Influence of Temperature and Method of Inoculation on the Response of Faba Beans (*Vicia faba*) to *A. euteiches*

by

Paul Wing-ming Pi

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FORWARD

The materials, methods and results in this thesis are presented in the form of two manuscripts intended for publication in the Canadian Journal of Plant Pathology. The style as well as the preparation of tables and figures comply with requirements of the Journal. A general discussion of the results is included after the manuscripts.

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GENERAL ABSTRACT

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Influence of Temperature and Method of Inoculation on the Response of Faba Beans (Vicia faba) to A. euteiches.

Major Professor: Dr. C. C. Bernier

The response of faba beans (Vicia faba L.) to infection by Aphanomyces euteiches Drechs. in the greenhouse and under controlled temperature was evaluated by using two susceptible cultivars and six mass selected populations (MSP) with resistance to the pathogen in the field. A temperature of 28 C was found to favor mycelial growth of the Manitoba isolate of A. euteiches recovered from infected faba bean seedlings. Plant vigor as expressed by stem dry weight was significantly greater at 12 than at 28 C. Experiments were designed to subject inoculated plants to 12 regimes under controlled temperature. Each regime consisted of two phases: a first phase at a higher temperature to favor infection and disease development and a second phase at a lower temperarture to enhance host growth and recovery. Temperature and duration in each phase ranged from 10 to 28 C and from 1 to 14 days respectively. Disease severity ratings (DSR) were based on a scale of 0 to 4. Correlation analy-

sis was conducted between DSR for each temperature regime and DSR obtained from 1984 field plots. Six of the twelve temperature regimes correlated highly with DSR in the field ($r>0.7$). The two temperature regimes with the highest correlation coefficients were: 14 days at 28 C followed by 14 days at 12 C ($r=0.8543$), and 7 days at 28 C followed by 21 days at 12 C ($r=0.8459$). Both regimes provided satisfactory separation of the susceptible cultivars from the resistant MSP and separation of the MSP with resistance into a high and a moderate resistant class. This technique should prove useful to plant breeders and plant pathologists attempting to improve resistance in faba beans to A. euteiches.

Relationship between plant/root vigor and DSR of faba bean cultivars and MSP was also investigated. Correlation coefficient between total dry weight (TDW) and DSR from greenhouse experiments was negative and high at 28 ($r=-0.7791$) and at 35 days ($r=-0.7821$). Relative root dry weight loss (RRL) was also highly correlated with DSR at both 28 ($r=0.7050$) and 35 days ($r=0.9027$). There was no significant correlation between root dry weight (RDW) of the uninoculated plants and DSR of the inoculated plants.

The DSR at any of the sampling dates in the greenhouse experiments could not reflect the ranking order of the susceptible cultivars and the resistant MSP in the field. Inoculation of stem and leaf tissues failed to separate the resistant MSP from the susceptible cultivars. However, infection of the lower tap root provided satisfactory separation of the most susceptible cultivars from the resistant MSP.

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Chapter I

GENERAL INTRODUCTION

Aphanomyces euteiches Drechs. was found to be a causal agent for pea root rot as early as 1925 (Jones and Drechsler, 1925). A field infested with A. euteiches is still a major limiting factor for pea cultivation. Oospores in the infested fields remained viable after 8 years, and subsequent cropping with peas leads to further increase in the inoculum potential (Temp and Hagedorn, 1964 , 1967).

Early surveys indicated that Fusarium spp., Rhizoctonia solani Kuehn, and Sclerotina sclerotiorum (Lib.) de Bary were associated with root rot of faba beans in the province of Manitoba (Platford and Bernier, 1973), and that disease incidence was generally low. However, severe root rot and seedling blight have been observed recently at the University of Manitoba campus farm (Lamari, 1982) and highly susceptible plants were either killed at early seedling stage or stunted. The lower leaves were yellow and much of the discolored root and stem tissues were macerated. A. euteiches was shown to be the causal agent (Lamari, 1982). In the field, the level of resistance found in some accessions was improved over 4 years of testing and screening (Lamari et al., 1984). Plants from resistant mass selected populations (MSP) were not stunted and did not show any yellowing of the lower leaves or blackening of the root system. The fact that A. euteiches was isolated from these plants showed resistance to be imcomplete.

A. euteiches is favored by high levels of soil moisture and by temperatures of about 28 C. By allowing pea lines infected with A. euteiches to recover at a lower temperature, an environment unfavorable to the pathogen, a technique based on environment-shift was developed to evaluate host response to infection (Shehata et al., 1976). Faba bean is a cool-season crop and therefore low temperature would be expected to favor host recovery after infection. Such a technique was used in this study to establish conditions under which the response of resistant faba bean MSP and susceptible cultivars would be similar to their disease reactions in the field. Inoculation in phase one under high temperature would enhance initial infection while a subsequent low temperature phase would allow the host to recover.

In peas, resistance was shown to be related to other traits such as the ability of the plant to regenerate new roots (Johnson, 1954), vigorous plant growth (Haglund, 1960), and the development of adventitious roots at seedling stage (Shehata et al., 1983).

A unique and quick way of assaying the effects of systemic fungicides in soybean seedlings against Phytophthora megasperma Drechs. involved inoculating the upper portion of the hypocotyl with a droplet of zoospore suspension (Lazarovitz et al., 1980). Differences in discoloration around the infection site were related to the effectiveness of the fungicides incoporated in the plant growth medium. In an attempt to relate the extent of tissue discoloration and decay to resistance, faba bean seedlings from mass selected populations (MSP) with resistance in the field were inoculated at various sections of the root system, on the stem, and on the leaf surfaces.

LIERATURE REVIEW

1. Host

1.1 Host Names

Vicia faba L. is the botanical name of faba beans. Bond (1970) indicated that Vicia faba minor and Vicia faba equina were usually referred to as field beans in the UK and Europe, while the large seeded major types were commonly known as broad beans. Of the field beans, the medium seeded var. equina may be known as horse beans, and the small seeded var. minor, can be referred to as tick beans. In the USA, Phaseolus vulgaris L., not Vicia faba, is known as field beans. In order to avoid the confusion arising from these common names, the term 'faba bean' is now commonly used to represent the whole species.

1.2. Major Areas of Production

Beside being an important food crop in ancient Egypt, faba beans were also considered to have medicinal value. Faba beans have been found in the fifth dynasty funerary temple of Sahuire (c. 2400 BC) and in the tombs at Dra, possibly dating back to the twelfth dynasty (2140-1785 BC) (Darby et al., 1977).

Ancient Greek literature contains many references to faba beans. In the Iliad by Homer (800 BC), black-seeded faba beans were mentioned as a cultivated crop.

Faba beans were the only beans known in Europe in the pre-Columbian era before the introduction of the Phaseolus beans from the New World (Hawtin and Hebblethewaite, 1982). Faba beans were a common food crop in the poorer households throughout the Middle Ages (Tannahill, 1973). However, there has been a steady decline in the production of the crop over the past ten years in Europe, probably due to the fact that the more recent use of the crop, primarily as animal feed, has been replaced by cheaper imported proteins (Hawtin and Hebblethewaite, 1982).

In other major areas of production such as North Africa, the Middle East and China, faba beans are still grown mainly for human consumption. In North Africa, the major types are preferred and up to 80% of the crop is grown for green pods and seeds. Early pods are harvested for green vegetables when prices are high, and late-set pods are harvested dry (Hawtin and Omar, 1981).

Faba beans rank the highest among the important pulse crops in Ethiopia and occupy about 1.5% of the total cultivated area (Hawtin and Hebblethewaite, 1982). With a rapidly increasing population, countries along Nile river such as Egypt and Sudan have become net importers of faba beans (Watson, 1981). In the Middle East countries with harsh winter such as Iran and Iraq, the major types are preferred and a significant proportion of the crop is harvested green.

China is by far the world's largest producer of faba beans and most common is the large seeded type which is harvested mainly for human consumption as a supplementary diet to rice. Most of the crop is cultivated along the Yangtze river (Tao, 1981), but recent decline in crop produc-

tion is due to the fact that faba bean is not an important crop for the export market.

Faba bean is not an important crop in North America: in the USA, only a few Northern States such as Montana have a limited area of production. However, both minor and equina types are becoming important in the Prairie Provinces of Canada, since such types are found to be well adapted to moist areas in these Provinces (Evans et al 1972). Between 2000 to 5000 ha. of faba bean silage per year has been grown in Alberta, but it was estimated that about 1/3 of faba bean production in Saskatchewan and most of the production in Alberta was irrigated (Slinkard and Buchan, 1980).

Faba bean has been an important crop in several areas of the world for thousands of years, and still is, even though production has declined in recent years. Faba bean is also important because of its ability to fix atmospheric nitrogen (Candlish and Clark, 1975), and will therefore remain important in areas of low soil fertility. Research underway in the UK, the Middle East, and Canada to overcome problems such as unstable yields and disease susceptibility, and to improve production practices, is likely to maintain or increase this crop's importance in future years.

2.1. Root Rot of Faba Beans

Root diseases, caused by a variety of soil-borne pathogens, are thought to be a serious problem in some major areas such as North Africa and West Asia (Hawtin and Stewart, 1979). These soil-borne pathogens can

cause a wide range of diseases such as: root rot in which the root cortex and lateral roots turn black and rot away; stem rot in which the stem base becomes diseased; vascular wilt in which the vascular system is damaged; and damping-off in which the hypocotyls are attacked and young seedlings are killed.

Root rot of faba beans can be caused by a number of pathogens. Early surveys of root rot of faba bean in Manitoba indicated that Fusarium spp., Rhizoctonia solani and Sclerotinia sclerotiorum (Lib.) de Bary were the main causal agents (Platford and Bernier, 1973) but the incidence of disease was generally low. However, subsequent to these surveys, it was noted that at the campus farm root rot and seedling blight of faba beans were severe, and pathogenicity tests indicated that Aphanomyces euteiches Drechs. was then the main causal agent (Lamari, 1982). Highly susceptible cultivars were killed at early seedling stage while those that survived were stunted, had yellowed lower leaves and the root systems and the stem bases were blackened.

Basically there are two kinds of root rot in faba beans: soft root rot with much decayed and macerated cortical tissues, and dry root rot with blackening, but no disintegration of, the cortical tissues (McKeen, 1952).

2.2. Soft Root Rot

Soft root rot is mainly caused by Oomycetes including members of the genera Pythium, Phytophthora, and Aphanomyces. There are many species of Pythium which can cause soft root rot in faba beans. The earliest

identified root rot pathogen associated with faba bean was Pythium debaryanum Hesse (Sideris, 1931), with Phytophthora megasperma Drechs. and Aphanomyces euteiches, which were earlier known were shown to infect soybeans and peas, more recently have also been shown to infect faba beans (Salt, 1980). There is evidence that faba beans are more resistant to these soft rot pathogens in cold and wet soil than, are peas (Schultz, 1950), and Phaseolus beans (Salt and Smalley, 1979).

