug of mycelium, from an Ascochyta hyalospora colony growing on 1/2PDA:1/2TYA, was inverted and placed onto the centre of each petri plate. The plates were placed in an incubator (12 hours darkness at 22C, 12 hours near UV & florescent at 24C) for 12 days before measuring the diameter of the colonies. An average of two perpendicular diameter measurements for each colony was calculated. Analysis of variance procedures were used to obtain main effect and interaction sum of squares. Since DPX-F6025 did not have a concentration level of 10000 ppm, two analyses were done. The first analysis consisted of the three herbicides at four concentration levels (1, 10, 100, 1000) whereas the second analysis consisted of atrazine and AC 263,499 at five concentration levels (1, 10, 100, 1000, and 10000 ppm). Orthogonal polynomial contrasts were used to fit equations to

describe the trends in the data.

Experiment #3 Effect of atrazine and Ascochyta on triazine resistant and triazine susceptible lamb's-quarters

An experiment was set up to observe the separate and combined effects of Ascochyta hyalospora and atrazine on triazine resistant and triazine susceptible lamb's-quarters plants. Triazine resistant lamb's-quarters seeds from Ripley, Ontario, were obtained from the University of Guelph. Plants were prepared using the soil germination technique (Section 4.1.2). Since triazine resistant seeds were limited in number, all seeds that germinated over a period of several days had to be used. The experimental units (7.5-cm pots containing one plant) were blocked according to stage of development. Plants having six to 12 leaves were placed in Block I, while plants with four to six leaves were placed in Block II, and plants with four leaves were placed in Block III. Seven treatments were evaluated in the experiment: 1) water, triazine susceptible plants (TSP); 2) water, triazine resistant plants (TRP); 3) Ascochyta, TSP; 4) Ascochyta, TRP; 5) Ascochyta + atrazine, TRP; 6) atrazine, TSP; 7) atrazine, TRP. The atrazine was applied at a rate of 2.2 kg active ingredient/ha while Ascochyta was applied at 1×10^8 conidia/m^2. The plants were placed in a 22C dew chamber after being sprayed using the turntable inoculation method (See Section 4.1.4). After a wetness period of 20 hours duration, the plants were placed in a growth chamber (See Section 4.1.2). Visual observations were made eight days after inoculation.

4.6.3 Results

Experiment #1 *Ascochyta* - *Phoma* combination experiment

Application of the pathogens had a significant effect on disease severity, height, and dry weight (Table 28). *Ascochyta* and *Ascochyta* + *Phoma* treatments caused the highest disease severity and greatest decrease in height and dry weight with the *Phoma* treatment, alone, causing less disease and less height and dry weight reduction (Table 29).

TABLE 28. Analysis of variance on the effects of *Ascochyta* - *Phoma* treatments on disease severity, height, and dry weight of lamb's-quarters.

	Disease Severity			Height			Dry Weight		
Source*	df	MS	Pr > F	df	MS	Pr > F	df	MS	Pr > F
R	4	53.8607	0.1595	3	8.1667	0.7367	3	0.0504	0.0552
TRT	2	487.0608	0.0008	3	942.8333	0.0001	3	0.5051	0.0001
ERROR	8	24.5151		9	19.0000		9	0.0136	

* R=block TRT=treatments

TABLE 29. Effect of *Ascochyta* - *Phoma* treatments on disease severity (%), height (cm/pot), and dry weight (g/pot) of lamb's-quarters.

Treatment	Disease Severity (%)[a]	Height Mean (cm/pot)[b]	Dry Weight Mean (g/pot)[b]
Water Control	-	53 a	1.21 a
Ascochyta	67.9 a	18 c	0.38 c
Ascochyta + Phoma	61.8 a	25 c	0.61 bc
Phoma	36.5 b	36 b	0.84 b

[a] Average of five replicates. Mean separation by Tukey's Studentized Range Test at the 0.05 level. Untransformed means presented in the table. [b] Average of four replicates. Mean separation by Tukey's Studentized Range Test at the 0.05 level.

