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DESCRIPTION AND STUDY OF A *PHOMA* SP., A NEW FUNGAL PATHOGEN OF LUPINES (*LUPINUS ALBUS* L.), IN QUÉBEC

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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ABSTRACT

Lupines (*Lupinus albus* L.) can provide high levels of good quality protein for human and animal nutrition. Unlike soybeans, lupine seeds do not need to be processed before consumption. Being a member of the legume family, lupines improve soil structure and fertility. Lupines also have the advantage of being able to grow in cooler climates and in fields of lower fertility than soybean. Lupine production is relatively new in Eastern Canada and new diseases limit production in this region. In 1990, a *Phoma* sp. was isolated from diseased lupine in Québec and Nova Scotia. This research was undertaken to acquire further knowledge about this lupine pathogen. The morphological characteristics of this fungus do not exactly fit the description of any other *Phoma* sp., but it is very close to *P. pinodella*. This *Phoma* sp. on lupines may be a new variety of *P. pinodella* affecting lupine in particular. In vitro, mycelium grew over a wide range of temperatures (5° - 35° C), the optimal temperature being 25° C. Spore germination occurred under different conditions of temperature, from 10° C to 35° C, the optimum being 25° C. Spores germinated at relative humidities from 94 % to 100 %, (-7.027 to 0 MPa) the optimum being between 98 % and 100 % (-2.768 to 0 MPa). In controlled environment, disease symptoms appeared over a wide range of leaf wetness durations (8 h - 36 h) and temperature regimes (15° C - 30° C). The most extensive disease symptoms occurred following 36 hours of leaf wetness duration at 30° C. This *Phoma* sp. survived over winter in infected stubble of lupines of the previous year crop. The infected crop residue may be another source of inoculum of this seedborne pathogen.

RÉSUMÉ

Le lupin, *Lupinus albus* L., est une excellente source de protéines de bonne qualité pour la nutrition humaine et animale. Contrairement au soya, le lupin ne nécessite aucune transformation particulière avant la consommation. Étant une légumineuse, cette plante améliore la fertilité et la structure des sols. Un autre avantage au lupin est son adaptation à des températures plus froides et des sols moins fertiles que le soya. La production du lupin est encore récente dans l'Est du Canada et certaines maladies inconnues jusqu'à ce jour peuvent limiter sa production dans notre région. En 1990, une nouvelle espèce de *Phoma* fut isolée sur des plants de lupins du Québec et de la Nouvelle-Écosse. Le but de cette recherche était d'augmenter nos connaissances au sujet de ce pathogène. Une étude des caractéristiques morphologiques et une description de ce champignon ont été effectuées. Il fut impossible de classer définitivement ce *Phoma* sp. dans une espèce particulière de *Phoma*, bien que beaucoup des caractéristiques de ce pathogène ressemblent à *Phoma pinodella*. Lors des tests in vitro, le mycélium démontrait des signes de croissance sur une gamme de températures (5° - 35° C) avec une température optimale de 25° C. De même, la germination des spores était maximale à 25° C cependant qu'elle pouvait se produire entre 10° C à 35° C. Les conditions d'humidité relative permettant la germination s'étendaient de 94 % à 100 % (-7.027 à 0 MPa) avec des conditions optimales entre 98 % et 100 % (-2.768 à 0 MPa). En environnement contrôlé, des symptômes d'infection sont apparus à différentes combinaisons de durée de mouillure (8 h - 36 h) et de température d'incubation (15° C - 30° C).

Les symptômes les plus considérables furent observés après 36 heures de mouillure des feuilles à une température de 30° C. Cette espèce de *Phoma* fut capable de survivre à la période hivernale dans les résidus de culture de la récolte de l'année précédente. Ces résidus pourraient être une source alternative d'inoculum pour ce pathogène qui est habituellement porté par les semences.

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INTRODUCTION

Legumes have a very important role in a crop rotation for sustainable agriculture. They have the advantage of improving soil structure, soil biology, and soil organic matter; they also increase water infiltration and help in preventing soil erosion (Gross, 1982).

Soybeans are widely used as a suitable rotational crop in cereal production and as a source of protein for human and animal consumption. Lupines can provide an interesting alternative where soybean production is more difficult due to cooler and damper conditions to which lupines are more adapted. Soybeans need to be processed before consumption due to the presence of anti-nutritional factors such as protease inhibitors. The absence of those anti-quality compounds in lupines makes them a good source of on-farm produced and readily available proteins (Gladstone, 1970). Lupines have been recently introduced in Eastern Canada, the first few hectares having been seeded in 1983 (Forrest, 1991). Lupines may develop new diseases that could become important under these different climatic conditions. In 1990, a fungus of the genus *Phoma* was isolated from infected lupine plants. The main objectives of this project were to first describe this fungal pathogen and to study its reactions

under different environmental conditions. This thesis is divided in four chapters. The first one is a literature review that describes lupines and lupine diseases in general. The second chapter describes the morphological characteristics of three different isolates of the fungus. The effects of environmental conditions on the fungus development in vitro and in plantae are discussed in the third chapter. The fourth chapter is a field study of the winter survival of the pathogen

.

LITERATURE REVIEW

1. Description of *Lupinus albus* L.

Lupinus albus is a member of the legume family *Fabaceae*. Lupines are native to the Mediterranean region, East Africa and the American continent. The *Lupinus* genus includes about 200 species, from the wild high alkaloids types to the cultivated sweet type and the beautiful ornamental varieties (Gladstone, 1982).

Lupine architecture is quite unique with a main stem and lateral branching orders. The first order of lateral branches starts when 10 to 40 leaves, depending on temperature and light conditions, have developed on the main stem. The second order develops from nodes of the first order of branches. This process may continue up to five orders in certain conditions (Field and Putnam, 1991). Harvest almost always originates from the main stem, and first

lateral orders. Up to 90-100% of seeds are situated on the main axis and the primary orders (Greenwood et al., 1975).

All orders of branches as well as the main stem terminate with a simple raceme type of inflorescence. Flowers are produced on the main stems first and develop later on the lateral orders. In cooler climates, flowering begins in late June and seeds are set in October. Under climatic conditions in Québec, the plant tends to have a more indeterminate growth habit; many orders are formed, up to three. Flowering is spread over a longer period of time making the plant more susceptible to adverse environmental conditions. In more determinate plants, main stem pods (which are set earlier) mature, but lateral pods do not. A more determinate growth is more desirable and results in earlier harvest dates, making the management operations easier (Clapham and Elbert-May, 1989).

Pods are all situated on the top part of the plant making it easy for mechanical harvest with a cereal harvester (Lopez-Bellido and Fuentes, 1986). Due to their strong stem and an upright growth habit, lupines are very resistant to lodging (Clapham and Elbert-May, 1989). Another favorable factor for lupines harvest is a lower seed shattering incidence, compared to other legumes, resulting in reduced losses (Gladstone, 1970). Costs to include lupines in a cereal rotation are very low since the same harvesting machinery can be used for both crops (Lopez-Bellido and Fuentes, 1986).

2. Climatic and soil requirements

Lupines will tolerate variable temperatures and low soil fertility. In general, it is a cool season crop, and it is considered as a summer annual in our cool temperate climates (Gladstone, 1970). Like small grain cereals it is cold tolerant, surviving temperatures as low as -6 to -9 C (Kaplan, 1988). However, this tolerance to cold varies with the stage of growth, with the highest tolerance at the early vegetative stage of up to four leaves (Lopez-Bellido and Fuentes, 1986). This permits its sowing in early spring, at the same time as small grain cereals.

The most successful areas in which to grow lupines should have a 5-month growing season free from moisture stress and monthly average temperatures between 15°-25° C (Gladstone, 1970). Rainfall levels should be above 350 mm during the growth cycle (Nelson et al., 1984). This plant tends to grow rapidly in cool moist weather and suffers from excessive heat and water stress. Lupines are especially sensitive at the flowering period where flowers can be killed, leading to a reduced amount of formed pods (Herbert, 1987). Another asset for this plant is a good resistance to soil waterlogging.

The best soil conditions for its production are a deep, fertile, well drained, acidic to neutral (pH 5.0 to 7.5), and medium to coarse texture soil (sandy to

sandy loam) (Meredith, 1991). When grown on clay, lupines exhibit greatly reduced vegetative growth and growing difficulties due to lack of root aeration; making them more susceptible to diseases. Chlorosis may occur in alkaline and highly calcareous soil (Gross 1982 in Lopez-Bellido and Fuentes, 1986). In Canada, lupines also perform relatively well at pH values up to 8 (Forrest, 1991).

Lupines have an excellent capacity to fix nitrogen (MacLeod et al., 1987) They have a symbiotic relationship with a slow growing bacterium *Rhizobium lupinus*, which forms nodules on lupine and other tropical legumes (Putnam et al., 1991). One of the advantages with lupines is that nitrogen fixation continues very late in the growth stage of the plant. Lupines usually fix nitrogen up to seed maturity and up to leaf drop (Lopez-Bellido and Fuentes, 1986). Nitrogen fixation by lupines is affected by temperature, water logging and soil nitrogen content. Lupine plants have a high capacity for nitrogen fixation and organic matter production, which makes it an attractive green manure crop. Lupines are able to fix up to 100-250 kg/ha of nitrogen per year at a pH as low as 5.4 (Jannasch, 1992). Lupines could bring marginal lands of Canada into production (Zahradnik, 1984). They can tolerate rocky acidic soils, and when properly inoculated they do not require the addition of nitrogen fertilizers (Putman et al., 1991). Furthermore, in case of low levels of available phosphorus, lupines produce supplementary feeder roots that enhance P uptake. These proteoid roots also secrete some organic acids which can help

mobilize otherwise unavailable phosphorus from the soil (Meredith, 1991).

3. Nutritional characteristics

The sweet white lupines that are grown in Québec produce large seeds with a very low concentration of alkaloid as compared to their wild ancestors, which were small seeded and with high alkaloid content (Brucher, 1976).

Nutritionally, lupines are very advantageous since they do not contain the trypsin inhibitors for which soybeans need to be micronized (Hill, 1977). Therefore lupines can be used directly on the farm. White lupines are an excellent source of quality vegetable protein with 34 to 45 % crude protein content (Pate et al., 1985). Amino acids are in good supply, with high contents of lysine, threonine and tryptophan (King, 1990). However, like other legume seeds, the sulfur containing amino acids cystine and methionine are found in low amounts (Wiseman and Cole, 1988). Lupine seeds have a high energetic value with lipid concentration between 10 and 15% (Guillaume et al., 1987). The nutrient composition of lupine seed is highly variable. Values may be anywhere between those values given above depending on species and cultivars (Hill, 1977).

4. Utilization

Lupines are used in very diverse forms of production and for very different purposes. They are used in a soil conservation perspective; as a catch crop after winter cereals; as a green manure crop to be incorporated before winter; in rotation with rye, oat, barley and potatoes where they provide the subsequent crop with an improved nutrient availability (Meredith, 1991); or in agroforestry to fight against erosion in deforested areas (Gross, 1982). Lupines are also used as a disease breaker in lupine/wheat rotation. Disease incidence is reduced in rotation compared to monocrops (Reeves, 1984).

Sweet lupines are also grown for their nutritional value. In animal production they are grown as forages, are sown in mixtures with oats or other cereals or with other forage legumes, and are grazed green or ensiled, but never used for hay (Meredith, 1991). Their seeds are also used as protein supplements in swine production. Lupine seeds could be used as a part of a soybean meal mixture to replace the more expensive protein sources such as meat meal, and fish meal (Batterham, 1979; Donavan et al., 1993). This legume can also be used in egg production where laying hens can be fed up to 20 % of their diet as lupine seeds while maintaining normal egg production (Hughes and Orange, 1976). In ruminants lupine seeds can be added to the diet as a part of the protein intake. They are appropriate for beef and sheep, while in dairy

production the proportion of lupine seeds in the ration must be evaluated carefully (Guillaume et al., 1987).

Lupines are a very good source of protein in a well balanced human diet. In regions where most of the population suffers from protein deficiency, they could become a very good supplement. In ancient times, lupines were favored in human nutrition due to their easy digestion and non-flatulent qualities. Lupines can be eaten in different forms such as cooked like chickpeas; canned and added to salads and various meals; and ground into a very fine flour to make high protein breads and cakes (Meredith, 1991). Many of the lupine species containing the alkaloids were used for medicine and as insecticides (Lopez-Bellido and Fuentes, 1986).

5. Constraints and diseases

Canopy development of lupines is very slow, making this crop very susceptible to weed competition that results in immediate yield losses. The problematic weeds are the late emerging broadleaf weeds such as common ragweed (*Ambrosia artemisiifolia*) and lambsquarters (*Chenopodium album*) (Ivany, 1991). Putnam et al., (1991) suggested that the best method for weed control is through prevention. Good cultural practices such as rotating crops and herbicides and avoiding sowing lupines in fields with heavy weed pressure

are important to avoid yield depression due to heavy weed infestation. Lupines are also affected by insect pests in many areas of the world. For example, aphids (that not only cause physical damage but are also carrier for diseases) are found on lupines in Australia, Brazil, Spain and New Zealand. Lupines tend to become more sensitive to pathogens in more humid conditions. The most widespread pathogens of lupines are: *Pleiochaeta setosa* (brown leaf spot) and *Colletotrichum gloeosporioides* (anthracnose) (Nelson et al., 1984). Brown leaf spot is very destructive especially in winter grown crops and it is mostly prevalent under cool, damp conditions. It is seed and residue borne, which could create problems in no-till types of agriculture (Gladstone, 1970). This disease was first recorded in North America on *L. albus* in Minnesota in 1989 by Kalis-Kuznia et al. (1991a) and in Canada by Paulitz and Atlin (1992). It is common in Western Australia, where lupines are used as a winter cover crop. The symptoms are dark brown sunken lesions on leaves, pods, stems, and roots of irrigated lupines and lesions only on leaves in the non-irrigated conditions. This disease has also been reported in other parts of the world including New Zealand, Europe, South Africa, and South America (Kalis Kuznia et al., 1991). Anthracnose is more prevalent in warm moist conditions. The symptoms are dark brown spots (acervuli) and cankers that form on cotyledons, stems, pods and seeds, and less often on leaves. This pathogen is also seedborne and there is low control efficiency with seed treatment (Gladstone, 1970). This disease was first reported in Canada in 1995 (Paulitz et al., 1995). The best

way to control disease spread and development is through cultural practices. especially crop rotations (Nelson et al., 1984).

Damping-off is very common in lupine production. Various fungi were isolated from rotting tissue *Rhizoctonia solani*, *Pythium* sp., *Fusarium* sp. and *Phytophthora* sp. are important disease causal agents. Rhizoctonia root rot is the most widely distributed and destructive rot disease of lupines. *R. solani* attacks seedlings before emergence and often kills a large number of seedlings due to infection at the soil surface after they emerged. Diseased plants have yellowish green foliage and may not set pods (Leach and Clapham, 1992).

Another fungal pathogen, *Phomopsis leptostromiformis*, only attacks the genus *Lupinus* and can cause some yield reduction on *Lupinus luteus* but causes visual symptoms only on mature and senescing tissues of *L. albus* and *L angustifolius* (Cowling et al., 1987). However this pathogen is well known for the mycotoxin it produces, which causes a very severe and potentially fatal disease in sheep and other herbivores known as lupinosis (Gardiner, 1975).

In Québec, where lupines have been recently introduced, diseases often have a detrimental effect on lupine stand and seed yield. By studying the diseases and knowing the factors favoring their occurrence and reproduction, one is batter able to develop methods to restrict these diseases. The main goal

of this research was to describe and study a new fungal pathogen a *Phoma* sp. that is known to affect the cultivated lupines in North America.

6. *Phoma*

Phoma is a genus containing more than 2000 described species. This genus traditionally refers to "simple stem inhabiting pycnidial fungi with small hyaline, unicellular conidia" (Sutton, 1980). Identification of a particular species is very difficult since there is no complete and easily workable key, due to the fact that many characteristics which are used in the identification are highly variable, depending on the environment (Sutton, 1980). *Phoma* is a pathogen affecting a wide variety of crops. *Phoma* is a very widespread pathogen around the world and it occurs on a broad range of plant species. For example, *P. glomerata* has been reported to be a pathogen on many crops such as grape, potato, India rubber, wheat, pear, mango, rice and other crops (Upadhyay et al., 1990). In Canada various crops suffer losses due to infection with *Phoma* species. Some important examples are *Phoma lingam* that causes the very serious black leg disease in canola. It is also found on other cruciferous crops such as cabbage, cauliflower and summer rape *P. medicaginis* var. *medicaginis*, causal agent of spring black stem of alfalfa, is found all over Canada; *P. medicaginis* var. *pinodella* causal agent of foot rot in peas and black stem in clover. All of these pathogens can overwinter in crop debris and are often

seedborne. Development of disease symptoms caused by these *Phoma sp.* will often be enhanced in wet and cool conditions (Martens et al., 1988). This genus shows a large pathogenic variation which often complicates the control of *Phoma* in legumes (Neabane and Ekpo, 1992).

The *Phoma* sp. used in this study is a fungal pathogen that is seedborne and probably residue-borne. Symptoms are reddish brown lesions, especially on stems and on mature pods (Paulitz and Coté, 1991). This disease can infect the pods and impede pod filling, thus causing high yield reduction.

II. MORPHOLOGICAL DESCRIPTION OF THREE ISOLATES OF PHOMA SP. PATHOGENIC ON WHITE LUPINE (LUPINUS ALBUS L.)

INTRODUCTION

Lupines production in Eastern Canada is very recent, less than 15 years old (Forrest, 1991). Some years, as in other part of the world, fungal diseases can be a limiting factor for seed yield and quality. Some important lupine diseases found worldwide are brown leaf spot caused by *Pleiochaeta setosa*, lupinosis caused by *Phomopsis leptostromiformis* (Gladstone, 1970; Wood and McLean, 1982; Kalis-Kuznia et al., 1991a), and anthracnose caused by *Colletotricum* spp. (Gladstone, 1970; Kalis-Kuznia et al., 1991b). Recently, however, a new fungal pathogen, *Phoma* sp. was found to infect lupine plants in Eastern Canada (Paulitz and Coté, 1991). Symptoms related to this *Phoma* disease are brown to black stem lesions enlarging as the plant grows and causing general browning of the stem. Lesions also appear on young pods stunting their development and infecting the seeds. As the plant grows, pycnidia are formed on the lesions and during moist conditions exude conidia.

Some species of *Phoma* have been reported on lupines other than *Lupinus albus* in North America. For example, *Phoma lupini* Ellis and Everh.

causing leaf spot was reported in Western North America, *Phoma lupinicola* Earle in Green producing stem symptoms was reported in Colorado, *Phoma minuta* was found on stems in Washington and Wyoming, and *Phoma pedicularis* was observed in Washington (Farr et al., 1986). When this new *Phoma* sp. was compared to these previously reported species, some characteristics were found to be different. Dr. J. White, an authority on Coelomycetes at Rutgers University, New Jersey, examined the isolates and suggested a similarity to *Phoma medicaginis*, causal agent of black stem rot of Fabaceae. There are no previous report of this *Phoma* affecting white lupines. This *Phoma* sp. can be confused with *Ascochyta* due to the presence of septate spores on infected plant tissue. However, in culture spores of *Phoma* are primarily aseptate, and septa resemble those according to the genus concept of *Phoma* (Boerema and Bollen, 1975). The objective of this study was to provide a description of this *Phoma* sp. attacking lupines in Eastern Canada. In terms of morphological characteristics and visual appearance during growth on different solid media.

MATERIALS AND METHODS

1. Inoculum production

For these experiments, three isolates of *Phoma* sp. were used: one from Nova Scotia, one from Prince Edward Island and one from Québec. These were collected from seeds of diseased lupine plants in the summer of 1992 and 1993, and were stored at 5° C in soil tubes (autoclaved soil + 1% ground rolled oats) for further use. Infected seeds from the Maritimes were provided by Dr. Gary Atlin, Nova Scotia Agricultural College, Truro, Nova Scotia. To recover the isolates, soil particles from each of the three isolates were sprinkled on fresh PDA (potato dextrose agar) plates containing 100 ppm chloramphenicol (ICN Biomedicals Inc.) and incubated for 10 days at 25° C with a 12 -hr photoperiod. Subcultures were made by transferring a piece of PDA + mycelium to fresh PDA plates.

2. Agar media

Four agar media were used to support the growth and observe the differences between the three isolates of *Phoma* sp. The agar media tested were: CMA (corn meal agar, Difco, St. Louis, MO) , MEA (malt extract agar, Difco, St. Louis, MO), OMA (oat meal agar, Tuite, 1969), and PDA (potato

dextrose agar, Difco, St. Louis, MO).

3. Culture incubation and observations

Subcultures were made by transferring a 7-mm plug (no. 3 cork borer) of mycelium from the edge of the colony on PDA to each of the four different media. These plates were then sealed with parafilm and incubated at 25° C. The colonies were measured every two days on two perpendicular axes. After 3 weeks, comparisons were made to a Rayner chart to determine colors (Rayner, 1970). Photographs were taken on a Zeiss (Wild Heebrugg) dissecting scope and an Olympus BH-2 compound microscope using an Olympus OM-4T camera with 35 mm Ektachrome color slide film, ASA 100. Measurements of pycnidia, spores and mycelium dimensions were taken with an ocular micrometer.

4. Culture identification

Cultures of the Phoma sp. of lupines were sent to National Identification Service of Economic Fungi at the Research Branch of Agriculture Canada for comparisons with other similar fungi.

RESULTS

1. Macroscopic characteristics

Corn Meal Agar

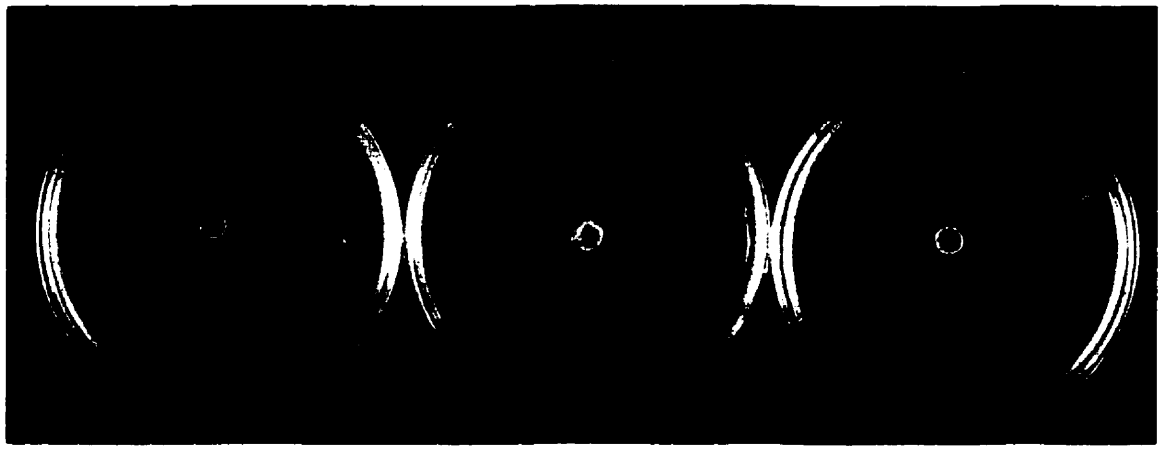
Growth of this *Phoma* sp. was initiated by enlargement of a white-beige to olivaceous mycelium followed by the formation of brown-black pycnidia, seven to ten days later at 25° C. Sparse pycnidia formed in concentrical zones were very small and of a very light brown beige color. Shortly after pycnidial formation a salmon-colored slime (no.41. Rayner, 1970) containing a spore suspension, started to be released from the ostioles. After 3 weeks at 25° C, colonies on CMA (Fig. 2.1) were very pale, of a whitish translucent coloration with no perceptible aerial mycelium. For all of the isolates, top side and reverse sides were similar in appearance. In the case of Prince Edward Island isolate, spore production was not initiated at the end of that 3-week period. After 7 days, the Nova Scotia isolate grew 62 mm, the Prince Edward Island isolate grew 66 mm and the Québec isolate grew 46 mm.

Figure 2.1

Mycelium of *Phoma* sp. isolated from lupines after 21 days of incubation on corn meal agar at 25° C. Top side view.

CMA

TOP SIDE



N.S. P.E.I. QUE.