All these pathogens can cause both seedling blight and root rot diseases. They attack the cortical tissues of roots causing a water-soaked appearance, which is followed by a soft rot, and the blackening then spreads throughout the root system and the stem base. Under dry conditions, symptoms are less severe, with infection being much reduced, and newly initiated root development occurs replacing that tissue which has been damaged (Salt, 1983).

2.3. Dry Root Rot

Fusarium species, Phoma species, and Rhizoctonia solani are the main agents of dry root rot in faba beans (Salt, 1982). These pathogens can cause seed rot and damping-off when the host is inoculated artifically (Clarkson, 1978). Under field conditions, root rot development is slow at the early seedling stage but progresses rapidly as plants mature (Eisa and Barakat, 1978). The aerial symptoms of leaf necrosis and death of older leaves are accompanied by dry blackening of the root system.

3.1. The Pathogen - A. euteiches - Historical

De Bary (1860) erected the new genus Aphanomyces, for a group of saprophytic and parasitic fungi with an aquatic habit. ' Aphanomyces ' in Greek means 'imperceptible'. This generic name was selected since these fungi were particularly difficult to detect. A. euteiches was first described in Jones and Drechsler (1925), and reported therein to be highly pathogenic to garden peas. Most of the knowledge on the pathogenicity of A. euteiches is drawn from root rot of peas (Walker and Musbach, 1939; Smith and Walker, 1941; Sparrow, 1960; Papavizas and Ayers, 1974). Aphanomyces root rot is still a major constraint to pea production in the USA as more than 80% of the cases of root rot in peas were attributed to this pathogen alone (Papavizas and Ayers, 1974).

3.2. Formation of Infection Unit - Zoospores

Like many other pathogenic water molds, A. euteiches is a diplanetic fungus producing two types of zoospores (Papavizas and Ayers, 1974). Primary zoospore cysts are produced asexually either by the formation of zoosporangia or, under favorable conditions, the whole thallus appears to be involved in asexual sporogenesis. Primary zoospore cysts remain attached to each opening and, after a period of encystment usually lasting a few hours, biflagellate secondary zoospores emerge from the primary zoospore cysts; the secondary zoospores can swim away and infect other healthy roots.

Zoospores are also formed within germ tubes of oospores under the influence of exudates from germinating host seeds. Sometimes, under high moisture, oospores may form a germ sporangium (Jones and Drechsler, 1925).

Apparently, primary zoospore production is approximately constant in number over a temperature range from 4 to 32 C (Sherwood, 1958), but the swimming ability of the secondary zoospore was inhibited at 32 C. However, 24 C was the optimum temperature for zoospore formation of three A. euteiches isolates studied by Llanos and Lockwood (1960).

Zoospore production can be induced by exposing agar-grown cultures (maltose-peptone medium) to several changes of distilled water; aeration further enhances the amount of zoospores produced during such treatments (Llanos and Lockwood, 1960; Mitchell and Mitchell, 1973). However, under field conditions, oxygen is not a limiting factor even when soil is saturated by flooding (Mitchell and Mitchell, 1973)

3.3 Zoospore Motility

A. euteiches is a biflagellated-zoosporic Oomycete. The motile, dispersive, secondary zoospores possess a pair of flagella one of which is a whiplash type and the other a tinsel type. Motility of most of the secondary zoospores ceased after a day in the soil , but a few spores can remain motile for up to 5 days (Yokosawa and Kuninaga, 1977).

3.4. Minimum Number of Zoospores for Infection

The minimum number of zoospores needed for infection of pea roots was shown to be related to the temperature. The average ED50 values were: 39, 710, and 985 motile zoospores at 28, 24 ,and 20 C respectively, and at 16 C infection did not reach 50% with any number of zoospores tested (Bhalla, 1968).

3.5. Attraction and Penetration by A. euteiches Zoospores

Attraction of zoospores to host roots can be attributed to either a specific stimulatory effect of the root exudates or to a non-specific effect of nutrient gradient. The roots of peas, as well as those of other non-host plants, displayed chemotaxis to zoospores of A. euteiches (Cunningham and Hagedorn, 1962a). However, the zoospores were attracted in greater number by pea root exudates than by other non-host plants (Morrison, 1972). In peas, the region strongly attractive to the zoospores was observed to be near the root cap (Cunningham and Hagedorn, 1962b). A. euteiches and Pythium aphanodermatum zoospores also showed preferential attraction to punctured wounds and to the stele exposed at cut ends of the root system (Royle and Hickman, 1964).

Acording to Cunningham (1961) the chemotactic response was not corrlated with resistance and susceptibility in peas to the pathogen. However, Morrison (1972) reported that fewer zoospores were attracted to exudates of a then newly developed resistant pea line, Minnesota 494-A-9 than to that of a susceptible cultivar, New Era. Resistance in this case can be related to the fact that fewer zoospores are attracted to the infection site; i.e. the initial inoculum potential is low.

Penetration is aided by the secretion of a pectic acid, endopolygalacturonase, which softens and macerates root tissues thus aiding mechanical penetration of germinating zoospores (Ayer and Papavizas, 1965). After one and half hours of exposure pea roots to a zoospore suspension, and presumably, the pectic enzyme, many zoospores attached to the root surface germinated; it then required another half hour for the

root epidermis to be penetrated by the germ tube (Cunningham and Hagedorn, 1962b). Generally, the germ tube peneration of root tissues is intercellular, but, occasionally, it can be intracellular. After eight hours, the fungus could be observed to have penetrated in as deeply as the seventh cell layer intracellularly, and after 24 hours, mycelium could be found in the endodermis.

4.1. Pathogen Growth - Oxygen Requirement

A. euteiches requires an adequate supply of oxygen even though its semi-aquatic habit allows it to thrive under high moisture levels. However, saturation of infested soil with water before seeding reduced the incidence of Aphanomyces infection in peas (Burke and Mitchell, 1978). The formation of zoospores was enhanced by a good supply of oxygen (Mitchell and Mitchell, 1973), but oxygen requirement for mycelial growth is extremely low (Sherwood and Hagedorn, 1961). The pathogen thrives under wet conditions because the water potential is too low for the growth of the pathogen in drier soil (Cook and Papendick, 1972).

4.2. Temperature and Growth

There have been a number of reports on the effects of temperature on disease development. An early study showed that the soil temperature favoring disease development was between 15 and 30 C when peas were inoculated artificially (Jones and Drechsler, 1925), while in another study using wider range of temperatures (12, 16, 20, 24, 28, and 32 C), found that 24 to 28 C was the optimum range for disease development

(Smith and Walker, 1941). Using a different set of temperatures (5, 10, 15, 20, 25, 30, 35, and 40 C), Cho and King (1963) showed that temperatures from 20 to 25 C were optimum for infection of excised root tips of a highly susceptible pea cultivar. This discrepancy in the reported optimum temperature range for infection cannot be resolved because different pea cultivars and different inoculation techniques were used in these studies. It is also possible that the different A. euteiches isolates employed might also have responded differently to temperature. When the pathogen was cultured on artificial medium, optimum temperature for mycelial growth was observed to be as high as at 34 C (Jones and Drechsler, 1925).

5. Pathogen Survival

Under non-competitive environmental conditions, mycelial growth was observed in dead pea tissues. However, A. euteiches requires organic nitrogen and reduced sulfur compounds and, under normal soil conditions, it can easily be out-competed by other saprophytes. A. euteiches might have a limited saprophytic phase, but the fungus cannot extend itself from a food base by mycelial elongation through natural soil (Lockwood, 1960b).

A. euteiches survives by means of thick-walled oospores when the fungus exhausts its mycelial growth potential. Determination of the number of oospores formed in excised root tips may be an accurate assessment of resistance or susceptibility in pea lines (King and Cho, 1963).

Oospores are long lived and were still viable after having been stored at -20 C for two years (Sherwood and Hagedorn, 1962). Under natural field conditions, oospores were observed to have survived for 8 years (Temp and Hagedorn, 1964).

Germination of oospores can be induced by root exudates of peas, soybeans, field beans, and sweet corn (Scharen, 1960). Significantly, a greater number of zoospores germinated next to the roots of peas than the roots of other plants. Germination can also be induced artificially by proteolytic enzymes (Yang, 1970). Two modes of germination were observed: oospores older than 4 weeks would germinate primarily by the formation of germ tubes whereas oospores younger than 4 weeks would germinate into germ sporangia.

6. Aphanomyces euteiches Races/Isolates

Beute and Lockwood (1967) were the first to attempt to determine whether discrete races of this pathogen existed. They used six pea varieties as differential hosts to test 15 single-zoospore isolates of A. euteiches, employing area under the disease curves (AUDC) as disease indices for comparison among these isolates. Fourteen of these isolates were determined to belong to one race, and the fifteenth to a different race. Using the same differential series, four additional races were identified from Norwegian field collections (Sundheim, 1972).

Sherwood and Hagedorn (1962) were able to show that isolates of A. euteiches from peas could also infect Phaseolus vulgaris L. Carley (1970) indicated that since most pea cultivars were highly susceptible

to the pathogen, bean varieties might be more suitable to differentiate A. euteiches races. Nine bean varieties were employed as differentials to confirm seven races of A. euteiches. However, a more recent study showed that an isolate of A. euteiches from field-grown beans was found to be pathogenic to beans but not peas (Pfender and Hagedorn, 1982). Two formae speciales were proposed: A. euteiches f. sp. pisi which could infect both peas and beans, and A. euteiches f. sp. phaseoli which could infect beans but not peas. These two formae speciales also differed from each other in growth rate at 32 C and in the diameter of their oospores (Pfender and Hagedorn, 1982).

Three pathotypes (AE1, AE2, and AE3) of A. euteiches were also isolated from naturally infected faba beans and peas in Manitoba (Lamari, 1982). Pathotype AE1, similar to a Wisconsin isolate P14, is the most common type isolated from infected faba beans at the University of Manitoba. Pathotype AE2 was separated from AE1 on a pea cultivar, Homsteader, to which AE1 was not aggressive. Pathotype AE3 was avirulent on faba beans and lentils but virulent on peas.

7. Disease Control

Effective control of pea root rot caused by A. euteiches is still not available and pea producers in the American Midwest still attempt control by avoiding fields with high disease risk (Jacobsen and Hopen, 1981). The difficulty in controlling the disease is due to the building up of inoculum potential during cultivation of the susceptible hosts.

In the 1960's and 1970's, considerable research was conducted into the control of pea root rot through chemical means. Most of the fungicides tested either failed completely or gave unsatisfactory results in the field, and only limited success has been achieved in the greenhouse and in small scale field trials (Lewis, 1973; Mitchell and Hagedorn, 1969; Papavizas, 1967; Alconero-Pivaral, 1967). The limited success has been associated with application of fertilizers containing Al, Cu and Zn compounds which may be toxic to the pathogen in the soil. Nitrogen fertilizer in the form of nitrate was also shown to reduce pea root rot in the field while nitrogen in the form of ammonium would enhance it (Carley and King, 1968).