Experiment #2 Effect of herbicides on Ascochyta hyalospora growth

At herbicide concentrations of 100 ppm there was increased growth in comparison to the lower rates and to the control (42.5 mm mean diameter). This was particularly true with DPX-F6025 (Figure 31). Analysis of variance (Table 30) of three herbicides at four concentration levels revealed the presence of interaction (P = 0.0001). Use of orthogonal polynomials indicated cubic trends for the three herbicides over the range of concentrations that were used (Table 31). Only quadratic equations, however, were fitted (Figure 31), since the means at the tested points suggested a quadratic response. Analysis of variance (Table 32) for atrazine and AC 263,499 at five concentration levels indicated the presence of interaction (P = 0.0001). The use of orthogonal polynomials (Table 33) indicated the presence of quartic trends but only quadratic equations were fitted and shown in Figure 32. In the case of all three herbicides, pycnidium formation was observed to decrease as the herbicide concentration increased.

TABLE 30. Analysis of variance on the effects of three herbicides (AC 263 499, atrazine, and DPX-F6025) and four herbicide concentrations (1,10,100, and 1000ppm) on colony diameter of Ascochyta hyalospora.

Source*	df	MS	Pr > F
H	2	30.5156	0.0001
C	3	660.7917	0.0001
H*C	6	250.5365	0.0001
ERROR	36	1.2951	

* H=herbicide, C=concentration

TABLE 31. Orthogonal polynomial contrasts and fitted regression equations for colony diameter of *Ascochyta hyalospora* (mm).

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
C IN H					
C in H1	3	9.0573	0.0008	diameter = 42.25 - 3.8541667*C	0.68
C linear	1	0.0031	0.9611	+ 5.375*C^2 - 1.3958333*C^3	
C quadratic	1	13.1406	0.0030		
C cubic	1	14.0281	0.0022		
C in H2	3	85.2656	0.0001	diameter = 39.625 - 7.8125*C	0.90
C linear	1	17.5781	0.0007	- 12.375*C^2 - 3.4375*C^3	
C quadratic	1	153.1406	0.0001		
C cubic	1	85.0781	0.0001		
C in H3	3	1065.5417	0.0001	diameter = 40.375 - 19.85417*C	0.99
C linear	1	556.5125	0.0001	+ 34.4375*C^2 - 10.083333*C^3	
C quadratic	1	1914.0625	0.0001		
C cubic	1	732.0500	0.0001		
ERROR	36	1.2951			

* C=concentration, H1=AC 263 499, H2=atrazine, H3=DPX-F6025

TABLE 32. Analysis of variance on the effects of two herbicides (atrazine and AC 263,499) and five herbicide concentrations (1, 10, 100, 1000, and 10000 ppm) on colony diameter of Ascochyta hyalospora.

Source*	df	MS	Pr > F
H	1	255.0250	0.0001
C	4	184.8813	0.0001
H*C	4	83.7125	0.0001
ERROR	30	1.6667	

* H=herbicide, C=concentration

TABLE 33. Orthogonal polynomial contrasts and fitted regression equations for colony diameter of *Ascochyta hyalospora* (mm).

CONTRAST*	df	MEAN SQUARE	Pr > F	FITTED EQUATION	R^2
C in H					
C in H1	4	11.6688	0.0004	diameter = 42.25 - 8.041667*C	0.68
C linear	1	10.0000	0.0204	+ 13.052083*C^2 - 5.583333*C^3	
C quadratic	1	20.6429	0.0014	+ 0.69791667*C^4	
C cubic	1	0.0000	1.0000		
C quartic	1	16.0321	0.0042		
C in H2	4	256.9250	0.0001	diameter = 39.625 - 17.40625*C	0.97
C linear	1	511.2250	0.0001	+ 29.963542*C^2 - 13.03125*C^3	
C quadratic	1	429.0179	0.0001	+ 1.59895833*C^4	
C cubic	1	3.3063	0.1693		
C quartic	1	84.1509	0.0001		
ERROR	30	1.6667			

* C=concentration, H1=AC 263 499, H2=atrazine

Figure 31. Effect of increasing concentration of three herbicides on colony diameter of Ascochyta hyalospora on PDA. Symbols represent mean colony diameter at each concentration. Regression equations: AC 263,499 diameter = 41.83125 + 2.70625*C - 0.90625*C^2 R^2 = 0.33, atrazine diameter = 38.59375 + 8.34375*C - 3.09375*C^2 R^2 = 0.60, DPX-F6025 diameter = 37.35 + 27.5375*C 10.9375*C^2 R^2 = 0.77 .