Malt Extract Agar

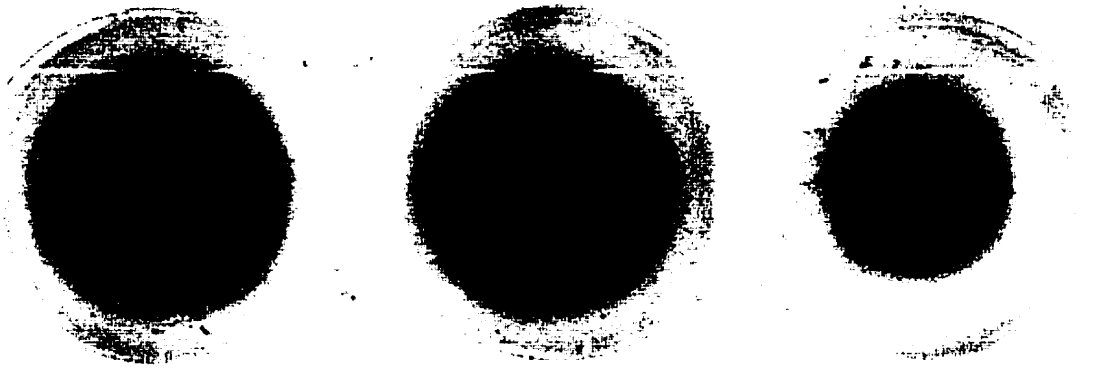
Colonies on MEA (Fig. 2.2), were denser and darker than on CMA. For the Nova Scotia isolate, mycelium color was between gray olivaceous (no.107, Rayner, 1970) and olivaceous black (no.108, Rayner, 1970), with the darker coloration being at the colony centre and becoming lighter toward the edges. The margins (outer 3-4 mm) were olivaceous buff (no.89, Rayner, 1970) to buff (no.45, Rayner, 1970). In the case of Prince Edward Island isolate, the coloration also varied between olivaceous black (no.108, Rayner, 1970) to gray olivaceous (no.107, Rayner, 1970) in the centre of the colony with pale buff (no.45, Rayner, 1970) colored margins. For the Québec isolate, the centre was also darker; olivaceous black (no. 108, Rayner, 1970) gradually becoming pale olivaceous (no.48, Rayner, 1970) toward the edges. Margins (outer 5-6 mm) were buff colored (no.45, Rayner, 1970). The reverse side of those cultures had a more uniform appearance and the three isolates looked very similar (Fig. 2.2). For all isolates, the central portion was olivaceous black (no.108, Rayner, 1970) surrounded by a lighter margin, honey (no.64, Rayner, 1970) in the case of the Nova Scotia isolate and buff (no.45, Rayner, 1970) for Prince Edward Island and Québec isolate. After 7 days, the Nova Scotia isolate grew 62 mm, the Prince Edward Island isolate grew 58 mm and the Québec isolate grew 45 mm.

Figure 2.2

Mycelium of *Phoma* sp. isolated from lupines after 21 days of incubation on malt extract agar at 25° C. Top and reverse side view.

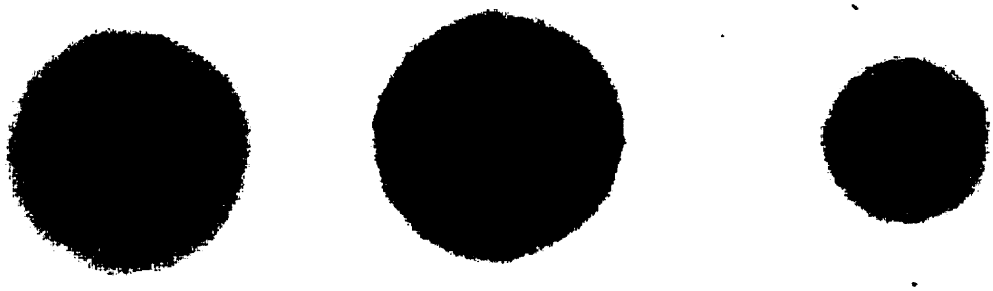
MEA

TOP SIDE



N.S. P.E.I. QUE.

REVERSE SIDE



N.S. P.E.I. QUE.

Oat Meal Agar

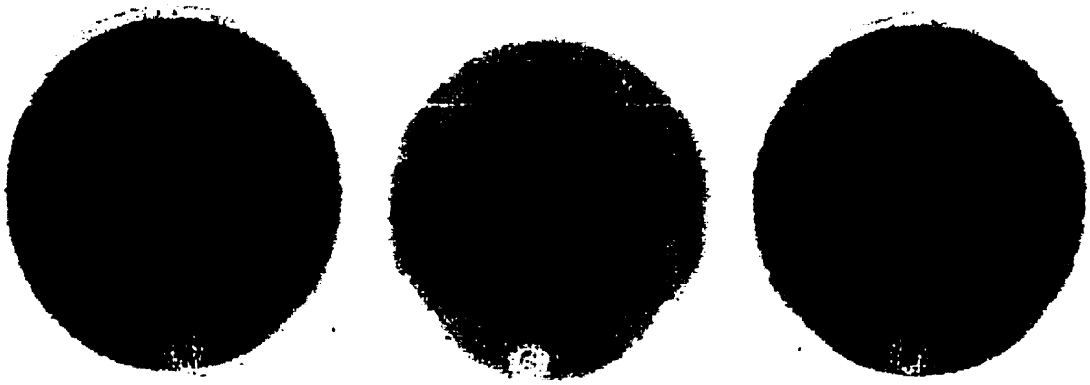
Oat meal agar is a very rich darkly pigmented medium, with a honey pigmentation (no.64, Rayner, 1970). For all isolates, colonies had a very dense and thick aerial mycelium (Fig. 2.3). Nova Scotia isolate was covered with cinnamon colored (no.62, Rayner, 1970) aerial mycelium, powdery in texture, surrounded by rings of olivaceous black (no.108, Rayner, 1970) submerged mycelium, covered with pycnidia. The isolates originating from Prince Edward Island consisted of a central aerial mycelium gray olivaceous colored (no.107, Rayner, 1970) surrounded by a ring of mycelium olivaceous black colored (no.108, Rayner, 1970) circled by another very thin ring of gray olivaceous (no. 107, Rayner, 1970) mycelium. The Québec isolate had concentric olivaceous black colored (no.108, Rayner, 1970) zones of mycelium. Colors on the reverse side were slightly different due to the pigmentation of OMA which acted like a color filter (Fig. 2.3). Reverse side of Nova Scotia isolate looked almost uniform isabelline (no.65, Rayner, 1970). The reverse side of Prince Edward Island isolate was also more uniform in term of color distribution and the centre was dark buff (no.45, Rayner 1970) surrounded by isabelline (no.65, Rayner, 1970). The reverse of the Québec isolate showed margins of honey colored agar and a darker ring of isabelline (no.65, Rayner, 1970) surrounding a dark buff centre, the transition between both colors was very diffuse. After 7 days, the Nova Scotia isolate grew 67 mm, the Prince Edward Island isolate grew 59 mm and the Québec isolate grew 61 mm.

Figure 2.3

Mycelium of *Phoma* sp. isolated from lupines after 21 days of incubation on oatmeal agar at 25° C. Top and reverse side view.

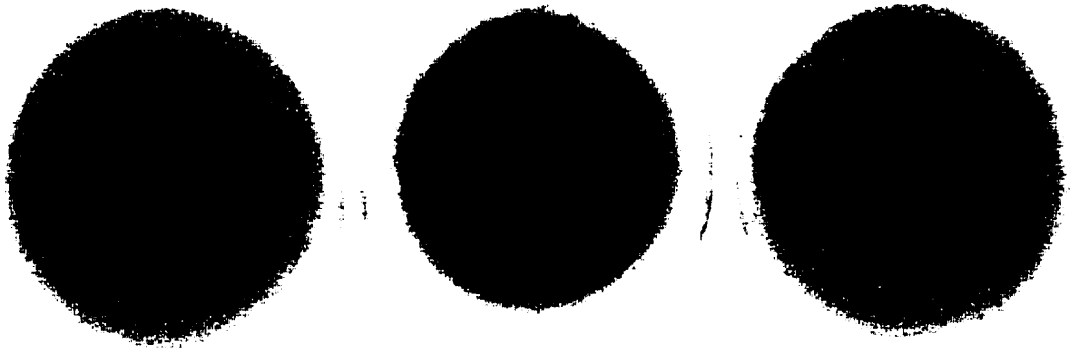
OMA

TOP SIDE



N.S. P.E.I. QUE.

REVERSE SIDE



N.S. P.E.I. QUE.

Potato Dextrose Agar

Colonies on PDA were not very different from those on MEA (Fig. 2.4). For the Nova Scotia isolate, the colony consisted of a dark centre, solid olivaceous black (no.108, Rayner, 1970) surrounded by very close rings of olivaceous black (no.108, Rayner, 1970) alternating with cinnamon colored (no.62, Rayner, 1970) rings of mycelium more aerial in nature. For the Prince Edward Island isolate, the top side was mostly formed of aerial mycelium which was smoke gray colored (no.105, Rayner, 1970) in the centre and gradually changed to a more pinkish color (buff, no.45, Rayner, 1970) toward the margins, on a dark honey pigmented agar. In the case of the Québec isolate, the colonies looked like two distinct olivaceous black (no.108, Rayner, 1970) rings surrounding a middle section of fluffy aerial light smoke gray colored (no.105, Rayner, 1970) on a honey (no. 64, Rayner, 1970) pigmented agar. The reverse sides were quite similar to the top sides (Fig. 2.4). The reverse side of the Nova Scotia isolate showed a dark olivaceous black centre (no.108, Rayner 1970) surrounded by rings of the same color alternating with rings of a dark honey. The reverse side of Prince Edward Island isolate was darker and resembled the Nova Scotia isolate, with a olivaceous black (no.108, Rayner, 1970) centre surrounded by dark honey margins. The reverse side of the Québec isolate was olivaceous black (no.108, Rayner, 1970) surrounded by a honey colored (no 64 , Rayner, 1970) ring. After 7 days, the Nova Scotia

Figure 2.4

Mycelium of *Phoma* sp. isolated from lupines after 21 days of incubation on potato dextrose agar at 25° C. Top and reverse side view.

PDA

TOP SIDE

N.S. P.E.I. QUE.

REVERSE SIDE

N.S. P.E.I. QUE.

isolate grew 64 mm, the Prince Edward Island isolate grew 60 mm and the Québec isolate grew 48 mm.

Table 2.1. Growth of three isolates of *Phoma sp.* after 7 days of growth on 3 different media

	ISOLATE		
	Nova Scotia	Prince Edward Island	Québec
MEDIA			
CMA	62[A]	66	46
MEA	62	58	45
OMA	67	59	61
PDA	64	60	48

A. Colony diameter in mm

2. Microscopic characteristics

Pycnidia were produced on all media, but they were most abundant on PDA and were distributed in a more or less concentric fashion. Pycnidia were generally light brown colored (almost rosy buff no.61, Rayner, 1970) darkening to olivaceous black (no.108 Rayner, 1970) with age. The diameters varied from 49 to 260 µm, with an average of 140 µm. Pycnidia were usually globose eand often flask shaped (Fig. 2.5 a and b). Pycnidia were mostly solitary but frequently gregarious in groups of 4 or 5 (Fig. 2.6 a), superficial or partially

immersed with a short neck and well defined ostiole and often multiostiolate (Fig. 2.6 a). The pycnidial wall was composed of several layers of irregular thick, brown- walled pseudoparenchymous cells especially in the outer layers (Fig. 2.6 b). In vitro, conidia were very abundant, hyaline, smooth, oblong to short cylindrical, obtuse at each end, unicellular often biguttulate, 2.25-3.5 X 5.5-9 µm in size (Fig. 2.7 a). Conidia were exuded in a salmon colored mucigel (no.41, Rayner, 1970). Chlamydospores were observed in older cultures, commonly present in 3-week-old colonies on PDA, MEA and OMA. Chlamydospores were intercalary, rarely solitary usually in chains of 5 to 7, mostly globose, smooth, dark brown, and 10-13.5 µm in diameter (Fig. 2.7 b).

Figure 2.5

Pycnidia produced by *Phoma* sp. on potato dextrose agar. Pycnidial diameter was between 49-260 µm. A) Pycnidia were often flask shaped with a short neck. Magnification 200 X. Bar = 10 µm. B) Pycnidia had a globose shape. Magnification 200 X. Bar = 10 µm.


A


B

Figure 2.6

Pycnidia produced by *Phoma* sp. on potato dextrose agar. A) Pycnidia were often in clusters of 4 or 5 and multiostiolate. Arrow points to one of the ostioles, the black dot on the pycnidia. Magnification 120 X. Bar = 100 µm. B) Pycnidial wall composed of several layers of pseudoparenchymous cells. Magnification 400 X. Bar = 5 µm.

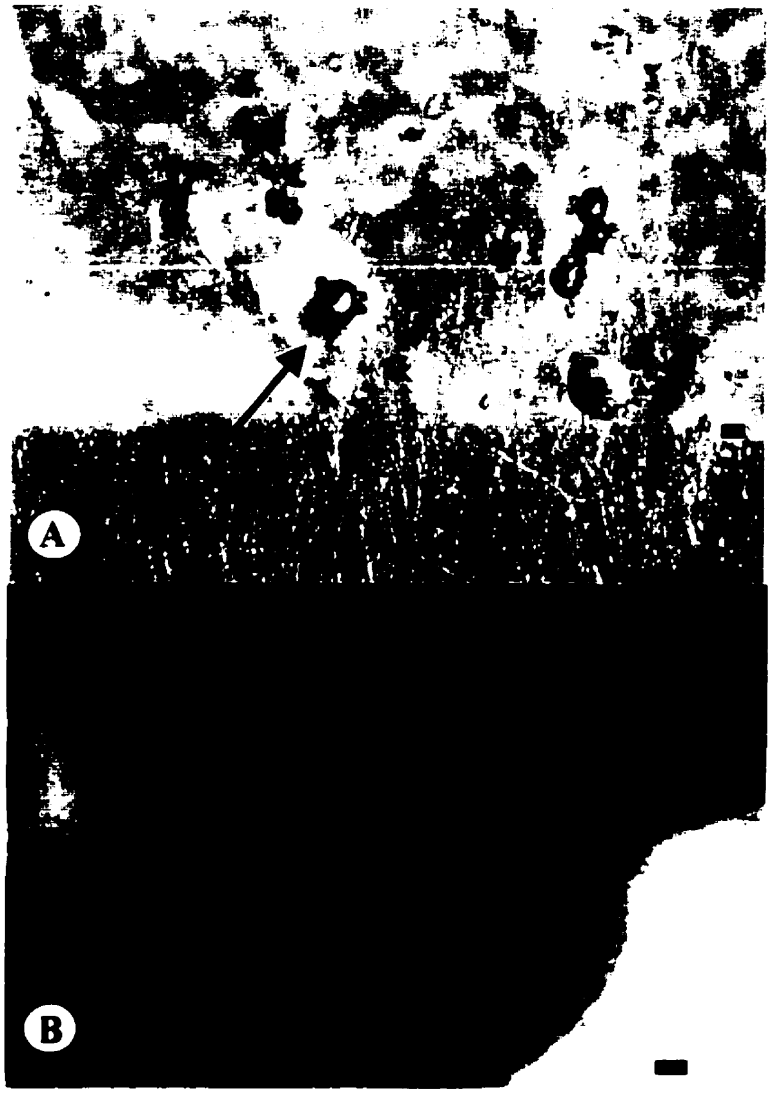
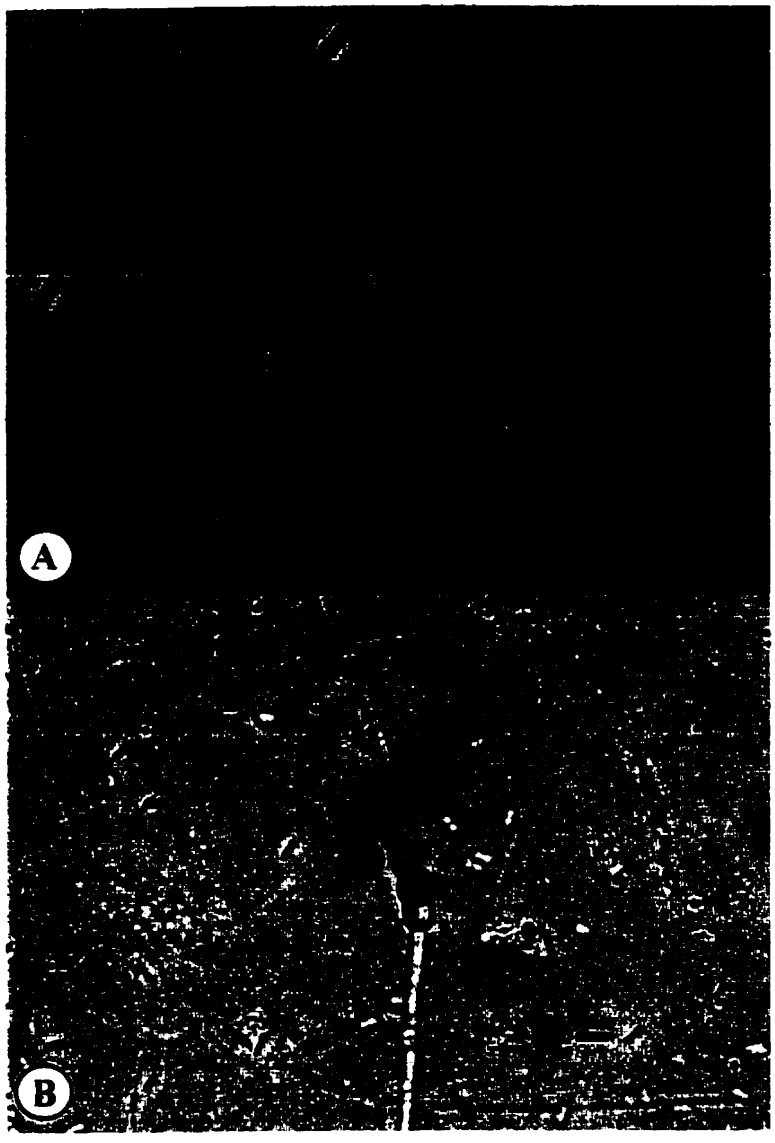

A
B

Figure 2.7

Conidia and Chlamydospores produced by *Phoma* sp. on potato dextrose agar. A) Conidia were very abundant, unicellular oblong to short cylindrical, obtuse at each end. Magnification 1000 X. Bar = 10 µm. B) Chlamydospores were intercallary. Magnification 200 X. Bar = 10 µ.


A
B

DISCUSSION

This research describes the *Phoma sp.* found on seed and stem lesions of *Lupinus albus* L. When cultures of this *Phoma sp.* were compared to authentic material of *Phoma lupini* Ell. & Ev. and *Phoma lupinicola* Earle by the National Identification Service of Economic Fungi at the Research Branch of Agriculture Canada, it was found that the strain under study was neither one of these species. *Phoma macrostoma* Mont. was proposed as a possible species due to some morphological similarities (Dr. John Bissett, personal communication). However, there were differences in conidial morphology, especially in size, shape and culture appearance. *P. macrostoma* produces a red pigment in the media (Rai and Rajak, 1993) while the *Phoma* sp. used here did not. Therefore this species was rejected as a possible identification for the pathogen used in this investigation, and no further comparisons will be made with this species. Similarly, the *Phoma* sp. under study was later also proposed to be similar to *Phoma medicaginis* Malbr. & Roum.(Dr. Jim White, personal communication). Two varieties of *P. medicaginis* have been recognized in the literature: *P. medicaginis* var. *medicaginis* Malbr. & Roum. and *P. medicaginis* Malbr. & Roum. var. *pinodella* L.K. Jones. The former was previously named both *Ascochyta imperfecta* Pk. and *Phoma herbarum* Westd. The present valid name is *Phoma medicaginis* Malbr. & Roum. The latter had been named both *Ascochyta pinodella* L. K. Jones and *Phoma trifolii* E.M. Johnston & Valleau.

The valid name for this species is now *Phoma pinodella* (L.K.Jones) Morgan-Jones & Burch. For simplicity in this text, only the valid names will be used. *P. medicaginis* and *P. pinodella*, causal agents of black stem diseases in legumes, were once considered to be varieties of the same species, however due to constant differences they were divided into two separate species.

Comparisons of *Phoma* species

Characteristics of *Phoma* sp. were compared with similar observations reported for two related pathogens, *P. medicaginis* and *P. pinodella* (White and Morgan-Jones, 1987; Morgan-Jones and Burch, 1987) On malt extract agar, the *Phoma* sp. under study varied between gray olivaceous and olivaceous black surrounded by a paler buff to olivaceous buff margin. This is quite similar to *P. medicaginis* colonies, which are usually olive green with a wide colorless marginal zone and *P. pinodella* which are olivaceous with a whitish marginal zone. Colonies on PDA seem to show more differences. In this case, *P. medicaginis* is usually olive gray to dark olive gray with a 3-4 mm wide whitish to pale buff margin. With age, the coloration becomes darker and sometimes concentrically zoned, eventually becoming carbonaceous black, compared to *P. pinodella* which is more variable in appearance. *P. pinodella* has dense mycelium light greenish green at the centre when young and becoming gray olivaceous or blackish at the centre with age with broad pale cream to whitish

margin. The *Phoma* sp. from lupine when grown on PDA differed in appearance depending on the isolate. The Nova Scotia isolate usually consisted of olivaceous black concentric rings very similar to *P. pinodella*, which also has numerous dark radial patches or small sectors surrounding the centre portion, as described by White and Morgan-Jones (1987). For Québec and Prince Edward Island isolates, colonies appeared somewhat different, with a smoke gray aerial mycelium in the centre portion surrounded by olivaceous black mycelium for the Québec isolate and becoming lighter toward the margins for Prince Edward Island isolate. The reverse side in younger colonies of *P. medicaginis* is uniformly olive black becoming darker toward the margins often with one or more black concentric rings with age. The reverse side of *P. pinodella* is usually brownish in the centre progressively lighter toward the margins and darker underneath radial sectors. The *Phoma* sp. from lupine has a reverse side consisting of a olivaceous black middle section surrounded by alternately olivaceous black and dark honey rings. *P. pinodella* produces abundant crystals in malt extract agar, while these formations are only occasionally produced in older cultures of *P. medicaginis* (White and Morgan-Jones, 1987; Morgan-Jones and Burch, 1987). These were not observed during these studies of *Phoma* sp. isolated from lupines.

Phoma medicaginis grows much faster on MEA than on PDA and *P. pinodella* does not show a difference in rate of growth between these two media.

In general, the rate of growth of *P. medicaginis* is slower than for *P. pinodella*. At 25°C on PDA, the growth of *P. medicaginis* is 39 mm after 7 days and for *P. pinodella* is 55 mm after 6 days (data not shown). This compares to the *Phoma* sp. of lupines, which grew 62 mm for Prince Edward Island and Nova Scotia isolates, and 48 mm for the Québec isolate after 7 days. On MEA, *P. medicaginis* grew 37 mm after 7 days, and *P. pinodella* grew 55 mm after 6 days. Our species grew 45 mm for the Québec isolate and 60 mm for the Nova Scotia and Prince Edward Island isolates after 7 days. As will be shown later, optimal growth temperature for *Phoma* sp. on lupine was 25° C, compared to the optimum for *P. medicaginis* , which is between 20°-25° C and *P. pinodella* which was closer to 20° C.

For the three species of *Phoma (P. medicaginis, P. pinodella* and *Phoma* sp. from lupines) conidial characteristics are very similar, for example sizes are comparable; 6-10 X 2.5-4 µm in the case of *P. pinodella*, 7-11 X 2-3.5 µm for *P. medicaginis* and 5.5-9 X 2.25-3.5 µm for the species under study. In these three species, conidial masses were whitish to pale pink-buff colored, and conidial septation occurred occasionally. For the *Phoma* sp. occurring on lupines, septation was sometimes observed in nature whereas none was observed in culture; the opposite was observed by Edmunds and Hansen (1960) for *P. medicaginis*. As will be shown later spores of *P. pinodella* germinated well at 35° C and very slowly at 5° C, compared to *P. medicaginis* which did not show

any germination at those extremes. The *Phoma* sp. in the present study showed very good germination when incubated at 35° C. However at 5°C and 24 hours of incubation no germination was observed.

Chlamydospores are observed in these three species of *Phoma*. However, in *P. medicaginis*, they are only produced in older cultures. This is different from *P. pinodella* and *Phoma* sp. from lupines which produced abundant chlamydospores on younger cultures. Other characteristics such as size, color, placement are all quite similar (data not shown).