In Russia, pea seeds treated with hydroxyisoxazole gave relatively good results in controlling the disease (Kotova and Tsvetkova, 1980). Some herbicides were also reported to have significant disease reduction (Grau and Reiling, 1977; Jacobsen and Hopen, 1981). Vapours from several sulfur-containing compounds adversely affect various processes in the life cycle of A. euteiches (Lewis and Papavizas, 1971). Cruciferous amendments, such as leaves and stems of cabbage, kale, and mustard at 0.5% of the dry weight soil as source of sulfur-containing compounds, gave considerable reduction of Aphanoymces root rot in the greenhouse (Papavizas and Lewis, 1971).

Oospores can be parasitized by a number of soil microbes, but only Penicillium species showed some promise in the biological control of the disease (Sneb et al., 1977).

8. Partial Resistance to A. euteiches in Peas and Faba Beans:

Complete resistance to A. euteiches could not be found among the 850 pea introductions evaluated (Lockwood, 1960a; Lockwood and Ballard, 1960), but a few showed reduced indices, and they were also believed to possess resistance useful in plant breeding programs. Attempts to improve peas for resistance to the pathogen have led to some success with the identification of cultivars Minnesota 108 and Minnesota 494-A11 (Davis et al., 1976; King et al., 1981). The resistance in both of these cultivars was confirmed by using the environment shift technique (EST) (Shehata et al., 1976, 1983). The technique involves shifting infected pea plants from an environment favorable to A. euteiches to an environment favoring host recovery and, therefore, resistant pea cultivars could be separated from the susceptible ones.

The only genetic study of the response in peas to A. euteiches showed that "tolerance" was associated with 3 dominant wild-type unlinked loci, and that substitution of the recessive, horticulturally desirable alleles at these loci could lead to a decrease in "tolerance" (Marx et al., 1972).

Partial resistance in peas to A. euteiches has also been related to other agronomic traits such as the ability of the plant to regenerate new roots (Johnson, 1954), vigorous plant growth (Haglund, 1960), and the development of adventitious roots at an early seedling stage (Shehata et al., 1983).

Severe root rot of faba beans was observed in University of Manitoba field plots in 1978, with most of the plants being infected (Lamari, 1982). The susceptible plants were either killed at an early seedling

stage or became stunted with much discoloration of the root system and the stem base.

Variability in resistance to root rot was found in some 350 faba bean cultivars and accessions (Lamari, 1982) Through many years of testing and selection, some mass selected populations (MSP) showed high level of resistance (Lamari et al., 1984). The root system of these MSP was only slightly discolored, the lower leaves remained green and the plants were not stunted. However, A. euteiches was regularly isolated from the seedling of these MSP showing resistance to be imcomplete.

9. Host Reaction to Infection as Influenced by Environmental Factors

The severity of root rot and vascular wilt diseases depends very much on environmental factors such as high temperature, shortage or excess of water, nutrient deficiencies, adverse soil conditions and the interactions of other soil microbes. Stress induced by some of these factors can predispose faba beans to various root diseases (Salt, 1982). In the arid climate of the Northern Sudan, faba bean root rot caused by Fusarium species was less in crops which were irrigated weekly than in those irrigated at 2 or 3 week intervals. Also, faba beans seeded in early October when temperatures were still high had a higher incidence of root rot than later sowings under cooler conditions (Freigoun, 1980). Therefore, in order to interpret the host response to soil-borne pathogens, environmental factors must be taken into consideration.

Chapter II

RESULTS OF RESEARCH

RESEARCH 1: Relationship between Plant Vigor and Partial Resistance to *Aphanomyces euteiches* in Faba Beans and Influence of Temperature on Host Response to Infection

ABSTRACT

Root dry weight (RDW), total dry weight (TDW),and relative root dry weight loss (RRL) due to disease in two susceptible faba bean cultivars and six resistant mass selected populations (MSP) were poorly correlated with disease severity ratings (DSR) in the field 17 days after planting. At 35 days after planting, TDW and RRL were highly correlated with DSR in the greenhouse (r=0.7821 and r=0.9201 respectively). However, RDW did not show a high correlation coefficient with DSR. DSR of the faba bean cultivars and MSP at 8 sampling dates did not reflect the ranking order of the susceptible cultivars and the resistant MSP in the field.

Mycelial growth of the isolate of *A. euteiches* recovered from infected faba beans in Manitoba was favored by a high temperature of 28 C whereas, stem dry weight of all faba bean cultivars and MSP was greater at 12 than at 28 C. Thus, host response was evaluated in experiments

whereby the inoculated plants were first placed at a temperature favoring the development of the pathogen and then at a temperature favoring host growth and recovery. The temperature and duration of each phase ranged from 10 to 28 C and from 1 to 14 days respectively. DSR from six of the 12 regimes evaluated were significantly correlated with DSR of the susceptible cultivars and the resistant MSP in the field. The two regimes with the highest correlation coefficients were: 14 days at 28 C followed by 14 days at 12 C ($r=0.8543$), and 7 days at 28 C followed by 21 days at 12 C ($r=0.8459$). Both regimes provided satisfactory separation of the susceptible cultivars from the resistant MSP, and separation of the MSP with resistance into highly and moderately resistant classes. This technique should prove useful to plant breeders and plant pathologists attempting to improve resistance of faba beans to A. euteiches.

INTRODUCTION

Aphanomyces euteiches Drechs. has been found to be associated with a severe root rot of faba beans (Vicia faba L.) at the University of Manitoba campus farm. Several faba bean cultivars and accessions showed various levels of resistance in the field over four years of testing and selection (Lamari, 1982; Lamari and Bernier, 1984). Plants from mass selected populations (MSP) with resistance were not stunted and did not show any yellowing of the lower leaves. Discoloration of the root system remained slight and superficial, and many new white lateral roots were present. In contrast, roots of susceptible cultivars showed extensive dark discoloration. A. euteiches was isolated from MSP showing resistance in the field, confirming that resistance is imcomplete. In preliminary experiments in the greenhouse, there were no differences between MSP with resistance and susceptible cultivars, and all showed a progressive blackening of the root system and the stem base. However, the level of resistance of many MSP in field tests was judged adequate and useful, as the seed yield of these MSP was greater than that of commercial cultivars in infested soil and about 2/3 that of the same cultivars in non-infested soil (Lamari, 1982).

In similar host-parasite systems, resistance has been attributed to plant or root vigor. Johnson (1954) in an early report, speculated that the ability of foreign pea introductions to withstand A. euteiches was apparently related to their ability to produce new roots. A recent study showed that Minnesota 108, a pea line moderately resistant to A. euteiches, developed more adventitious roots at the seedling stage as com-

pared to four other susceptible pea cultivars (Shehata et al., 1983). Rowlinson and Colhoun (1970) showed that oat seedlings with reduced vigor were prone to many soil-borne pathogens. Zerlik (1979) concluded that in pea lines, resistance to foot rot caused by F. solani was related to the amount of root branching and the number of adventitious roots could be the basis of a quick method of screening for resistance. Bruehl (1983) indicated that many root diseases caused by Pythium species could be greatly reduced by increasing host vigor as in the case of the reduction of root rot in sugar cane by hybrids.

Partial resistance to A. euteiches was better expressed when pea lines and cultivars were grown in an environment unfavorable to the pathogen (Shehata et al., 1976, 1983). Under dry soil conditions which were unfavorable to A. euteiches, symptoms of faba bean root rot were less severe as new root development took place when infection was reduced (Salt, 1983).

Of the environmental factors affecting host reaction to a pathogen, temperature is the easiest to study and to control in the greenhouse. Temperature can influence the activity of the pathogen within the host, either directly or indirectly, through the host's reaction to temperature. Root rot symptoms on peas developed more slowly at 16 C than at 20, 24 or 28 C, but the incidence of A. euteiches root rot was nearly the same at each temperature (Burke et al., 1969). In naturally infested soil, the production of zoospores as the inoculum source was optimal at 24 C (Llanos and Lockwood, 1960). However, at temperatures below 16 or above 32 C, the number of zoospores produced was greatly reduced. Therefore, under field conditions, temperature could affect host reaction by

influencing the level of inoculum in the soil and by affecting the development of the fungus within the host plant.

Jones and Drechsler (1925) showed that the optimum temperature for mycelial growth of A. euteiches on an artificial medium appeared to be between 15 and 34 C whereas temperatures between 15 and 30 C favored infection. The difference in the effects of temperature may be due to the fact that after penetration, the fungus produces an endopolygalacturonase enzyme which was recently shown to be sensitive to temperatures above 30 C (Ayers et al., 1969).

In screening for resistance in peas to A. euteiches , an environment shift technique (EST) was successfully used by Shehata et al., (1983). Following initial growth in a growth chamber environment infection by the pathogen (100% RH at 26 +/- 2 C for 7 days), inoculated plants were transplanted to a greenhouse bench bed at a soil temperature between 10-12 C to favor host recovery. High level of moisture was maintained throughout the entire experiment. This technique proved useful in separating resistant pea line 494A11 from other susceptible cultivars.

In this study, attempts were made to relate susceptibility and resistance to A. euteiches, in faba bean populations selected in the field, to differences in plant/root vigor, and to establish environmental conditions under which the response of suceptible cultivars and resistant MSP would be similar to their disease reactions in in the field.

MATERIALS AND METHODS

Two faba bean cultivars Pi222128 and Aladin and six mass selected populations (MSP) from accessions 2N19, 2N37, 2N94, 2N114, 2N134, and I12 were chosen for host vigor determination and evaluation of response to infection under controlled temperature conditions. Both Pi222128 and Aladin were shown to be highly susceptible while the six MSP showed various levels of resistance in the field (Lamari, 1982).

All seeds were surface sterilized in 2.5% sodium hypochlorite for 5 minutes and rinsed in tap water before they were allowed to germinate in petri dishes on filter paper moistened with distilled water. After one day, all germinated seeds were planted in steamed soil:sand: vermiculite (2:1:1, v/v/v) medium, one seed per 13 cm clay pot; high levels of moisture in each pot was maintained by watering daily.

Inoculum Preparation and Inoculation Procedure

Zoospores of A. euteiches were isolated and produced by the method described by Lamari (1982) (Appendix 3) with a slight modification: cornmeal agar instead of water agar plates were used. Zoospore concentration was determined with a haemocytometer, an average of six samples, and inoculum concentration was adjusted with distilled water to 2.5×10^5 zoospores/mL. All seedlings were watered thoroughly before inoculation. One and a half mL of zoospore suspension was pipetted next to the stem base of each seedling.

Root and Total Dry Weight of Uninoculated Faba Bean Plants

Twenty seedlings of each faba bean cultivar and MSP were employed in each of the three sampling dates: 17, 28, and 35 days after planting in the greenhouse at a day temperature of 20 to 22 C and a night temperature of 15 to 17 C. A minimum 16 hours of light was provided by supplementary fluorescent lighting. At the end of each experiment, all plants were carefully uprooted and washed until all soil particles were removed. Each plant was separated at the base of the hypocotyl into stem/leaf and root sections and then were placed in separate envelopes and dried at 93 C. Twenty-four hours later, stem and root dry weight were measured and recorded.