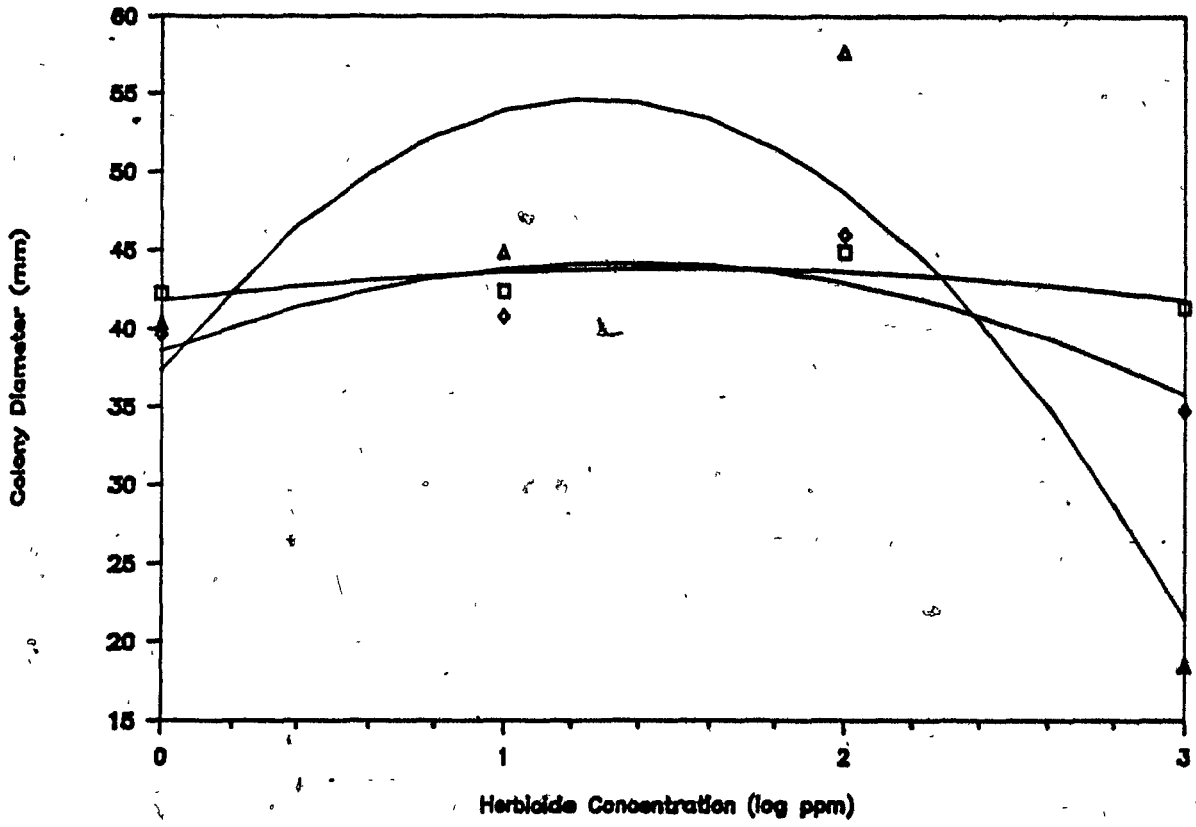
Herbicide

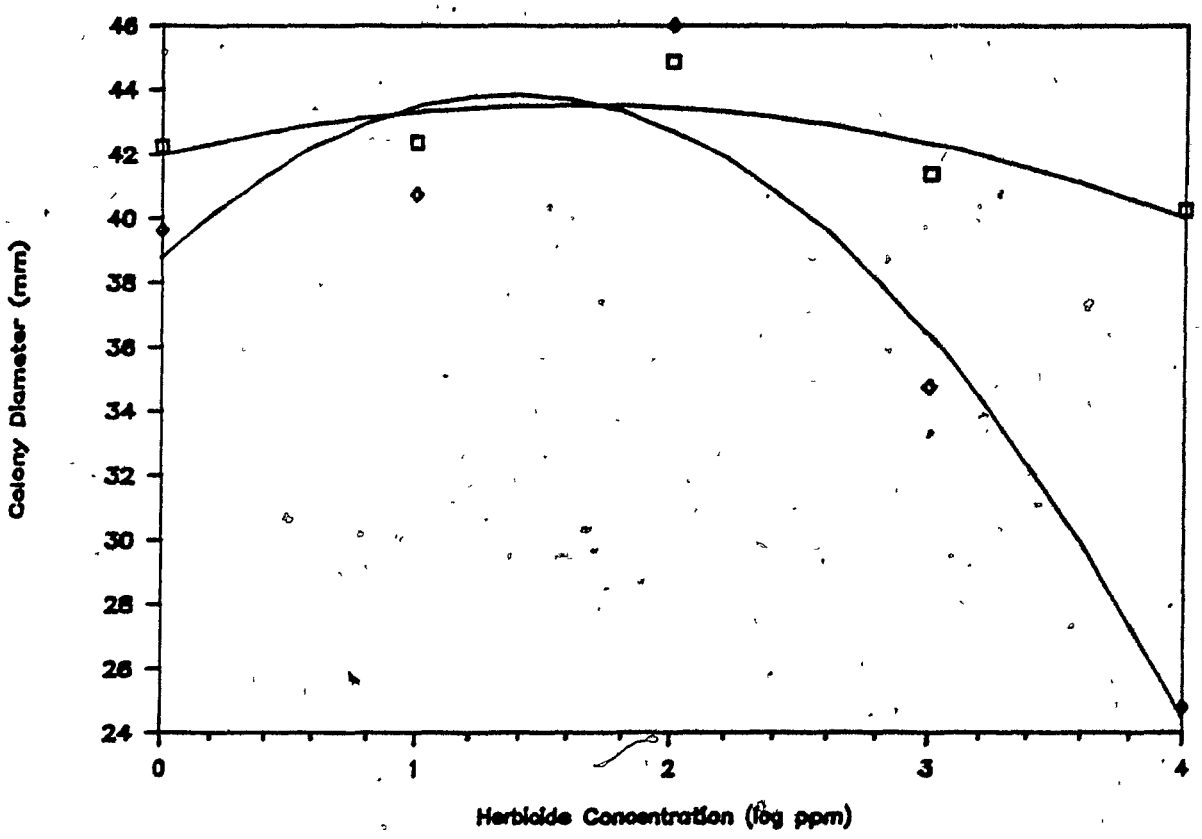
□ AC 263,499 ◇ atrazine ▲ DPX-F6025

Figure 32. Effect of increasing concentration of two herbicides on colony diameter of Ascochyta hyalospora on PDA. Symbols represent mean colony diameter at each concentration. Regression equations: AC 263,499 diameter = 42.01071429 + 1.92857143*C - 0.60714286*C^2 R^2 = 0.45, atrazine diameter = 38.789285 + 7.4964286*C - 2.76785714*C^2 R^2 = 0.89 .

Herbicide

□ AC 263,499 ◇ atrazine


Colony Diameter (mm)
60
55
50
45
40
35
30
25
20
15
0
1
2
3
Herbicide Concentration (log ppm)


Colony Diameter (mm)
46
44
42
40
38
36
34
32
30
28
26
24
0
1
2
3
4
Herbicide Concentration (log ppm)

Experiment #3 Effect of atrazine and Ascochyta on triazine resistant and triazine susceptible lamb's-quarters.

As expected, the triazine resistant plants were not affected by atrazine, whereas the triazine susceptible plants were affected (Table 34). Ascochyta hyalospora was capable of infecting both triazine resistant and susceptible lamb's-quarters and was also capable of infecting the plant when used in combination with atrazine.

TABLE 34. Effect of atrazine and Ascochyta hyalospora on triazine resistant and triazine susceptible lamb's-quarters plants.

Treatment	Observations (eight days post-inoculation)
	Block I, II, and III
1. water, TSP	healthy
2. water, TRP	healthy
3. Ascochyta, TSP	heavy Ascochyta infection on lower leaves
4. Ascochyta, TRP	heavy Ascochyta infection on lower leaves
5. Ascochyta+ atrazine, TRP	heavy Ascochyta infection on lower leaves
6. atrazine, TSP	plant severely affected, leaves dry, different from Ascochyta infection
7. atrazine, TRP	healthy*

* in block II & III, there were two small lesions on one leaf

4.6.4 Discussion

Based on the experiment reported in this section, there is no advantage in combining Ascochyta hyalospora and Phoma. The results of this experiment contrast to observations collected in

the field. During field surveys, Phoma was found to occur more frequently than Ascochyta, but in laboratory experiments Ascochyta had a greater effect on lamb's-quarters.