Pycnidia are abundant in *P. medicaginis*. However, no defined ostioles are observed compared to *P. pinodella* and *Phoma* sp. from lupines which both show well defined ostioles. Secondary pycnidia, produced atop primary ones, are present in *P. pinodella*, and were also observed in the *Phoma* sp. under study. There is no reference to such pycnidia in *P. medicaginis*. Pycnidial size and colors were very similar for these three species. Pycnidial cell walls can also be a determinant characteristic to differentiate between different species. Walls are made of different layers of cells which show different wall thickness, coloration, sizes and shapes depending on the species under study (White and Morgan-Jones, 1987). However, due to a superposition of layers it is very difficult to observe specific cell layers and this comparison was not used in this study. Differences between *Phoma* spp. are not only based on morphological

characters. Pathogenicity can also be a criterion to identify the appropriate species. *P. medicaginis* is constantly associated with black stem disease of alfalfa and *P. pinodella* to black stem disease in red clover and footrot of peas. Although these fungi can be cross inoculated on other hosts, symptoms of pathogenicity are always stronger on the original hosts (Boerema et al. 1965). When the *Phoma* sp. from lupine was cross inoculated on other members of the Fabaceae family, no pathogenicity symptoms were observed (Paulitz, unpublished).

The description and identification of a new species of *Phoma* is a very difficult task. More than 2,000 species of *Phoma* are reported in nature (Sutton, 1980) and no extensive keys have been published to date. Many names have been attributed to the same *Phoma* species, which complicates the task further. Variability in cultures of the same species is also a problem for the identification of species of *Phoma*. Another factor related to this problem is the fact that methodology differs from one researcher to another and the same characteristics are not always observed under the same conditions.

Considering morphological characteristics, the species of *Phoma* from lupines most closely resembles *P. pinodella*, based on colony morphology, growth rate on PDA and MEA, temperature profiles, chlamydospore formation, and well defined ostioles. However, the *Phoma* sp. from lupines did not form

crystals in MEA, and is not pathogenic on peas. It appears that the *Phoma* sp. used here may be a new variety of *P. pinodella*, but further pathogenicity tests are needed, along with molecular comparisons using isozymes or RFLP studies.

III. EFFECTS OF THE ENVIRONMENT ON IN VITRO AND IN PLANTA GROWTH AND GERMINATION OF *PHOMA* SP.

INTRODUCTION

A combination of three factors is required for infection to occur: 1) a suitable host, 2) an infective pathogen, and 3) suitable environmental conditions. Of these, environment is the factor that can vary the most. Even during a single day, it could be the determinant for infection. Fungi as well as higher plants are affected by environmental conditions. These conditions can determine whether or not a disease will occur. Environmental conditions can affect microorganism growth and reproduction by acting on the metabolism and on the different chemical and physical reactions usually present in any living organism. Environmental conditions will affect the incidence and development of plant diseases by working at two levels: 1) they will affect pathogen development and reproduction and, 2) they will predispose the plant to diseases (Colhoun, 1979).

Predisposition and susceptibility of a plant will first depend on the plant itself; age of the plant, age of the affected plant part (i.e. young versus old branch) and by its location within the plant canopy (Populer, 1978). Morever,

many environmental factors may cause plant predisposition. Temperature and moisture are the most important; however, other factors such as light, nutrition, soil pH, radiation, chemicals, presence of other organisms and interaction of all those factors may all be taking part in plant susceptibility to disease (Colhoun,1979).

Minimum conditions of temperature are required for any biological activity to occur. Minimum temperature is required both to permit plant growth as well as to permit pathogen development. For example, late fall, winter and early spring are usually too cold to allow active pathogen development. Consequently during these periods of the year diseases are not initiated and pathogen development is stopped (Agrios, 1988). During the warmer seasons (late spring, summer, and early fall) temperature will also be important in determining infections. Sub optimal temperature will affect the rate of incidence of disease by diminishing the quantity of spores that will germinate and the rate of germ tube growth. Extreme temperature will also decrease the rate of mycelium growth and development therefore also affecting spore production thus increasing the length of time between infections. Generally, the range of temperature permiting growth is broader than that allowing for sporulation (Griffin, 1994).

There are a multitude of factors that temperature may affect and that will permit diseases incidence with varying degrees of severity. Some of these factors are: 1) pre-inoculation effects of temperature on plant (by increasing susceptibility to diseases), 2) temperature effect on pathogen survival (during one season or from one season to the next), 3) temperature effect on the length of incubation period (may affect the number of cycles present in one season), 4) temperature effect on spore germination, 5) temperature effect in relation to plant and fungus stage of development, and 6) temperature effects on spore production and discharge (Colhoun, 1979).

Water is the second most important factor for disease development. The most important impact of moisture is probably at the stage of spore germination and penetration of the host by the germ tube. As with temperature, moisture level will also affect the plant by increasing tissue succulence and therefore decreasing resistance to pathogen invasion. Limits for fungal growth in terms of water potential are between 0 MPa which is pure water and -81 MPa which is when DNA degenerates (Griffin,1994).

A minimum level of moisture is usually required for diseases to become established. A film of moisture at the leaf surface is generally necessary for spore germination. Not only is moisture important, but also is the length of the period of leaf wetness, and may be the determinant of disease incidence and

severity. Further, not only is water important at the infection stage of the pathogen, but also in many cases water is the main dispersal agent from one plant to another by splashing of rain drops which dislodge spores from infected tissues and distribute them further. Irrigation water will also serve the same function by carrying infectious propagules to other sites (Rotem, 1978).

The goals of the following experiments were to determine how temperature affects three isolates of Phoma sp. isolated from lupines in terms of mycelial growth, spore germination and how relative humidity affects the incidence of spore germination of *Phoma* sp. isolated from lupine at constant temperature in vitro; and to observe how temperature and dew period affect disease development in lupine plants.

MATERIALS AND METHODS

1. Effects of temperature on mycelial growth

1.1 Inoculum production

Inoculum was prepared using the same method as described in section 1 of the materials and methods in chapter 2.

1.2 Inoculation and incubation

Two isolates from each of the three locations (Québec, Prince Edward Island and Nova Scotia) were used. Potato dextrose agar (PDA) plates were inoculated with a 0.7 cm-diam-plug (no.3 cork borer). Plates were then sealed with parafilm and stacked into piles containing each of the isolates. The stacks were wrapped in aluminum foil to avoid any light effect. Each one of the six isolates was incubated under seven different temperatures: 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. Every two days, the diameter of the colonies was recorded on two perpendicular axes of the colonies. This test was organized as a randomized complete block design with four replicates per isolate. The experiment was conducted twice.

2. Effects of temperature on spore germination

2.1 Inoculum production

Inoculum was prepared using the same method as described in section 1 of the materials and methods in Chapter 2.

2.2 Spore germination

Spores produced by the three isolates were collected by pouring 5 ml of a 0.1% agar solution containing 100 ppm DL-asparagine (Sigma, St. Louis, MO), and 1,000 ppm glucose over cultures grown on PDA. A serial dilution was then performed with this same solution until a concentration of 5×10^5 spores per ml was reached. Spore concentration was evaluated with a haemocytometer One 10-µl drop of the spore suspension was then deposited at both ends of microscope slides. These slides were then transferred to petri dishes with moist filter paper (no.3 Whatman) at the bottom. The petri dishes were later sealed with parafilm and stacked in piles of 6 (one petri dish/per time period). Each pile was wrapped in aluminum foil and then incubated at its respective temperatures Each one of the three isolates was incubated at seven temperatures: 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. Germination was measured at six time periods: 6, 8, 12, 24, 36, and 48 hours. This experiment was a 7 X 3 factorial model with two replicates per isolate. The experiment was conducted twice.

3. Effects of osmotic potentials on spore germination

3.1 Inoculum production

Inoculum was prepared using the same method as described in section 1 of the materials and methods section in Chapter 2. However, only the Québec isolate was used.

3.2 Spore collection

Ten ml of a solution containing sterile distilled water , 100 ppm DL-asparagine (Sigma, St. Louis, MO) and 1,000 ppm of glucose was poured over an actively sporulating culture of *Phoma*. A flame sterilized glass rod was used to liberate the spores from the pycnidia and to mix them with water. A serial dilution was then performed until a concentration of (5×10^5) conidia/ml was obtained.

3.3 Assessment of KCl concentration required to obtain the different osmotic potentials in PDA

A modification of the agar dish isopiestic equilibration technique described by Harris et al. (1970), was used to control water potentials. In this

experiment, nine osmotic potential values were studied. These osmotic potentials were chosen on the basis of their corresponding values of relative humidities derived from the following formulas:

Osmotic potential (bar) = 1370 ln aw (Griffin, 1994)

where:

aw is water activity

and relative humidity = 100 × aw (Griffin, 1994)

Required quantities of potassium chloride were determined from the following formula

Y= (-0.475) + (-0.057) x (adapted from Epiphane, 1996)

where:

Y= osmotic potential

x= concentration of KCl in g/L

This formula was derived from a reference curve of osmotic potential (MPa) in PDA as a function of KCl concentration (Epiphane, 1996). These calculations were used to determine KCl quantities required per litre of PDA (Difco, St.Louis, MO). Osmotic potentials of -1.377, -2.768, -4.173, -5.393, -7.027, -8.476, -12.920, -17.513 MPa corresponding to relative humidities (R.H.) of 99, 98, 97, 96, 95, 94, 91, 88 % , were obtained using 16, 40, 65, 90, 115, 140, 218, 299 g/L of KCl.

3.4 Conidial germination

A 0.5 ml-drop of the 5 X 10^{5} spores/ml conidial suspension was spread on the different agar petri plates using a flamed sterilized glass rod. The petri plates were then sealed with three layers of parafilm. Four piles, each containing a plate of every R.H. treatment were wrapped in aluminum foil and then incubated at 25° C for 24 hours.

After incubation, germination was stopped by transferring the agar petri dishes to incubators at 5° C. Observation of spore germination was then performed within 24 hours. Spores were observed under a microscope and counted directly on the agar petri dish without transfer to a microscope slide. The experimental design was a completely randomized design with four replicates, the replicates being each of the piles wrapped in aluminum foil, containing the nine relative humidity plates arranged in a random order in the pile. The experiment was conducted twice.

4. Effects of dew period and temperature on seedling infection

4.1 Seedling production

Seeds of *Lupinus albus* cv. Amiga were planted in 12.7 cm diameter plastic pots, containing promix (PRO-MIX, Premier Horticulture Inc.). Four seeds were evenly planted in each pot. The pots were placed in a growth chamber maintained at 22° C/18° C day/night temperature, with an intensity of 250 micromoles $m^{-2} sec^{-1}$ on 16h/8h light/dark cycle. Plants were watered when needed, usually every two days. After emergence, plants were thinned to one seedling per pot.

4.2 Seedling inoculation

Two-week-old plants were then used for inoculation. A scalpel was used to wound the stems at about 2 cm from the soil line. Wounded plants were evenly sprayed with 8-ml of a spore suspension adjusted to 5×10^4 conidia/ml. The seedlings were covered with 20 x 38 cm polyethylene bags with the inside sprayed with distilled water to enhance humidity.

4.3 Seedling incubation

Plants were incubated in total darkness in four different growth chambers 15°, 20°, 25°, 30° C. The polyethylene bags were removed after 8. 12. 24. 36 hours to create four different dew period durations. Five replicates were inoculated for every temperature x dew period duration.

4.4 Spore production

Inoculum was prepared using the same method as described in section 1 of the materials and methods in Chapter 2. However, only the Québec isolate was used.

4.5 Disease rating

Observations and measurement of stem lesion size were performed two days (48 hours) after inoculation and every second day for the following 18 days. and after 24 , and 30 days. This experiment was a 4 X 4 factorial experiment with two treatments each containing 4 levels. The experiment was conducted twice.

RESULTS

1. Effects of temperature on mycelium growth

1.1 Data analysis

Statistical analysis were performed using the SAS (SAS Institute, version 6.11) statistical tool. Due to some discrepancies from normality or the residuals, logarithmic transformations were performed on the data of both runs. However, all the tables and figures show the non-transformed data. For both runs, a few sampling dates did not show normality at the $P<0.01$. However, the stem and leaf figure of the SAS output showed a quasi normal unimodal bell shaped distribution. The data set was then considered normal and repeated measures tests were performed. When some factors showed statistical differences, means were compared using the SNK (Student, Newman, Keuls) test. For both runs, there was a large temperature effect ($P<0.01$), there was a highly significant isolate effect ($P<0.01$), and the block effect was not significant ($P>0.01$). Time also affected mycelial growth ($P<0.01$) as well as time*isolate interaction. Interactions of temperature*block, time*block and time*temperature*block, were not significant ($P>0.01$).

1.2 Effect of temperature

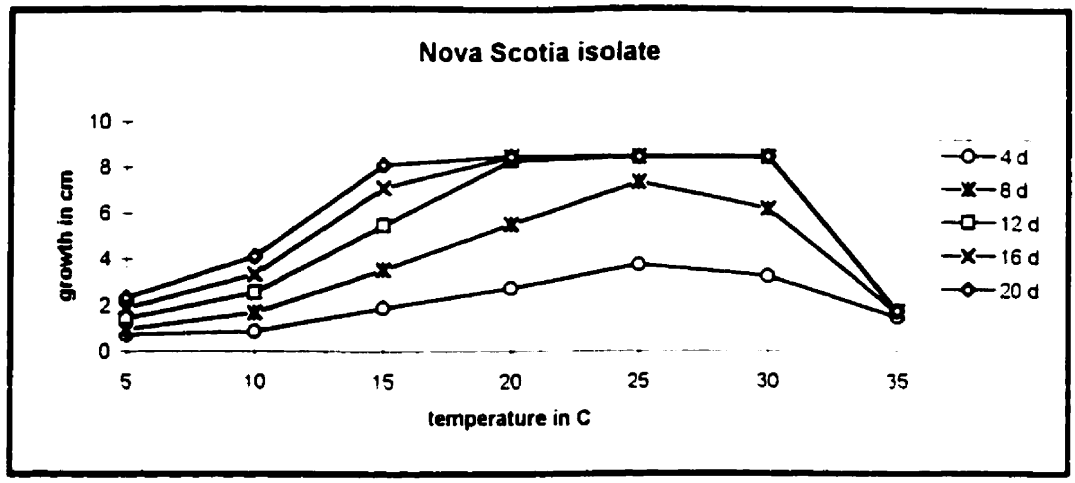
Temperature had a significant effect on mycelial growth ($P<0.01$). For both trials, two days after transfer, mycelium had started to grow at temperatures higher than 15° C. Colonies incubated at 5°C started to grow slowly after 4 days but at a very slow rate (Fig. 3.1.1 and 3.1.2). After 22 days of incubation, the average measured colony diameter was 26 mm for the first trial. Six days were required in the second trial to see any measurable growth at 5° C, and the maximum diameter observed after 22 days of incubation was 29 mm. Cultures incubated at 35° C started to develop only two days after transfer for the first trial and only 14 days after transfer for the second trial. Mycelium was also very slow to develop at 35° C, the maximum average size attained was only 14.6 mm diameter for the first trial and 14.8 mm diameter in the second trial.

Maximum average size (observed when the mycelium reached the sides of the petri dishes) was attained after 18 days of incubation at 25° C for the first trial (84.9 mm diameter). For the second trial, the maximum average size (85 mm diameter) was measured after 22 days.

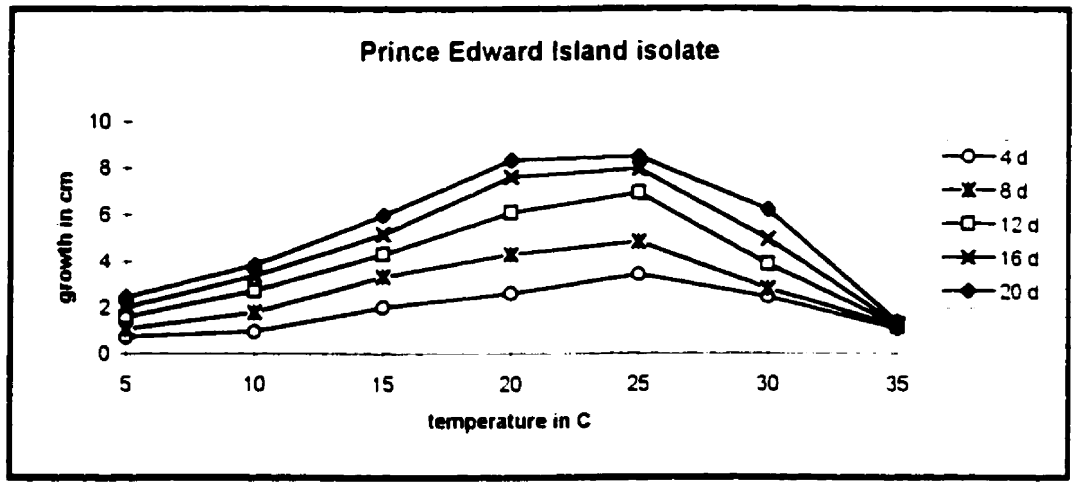
Figure 3.1.1

Effects of temperature on mycelium growth over time for the first trial. A) Nova Scotia isolates. B) Prince Edward Island isolate. C) Québec isolate.

A.


Nova Scotia isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

B.


Prince Edward Island isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

C.

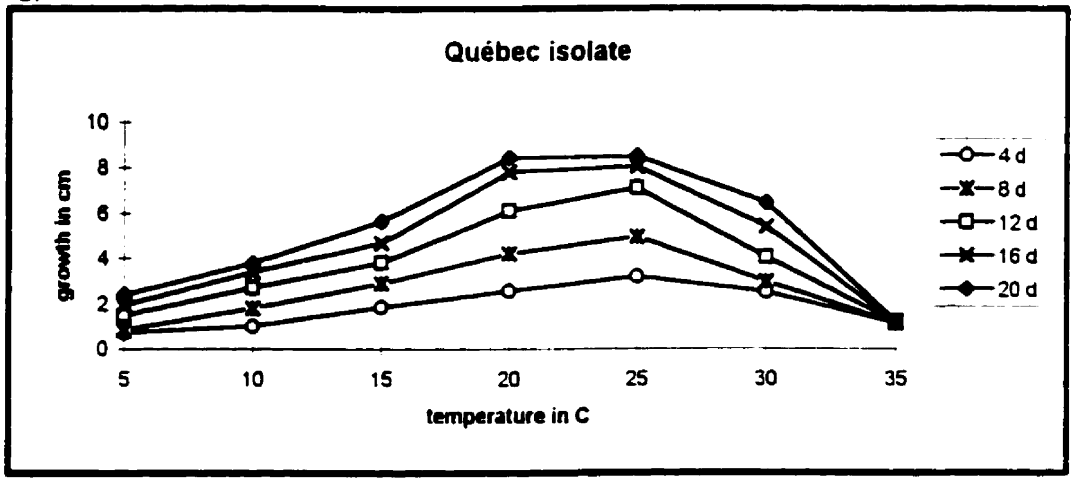
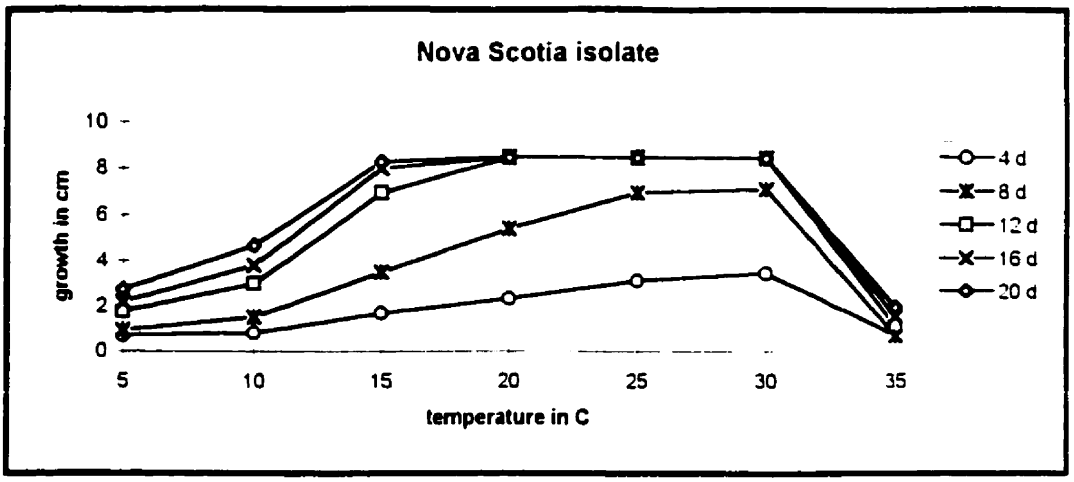

Québec isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

Figure 3.1.2

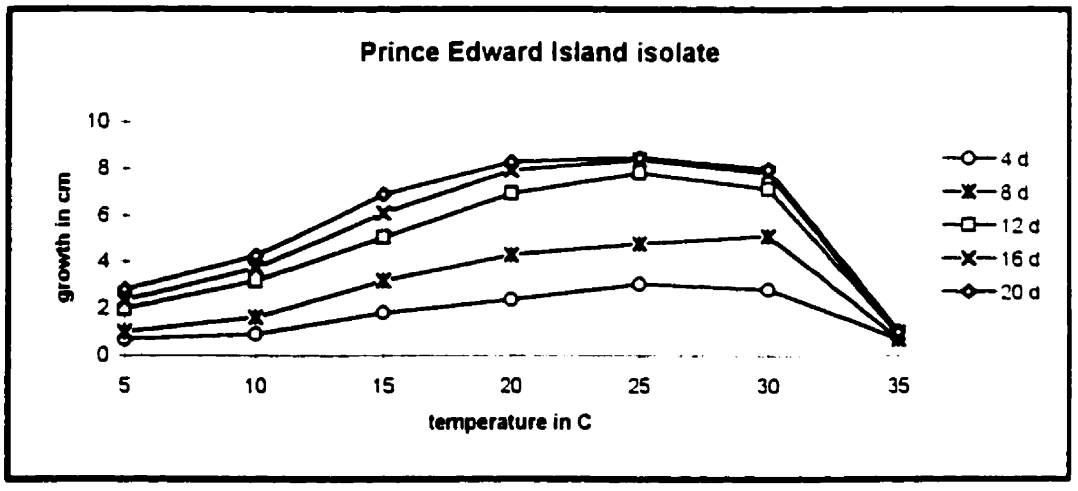
Effects of temperature on mycelium growth over time for the second trial.

A) Nova Scotia isolate. B) Prince Edward Island isolate. C) Québec isolate.

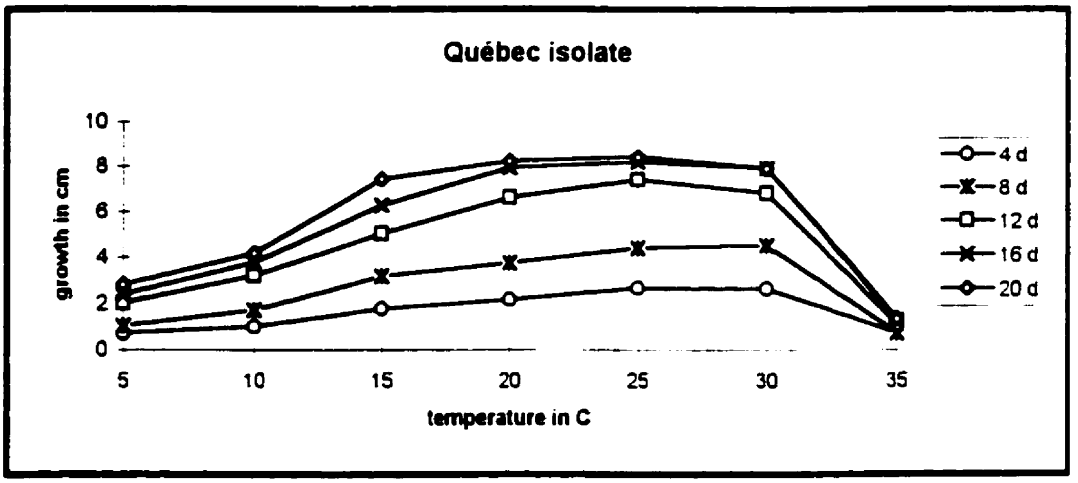
A.


Nova Scotia isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

B.


Prince Edward Island isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

C.