Disease Severity Ratings at 17, 28, and 35 Days after Planting

Faba bean seedlings of each cultivar and each MSP were inoculated at the 2 to 4 leaf stage (7 to 10 days after planting) employing 1.5 mL zoospore suspension (2.5×10^5 zsp/mL) deposited at the stem base. DSR were assessed at each of the sampling dates (17, 28, and 35 days after planting).

Disease severity was assessed by rating individual plants on a scale of 0 to 4 as followed:

0 = no discoloration due to infection on hypocotyl or root;
0.5 = light brown discoloration on the upper portion of the tap root or a small lesion on hypocotyl;
1.0 = light brown discoloration on both tap root and lateral roots not exceeding 1/2 of the circumference of the stem base;

1.5 = extensive dark brown discoloration throughout the upper portion of the root system not exceeding 3/4 of the circumference on the base of the stem;

2.0 = extensive dark brown discoloration throughout the root system but no decay, and blackening of the stem base not exceeding 1/2 cm in length;

2.5 = same as 2.0 but with limited decay in the root system, and blackening of the stem base not exceeding 1 cm in length;

3.0 = same as 2.5 but decay or water-soaked tissue observed in the stem base;

3.5 = extensive decay in root system and stem base with wilting symptoms and some yellowing of the lower leaves;

4.0 = plant severly wilted or death occurred within 4 weeks from inoculation.

Relative Dry Root Loss (RRL) Calculation

After DSR were assessed at each sampling date, infected roots of the same plants were carefully washed and dried at 93 C for 24 hours before weighing. The amount of mascerated root tissues of each cultivar and each MSP were expressed in terms of the percent relative root dry weight loss (RRL) which was calculated by the following formula:

$$\frac{\text{(Disease Free Root Dry Weight - Diseased Root Dry Weight)} \times 100\%}{\text{Disease Free Root Dry Weight}}$$

Comparison of Stem Weight of Unioculated Plants and A. euteiches Mycelial Growth at 12 and 28 C

Twenty uninoculated seedlings of each cultivar and each MSP were allowed to grow for 6 weeks under constant temperature at 12 and 28 C. The aerial portion of each plant was cut and put into a labelled bag and dried at 93 C for 24 hours before weighing.

Ten sealed petri dishes each containing 10 mL cornmeal agar were inoculated at the center with mycelium of A. euteiches on agar disks (5 mm in diameter) were placed with plant materials in a growth cabinet at each of the two temperature regimes (12 and 28 C). The diameter of the mycelial colony under both temperatures was measured daily for four days.

1984 Field Trials

In the second week of May, 180 seeds of each cultivar and each MSP were planted in field infested with A. euteiches (Block 18) at the University of Manitoba campus farm. In the last week of July, at the flowering stage, all plants were uprooted and disease severity was assessed. A randomized complete block design was used in this trial.

Disase Severity of Plants Under Controlled Temperature Regimes:

Ten seedlings of each cultivar and each MSP were inoculated at the 2 to 4 leaf stage (7 to 10 days after planting) employing 1.5 mL zoospore suspensions (2.5×10^5 zsp/mL) deposited at the stem base, and placed in a growth cabinet at a photoperiod of 16 hours.

Table 1: Outline of temperature regimes used to evaluate susceptible faba bean cultivars and mass selected populations (MSP) with resistance to A. euteiches

Regime	1st Phase		2nd Phase	
	Temp* (C)	Duration (Days)	Temp (C)	Duration (Days)
E1	28	14	12	14
E2	28	7	12	21
E3	28	5	12	23
E4	28	3	12	25
E5	28	1	12	27
E6	25	10	12	18
E7	25	7	12	21
E8	25	5	12	23
E9	12	7	28	21
E10	12	14	12	14
E11	28	14	28	14
E12	28	7	10	21

* within 1 C accuracy under growth cabinet condition

Plants were grown under a series of 12 temperature regimes (Table 1) with each regime consisting of 2 phases: one at a temperature and duration favoring infection and a second favoring host growth and recovery. DSR of all plants in each temperature regime were assessed at 28 days after inoculation, and the DSR scale was the same as previously described. Both temperature regimes, E1 and E2, were repeated two times, and the remaining 10 temperature regimes were not repeated.

Disease Severity Assessed Under Greenhouse Condition:

Methods of inoculation of seedlings of each cultivar and each MSP were the same as in the previous temperature regime study. Disease severity ratings of 20 seedlings of each cultivar and each MSP were assessed at each of the six sampling dates (22, 25, 42, 50, 60 and 65 days after planting). Two uninoculated plants of each cultivar and each MSP served as controls at each sampling date.

RESULTS

There were no significant differences in root dry weight among uninoculated plants of the 2 faba bean cultivars and 6 MSP up to 35 days after planting (Appendix 1). However, the mean dry root weight of the susceptible cultivar Pi222128 was the lowest, and increased only slightly (0.02 gm) from day 28 to day 35 after planting, whereas values for all other cultivars and MSP increased substantially.

Differences in total dry weight among the uninoculated cultivars and MSP were significant at 35 but not at 28 days after planting with MSP 2N37, 2N94, and 2N114 being the heaviest (2.51 to 3.02 gm) and MSP I12, cultivar Pi222128 and Aladin the lightest (1.51 to 1.87 gm) (Appendix 2).

The ranking of individual cultivars and MSP on the basis of DSR of infected plants grown in the greenhouse was essentially the same at 17 and 35 days after planting with cultivar Pi222128 and Aladin having the highest , MSP 2N37, I12, and 2N19 having an intermediate, and MSP 2N94, 2N114, and 2N134 having the lowest DSR (Table 3). However, the ranking order at 28 days showed MSP I12, having the highest DSR of 3.8 and Pi222128 having one of lowest DSR of 1.8. Susceptible cultivars, Pi222128 and Aladin could be separated from the MSP with resistance at 35 days after planting. However, the ranking order of the MSP was not the same as in the field as well as the level of infection was too low for visual separation.

Table 2: Relative Root Dry Weight Loss in % (RRL)
of Faba Bean Cultivars and Mass Selected Populations (MSP)
due to Infection by A. euteiches in the Greenhouse

Cv/MSP*	Loss(%)***	
Pi**	42.6	a****
Aladin	35.3	a b
2N134	29.2	a b
2N94	25.8	a b
2N114	22.6	b
I12	22.5	b
2N19	20.9	b
2N37	20.5	b

*Cv = cultivars = Pi222128 and Aladin, MSP = numbered entries.
** 20 seedlings of each cultivar and MSP were inoculated with A. euteiches zoospore suspensions and the same number of seedlings served as uninoculated controls for use in the following calculation,
*** Loss % = (disease free root dry weight - diseased root dry weight) x 100 divided by (disease free root dry weight)
**** Numbers followed by the same letter are not significantly different at p=0.05 (Duncan's Multiple Range Test).

Table 3: Mean Disease Severity Ratings (DSR) on Faba Bean Cultivars and Mass Seleced Populations (MSP) Inoculated with A. euteiches Zoospore Suspensions in the Greenhouse

No. of Days After Planting												
17				28				35				
Cv/MSP*	DSR***			Cv/MSP	DSR			Cv/MSP	DSR			
Pi**	4.0	a****		I12	3.8	a		Aladin	2.8	a		
Aladin	3.7	a	b	2N94	3.4	a		Pi	2.7	a		
2N37	3.1		b	c	Aladin	3.2	a		2N37	1.5		b
I12	3.0		b	c	2N37	2.9	a		I12	1.4		b
2N19	2.9		b	c	2N19	2.9	a		2N19	1.1		b c
2N114	2.7			c	2N134	2.8	a		2N134	0.9		b c
2N134	2.6			c	Pi	1.8		b	2N94	0.8		b c
2N94	2.4			c	2N114	1.7		b	2N114	0.3		c

** 20 seedlings of each cultivar and MSP were used.
*** DSR = Disease Severity Ratings based on a scale of 0 (no infection) to 4 (extensive blackening of the root system and the stem base).
**** Numbers followed by the same letters in the same column are not significantly different at p=0.05 (Duncan's Multiple Range Test).

The DSR of individual faba bean cultivars and each MSP varied considerably at the three sampling dates (Table 3). The highly susceptible cultivar Pi222128 showed the highest DSR of 4.0 at 17 days after planting. However, the same cultivar showed DSR of 1.8 at 28 days after planting and was considered resistant. On the other hand, the MSP from 2N94 with a high level of field resistance had DSR of 3.4 at 28 days while it was considered resistant at 17 and 35 days with DSR of 2.4 and 0.8 respectively.

All except MSP but 2N94 were rated highly susceptible at 17 days after planting (DSR>2.5) indicating that at this early stage, symptoms on the young seedlings did not reflect the field performance of the MSP with resistance. Most of the MSP with resistance were also rated highly susceptible at 28 days. In general, the DSR at 35 days more accurately reflected the disease reaction of the cultivars and MSP in the field as both suceptible cultivars, Pi222128 and Aladin were rated highly susceptible (DSR>2.5) while all the MSP with resistance had DSR less than 2.0.

Cultivars and MSP differed in relative root dry weight (RRL) loss due to infection by A. euteiches (Table 2). The greatest loss at 42% occurred in the susceptible cultivar Pi222128. The cultivar Aladin, and MSP 2N134 and 2N94 suffered losses ranging from 25.8 to 35.3% whereas minimum losses ranging from 20.5 to 22.6% occurred in MSP 2N114, I12, 2N19 and 2N37.

Correlation analysis was conducted among root dry weight (RDW), total dry weight (TDW), relative root dry weight loss (RRL), and disease severity ratings (DSR) from the greenhouse experiments (Table 4). The correlation coefficients at day 17 were low and not significant. As the plants matured (28 and 35 days after planting), correlation coefficients increased and became significant.

At 28 days after planting, RDW was significantly correlated with TDW, r=0.7575, and TDW was negatively and significantly correlated with DSR, r=-0.7792. The correlation coefficient between RRL and DSR was significant , r=0.7050.

The correlation coefficient between RRL and DSR was positive and high (r=0.9027) at day 35. TDW was also correlated significantly with RRL (r=-0.82648), and with DSR (r=-0.78210). The correlation coefficient between RRL and TWD increased in significance from day 17 to day 35 (-0.4483 at 17 days , -0.6515 at 28 days and -0.8265 at 35 days). As plants matured, the root dry weight loss due to infection was less. The correlation coefficient between RDW and TDW was the lowest at 35 days indicating that as the plants became more mature root weight played a lesser role in the determination of the total weight.

Generally, stem and leaf dry weight of plants of faba bean cultivars and MSP were heavier and developed shorter and thicker stems at 12 than at 28 C (Fig. 1 and Fig. 2). Mycelial growth at 28 C was faster than at 12 C. After four days, the diameter of the mycelial colony was 80 mm at 28 C, whereas at 12 C it was only 42 mm (Fig. 3).