It appears that Ascochyta is compatible with those herbicides tested and tank mixes of Ascochyta and chemical herbicides may be possible. Colony growth increased at lower rates, particularly with DPX-F6025. At higher rates, the herbicides decreased growth, but AC 263,499 had minimal effect. The pattern of growth caused by the other two herbicides has previously been shown to occur in other fungus-herbicide combinations. Atrazine was found to have opposite effects on Fusarium solani (Mart.) App. & Wr. and Fusarium sporotrichioides Sherb., by stimulating growth at low concentrations and depressing the growth at high concentrations (Richardson, 1970). Similarly MCPA (2-methyl-4-chlorophenoxyacetic acid) promoted growth of Aspergillus niger van Tiegh. at low concentrations but suppressed growth at high concentrations (Smith & Shennan, 1966).

It appears that not only can Ascochyta hyalospora grow well in the presence of of some herbicides, but it can also infect the plant when "tank-mixed" and applied with the herbicide atrazine, as in Experiment #3. The other important aspect of the atrazine experiment was the indication that Ascochyta hyalospora was able to infect both triazine resistant and triazine susceptible plants.

These studies show the need for further research to evaluate the use of A. hyalospora in integrated weed management. More specifically, it may be possible to tank-mix the pathogen with atrazine (at low rates?) for control of triazine-resistant

lamb's-quarters. In Experiment #2, the ability of the conidia to germinate in the herbicide concentration series was not tested. Experiments with COLLEGO®, have indicated that some herbicides can depress the germination of the conidia (COLLEGO® technical manual). Therefore, further studies should examine the effect of herbicides on germination. Additional field experiments with Phoma-Ascochyta combinations would also be of interest in that it might help determine the reason for the disparity between the relatively weak effect of Phoma that was observed in the growth chamber experiment and its apparent prevalence in the field compared to Ascochyta hyalospora.

V CONCLUSIONS

Several conclusions, and suggestions for future work, can be drawn from the results of the research:

(1) Ascochyta hyalospora was found to be the best candidate for further testing as a bioherbicide for lamb's-quarters. This conclusion was based on its virulence and conidium production, which were found to be superior to those of the other plant pathogens collected during surveys. Further surveys, based on wider geographical areas might be beneficial in providing a wider genetic base for an Ascochyta hyalospora bioherbicide.

(2) Ascochyta hyalospora would appear to be most virulent on plants in the family Chenopodiaceae, especially the genus Chenopodium. Although there was limited testing on plants outside the Chenopodiaceae, all plants tested were immune. Future host range testing should include a larger number of species in the Chenopodiaceae as well as other families.

(3) Older plants (>six leaves) were not damaged as much by

disease as were younger plants (<four leaves), but the amount of damage could be increased by applying a higher concentration of conidia. The amount of damage sustained by a plant appeared to be more related to stem infection rather than leaf infection.

(4) Wetness period temperatures of 18C and 24C were found to be optimum for disease development and subsequent damage to lamb's-quarters plants. Plants at 12C and 30C needed longer wetness periods to attain a similar amount of disease development and damage that was obtained with optimum temperatures (and shorter wetness periods).

(5) Interruption of a wetness period by a period of dryness was found to hinder disease development and subsequent damage to inoculated plants. Although the germination process on dialyzing membrane could be resumed after periods of dryness, inoculated plants developed more disease (and sustained more damage) after 20 hours of continuous wetness than plants kept under interrupted moisture conditions for 20 hours. Modifications to these experiments might give clearer results. Quantitative measurements, in addition to qualitative observations, might show treatment differences in the dialyzing membrane experiment. If further work is done on the effect of interrupted wetness periods on inoculated plants, trends might be indicated more readily by using treatments with increasing numbers of dry periods, instead of the single dry period that was used in the experiments reported here.

(6) Ascochyta hyalospora grew on PDA mixed with atrazine, AC 263 499, and DPX-F6025 herbicides. When "tank-mixed" and sprayed with atrazine, the pathogen incited disease on triazine resistant lamb's-quarters. These preliminary results would

indicate that Ascochyta hyalospora could be integrated into a weed management system where chemical herbicides are used. More extensive testing with triazine and other types of herbicides is needed.

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