Québec isolate
growth in cm
10
8
6
4
2
0
5
10
15
20
25
30
35
temperature in C
4 d
8 d
12 d
16 d
20 d

For both trials, 25° C was the overall optimal temperature for mycelial growth. It was closely followed by 20° and 30° C, depending on the number of days after transfer. In the first trial, 25° C was the optimal temperature until 12 days after transfer, when 20° C and 25° C resulted in the same growth rate, for the remainder of the experiment. For the second trial of the experiment, 25° and 30° C gave equal growth until day 18, when 25° C became the sole optimal temperature.

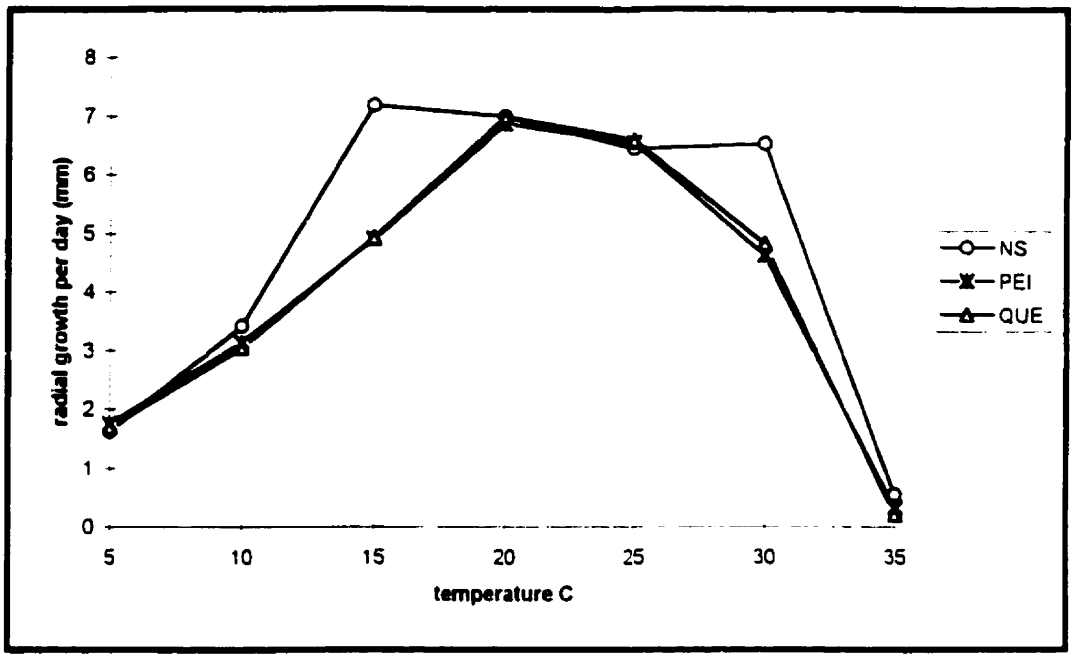
1.3 Effect of isolate

Isolate also had a significant effect on mycelial growth. In the first trial, growth from the Nova Scotia isolate was always greater than growth from the Québec and Prince Edward Island isolates which were not statistically different from each other (Fig. 3.1.3 a). In the second trial, an isolate effect was observed 4 days after transfer where Nova Scotia isolate was different from Québec isolate but showed similar growth to Prince Edward Island isolate (Fig. 3.1.3 b). Six days were required to observe the same results as in the first trial.

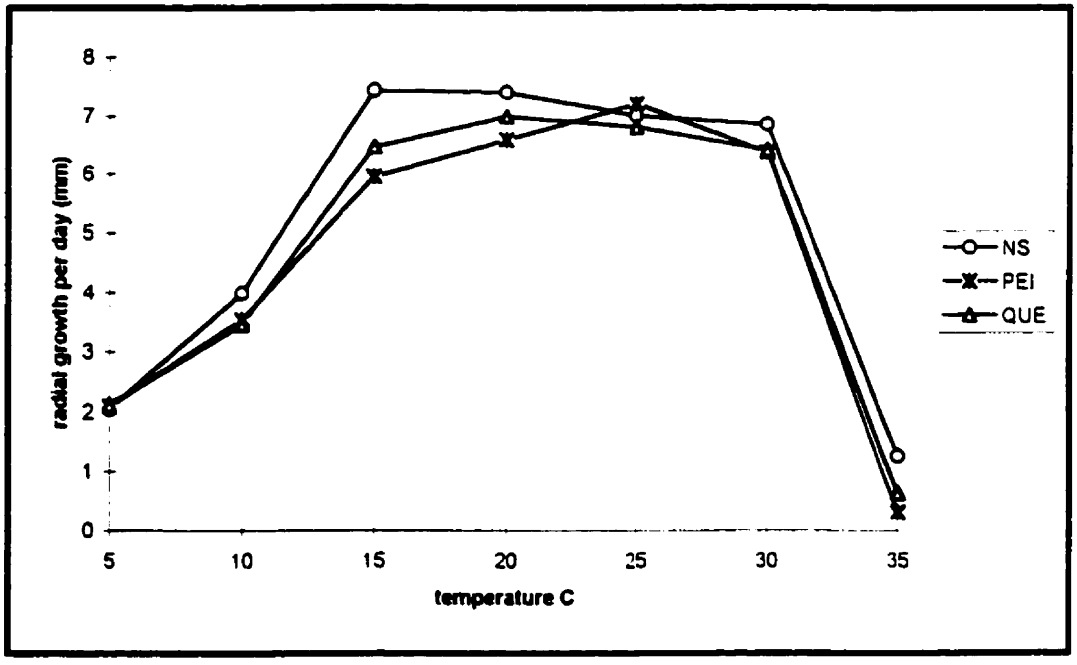
Figure 3.1.3

Rate of mycelium growth in mm per day for each location (average of two isolates per province) as a function of temperatures. A) First trial. B) Second trial.

A.


radial growth per day (mm)
0
1
2
3
4
5
6
7
8
5
10
15
20
25
30
35
temperature C
NS
PEI
QUE

B.


radial growth per day (mm)
0
1
2
3
4
5
6
7
8
5
10
15
20
25
30
35
temperature C
NS
PEI
QUE

2. Effects of temperature on spore germination

2.1 Data analysis

Statistical analyses were performed using the SAS (SAS institute, 1995) statistical tool. To fulfill the normality assumption, the data were square root and arcsin transformed. The data were analysed using the repeated measures statement of SAS. When factors showed statistical differences, means were compared using the SNK (Student, Newman, Keuls) test. For both runs, there was a large temperature effect ($P<0.01$). There was no significant isolate effect at ($P> 0.01$) . Time also affected germ tube growth ($P<0.01$). There were time * temperature interactions ($P<0.01$). There were no time * isolate interactions.

2.2 Spore germination

In general, at 5° C no spore germination occurred, even after 48 hours of incubation. At the other extreme of the temperature scale (35° C) germination was observed only after 12 hours of incubation. After 24 hours at temperatures between 15° and 30° C, a high percentage of spores germinated (>80%).

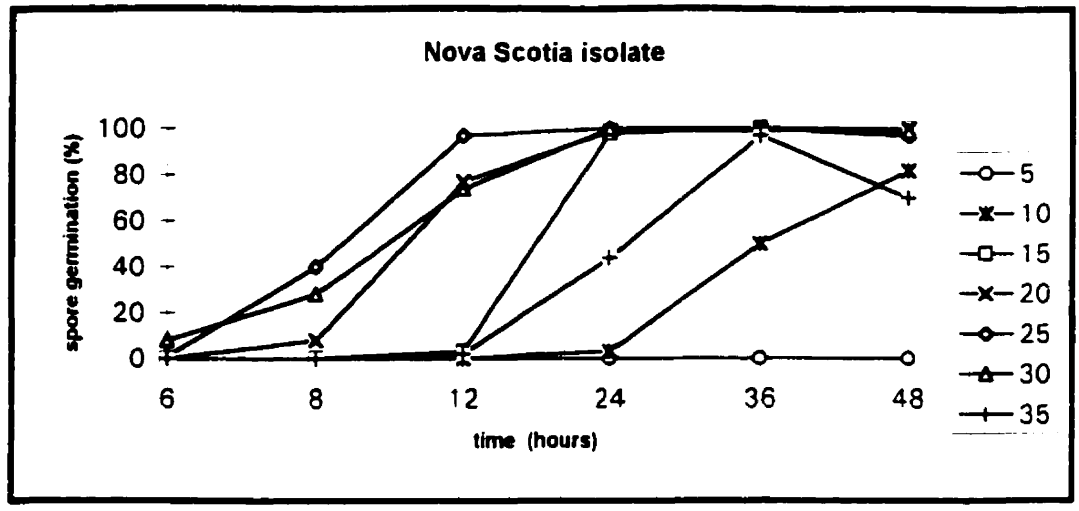
From Figure 3.2.1 and 3.2.2 it can be observed that conidia germinated at temperatures ranging from 10° to 35° C. Germination was minimal after 6 hours with a pooled average of germination of less than 3 %. After 12 hours, total germination reached about 20 %. After 24 hours, for both runs, germination was close to its maximum for 20°, 25° and 30° C. In the first run of the experiment, maximum germination was very close to 100 %. However in run 2, this maximum germination was lower than 100 % and varied somewhat depending on the isolates.

The optimal temperature for spore germination was 25° C, followed closely by 30° and 20° C. At 35° C, germination was similar to that of 15° C and these were significantly lower than those 30° C.

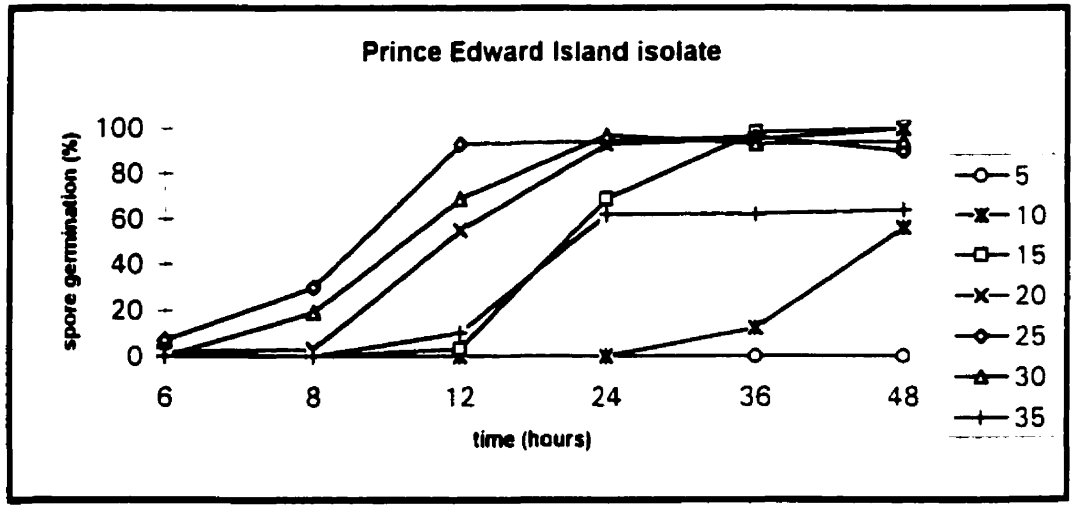
Figure 3.2.1

Spore germination of three isolates of *Phoma* sp. isolated from lupine plants as a function of time incubated at 7 temperatures. First trial. A) Nova Scotia isolate. B) Prince Edward Island isolate. C) Québec isolate.

A.


Nova Scotia isolate
spore germination (%)
100
80
60
40
20
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

B.


Prince Edward Island isolate
spore germination (%)
100
80
60
40
20
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

C.

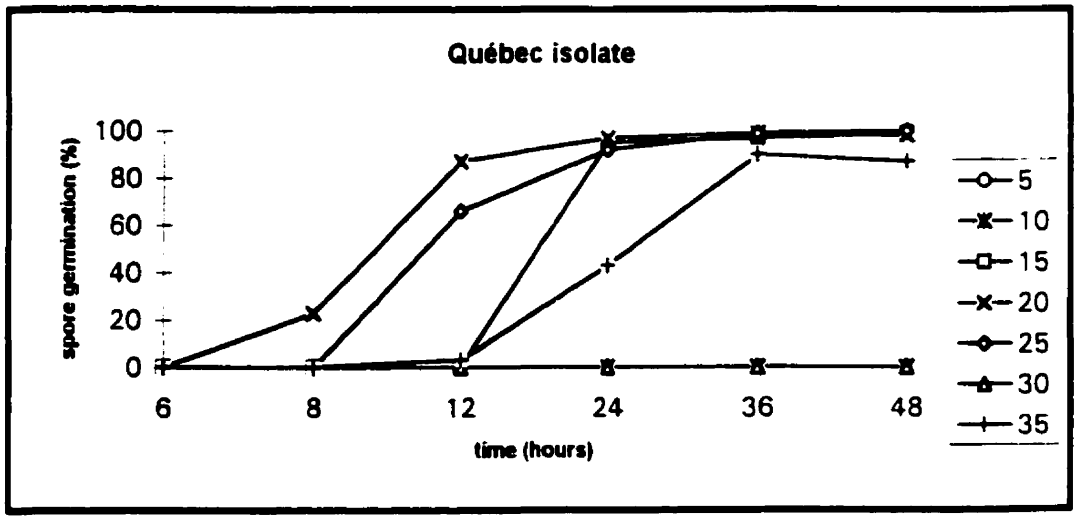
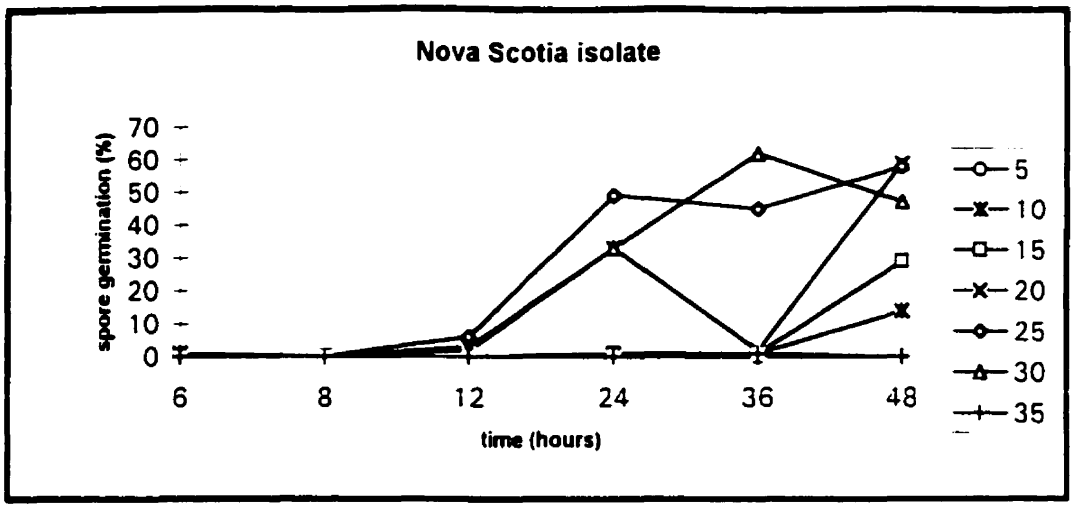

Québec isolate
spore germination (%)
100
80
60
40
20
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

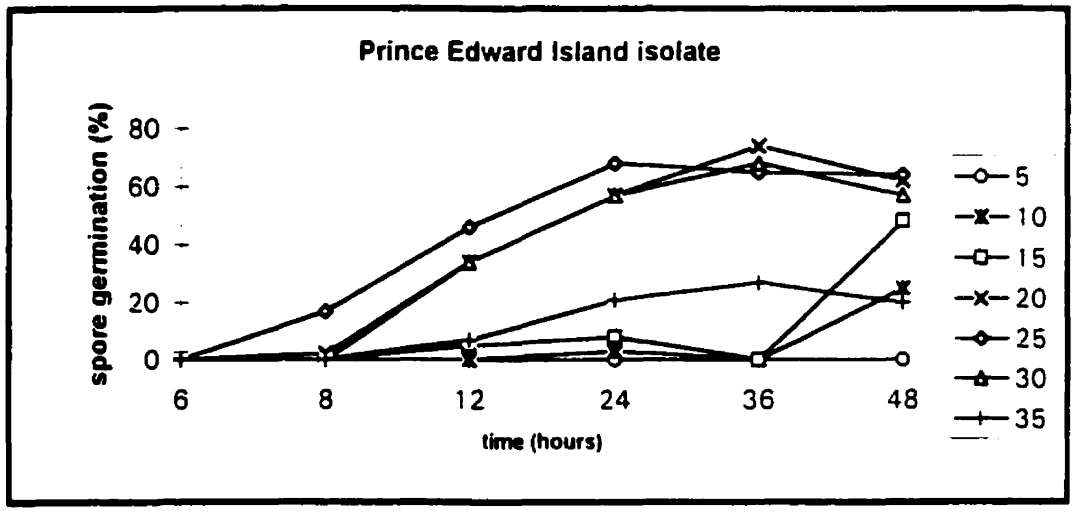
Figure 3.2.2

Spore germination of three isolates of *Phoma* sp. isolated from lupine plants as a function of time and incubated at 7 temperatures. Second trial. A) Nova Scotia isolate. B) Prince Edward Island isolate. C) Québec isolate.

A.


Nova Scotia isolate
spore germination (%)
70
60
50
40
30
20
10
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

B.


Prince Edward Island isolate
spore germination (%)
80
60
40
20
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

C.

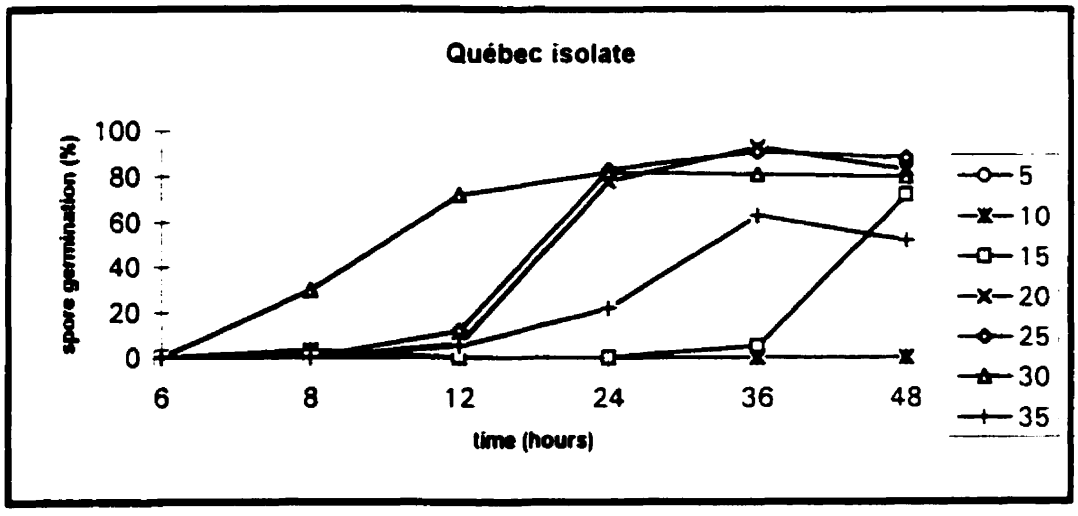
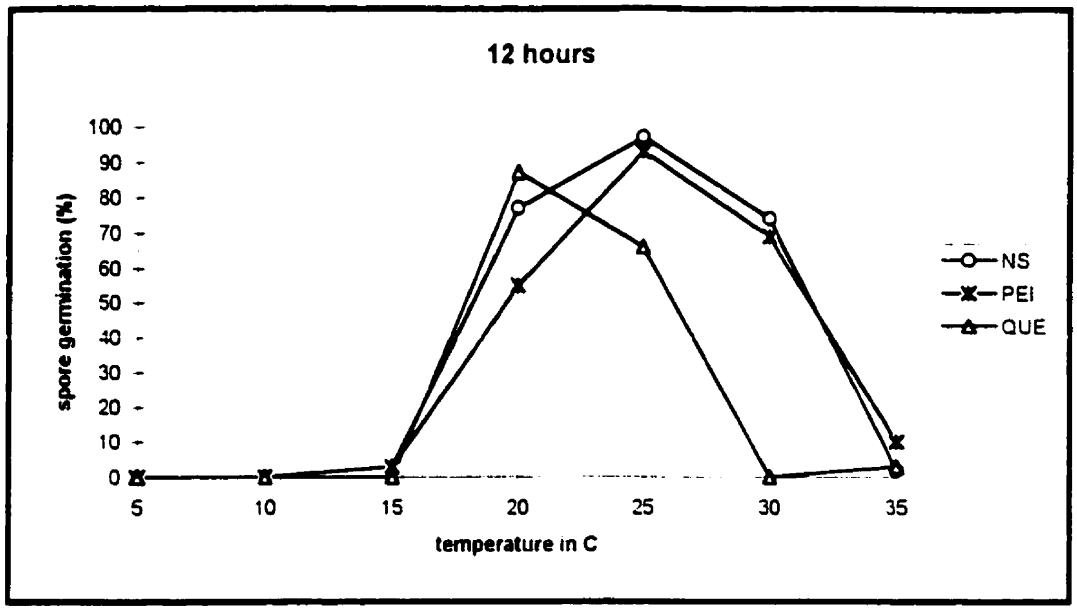

Québec isolate
spore germination (%)
100
80
60
40
20
0
6
8
12
24
36
48
time (hours)
5
10
15
20
25
30
35

Figure 3.2.3

Effect of 7 temperature regimes on spore germination of three isolates of *Phoma* sp. isolated from lupine plants. First trial. A) Germination after 12 hours. B) Germination after 24 hours.

A.


12 hours
spore germination (%)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
temperature in C
NS
PEI
QUE

B.