Table 4: Correlation Analysis Conducted Among Root Dry Weight (RDW), Total Dry Weight (TDW), Relative Dry Root Loss in % (RRL), and Disease Severity Ratings (DSR) on Faba Bean Cultivars and Mass Selected Populations (MSP) in the Greenhouse

Days After Planting		TDW***	RRL****	DSR*****
17				
	RDW	0.6233	0.1667	0.1628
	TDW		-0.4483	-0.3530
	RRL			0.4278
28				
	RDW	0.7575*	-0.3241	-0.2463
	TDW		-0.6515	-0.7792*
	RRL			0.7050*
35				
	RDW	0.4878	-0.4248	-0.5963
	TDW		-0.8265**	-0.7821*
	RRL			0.9021**

* level of significance p=0.05
** level of significance p=0.01
*** TDW = total dry weight of 20 seedlings of each of the 2 faba bean cultivars and 6 MSP.
**** RRL = % relative root dry weight loss is expressed by the difference in root dry weight of the uninoculated and inoculated seedlings of each cultivar and MSP multiplied by 100% and divided by root dry weight of the uninoculated seedlings.
***** DSR = disease severity ratings of each cultivar and MSP

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Fig. 1: Comparison of Faba Bean Cultivar Pi 222128 and Mass Selected Populations (MSP) 2N19 and 2N114 grown at 12 (green label) and 28 (red label) C

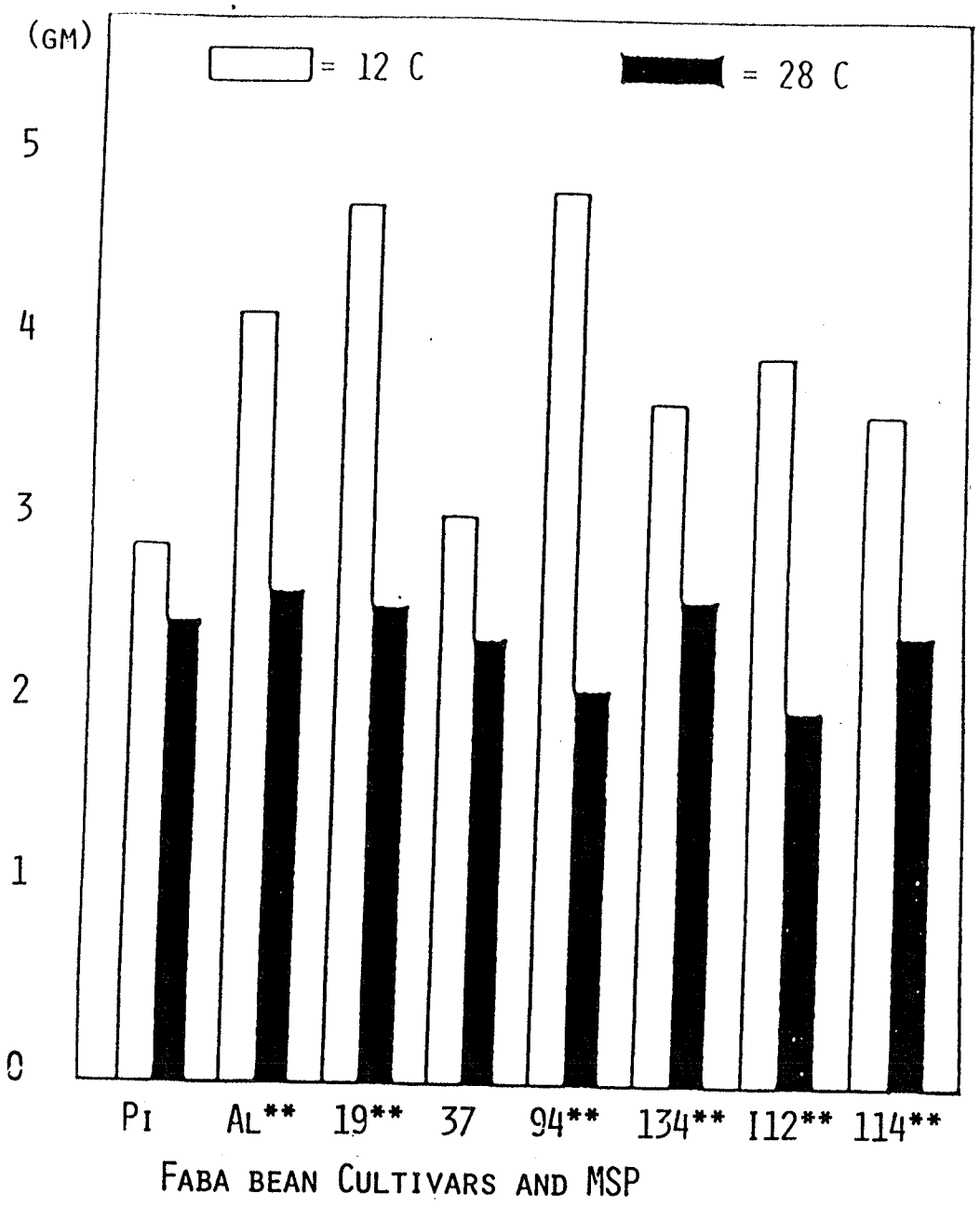

Fig. 2: 'Comparison of Mean Stem/Leaf Dry wt. of 2 Faba Bean Cultivars and 6 Mass Selected Populations (MSP) at 12 and 28 C (* significant at 0.05; ** significant at 0.01)'

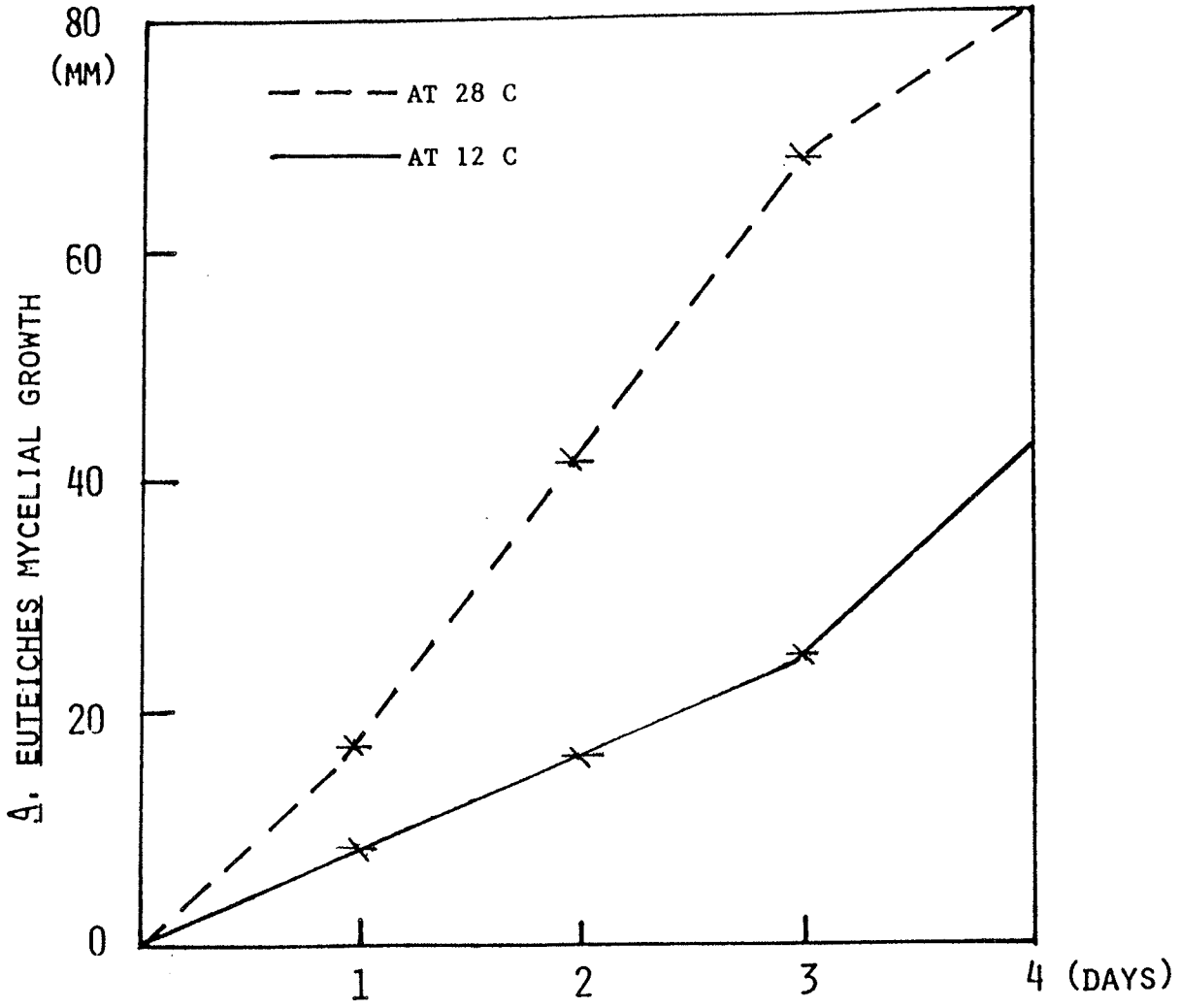

Fig. 3: Measurement of Mycelial Colony Diameter of the Manitoba Isolate of Aphanomyces euteiches cultured on cornmeal agar at 12 and 28 C over a period of four days

The two cultivars and six MSP were planted in naturally infested soil in the field at the University of Manitoba campus farm. Both cultivars Pi222128 and Aladin were rated as susceptible ,DSR>3.0, while MSP 2N114, 2N37, and 2N94 were rated as highly resistant ,DSR<2.0 (Table 5). Most plants of the susceptible cultivars were either killed at early seedling stage or were stunted with extended blackening of the root system and the stem base. Resistant expression in MSP 2N94, 2N114, and 2N37 was characterized by a light discoloration of the root system without much decay of root or stem tissues. However, plants of the MSP from 2N19 were all highly diseased and extremely stunted.

The Disease severity ratings and the ranking order of the cultivars and the MSP varied considerably between the 12 temperature regimes (Table 6). Separation of resistant MSP from susceptible cultivars was not possible when the temperature in phase one and phase two were the same. All plants of susceptible cultivars and resistant MSP were rated highly susceptible (DSR >2.5) when the temperature was kept constant at 28 C in both phases (E11). On the other hand, when both phases were kept at a constant low of 12 C (E10), all but Pi222128 were rated resistant.

There was a tendency for the DSR to be higher on all cultivars and MSP as the duration of phase one at 28 or at 25 C increased. At 25 C in phase one, the DSR ranged from a maximum of 2.5 in E8 (5 days) to 4.0 in E6 (10 days). Similarly, at 28 C in phase one, the DSR were lower in E5 (1 day) than in E1 (14 days), and E2 (7 days).

Table 5: Disease Severity Ratings of Faba Bean Cultivars and Mass Selected Populations (MSP) Grown in a Field Naturally Infested with A. euteiches in 1984

Cv/MSP*	DSR	
Pi	3.88**	a***
Aladin	3.23	b
I12	2.33	c
2N134	2.19	d
2N114	1.96	de
2N37	1.94	de
2N94	1.57	e

*Cv = cultivars = Pi222128 and Aladin, MSP = numbered entries. 2N19 was not used because of inconsistent performance in the field
** mean disease severity ratings on 180 seedlings
*** numbers followed by the same letter are not significantly different at p=0.05 (Duncan's Multiple Range Test)

Table 6: Mean Disease Severity Ratings on Faba Bean Cultivars and Mass Selected Populations (MSP) in a Growth Cabinet at 12 Temperature Regimes

Cv/MSP*	E1****	Cv/MSP	E2	Cv/MSP	E3
Pi	3.77** a	Aladin	3.92 a****	2N114	3.75 a
Aladin	3.22 a	Pi	3.73 a	Aladin	3.38 ab
2N134	2.89 bc	2N134	3.44 a	2N134	3.00 bc
2N19	2.65 c	I12	2.50 b	Pi	2.38 bcd
I12	2.55 c	2N114	2.45 b	2N94	2.00 bcd
2N37	2.38 c	2N37	2.45 b	2N19	1.61 cd
2N94	2.33 c	2N94	2.00 b	2N37	1.71 d
2N114	1.60 d	2N19	1.13 b	I12	1.13 d

Cv/MSP	E4	Cv/MSP	E5	Cv/MSP	E6
2N94	1.70 a	Aladin	3.00 a	Aladin	4.00 a
2N37	1.60 ab	Pi	1.10 b	Pi	4.00 a
2N19	1.40 ab	2N19	1.10 b	2N134	3.80 a
I12	1.10 abc	2N37	0.90 b	2N19	3.60 abc
Pi	0.80 abc	2N94	0.80 b	2N37	2.85 bc
Aladin	0.60 abc	2N114	0.30 b	2N114	2.80 bc
2N114	0.50 bc	I12	0.00 b	I12	2.25 c
2N134	0.20 c	2N134	0.00 b	2N94	1.15 d

Cv/MSP	E7	Cv/MSP	E8	Cv/MSP	E9
Aladin	2.40 a	2N114	2.15 a	Pi	3.71 a
Pi	2.00 ab	Pi	1.15 b	Aladin	3.70 a
2N94	1.40 bc	2N37	1.15 b	2N134	3.63 a
2N114	1.20 bcd	2N134	1.50 b	2N19	3.20 ab
2N19	0.85 cd	2N94	1.40 b	2N37	3.14 abc
2N37	0.45 cd	Aladin	1.40 b	2N114	2.78 bc
I12	0.44 cd	I12	1.10 bc	I12	2.56 cd
2N134	0.10 d	2N19	0.68 c	2N94	2.17 d

Cv/MSP	E10	Cv/MSP	E11	Cv/MSP	E12
Pi	3.25 a	2N37	4.00 a	Pi	3.23 a
2N114	1.60 b	Pi	4.00 a	I12	3.00 ab
2N134	1.38 bc	2N134	3.83 ab	2N19	2.25 bc
Aladin	1.25 bc	Aladin	3.75 abc	Aladin	2.10 c
2N19	0.92 cd	I12	3.56 bc	2N37	2.00 c
2N94	0.60 de	2N94	3.40 c	2N134	2.00 c
I12	0.50 de	2N114	3.00 d	2N114	1.67 c
2N94	0.00 e	2N19	2.60 e	2N94	0.57 d

*Cv = cultivars = Pi222128 and Aladin, MSP = numbered entries.
**mean DSR on 20 seedlings: scale 0=no infection to 4=max. infection
*** numbers followed by the same letter in the same same column are not significantly different at p=0.05 (Duncan's Multiple Range Test).
**** E1 to E12 = 12 temperature regimes with each regime divided into two phases varying in temperature and duration as outlined in Table 1.

A short period (7 days) at 12 C prior to exposure at 28 C for 21 days (E9) did not reduce DSR appreciably since the DSR in E9 were similar to those in E11 where temperature was kept constant at 28 C in both phases. Regimes E2 and E12 were identical in phase one (28 C for 7 days) but different in phase two (E2 at 12 and E12 at 10 C), and yet the DSR in E12 indicated all the entries except cultivar Pi222128 and MSP I12 were resistant. Growth of A. euteiches at such a low temperature of 10 C may be greatly reduced.

The two susceptible cultivars were readily separated from the MSP with resistance in three temperature regimes, E1, E2, and E7. These three regimes had in common a high temperature (25 or 28 C) in phase one lasting from 7 or 14 days followed by a temperature of 12 C for 14 or 21 days in phase two. In E7, Pi222128 and Aladin could be separated easily from the resistant MSP even though they had only a DSR of 2.0 and 2.4 respectively, and were considered resistant. Correlation analysis was conducted between the DSR from the field and the DSR from each of the 12 temperature regimes (Table 7). The highest correlation coefficients were associated with E1 (28 C for 14 days followed by 12 C for 14 days) and E2 (28 C for 7 days followed by 12 C for 21 days). Both E1 and E2 reflected the response of the resistant MSP and susceptible cultivars in the field as Pi222128 and Aladin were rated greater then 3.0 while the MSP with resistance were rated less than 2.5.

The DSR and the ranking order of the cultivars and the MSP were inconsistent over the six sampling dates in the greenhouse experiment (Table 8). When plants were rated at 22 days after planting, the DSR were low, and all cultivars and MSP were resistant. When DSR were made on 25, 42, 60, and 65 days after planting the susceptible cultivar, Aladin, was rated resistant (DSR<2.50). MSP I12 with resistance was rated susceptible at 50 days after planting.

Table 7: Correlation Analysis between Disease Severity Ratings on 2 Faba Bean Cultivars and 5 MSP Grown in Naturally Infested Soil in the Field and Inoculated with A. euteiches in the Growth Cabinet at the 12 Temperature Regimes

	E1***	E2	E3	E4
Field	0.8542**	0.8459*	0.1690	-0.3850

	E5	E6	E7	E8
Field	0.5234	0.7480*	0.6343	-0.1370

	E9	E10	E11	E12
Field	0.7620*	0.7370*	0.7485*	0.5330

* Significant at 0.05 level
** significant at 0.01 level
*** E1 to E12 = 12 temperature regimes with each regime divided into 2 phases varying in temperature and duration as outlined in Table 1.

Table 8: Mean Disease Severity Ratings on Faba Bean Cultivars and Mass Selected Populations (MSP) Inoculated with *A. euteiches* Zoospore Suspensions in the Greenhouse

Cv/MSP*	(22)**		Cv/MSP	(25)	
Aladin	2.10***	a****	Pi	3.40	a
Pi	2.00	a	2N134	2.63	b
I12	1.62	ab	I12	1.90	c
2N134	1.15	bc	2N114	1.87	cd
2N94	1.15	bc	2N94	1.60	cd
2N37	0.90	bcd	Aladin	1.42	cd
2N114	0.70	cd	2N37	1.09	d
2N19	0.15	d	2N19	1.08	d

Cv/MSP	(42)		Cv/MSP	(50)	
Pi	3.14	a	Pi	3.80	a
2N114	1.79	bc	I12	3.25	b
I12	1.60	bc	Aladin	3.00	b
Aladin	1.25	c	2N94	2.93	bc
2N134	0.67	d	2N19	2.50	c
2N37	0.44	de	2N134	2.00	d
2N94	0.25	de	2N37	1.75	de
2N19	0.10	e	2N114	1.30	e

Cv/MSP	(60)		Cv/MSP	(65)	
I12	3.63	a	Pi	4.00	a
Pi	3.78	a	Aladin	2.40	b
2N94	2.67	b	I12	2.40	b
2N114	2.64	b	2N114	2.38	b
2N37	2.30	bc	2N94	2.15	b
2N19	2.29	c	2N134	1.95	b
2N134	1.78	c	2N19	1.58	bc
Aladin	1.75	c	2N37	0.68	c

*Cv = cultivars = Pi222128 and Aladin, MSP = numbered entries.
** days after planting.
*** mean disease severity ratings on 20 seedlings; scale from 0 (no infection) to 4 (extensive blackening of the root system and the stem base.
**** numbers followed by same letter in the same column are not significantly different at p=0.05 (Duncan's Multiple Range Test).

DISCUSSION

The relationship between plant/root vigor (in terms of dry weight) and the disease reactions in the field of plants from susceptible cultivars and resistant MSP grown in soil infested and non-infested with A. euteiches, was investigated by comparing correlation coefficients between various measurements of plant total dry weight (TDW), root dry weight (RDW), relative root dry weight loss (RRL) and disease severity ratings (DSR). The correlation coefficient between TDW and DSR was significant and negative at 28 ($r=-0.7792$) and at 35 ($r=-0.7821$) days after planting. It was obvious that less infection on a plant would allow for better plant growth. On the other hand, a heavier or more vigorous plant might also be able to withstand infection better, and therefore might appear to be more resistant.

Loss in root weight due to root rot was clearly related to the disease reactions of the susceptible cultivars and the resistant MSP in the field (Table 4). The susceptible cultivars, Pi222128 and Aladin, both suffered the heaviest losses while the least losses were associated with the resistant MSP. However, the procedure could not separate the resistant MSP into more or less resistant classes. Furthermore, the measurement of RRL might not be practical when a large number of plants are involved.

The environment-shift technique was developed to screen for resistant pea lines to A. euteiches (Shehata et al., 1976). The concept was based on the idea that a heavily infected but resistant pea line would have a chance to recover and be identified in an environment favorable

to the host but not the pathogen. Similarly, the response of the susceptible faba bean cultivars and MSP with resistance was evaluated under an environment favoring host recovery but not pathogen development.

The initial determination of the duration of phase one favoring the pathogen at 28 C was based on the time required for the first plant of the cultivar Pi222128 to die. Temperature was then shifted to 12 C to constitute a second phase favoring host recovery but not pathogen development. Both E1 (28 C for 14 days followed by 12 C for 14 days) and E2 (28 C for 7 days followed by 12 C for 21 days) not only had the highest correlation coefficients with the DSR in the field, but also were able to reflect the disease reaction of the susceptible cultivars and the resistant MSP as well as to identify MSP with the highest level of resistance. It was not possible to separate the susceptible cultivars from the resistant MSP if the temperature was kept constant in both phases; all cultivars and MSP became highly susceptible at 28 C, while all but P1222128 were highly resistant at 12 C.

The susceptibility of resistant MSP 2N19 in the field in 1984 may be due to the fact that the original seed source was replaced by a more advanced mass selected population of 2N19 which was less vigorous.

The DSR and the ranking order of the susceptible cultivars and the resistant MSP varied considerably over the six sampling dates in the greenhouse experiment. DSR at any of the sampling dates could not reflect the ranking order in the field. Separation of the susceptible cultivars from the MSP was achieved 35 days after planting (Table 3) but however, both I12 and 2N134 were not separated from 2N37, 2N114 and 2N94

as observed in the field (Table 5). It seemed that 2 to 3 week old faba bean seedlings in general were more susceptible to A. euteiches. Subsequently seedlings that had not been killed were able to recover so that the symptoms of the disease were less severe.