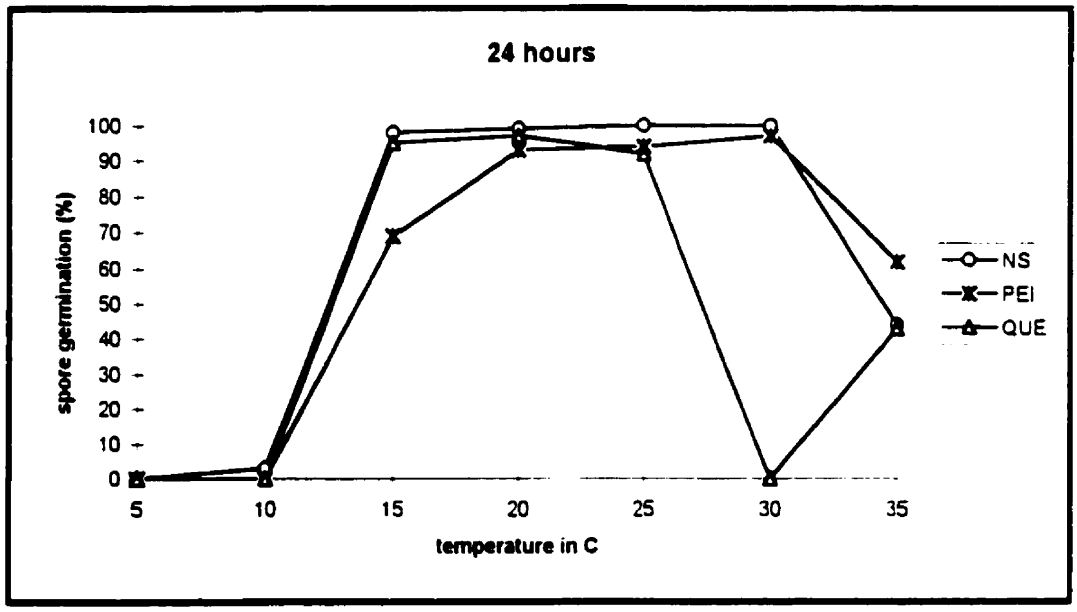
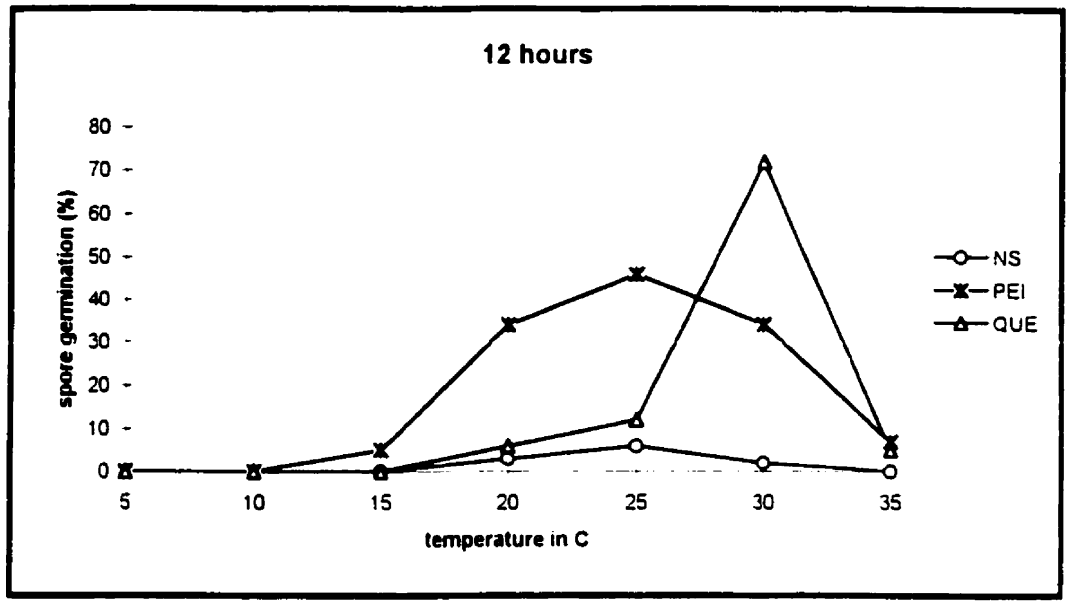

24 hours
spore germination (%)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
temperature in C
NS
PEI
QUE

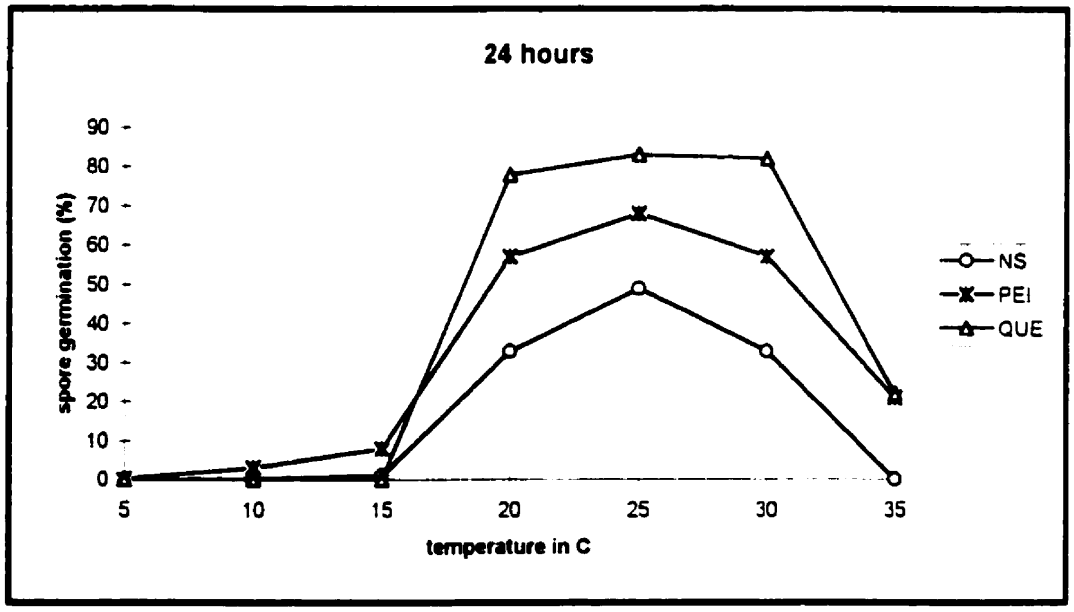
Figure 3.2.4

Effect of 7 temperature regimes on spore germination of three isolates of *Phoma* sp. isolated from lupine plants. Second trial. A) Germination after 12 hours. B) Germination after 24 hours.

A.


12 hours
spore germination (%)
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
temperature in C
NS
PEI
QUE

B.


24 hours
spore germination (%)
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
temperature in C
NS
PEI
QUE

3. Effects of osmotic potential on spore germination

3.1 Data analysis

The variances and standard deviations of both trials were compared using the compare data option in Statgraphics (Statgraphics Plus for Windows, version 1). No statistical differences were observed between the two populations. Both data sets were then pooled for subsequent analysis. A logistic regression was found to be the most appropriate model and was used for the analysis.

3.2 Conidial germination

Conidial germination was affected by variations in osmotic potentials (Figure 3.3.1) Germination decreased with decreasing water potentials However, at -2.768 , -1.377 and 0 Mpa (98, 99 and, 100 % R.H.) germination was similar and averaged 100%. At lower water potential values -17.513 and -12.920 MPa (88 and 91 %), there was not enough free moisture and no germination occurred . After 24 hours, -8.476 MPa (94 % R.H.) was the minimum required to stimulate germination. Percentage of conidial germination continued to increase with increasing water potential until its maximum 100 % germination from -2.768 to 0 MPa (98 % to 100 % R.H.).

It was also observed (data not shown) that germ tube length was affected by relative humidity. At -8.476 (94 % R.H.), germ tube length was similar to spore length. At -1.377 and 0 MPa (99% and 100 % R.H.), the tube had become branched mycelium and it became very difficult to distinguish between spores.

The effect of variable relative humidities on conidial germination at 25° C after 24 hours of incubation was described by the following logistic regression equation:

$Y = e^{(x)} / 1+e^{(x)}$

where:

Y= germination

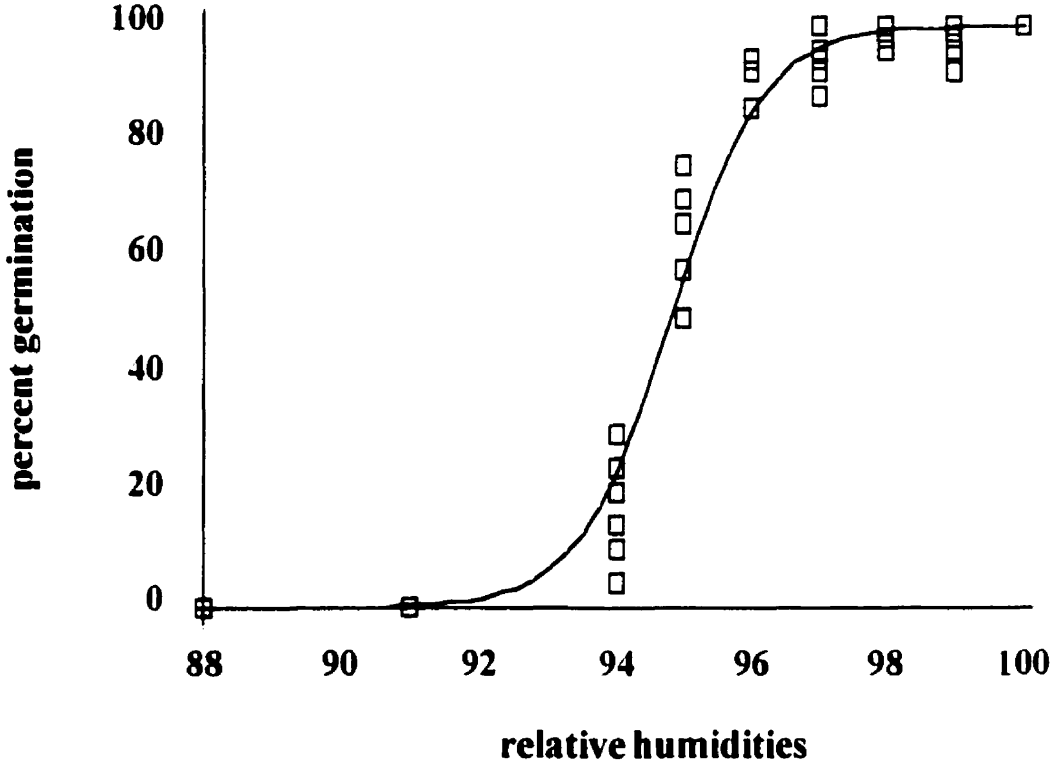
X= -140.783 + 148.476 * R.H.

Equivalent to R^2 adjusted: 0.89

For logistic models, R^2 statistic is not directly used, another similar statistic is used: percentage of deviance in germination explained by the models. It has the same significance as R^2 adjusted for comparing models. A Chi square test for goodness of fit was then performed and the model was considered adequate at the 90 % or higher confidence level.

Figure 3.3.1

Influence of relative humidity on germination (%) of spore of Québec isolate of *Phoma* sp. on PDA osmotically adjusted with KCl incubated for 24 hours at 25° C.


100
80
60
40
20
0
percent germination
88
90
92
94
96
98
100
relative humidities

4. Effects of dew period and temperature on seedling infection

4.1 Data analysis

Stem lesion enlargement over the duration of the experiment was used to calculate the area under the disease progress curve (AUDPC). These AUDPC data were analysed with the ANOVA function of SAS (SAS Institute, version 6.11). The Student-Newman-Keuls (SNK) test was used for mean separation. All analyses were performed on logarithmic transformed data to meet the normality assumption of ANOVA.

Incubation temperatures had a significant effect on stem wound enlargement ($P<0.01$ for the first trial and $P<0.05$ for the second) and the incubation period also had a significant effect on wound enlargement ($P<0.01$). However no interactions were observed.

4.2 Stem wound enlargement

In Figure 3.4.1(a) representing the SNK means, an increase in length of leaf wetness duration period (LWD) seemed to permit better stem wound enlargement, and higher temperature seemed to promote stem wound

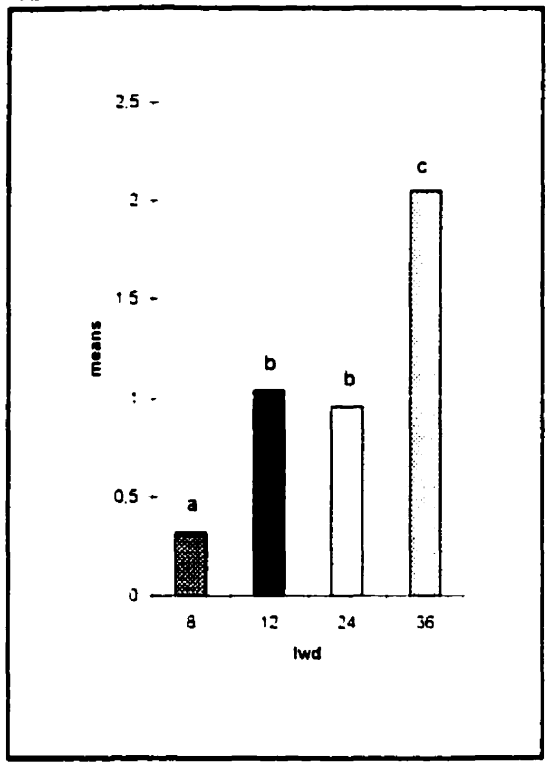
enlargement (Fig. 3.4.1 b). In the second trial of the experiment, the effects seemed to be slightly different. Leaf wetness duration significantly promoted stem wound enlargement (Fig 3.4.1 c) and temperature had a significant effect on stem wound enlargement at the 95 % confidence level (Fig. 3.4.1 d). Figures 3.4.2 to 3.4.9 present the result for each combination of leaf wetness duration and temperature. In both trials incubation at 15 °C was sufficient to promote some infection and stem wound elongation. In the first trial elongation was mainly observed after a long period of incubation (36 hours) (Fig. 3.4.2), and in the second trial some elongation was observed after short periods of incubation (8h, 12h). No elongation was observed after 24 hours and some after 36 hours (Fig. 3.4.3). At 20 °C, it can be observed that for the first trial some wound enlargement was visible at all combinations of temperature and leaf wetness duration (Fig. 3.4.4). In the second trial (Fig. 3.4.5) 8 hours of incubation was not sufficient to permit wound enlargement and 12 hours of incubation caused some wound elongation but symptom appearance was delayed. At 25 °C, for the first trial, lesions are observed only when at least 24 hours of incubation are provided (Fig. 3.4.6). In the second trial for the same temperature, lesions were observed under all periods of incubation (Fig. 3.4.7). At 30 °C, for the first trial, all values of leaf wetness duration promoted stem wound enlargement. However, at the shortest period (8 h) symptom appearance took more time than when longer period of incubation were present (Fig. 3.4.8). In the second trial the same pattern was observed (Fig. 3.4.9). From all these figures, it can be

observed that in both cases, 30 °C and a period of 36 hours of leaf wetness duration were the optimal condition for infection to develop. From these figures it can also be observed that at short periods of leaf wetness duration stem wound enlargement is very often delayed compared to longer periods of leaf wetness duration.

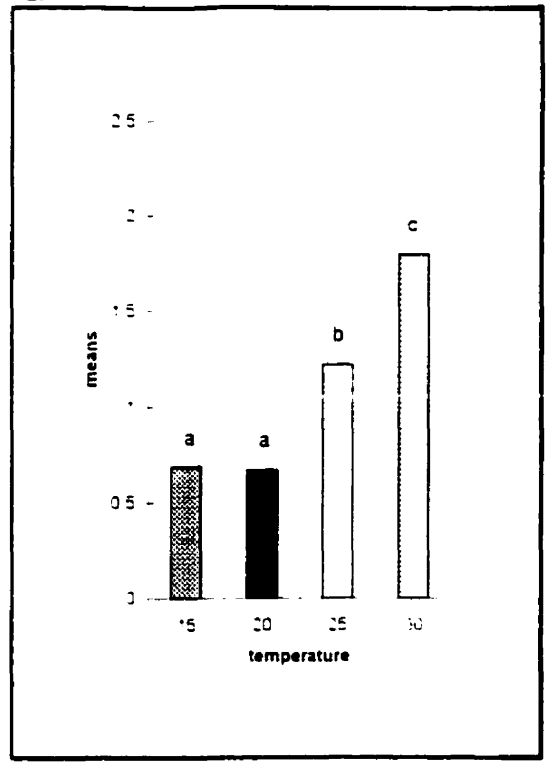
Figure 3.4.1.

Effect of incubation temperature and leaf wetness duration on lupine stem wound enlargement (AUDPC values). A) SNK means for leaf wetness duration over all temperatures, first trial. B) SNK means for temperature over all leaf wetness durations, first trial. C) SNK means for leaf wetness duration over all temperatures, second trial. D) SNK means for temperature over all leaf wetness durations, second trial. Bars with same letters are not statistically different.

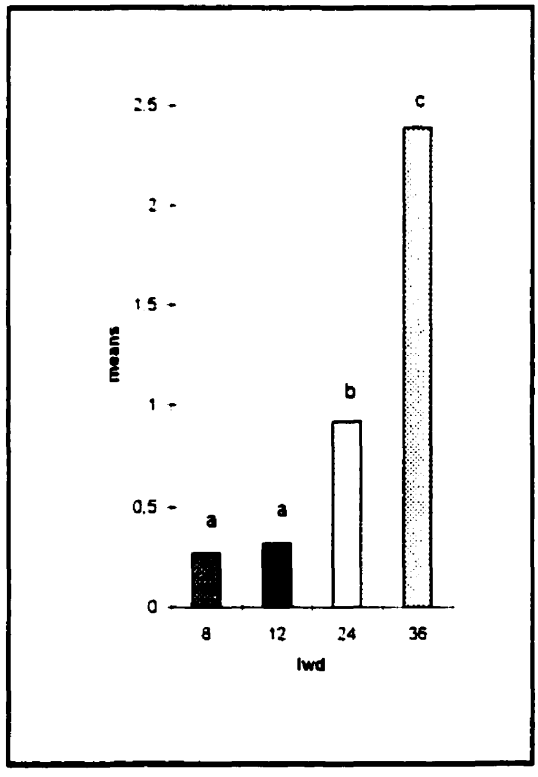
A.


2.5
2
1.5
1
0.5
0
means
a
b
b
c
8
12
24
36
lwd

B.


2.5
2
1.5
1
0.5
0
means
a
a
b
c
15
20
25
30
temperature

C.


2.5
2
1.5
1
0.5
0
means
a
a
b
c
8
12
24
36
lwd

D.

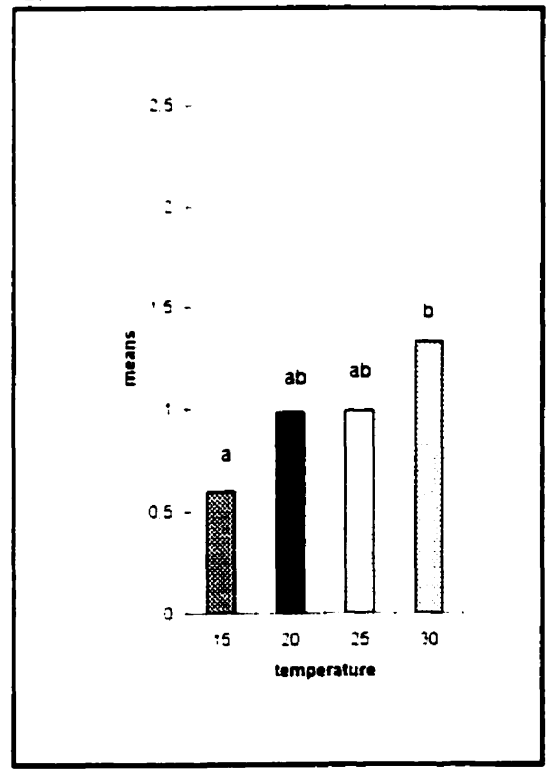
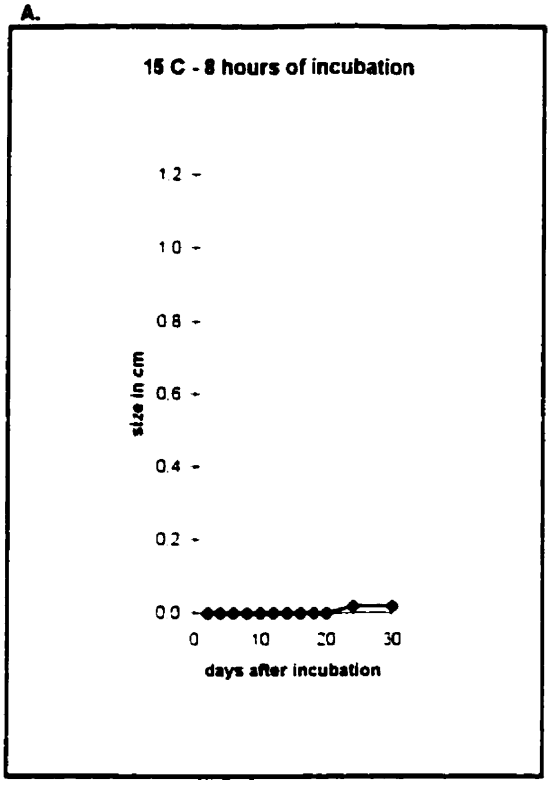

2.5
2
1.5
1
0.5
0
means
a
ab
ab
b
15
20
25
30
temperature

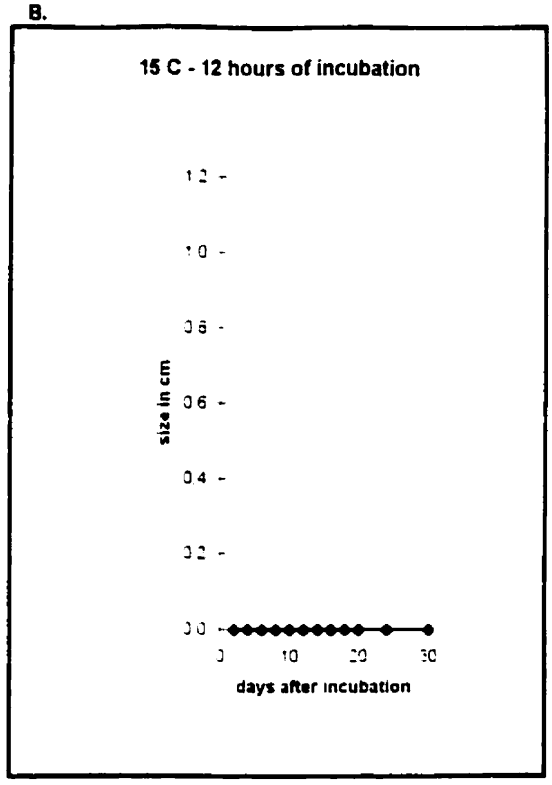
Figure 3.4.2

Effect of leaf wetness duration on lupine stem wound enlargement at 15° C for the first trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

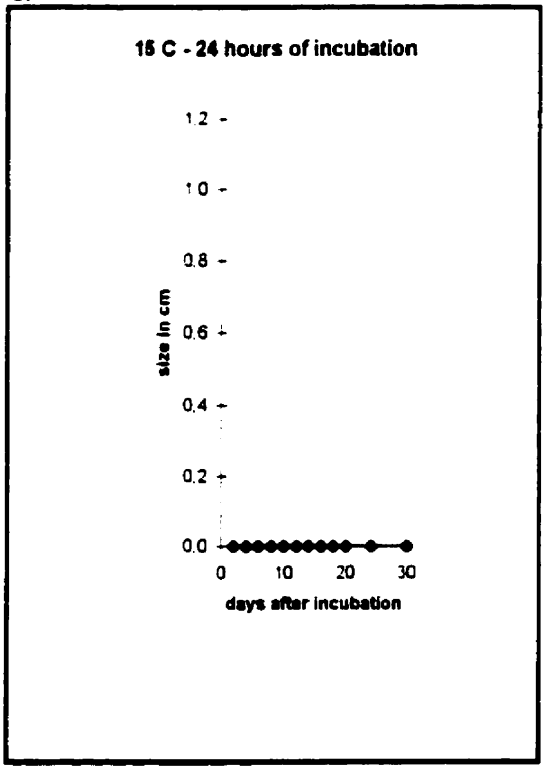
A.


15 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

B.


15 C - 12 hours of incubation
size in cm
days after incubation

C.


15 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.

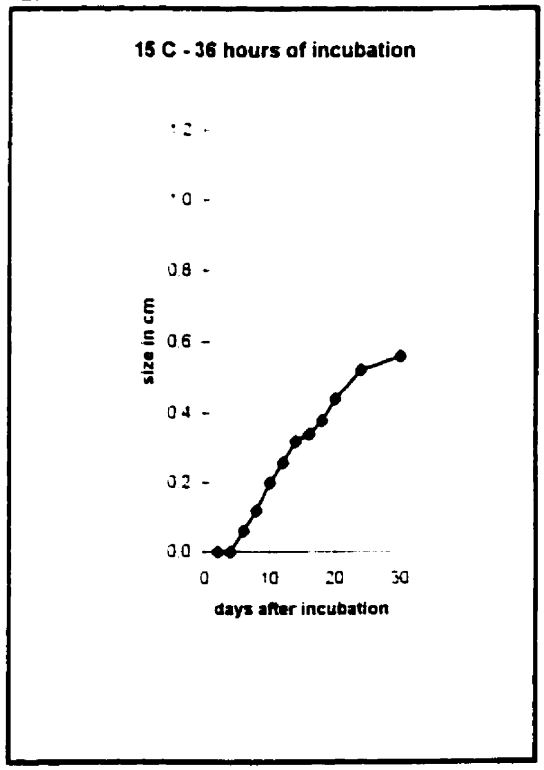
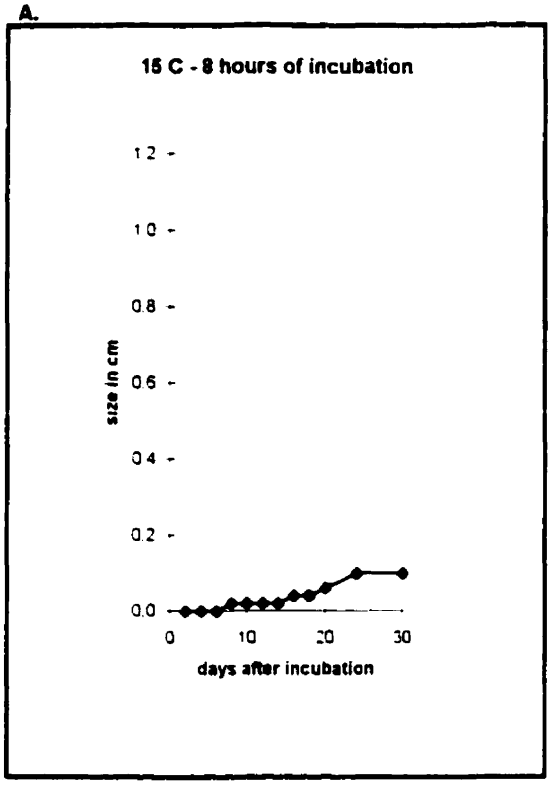

15 C - 36 hours of incubation
size in cm
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

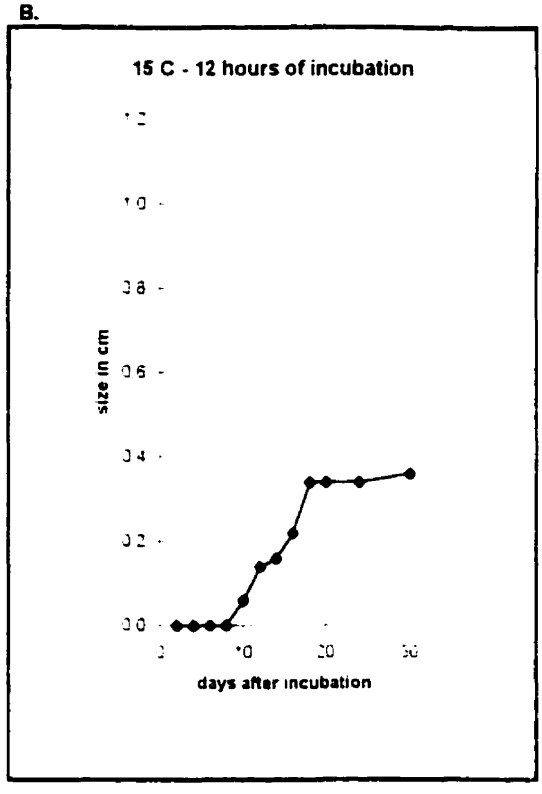
Figure 3.4.3

Effect of leaf wetness duration on lupine stem wound enlargement at 15° C for the second trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

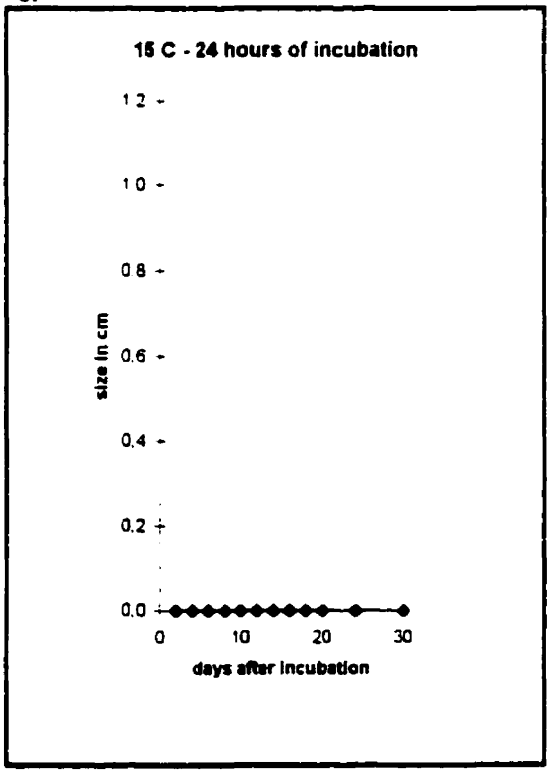
A.


15 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

B.


15 C - 12 hours of incubation
size in cm
days after incubation

C.


15 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.

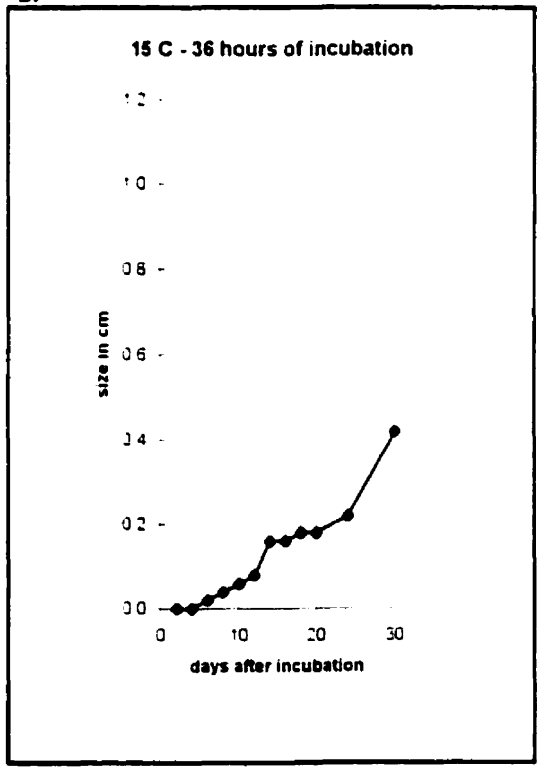
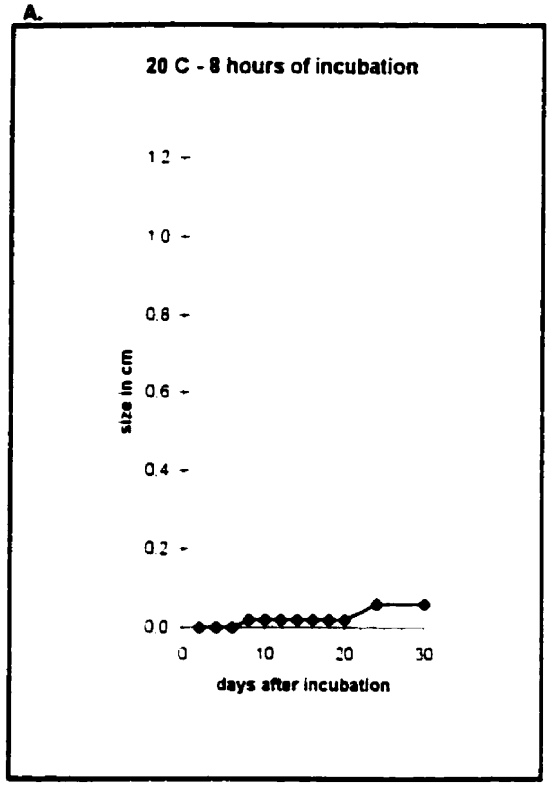

15 C - 36 hours of incubation
size in cm
0
10
20
30
days after incubation

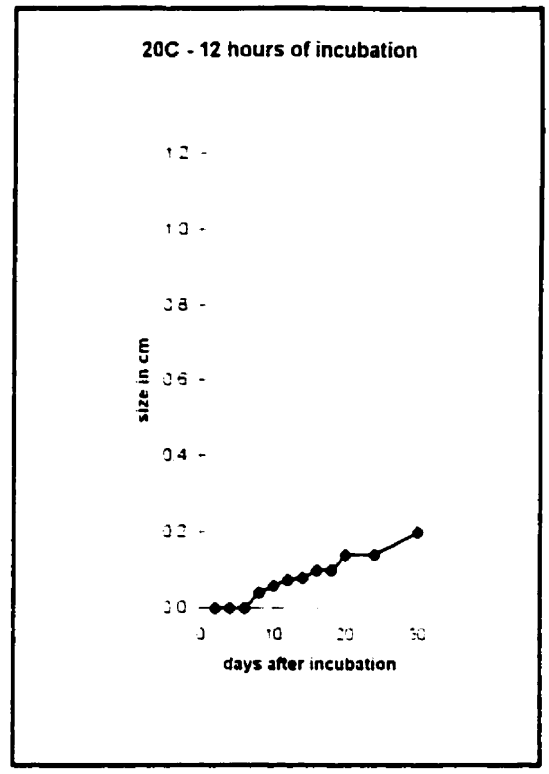
Figure 3.4.4

Effect of leaf wetness duration on lupine stem wound enlargement at 20° C for the first trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

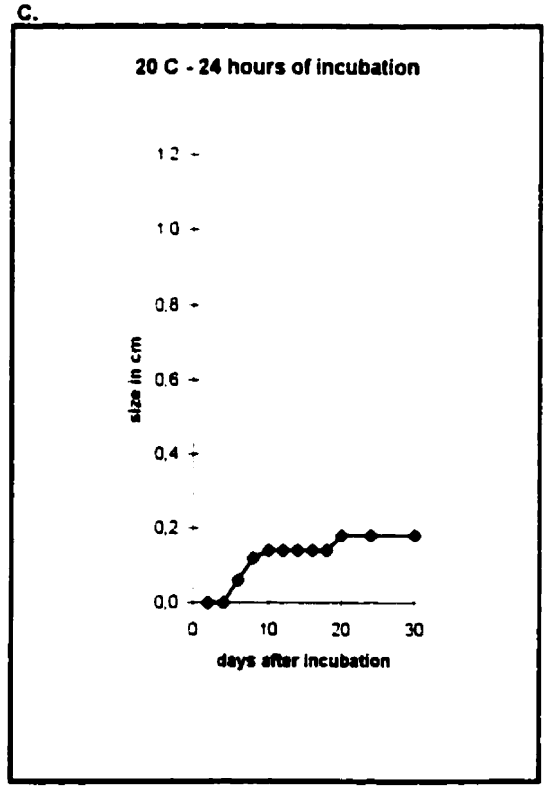
A.


20 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

B.


20C - 12 hours of incubation
size in cm
days after incubation

C.


20 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.

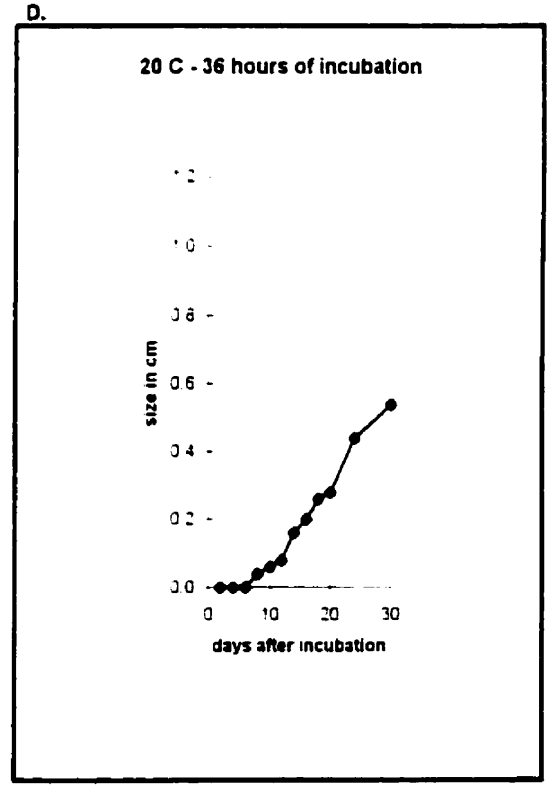
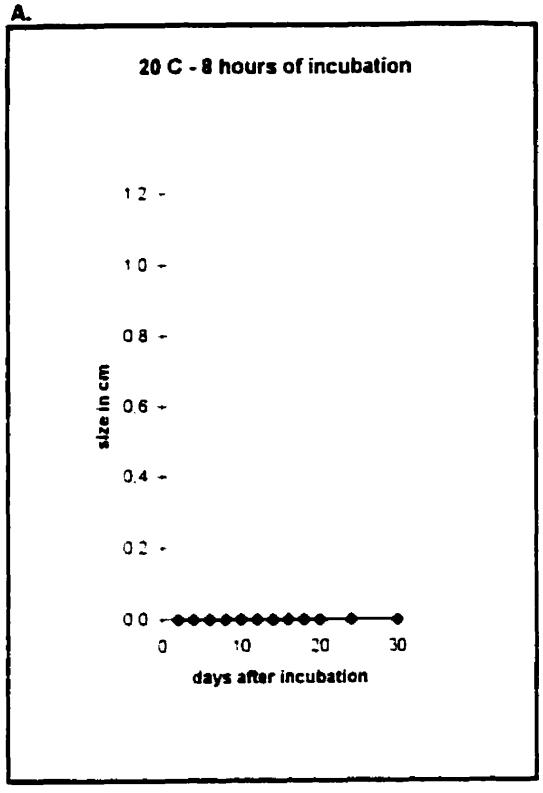

20 C - 36 hours of incubation
size in cm
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

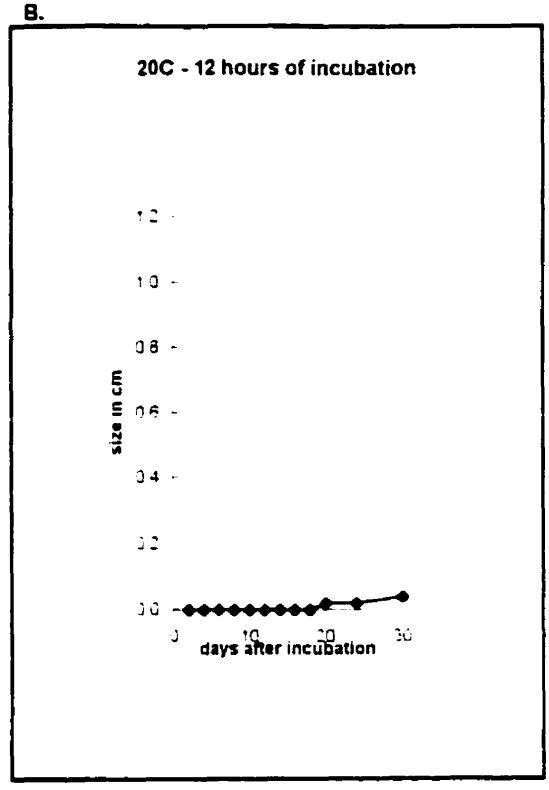
Figure 3.4.5

Effect of leaf wetness duration on lupine stem wound enlargement at 20° C for the second trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

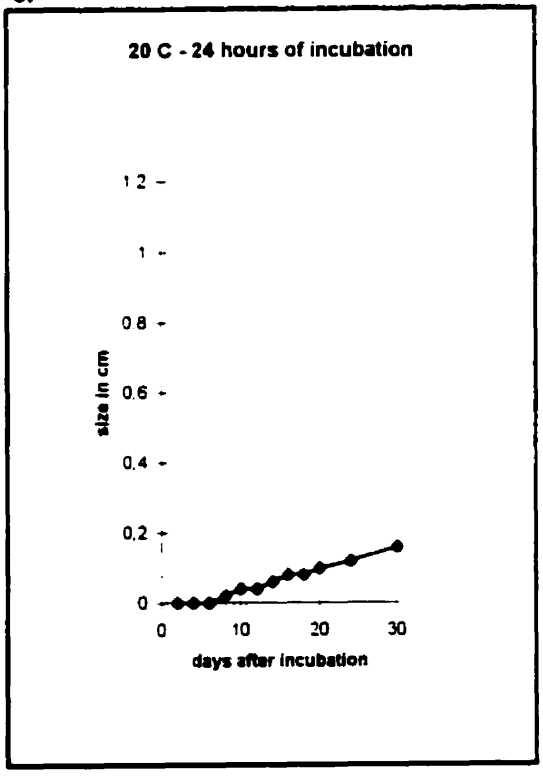
A.


20 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

B.


20C - 12 hours of incubation
size in cm
days after incubation

C.


20 C - 24 hours of incubation
size in cm
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
days after incubation

D.

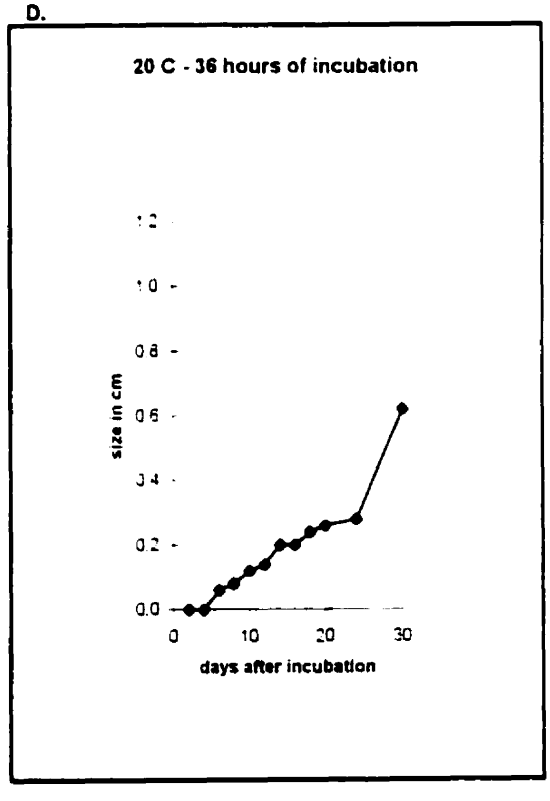
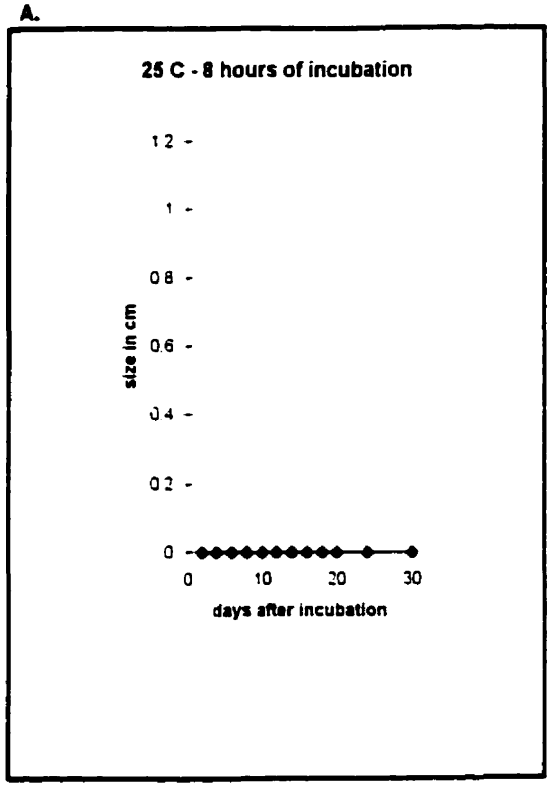

20 C - 36 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

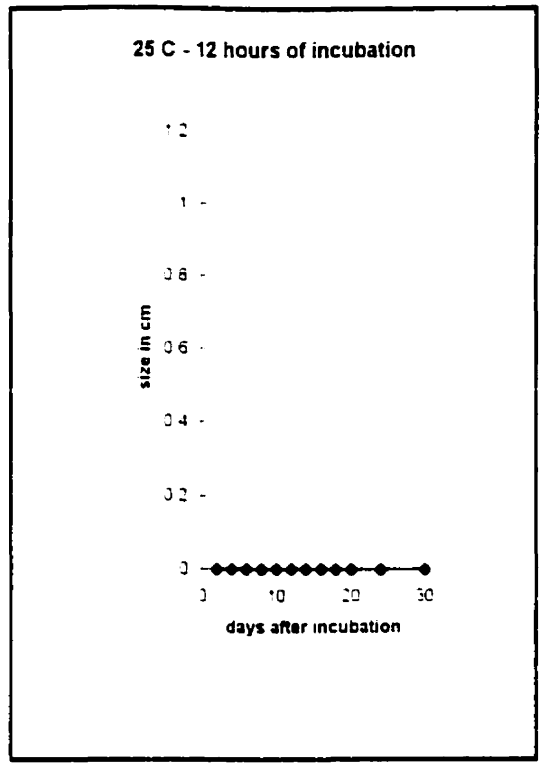
Figure 3.4.6

Effect of leaf wetness duration on lupine stem wound enlargement at 25° C for the first trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

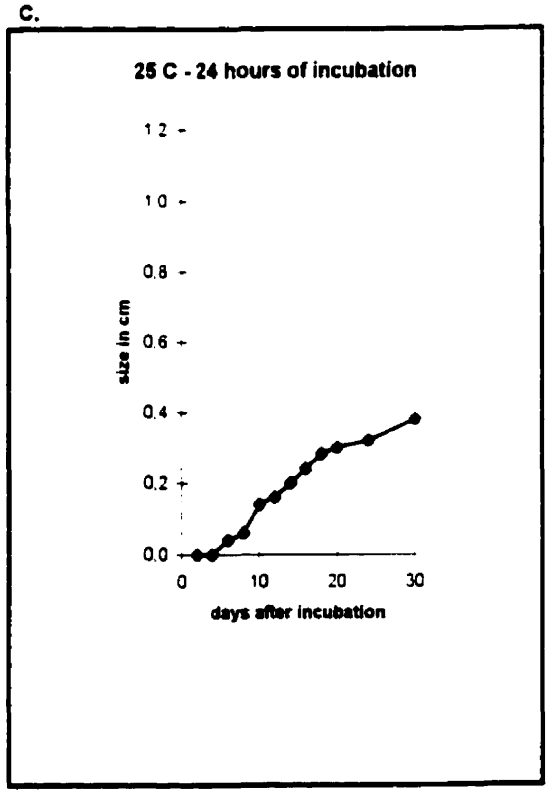
A.


25 C - 8 hours of incubation
size in cm
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
days after incubation

B.


25 C - 12 hours of incubation
size in cm
days after incubation

C.


25 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.

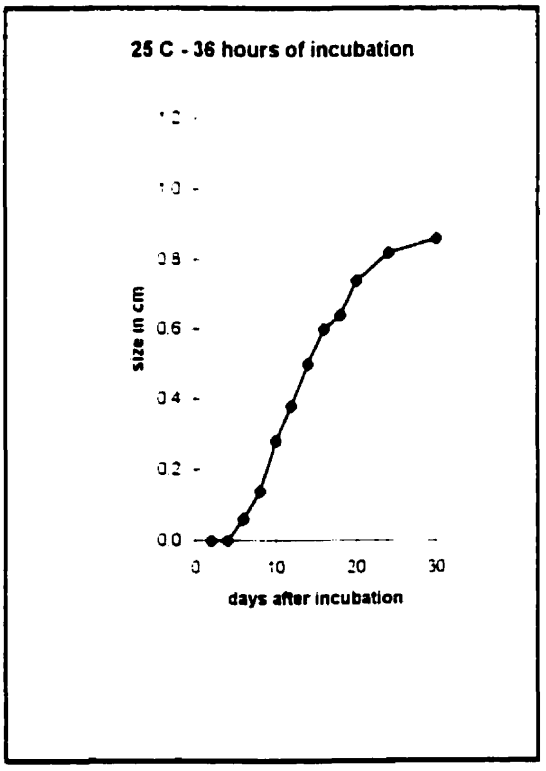
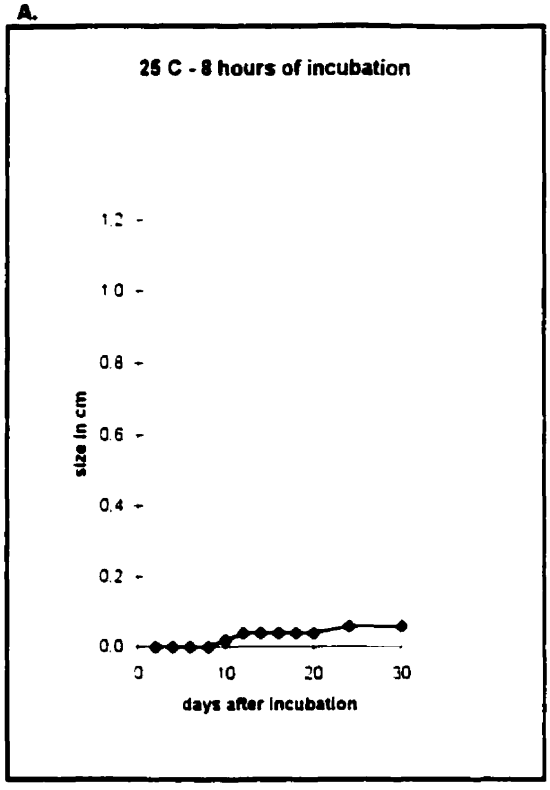

25 C - 36 hours of incubation
size in cm
days after incubation

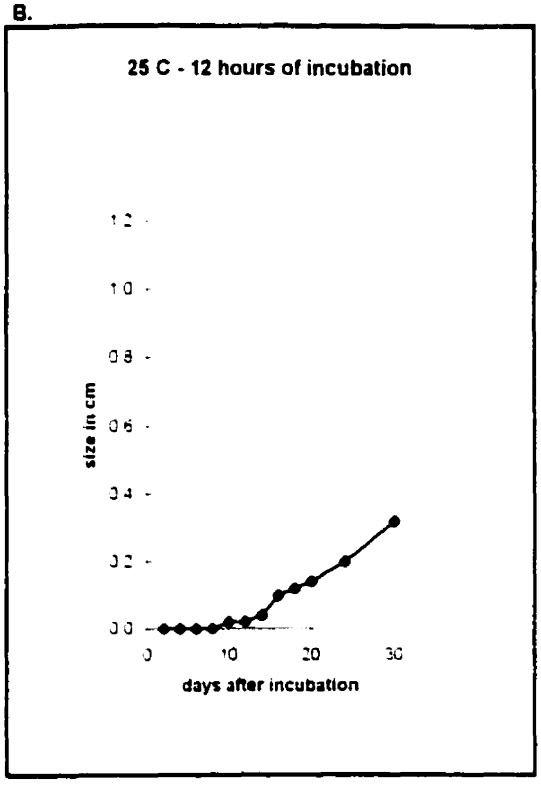
Figure 3.4.7

Effect of leaf wetness duration on lupine stem wound enlargement at 25° C for the second trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

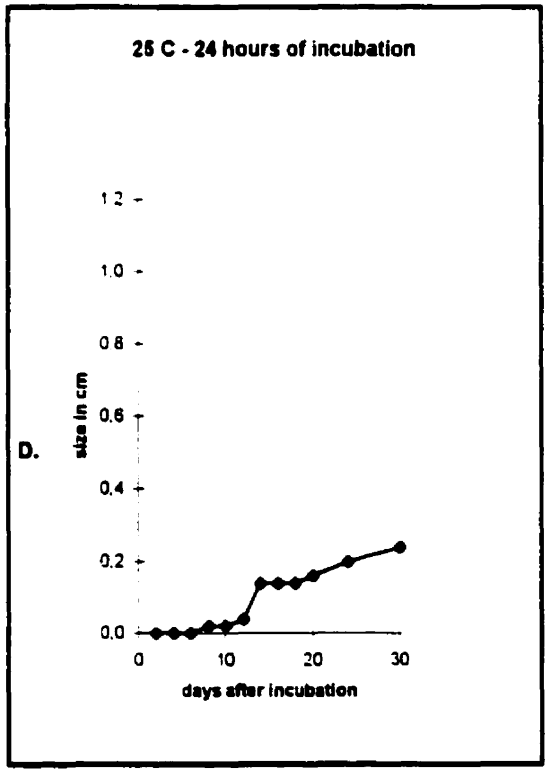
A.