The best procedure for evaluating faba bean cultivars and MSP for resistance to A. euteiches indoors would appear to be the temperature-shift technique whereby inoculated plants are grown at a temperature favoring pathogen development followed by a temperature favoring host recovery and growth.

Chapter III

RESEARCH II

RESEARCH II: Some Observations on Aphanomyces euteiches Root Rot Development and Host Response in Faba Beans

ABSTRACT

Three inoculation techniques with Aphanomyces euteiches were evaluated on two faba bean cultivars and six mass selected populations (MSP). In the first experiment, three seedlings of each cultivar and MSP were grown in test tubes containing sterilized water adjusted to one of the three levels: to the tap root tip, to the middle section of the tap root, and to the base of the epicotyl. A zoospore suspension (2mL) was added to each test tube. Zoospores were observed to swim near the water surface and roots were infected at the surface of each water level. All cultivars and MSPs were infected but the blackening of root tissues only progressed upward while tissues beneath the point of inoculation always remained white. There were no differences in the extent of tissue discoloration among the susceptible cultivars and the resistant MSP.

The ability of A. euteiches to infect the lower tap root of faba bean seedlings was evaluated by placing pressed peat pots with 10-14 day

old seedlings over 6 in. pots containing soil naturally infested with the pathogen. The tap root was exposed to the fungus as it grew into the infested soil. Examination of the entire root system after 3 weeks showed that roots of all Pi222128 plants were heavily infected while roots of 10 of the 12 plants from MSP 2N94 showed slight discoloration. This technique was effective in separating resistant MSP from susceptible cultivars but did not allow the separation of resistant MSP into more than one resistant class.

When leaves and stems were inoculated with A. euteiches, no infection was observed on any of the cut leaves, and the fungus could not penetrate stem tissues without wounding. Symptoms developed only on a few stems and four of the 12 susceptible Pi222128 plants were not infected.

INTRODUCTION

Seedling blight and root rot of faba bean (Vicia faba L.) in Manitoba were found to be caused by Aphanomyces euteiches Drechs., Rhizoctonia solani Kuehn and Fusarium avenaceum (Fr.) Sacc. (Lamari, 1982). Pathogenicity tests indicated that A. euteiches was the main pathogen at the campus farm of the University of Manitoba.

Root rot of faba beans is characterized by cortical necrosis of the hypocotyl and the root. The typical symptom on the shoot is slow acropetal yellowing of the older leaves. Extensive blackening of the stem tissues in highly susceptible cultivars will lead to rapid death of the seedlings. In contrast, the root systems of the mass selected populations (MSP) with resistance were slightly discolored and plants had normal green leaves and were not stunted (Lamari et al., 1984).

In a similar host-parasite system, Phytophthora root rot of soybeans, a rapid assay for the evaluation of systemic fungicides against the pathogen was developed by inoculation of the hypocotyl with a droplet of zoospore suspension of Phytophthora megasperma Drechs. (Lazarovits et al., 1980). Differences in the discoloration around the infection site were related to the effectiveness of the fungicides mixed with the plant growth medium. The discoloration symptom can be seen throughout the faba bean root system but the discoloration symptom on the stem is only limited at the stem base. The present research was designed to determine whether susceptibility and levels of resistance to A. euteiches in faba beans might be identified by the amount of discoloration on various sections of the root systems, on punctured stems, on cut leaf surfaces.

MATERIALS AND METHODS

1. Test Tube Inoculation

Seeds of two faba bean cultivars (Pi222128, Aladin) and six mass selected populations (MSPs) from accessions 2N19, 2N37, 2N94 2N114, 2N134, and I12 were surfaced sterilized in 2.5% sodium hypochlorite solution for five minutes. After rinsing thoroughly in distilled water, seeds were allowed to germinate in petri dishes filled with distilled water. A day later, three germinated seeds of each cultivar and MSP were held in place inside sterilized test tubes (one seed per test tube) with folded filter papers (Fig. 1).

Water in each test tube was adjusted to one of the three levels : to the tip of the tap root, to the middle of the tap root, and to the base of the epicotyl.

The bottom half of all test tubes was wrapped with aluminium foil to prevent light from influencing the root systems. All plants were inoculated when the tap roots reached 6 to 10 cm in length. Inoculum preparation and concentration were as described in Thesis Section 1 and inoculation was effected by pipetting 2 mL zoospore suspension into the water through a small plastic tube (Fig 1).

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Figure 1: Inoculation of faba bean seedlings in test tubes with a zoospore suspension (2 mL at 2.5 x 10^5 zoospores/mL) of A. euteiches pipetted through small plastic tubes

In a preliminary experiment, a young faba bean seedling was submerged in a zoospore suspension diluted with water and 24 hours later, zoospores could not be observed under the dissecting microscope. Presumably all zoospores had been attracted to the root surface. Thus, in subsequent experiments, all test tubes were filled with water 24 hours after inoculation and free swimming zoospores were assumed not to be present for infection on portions of the root system above the initial water level.

2. Infection of the Lower Tap Root

Seeds of the same cultivars and mass selected populations (MSP) used in the previous experiments were surface sterilized and planted in steamed soil:peat:sand (2:1:1, v/v/v) mix. Twelve seeds from each cultivar and MSP were grown seven to ten days in 8 cm pressed peat pots at which time the tap roots began to extrude about 1 cm through the bottom of the pots.

All peat pots were then placed on soil collected in 1982 from a field infested with A. euteiches at the the University of Manitoba campus farm. The soil was stored in a cold room (at 5 C) for about 12 months. The infested soil was than mixed with sand in a ratio of 2:1 (v/v) for ease of handling. Prior to conducting the tests, the presence of the pathogen (in the form of oospores) was confirmed by the infection of seedlings of the highly susceptible check cultivar Pi222128.

Three weeks later, the root system was well established within the naturally infested soil (Fig 2), and the number of infected plants with decayed root tissues from each cultivar and MSP were recorded.

3. Leaf Inoculation

An equal number of plants of each faba bean cultivar and MSP used in the previous experiment were planted in 8 cm pressed peat pots. Inoculum preparation and concentration were as described in Thesis Section 1. A zoospore suspension (2 mL) was added to a 50 mL beaker containing 30 mL distilled water.

The whole peat pot was enclosed within a small plastic bag in which a small hole was cut permitting exposure of the shoot. Half of the top leaflet of the seven to ten days old seedling was cut off. The entire seedling was inverted into the beaker containing the zoospore suspension for 12 hours. Two seedlings of each cultivar and each MSP were inverted into beakers with tap water to serve as controls. An equal number of plants of each cultivar and MSP with normal leaves was also inverted into beakers containing zoospore suspension. The peat pot was supported over the beaker by a piece of hard card-board (Fig. 3).

4. Stem Inoculation:

An equal number of plants of each cultivars and MSP were employed as in the previous experiment. All plants were sown in steamed soil:vermiculite:sand (2:1:1 v/v/v) mix, one plant per 13 cm clay pot. The production and concentration of zoospore suspension were as outlined in Thesis Section 1. A small hole was punctured at the base of the lowest leaf-node with a toothpick and a drop of the zoospore suspension (>0.5 mL) was deposited at the base of the lowest leaf-node over the wounded area and also at the base of the second lowest intact leaf-node.

A drop of distilled water was deposited at the base of the lowest punctured leaf-node of a similar plant to serve as control.

5. Assessment of Root Rot Disease in Faba Beans

Disease severity was rated on a scale of 0 to 4 where 0 = no infection and 4 = plant severly wilted or death occurred as outlined in Thesis Section 1. A host was considered resistant when the root system and cortical tissues were only slightly discolored and were not decayed. This is equivalent to a DSR less than 2.5 in Thesis Section 1. A susceptible host shows extensive blackening of the root system and much decay was observed at the tip of the lateral roots and at the stem base. This is equivalent to a DSR of 2.5 or greater.

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Figure 2: The placement of a pressed peat pot with a faba bean seedling over a pot containing soil naturally infested with A. euteiches

Figure 3: Leaf Inoculation: a faba bean seedling placed upside down over a beaker containing a zoospore suspension (2mL of 2.5×10^5 zoospores/mL) of A. euteiches

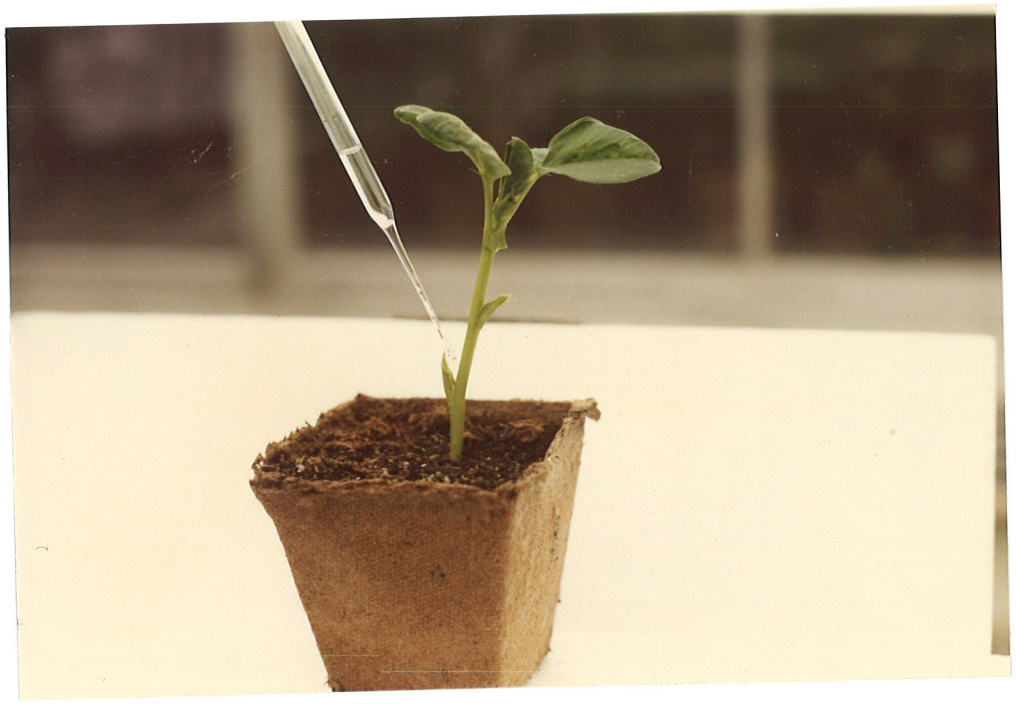

Figure 4: Stem inoculation at the lowest leaf-node of a faba bean seedling with a zoospore suspension (2mL of 2.5×10^5 zoospores/mL) of A. euteiches

RESULTS

1. Test Tube Inoculation

All faba bean seedlings were infected at the point of inoculation; i.e. at the surface of each of the three water levels. Tissue discoloration extended progressively upward from the point of inoculation in all three treatments. Root tissues beneath the point of inoculation remained white throughout the entire period of the experiment. Within seven to ten days of inoculation, most seedlings were dead. The discoloration on the tap roots could be observed within 4 to 7 days after inoculation and was extensive on all MSP. Therefore, separation between susceptible cultivars and resistant MSP was not possible.