25 C - 8 hours of incubation
1.2
1.0
0.8
0.6
0.4
0.2
0.0
size in cm
0
10
20
30
days after incubation

B.


25 C - 12 hours of incubation
size in cm
days after incubation

C.


25 C - 24 hours of incubation
1.2
1.0
0.8
0.6
0.4
0.2
0.0
size in cm
0
10
20
30
days after incubation

D.

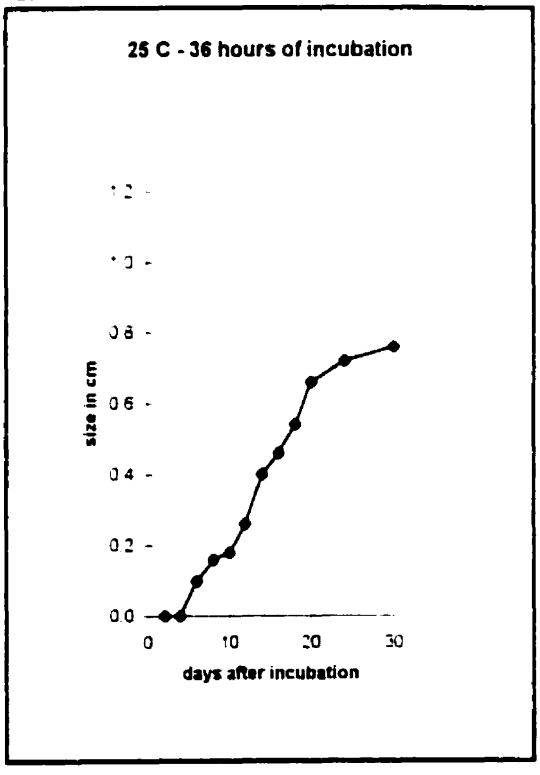
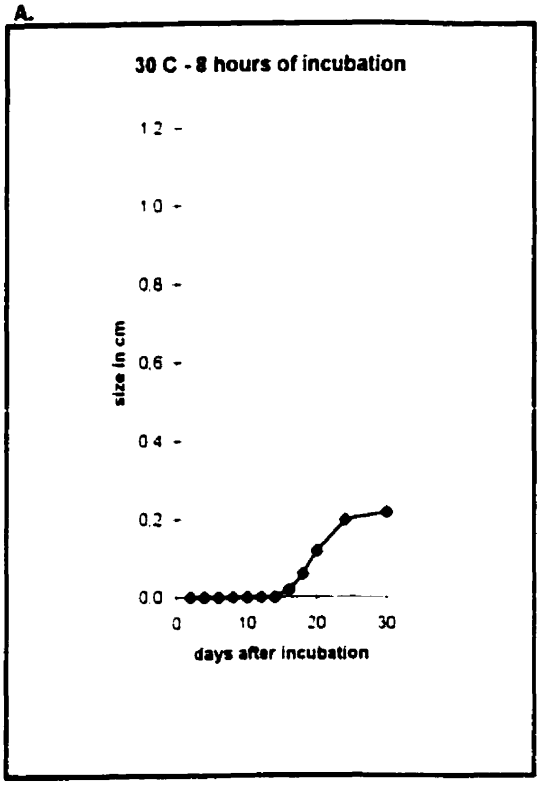

25 C - 36 hours of incubation
size in cm
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

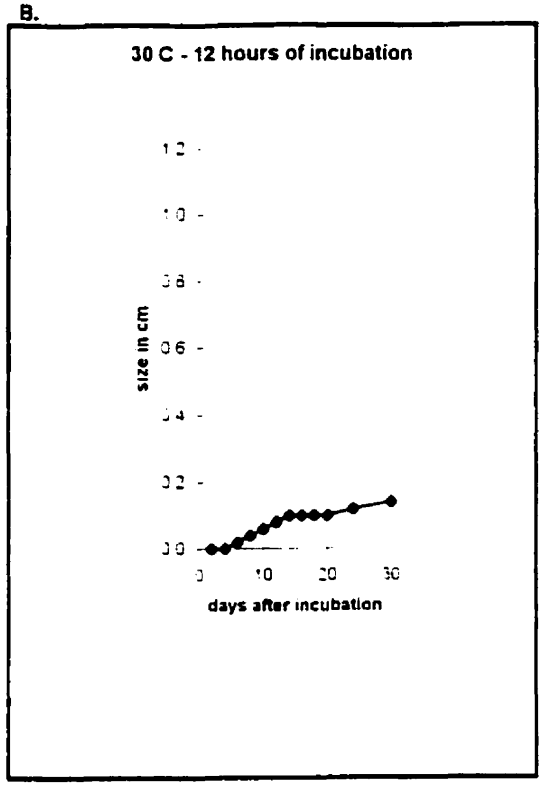
Figure 3.4.8

Effect of leaf wetness duration on lupine stem wound enlargement at 30° C for the first trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

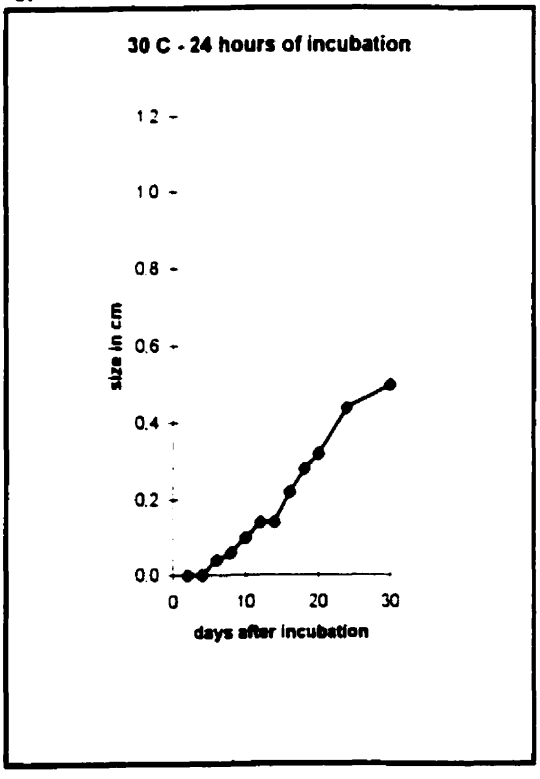
A.


30 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

B.


30 C - 12 hours of incubation
size in cm
days after incubation

C.


30 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.

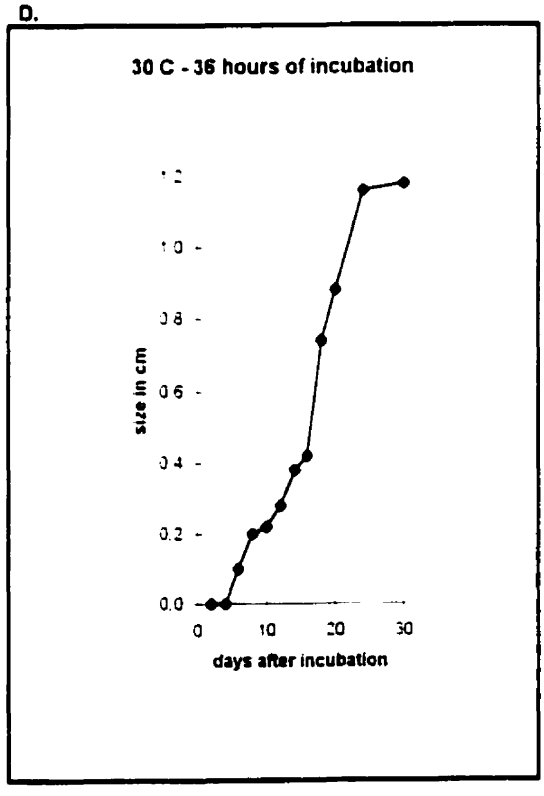
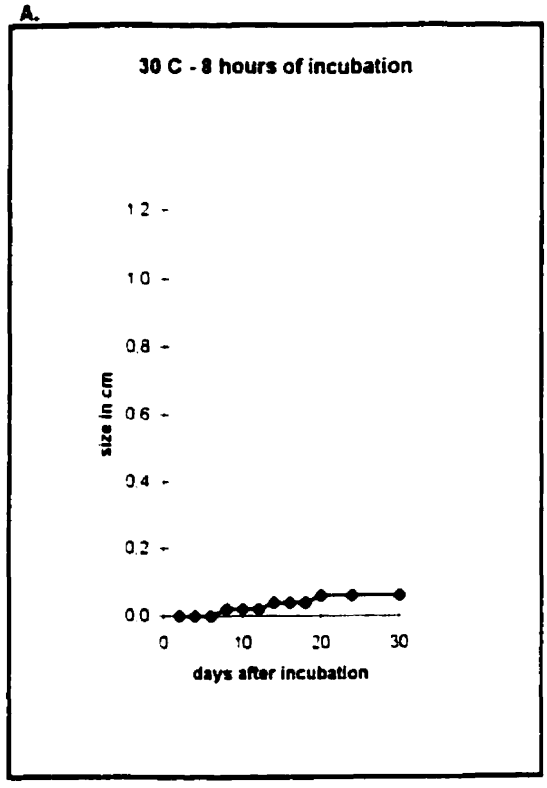

30 C - 36 hours of incubation
size in cm
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

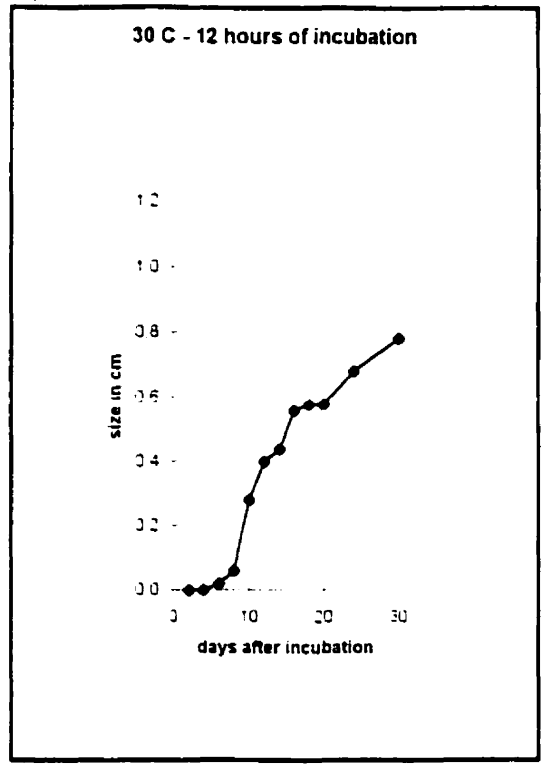
Figure 3.4.9

Effect of leaf wetness duration on lupine stem wound enlargement at 30° C for the second trial. A) After 8 hours of incubation. B) After 12 hours of incubation. C) After 24 hours of incubation. D) After 36 hours of incubation.

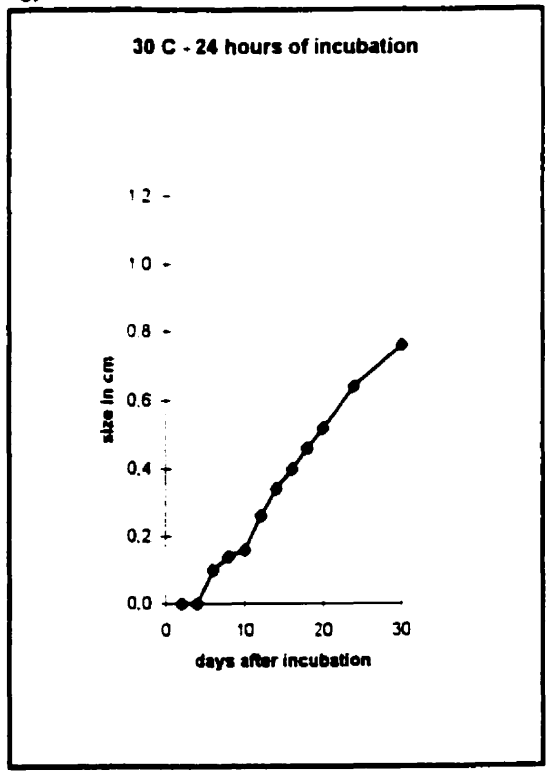
A.


30 C - 8 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

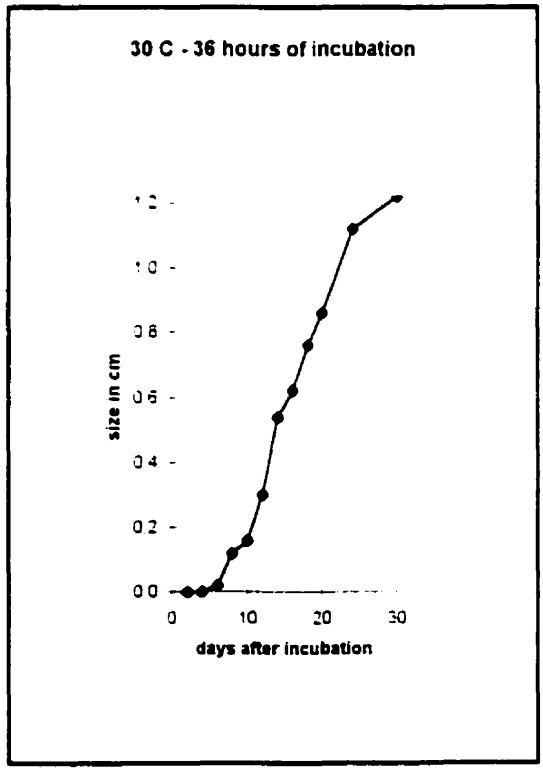
B.


30 C - 12 hours of incubation
size in cm
days after incubation

C.


30 C - 24 hours of incubation
size in cm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
days after incubation

D.


30 C - 36 hours of incubation
size in cm
0
10
20
30
days after incubation

DISCUSSION

Temperature had an impact on the mycelial growth and spore germination of the *Phoma* sp under study. Mycelium growth of *Phoma* sp. occurred from 5° to 35° C. This *Phoma* sp. seems to have a wider temperature range than other *Phoma* spp. (Nebane and Ekpo, 1992; Schenck and Gerdemann, 1956). Optimum temperature for mycelium growth was 25° C, which differs from the observation made by Schenck and Gerdemann (1956) who reported the optimal temperature for mycelium growth of *Phoma pinodella* to be 20° C. and between 20° and 25° C for *Phoma medicaginis*). In their experiments, for both pathogens, the maximum temperature where mycelium growth could be measured was 30° C, which differs from the *Phoma* sp. isolated from lupines which showed substantial growth at higher temperature (35° C).

For the temperature effect on mycelium growth, differences were observed among the three isolates. For both trials, Nova Scotia mycelial diameter was greater and its development was faster than Québec or Prince Edward Island isolates. However, mycelium growth for Québec and Prince Edward Island isolates were not statistically different.

These results clearly show that at 25° C germination was significantly higher ($P<0.01$) than at any other temperature. This could be compared to the

results of Schenck and Gerdemann (1956) who also obtained optimal germination at 25° C for *P. pinodella* and *P. medicaginis*. In their experiment. 8 hours after the beginning of incubation, for *P. pinodella trifolii* germination attained about 60 %, and for *P. medicaginis* germination attained 15 %. In the case of *Phoma* sp. of lupine, the data are much closer to the 15 % than the 60%, suggesting that *Phoma* sp. from lupine is closer to *P. medicaginis* than *P. pinodella* in this character. However, in their experiment, spores of *P. medicaginis* did not germinate at 5° and 35° C and *P. pinodella* germinated well at 35°C and slowly at 5° C. In the tests performed on *Phoma* sp. attacking lupines, spores germinated well at 35° C. In this case *Phoma* sp. from lupine more closely resembles *P. pinodella*. It is interesting to compare the temperatures at which this pathogen grows to those for the lupine plants (temperatures between 15 ° C and 25° C; Gladstone, 1970). The *Phoma* sp. under study germinates and grows well at temperatures of 25° C and higher. which is about the highest limit for lupine plant development. This could provide an advantage for the disease to develop at the beginning of pod fill when the temperatures are higher and the plants are weaker. Germination at temperatures lower than 15° C, which is the lower limit for lupine plant growth. benefits the pathogen permitting it to infect the plants early in the season and leaving the full summer season to reproduce and disperse itself.

Time also had a significant effect ($P<0.01$) on spore germination. As time goes by, germination increases until it reaches its maximum. The statistical analysis also showed that there was a significant time * temperature interaction effect. Generally no significant isolate effects were detected, except in the second trial where the Nova Scotia isolate reacted slightly differently ($P<0.05$) than the two other isolates and showed significantly lower germination at the seven temperatures tested.

Some of the difference in the two trials may be due to the fact that spores did not come from the same petri dish, and they were probably not exactly at the same maturity stage. Since the two trials were performed on three different days. The differences may also be due to the incubator conditions, which may have slightly varied from one trial to the other.

Conidial germination was affected by variations in water potential. Germination tended to increase with increasing water potentials. Moisture is necessary to permit spore germination. Water potential lower than -12.920 (R.H. of 91% and below), did not allow spore germination of *Phoma* sp. found on lupine. This could be related to other fungi in the literature such as *Fusarium* sp. in corn where spore germination is also affected by water potential and at 25° C, -17.513 to -14.434 MPa (88% to 90 % R.H depending on the isolate) are required for germination to occur (Marin et al. 1996). Jacome and co-workers

(1991) observed that at 25° C no germination of *Mycosphaerella fijiensis* var. *diformis* occurred at -17.513 MPa (88 % R.H.). And -11.423 MPa (92 % R.H.) stimulated low level of germination only in a few of the tested isolates.

Since germ tubes are much longer at the higher relative humidities after the same amount of time, it can be concluded that at higher humidities, germ tubes grow faster. This would influence penetration of the host tissue by the germ tube and favour the pathogen by permitting a shorter infection period under appropriate environmental conditions.

Stem wound enlargement was very variable depending on the plant observed. For some plants the needle wound did not change in color or in size compared to another replicate plant in the same treatment which showed some effects. In the seedling experiment, we were able to observe a trend where infection tended to develop more under the longest leaf wetness duration period tested. The same trend was also reported where the highest temperature of incubation led to increased disease symptoms. These reactions could be explained by the fact that these incubation temperatures and humidities may predispose the plant for infection. Colhoun (1979) reported that non optimal environmental effects, especially temperature, light and humidity decreased the plant resistance to pathogen attack. In the experiments performed on *Phoma* sp. from lupine, the highest temperature of 30° C is much more than the optimal

temperature for lupines growth thus weakening the plants. The absence of light during the incubation period may also have had a synergistic stress effect during the LWD period that was alleviated when the plants were returned to the cabinet, they were back to their normal light regime. This test was done on plants in controlled environment with large space between plants permitting good aeration. In natural stands, dense plant canopie will create a microclimate with higher humidity and less aeration within the plant canopy than the ambient air (Rotem, 1978). Because of this, the results could be different in nature and it would be important to collect the environmental data within the plant canopy and not on bare ground.

IV. SURVIVAL OF *PHOMA* SP. ON INFECTED LUPINE RESIDUE OVER THE WINTER

INTRODUCTION

Many environmental factors affect disease initiation, development and spread. As observed in previous chapters, temperature and relative humidity affect the pathogen during its development. However, some other factors may prevent disease initiation and development. Van der Plank, (1963) described three main categories of environmental management of diseases: 1) reduction of the initial amount of inoculum, 2) reduction of the spread of the pathogen and 3) practices that integrate both methods. Reduction of inoculum most often involves physical practices and a second approach of a more chemical nature Good cultural practices are the basis for good yields, other practices such as use of chemicals are only secondary and will be effective only if the basic requirements are met. Crop rotation is one of the best and easiest management practice to avoid inoculum build up in a particular area (Cook, 1977). Good sanitation is also useful to reduce the amount of inoculum present and prevent introduction of a pathogen into a region. Good sanitation is not only performed during the growing season, but also before the crop is planted. It means the use of clean planting material (disease free through certification programs), use of clean manure as a soil amendment (through composting), and the use of clean

machinery. During the growing season it may mean removal of diseased plants (roguing), and after the growing season it also means disposal and treatment of the crop debris and stubble (Zentmyer and Bald, 1977).

Residues coming from diseased plants of a previous crop can be a very important reservoir for large amounts of inoculum. This enables the pathogen to induce diseases early in the growing season when the host could be most vulnerable (Doupnik and Boosalis, 1980). These residues, often left on the ground, may be dealt with in several ways: by removing them if economical, incorporating them in the soil, burning them, and in some cases, when practical, flooding them (Palti, 1981)

In the case of lupines grown for their seeds, the stubble is of no real value neither for bedding nor for feed additive. It is usually left in the field and becomes an important shelter and nutrient source for pathogens, permitting their survival and initiation of diseases during the next growing season. Tillage is a well-known traditional management practice, widely used by farmers. Its first aim is mainly to construct a suitable seed bed through the incorporation of the residues in order to permit a faster microbial decomposition. It is indirectly a method for control of diseases by destroying the substrate, thereby reducing the amount of inoculum (Cook and Baker, 1983).

The objectives of this study are first, to verify if the pathogen *Phoma* sp. can effectively survive over winter in crop debris and second, to determine if burial and depth of burial of the debris affect the survival of this pathogen.

MATERIALS AND METHODS

1. Preparation of infected plant debris

For this experiment, lupine plants were grown in the field during the summer periods of 1994 and 1995 on the Horticulture Farm of Macdonald Campus of McGill University, Ste-Anne-de-Bellevue in 10 x 10 m plots. Inoculum of *Phoma* sp. was produced on autoclaved white lupine seeds in 1 l mason jars. The infected seeds containing mycelium and spores of *Phoma* sp. were randomly distributed in the field plot in mid June. The disease developed on the previously established lupine plants during the summer. In August, the stems containing pycnidia were harvested, air dried and stored at room temperature.

2. Field assay

Four pieces of the harvested and infected lupine stems (about 2 cm long) were put into pouches made of black fibreglass screening material, with a mesh

size of 1.5 mm. These screen pouches allowed the stems to be placed in contact with the soil, and facilitated their removal at the appropriate time. These pouches were cut and stapled to form 6 cm X 8 cm rectangular sachets. A few grams of soil from the field in which the stems were to be buried was added to the pouch to permit a better contact between the decomposing microorganisms present in the soil and the stem pieces. At the beginning of November, 1994 and 1995, pouches were buried at three different depths in the field: surface, 10 cm and 20 cm in four blocks of 1.5 m X 1.5 m on the Horticulture Farm of Macdonald Campus of McGill University. Four replicates per date and for each depth were used.

3. Estimation of pathogen survival

As soon as the soil had thawed, in March 1995 and April 1996, pouches were dug up and stem pieces retrieved. Pouches were retrieved once a month throughout the whole summer season, in the third week of each month. Stems were weighed and then ground in 200 ml of distilled water using a household blender at the maximum speed. Serial dilutions of 10^{-1}, 10^{-2}, 10^{-3} were done and 0.50 ml of this suspension was spread on ½ strength PDA (Potato dextrose agar, Difco, St.Louis, Mo) + 100 ppm chloramphenicol. The plates were incubated at room temperature under natural light for 2 weeks and then colonies were counted and colony-forming-units per grams of dry weight (cfu/g)

calculated. On this medium, colonies of *Phoma* sp. produced distinctive pycnidia, enabling them to be distinguished from other fungi present on the petri dish.

RESULTS

1. Data analysis

Data from both years were analysed using the repeated measure tests in Version 6.12 of SAS (SAS Institute, 1995). In both cases the data were normal. A logarithmic transformation of the data was performed due to high coefficients of variability in the GLM functions of both years.

2. Observations

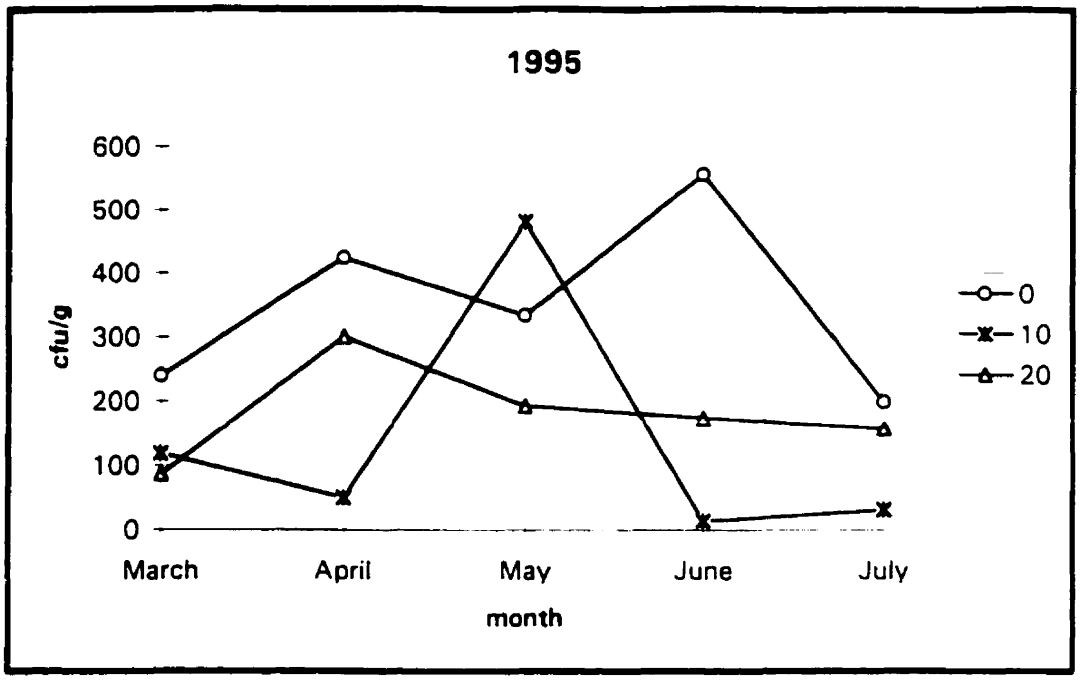
For both years, block had no significant effect. For 1995 and 1996 (Fig. 4.1 and 4.2) the depth at which the stem pieces were buried had a significant impact on propagule survival at the 95 % confidence interval ($P<0.05$). For both years, stem pieces left at the soil surface (0 cm) showed higher propagule survival than at 10 or 20 cm, however between 10 cm and 20 cm there was no statistical difference. The effects of period of time after burial depended on the

year. For 1995, the effect was close to being significant at the 95 % level (P= 0.06). For 1996 a significant time effect was observed (P<0.01). For the warmest period of the year, June and July, the survival of fungus on the stem seemed to decrease significantly. For both years there were no interactions of time * block nor time * depth.

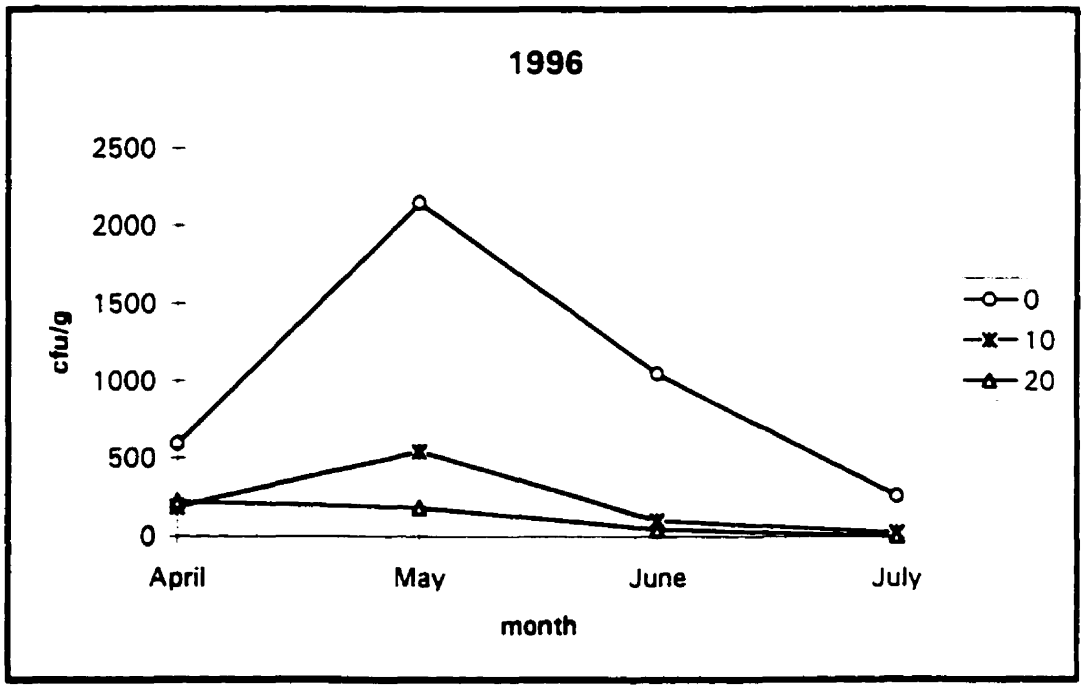
Figure 4.1

Effect of depth of burial (cm) and time on survival over winter of conidia of *Phoma* sp. pathogenic on white lupines. A) First year of experimentation. B) Second year of experimentation.

A.


1995
600
500
400
300
200
100
0
cfu/g
March
April
May
June
July
month
0
10
20

B.


1996
2500
2000
1500
1000
500
0
cfu/g
April
May
June
July
month
0
10
20

DISCUSSION

The results reported here suggest that *Phoma* sp. is able to overwinter on mulches and stubbles of the previous year's crop directly in the field. However, during the warmer temperatures of spring and summer, propagule survival seems to decrease as the season advances. This supports the observations of Cook and Baker (1983) who reported that during the warm period of the year (spring, summer and fall) saprophytes colonize and decompose the stubble, gradually reducing the amounts of available nutrients for *Phoma* sp. to survive on the lupine mulches.

Wiese and Ravenscroft (1975) observed that saprophytic survival of pathogen decreased with burial due to tillage. We observed similar results in the experiments performed using *Phoma* sp. isolated from lupine . In this study, stem pieces were buried in the soil at depth of 10 and 20 cm to mimic the burial effect of plowing which usually moves plant debris from the soil surface to a zone 10-30 cm deep and brings the deeper soil to the surface (Sumner et al., 1981). Buried stem pieces tended to show less colony-forming-units per gram of dried stem material than the pieces left at the soil surface. This again might be explained by the saprophytes which, due to soil mixing and residue fragmentation, have a higher chance of colonizing the pieces, thus increasing the rates of decomposition of residues by exposing more substrate surface to

saprophytes. Decomposers usually displace the pathogen which is often a weaker saprophyte, and this affects the survival of the pathogen. It has been observed by Cook and Baker (1983) that buried and fragmented stubble decompose faster therefore reducing survival of pathogens by destruction of their shelter and source of nutrients. Tillage will not only affect pathogen survival in relation to stubble but will also directly benefit the crop by improving root soil penetration and water infiltration. This creates healthier and more resistant plants which will be less sensitive to physical stresses that usually permit initiation of fungal diseases (Burke et al., 1972).

A reduction of propagule survival during the warmer summer period was also observed in these experiments. In 1996, the June and July sampling dates showed less cfu than the earlier May sampling. With June and July being a warmer period, the heat and the sun could destroy the pathogen on surface debris through desiccation (Palti, 1981). The earlier sampling date March for 1995 and April for 1996 showed lower colony-forming units than the later sampling dates; this could be explained by the fact that some fungi undergo dormancy (Allen, 1965; Sussman and Doutnit, 1973). At this early sampling date average temperatures are still below 0° C and dormancy is probably not broken for all the propagules. This could also be explained by the fact that later in the season, pynidia of this *Phoma* sp. on surface residues may have matured and exuded spores. These new spores may increase the cfu counts, and they serve

as a source of inoculum to be splashed onto plants to start new infections.

Cerkauskas (1987) reported that infected debris such as leaves, petioles and mulches are an important source of overwintering inoculum. Removal of the mulches and stubbles after harvesting of the seeds could be a way to reduce the incidence of field initiated infections for the following year. For lupines this may be difficult and non practical, this is why conventional tillage to depths between 10 and 30 cm may prove effective in reducing the amount of surviving inoculum. Plowing under debris will reduce initial inoculum by two main ways: 1) plowing under will permit better contact for the soil flora to suppress inoculum or 2) material will desiccate through heat and drought from the soil surface. Since this *Phoma* sp. is seed and residue borne, clean seed stock and good cultural practices through crop rotation and sanitation could keep diseases to a minimum (Palti, 1981).

Differences were observed between the two years of experimentation. The number of colony-forming-units observed was much higher in 1996 than in 1995. This could be explained by the fact that stem pieces that were used were grown and inoculated in the field, thereby causing a variability in the density of pycnidial coverage from plant to plant and from year to year. The weather conditions for both years were very different. The summer of 1996 tended to be cooler in general compared to 1995 (appendix). Rainfall distribution was also

very different. 1995 was very dry from the beginning of June to mid-July, compared to 1996 which had a more even rainfall distribution (appendix). The dryness of 1995 might also have helped in reducing the amount of colony-forming-units surviving on stubble throughout the season.

GENERAL SUMMARY AND CONCLUSIONS

This research has increased our general knowledge of a particular *Phoma* sp. causing disease symptoms on lupine plants. The morphological description provided sufficient information to classify this pathogen in the genus *Phoma*. It was however impossible to effectively characterize this *Phoma* sp. to the species level. From the results obtained, it can be concluded that this *Phoma* sp. is very similar to *P. pinodella* causing black stem in red clover and foot rot in peas. However, slight morphological differences between the *Phoma* sp. under study and *P. pinodella* , and the fact that this *Phoma* sp. was not pathogenic on peas, suggests that the *Phoma* sp. under study could be a separate variety of *P. pinodella*.

As with other living organisms, this *Phoma* sp. responded differently to various environmental conditions. Mycelium growth was influenced by temperature, mycelium expansion was observed over a broad range of temperature (between 5° C and 35° C), the optimal growth temperature occurring between 20° C and 30° C. Conidial germ tube elongation of this *Phoma* sp. was also affected by incubation under various temperatures. Low temperatures (5° C) did not allow germination. On the other hand, high temperatures (for example 35° C) permitted a small amount of germination. In all the tests, at least 12 hours of incubation were required for germ tube

elongation to occur. After 24 hours more than 80 % of the conidia had germinated when incubated between 15° C and 30°C. Optimal temperature for conidial germ tube elongation was 25° C. Humidity was an important factor for conidia to germinate. Relative humidities higher than 91 % (-12.920 Mpa) were required to stimulate and maintain conidial germ tube elongation. Drier conditions tended to inhibit germination. Optimal conditions for germination were present when relative humidity was between 98% and 100 % (-2.768 to 0 MPa) resulting in close to 100 % germination.

In controlled environment disease symptoms were observed under various conditions of temperature and leaf wetness duration. A period of 8 hours of leaf wetness duration was sufficient to cause symptoms. Stem lesion enlargement was also observed at temperatures as low as 15° C. However the most favorable conditions for stem lesion enlargement were 36 hours of leaf wetness duration at a temperature of 30° C.

Like many other pathogenic *Phoma* species, the *Phoma* sp. under study was able to effectively survive over the winter on lupine crop residues. This could provide inoculum for a subsequent lupine crop. This survival of the *Phoma* sp. on mulches of the previous year crop was extended to the summer period, when even at the end of July, spores present in pycnidia could still be isolated and cultured on artificial media. These results will permit to farmers to

plan management practices consequently.

It would be interesting to genetically identify this *Phoma* sp. RFLP methods. New keys could be created to facilitate the identification of *Phoma* species. This research provides the description of a particular *Phoma* sp. this will be helpful in the elaboration of a more extensive description key. Further research is required to develop effective control methods for this disease. Development and breeding of lupine cultivars resistant to *Phoma* and other diseases would provide farmers with more reliable harvest.

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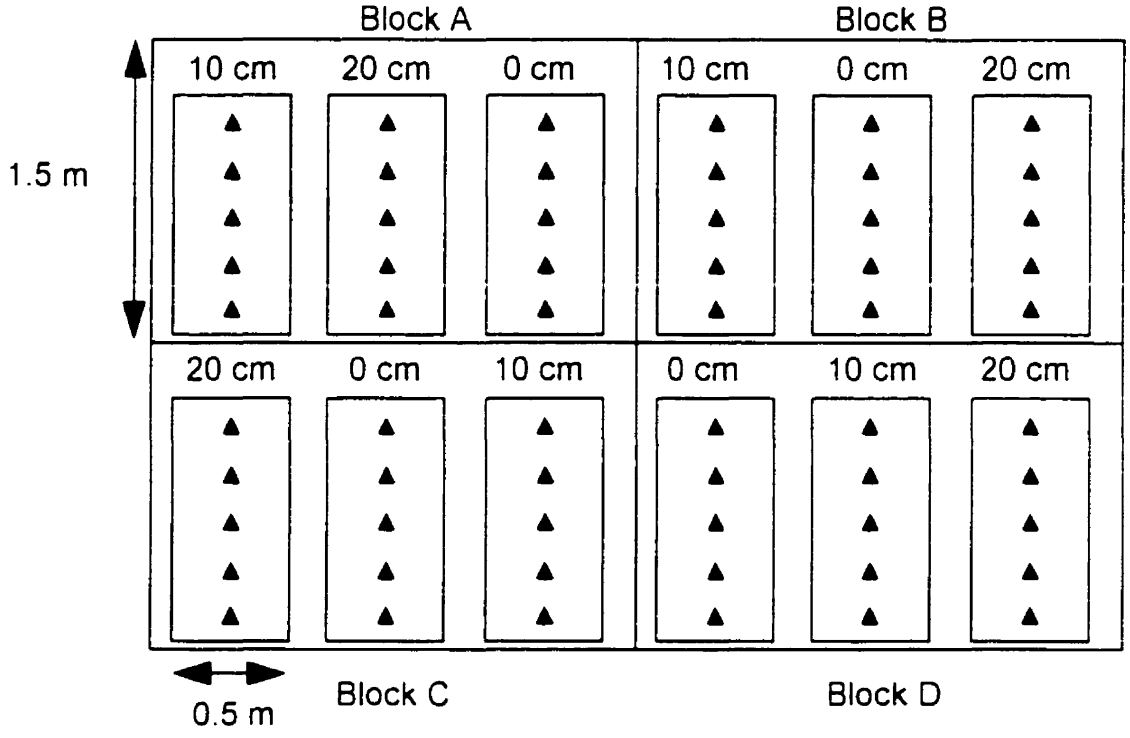
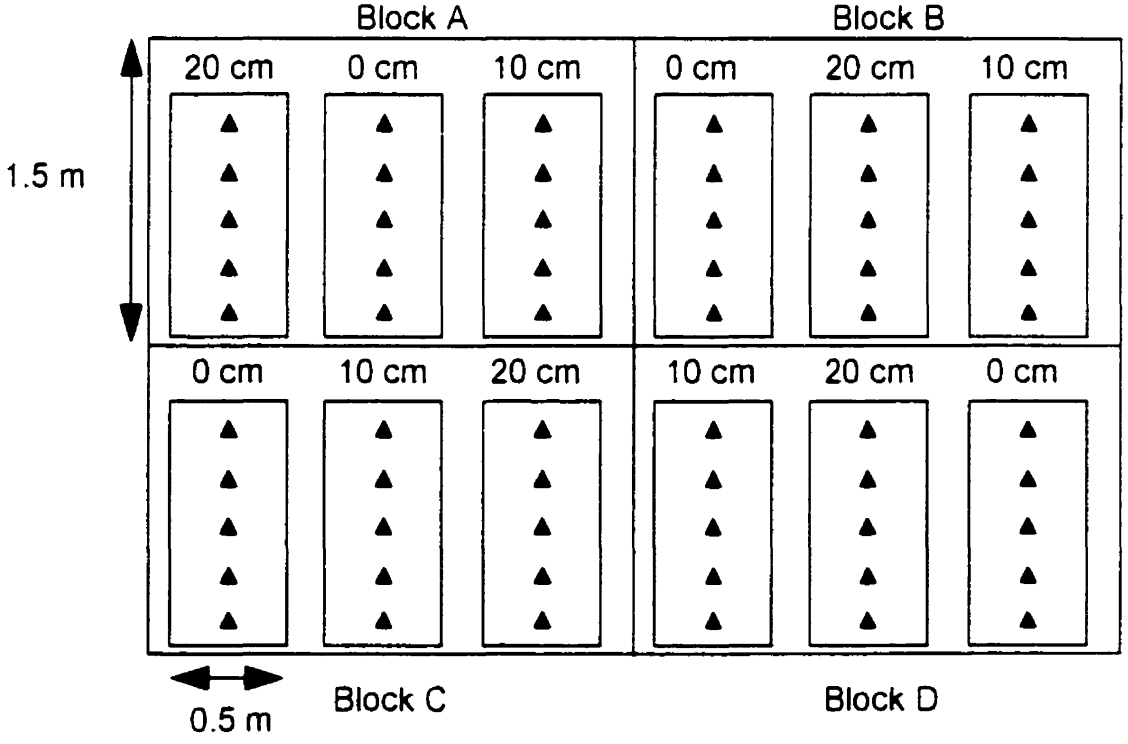
APPENDICES

APPENDIX 1. Field plans for overwintering experiments (Macdonald Campus, Horticulture Farm).

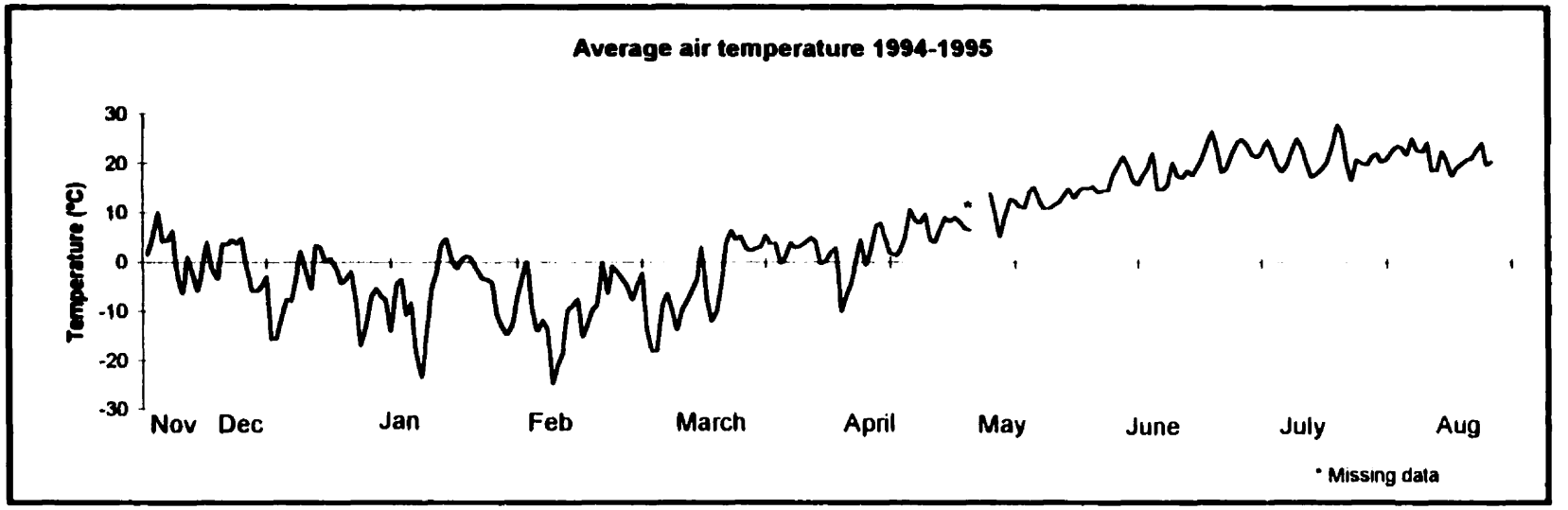
1994-1995

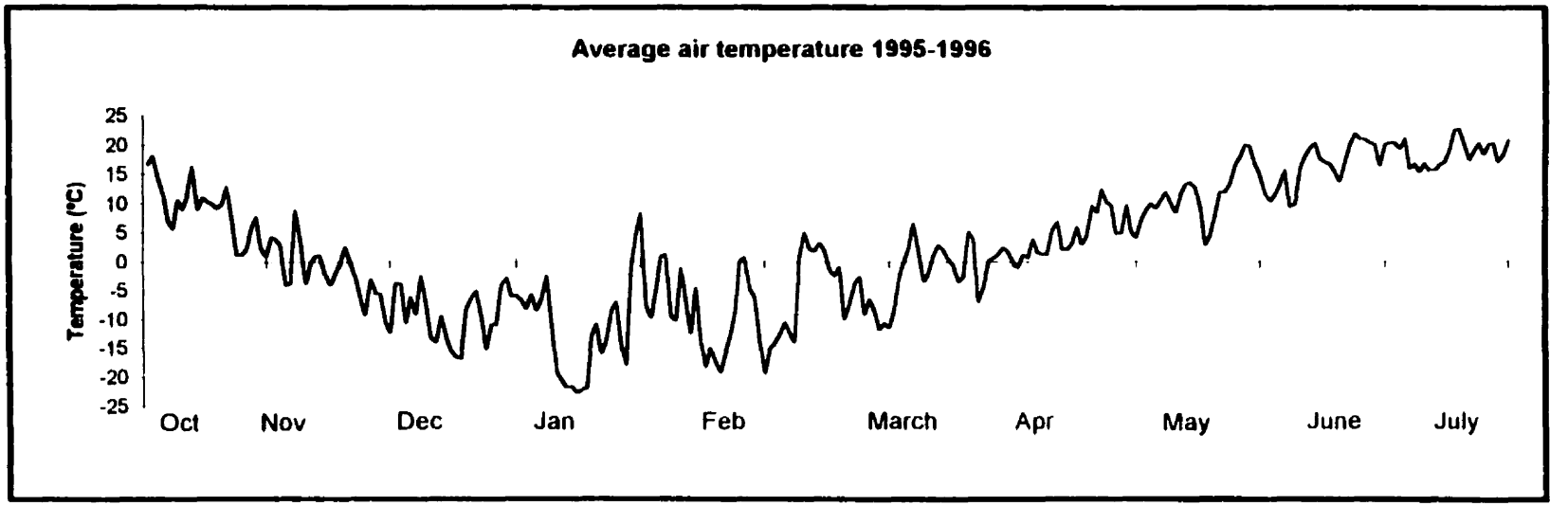


1995-1996



APPENDIX 2. Average air temperatures recorded during the two years of field experimentation (Macdonald Campus weather station).





APPENDIX 3. Precipitation recorded during the two years of field experimentation (Macdonald Campus weather station).