2. Infection of the Lower Tap Root

All cultivars and MSP evaluated were infected, with symptoms ranging from light discoloration on the lower tap root to extensive blackening of the entire root system and decay of the cortical tissues progressing from the tip of the roots.

All plants of cultivars Pi222128 and Aladin with the exception of one plant from cultivar Aladin were found to be susceptible with decayed root tissues (Table 1). Ten of the 12 inoculated plants from MSP 2N94 and eight of the 12 plants from each of the MSP I12 and 2N114 were rated as resistant and showed only slight discoloration of the root system. One half of the plants of MSP 2N19, 2N39 and 2N134 were resistant and the other half susceptible. These MSP might be more heterogeneous than the first three.

Table 1: Classification of plants from faba bean cultivars and mass selected popultations (MSP) based on disease severity ratings (DSR) of the lower tap root

Cv/MSP*	Susceptible Plants*** (DSR>2.0)	Resistant Plants**** (DSR<2.5)
2N94**	2	10
I12	4	8
2N114	4	8
2N37	6	6
2N19	6	6
2N134	6	6
Aladin	11	1
Pi	12	0

* Cv = 2 susceptible cultivars, Pi222128 and Aladin, MSP = others
** 12 seedlings of each cultivar and MSP in peat pots with steamed soil were placed on naturally infested soil to allow further growth of the root systems into the infested soil.
*** susceptible = extensive discoloration of the entire root system with decay of cortical tissues starting from the tip of the lateral roots.
**** resistant = slight discoloration through out the entire root system but no decay of any root tissues.

3. Infection on Leaf and Stem

A. euteiches could not penetrate the stem without wounding. However, not all punctured and inoculated stems showed symptoms of infection. Blackening of the stem over 2 cm in length was observed in two or three inoculated plants from each of the MSP with resistance while no symptoms were observed in four of the 12 plants of susceptible cultivar Pi222128. The blackening spread in both directions from the point of inoculation (at the base of the lowest leaf-node). The length of the blackened stem tissue varied extensively and therefore, did not reflect the response of susceptible cultivars and partially resistant MSP in the field. A. euteiches failed to infect cut leaf tissue, and the slight discoloration observed at the cut edge on the inoculated leaflets appeared to be the same as that on leaflets treated with water only. This slight discoloration might therefore be related to the healing process rather than a symptom of infection.

DISCUSSION

Attempts were made to determine the relationship between levels of resistance to A. euteiches in the MSP and the extent of stem and root tissue discoloration after inoculating plants at the tip of the tap root, at various sections along the root system, on the stem surface and on a cut leaflet.

Infection of the lower tap root as it grew from non-infested soil to the naturally infested soil appeared to reflect the response of the MSP with resistance in the field. Susceptible cultivars could easily be distinguished from the resistant MSP as decay of the cortical tissues which is an important criterion for susceptibility, was always accompanied by extensive blackening of the entire root system. Generally, no decayed tissues were found in the MSP with resistance. This technique proved useful in separating MSP with resistance from susceptible cultivars. Further improvements on the technique might be made by recording the length of tissue discoloration along the tap root at intervals of 2 days. This might provide better separation of the MSP into different resistant classes.

Inoculation of various sections of the root system, using test tubes as inoculation reserviors, failed to reflect the field response of the MSP with resistance. Plant nutrients were not added to the water medium, and all seedlings were weak in appearance and were dead within a relatively short period of time. The technique might be improved by growing the seedlings in an aerated medium supplied with adequate plant

nutrients. The seedlings might then survive longer and the extent of disease development along the root systems of faba bean cultivars and MSP might be greater in the susceptible cultivars than in the resistant MSP.

The results of stem inoculation showed that A. euteiches could only infect injured stem tissues. Most of the faba bean cultivars and MSP remained healthy and even four of the 12 plants of susceptible cultivar Pi222128 did not develop symptoms. In the few infected MSP plants, blackening extended in both directions from the point of inoculation (at the base of the lowest leaf-node). The poor results may be due to the method of inoculation: a water droplet containing the zoospore suspension may dry too rapidly or the zoospores may not to be attracted to the stem tissues. Improvement on the inoculation technique can be made by using A. euteiches mycelium as inoculum or the inoculated seedlings placed under high relative humidity. The variability observed may be due to the fact that faba beans are partially cross-pollinated and that the plant materials evaluated are not homozygous. The light discoloration observed along the edge of the cut leaf did not confirm infection.

We can summarize the above observations as follows:

1. Infection could take place at any point along the root system.
2. Disease progressed only upward from the point of inoculation.
3. Inoculation through the tip of the tap root was shown to be effective in separating the most susceptible cultivar Pi222128 from the most resistant MSP 2N94.
4. Stem infection was not possible without wounding.

5. A. euteiches failed to develop in leaf tissues.

Chapter IV

GENERAL DISCUSSION

Susceptibilty and resistance of faba bean cultivars and MSP were not correlated with root dry weight. The DSR of faba bean cultivars and MSP obtained from the greenhouse experiments did not reflect the host response to infection in the field. This may be due to the fact that faba beans usually grow better in the field than in pots in the greenhouse, i.e., growth conditions after infection of the resistant MSP in the greenhouse was not as favorable as in the field.

The susceptible cultivars and the resistant MSP could not be separated when plants were grown under a constant high (28 C) or low (12 C) temperature. On the other hand, the temperature-shift technique was able to reflect the field response of the susceptible cultivars and the resistant MSP.

Oospores as the resting stage are usually formed when the fungus reaches its limit of vegetative growth and they can be observed under the microscope in the infected tissues of the mature faba bean plant. It seemed that A. euteiches was able to infect and develop efficiently at the seedling stage of both peas and faba beans. King and Cho (1962) showed that the highest number of oospores were produced in seven days old pea seedlings, while Lamari (1982) could only isolate the fungus in agar medium from infected faba bean seedlings but not from mature

plants, and he also show that seedlings older than 21 days could not be infected.

The temperature-shift technique developed in this study enhanced the initial infection of faba bean seedlings by A. euteiches but allowed the resistant host to recover and grow favorably at a later stage when the vegetative growth of the fungus was replaced by the formation of the oospores.

Infection of the lower tap root provided separation between susceptible cultivars and resistant MSP. However, improvement of the experimental procedures is needed so that roots can be examined without being exposed to the air which causes faba bean roots to discolor (Lamari, 1982). Inoculating various sections of the root system in test-tubes allows direct observation on the disease development and reduces the amount blackening in healthy tissues. Performance of the susceptible cultivars and the resistant MSP might even be expressed better if seedlings are inoculated in the test tubes at a room temperature and then placed at a low temperature (12 C) as in the temperature-shift technique.

Evaluation of the response of faba beans to infection by A. euteiches might be improved by changing the scale of DSR. The '0 to 4' scale used in this study failed to reflect the difference between a killed plant and a heavily infected plant which is not killed. A new scale from 0 to 10 would better reflect the performance of plants with varying levels of resistance from the heavily infected but not killed plants, from plants killed at seedling stage, and from plants killed at the mature stage.

The assumption here is that the less resistant plants would be killed at an earlier stage by the pathogen. In a scale of 0 to 10, an '8' would thus be equivalent to a '4' of the previous scale. A '9' would indicate the infected plant killed at the mature stage while a '10' would show infected plant killed at the seedling stage.

REFERENCES

Alconero-Pivaral, R. A. 1967. Chemical control of Aphanomyces root rot of pea and the relationship of Pythium species to the disease. PhD Thesis. The University of Wisconsin. 97 pp.

Ayers, W. A., and G. C. Papavizas. 1965. An exocellular pectolytic enzyme of Aphanomyces euteiches. Phytopathology 55:249-253.

Ayers, W. A., and G. C. Papvizas. and R. D. Lumsden. 1969. Factors affecting the pectolytic activity of Aphanomyces euteiches in vitro and in infected tissue. Phytopathology 59:786-791.

Bary, A. de. 1960. Einige neue Saprolengnieen. Jahrb. wiss. Bot. 2:169-192. In Scott, 1961.

Bernier, C. C. 1975. Disease of pulse crops and their control. In "Oilseed and Pulse Crops in Western Canada", ed. J. T. Harapiak. A Symposium: Western Co-operative Fertilizers Ltd., Calgary, Alta. 439-454.

Beute, M. K., and J. L. Lockwood. 1967. Pathogenic variability in Aphanomyces euteiches. Phytopathology 57:57-60.

Bhalla, H. S. 1968. Number of zoospores of Aphanomyces euteiches required for infection on peas. (abstr.) Phytopathology 58:1043.

Bond, D. A. 1979. English names of Vicia faba broadbean, fieldbean or faba bean? FABIS 1:15.

Bruehl, G. W. 1983. Nonspecific genetic resistance to soil-borne fungi. Phytopathology 73:948-951.

Burke, D. W., and J. E. Mitchell. 1978. Temperature and moisture effects on infection of pea seedlings by Aphanomyces euteiches in soil. (abstr.) Phytopathology 68:1045.

Burke, D. M., Mitchell, J. E., and D. J. Hagedorn. 1969. Selective conditions for infection of pea seedlings by Aphanomyces euteiches in soil. Phytopathology 59:1670-1674.

Carley, H. E. 1970. Detection of Aphanomyces euteiches races using a differential bean series. U. S. Dept. Agr. Plant Dis. Rptr. 54:943-945.

Carley, H. E. and T. H. King. 1968. Influence of nitrogen form on the incidence and severity of Aphanomyces root rot of peas. (Abstr.) Phytopathology 58:1046.

Candlish, E. and K. W. Clark. 1975. Prelimary assessment of several faba beans grown in Manitoba. Can. J. Pl. Sci. 55:89-93.

Cho, Y. S. and T. H. King. 1963. Factors affecting infection and oospore formation of Aphanomyces euteiches in excised root tips of Pisum sativum The Minn. Academy of Sci. Proc. 30:123-127.

Clarkson, J. D. S. 1978. Pathogenicity of Fusarium species associated with foot rots on peas and beans. Pl. Path. 27:110-117.

Cook, R. J. and R. I. Papendick. 1972. Influence of water potential of soils and plants on root diseases. Ann. Rev. Phytopath. 10:349-374.

Cunningham, J L. 1961. The relationship of zoospores of Aphanomyes euteiches to the host root. Ph.D. Thesis. University of Minnesota.

Cunningham, J. L. and D. J. Hagedorn. 1962a. Attraction of Aphanomyces euteiches zoospores to pea and other plant roots. Phytopathology 52:616-618.

Cunningham, J. L. and D. J. Hagedorn. 1962b Penetration and infection of pea roots by zoospores of Aphanomyces euteiches. Phytopathology 52:827-834.

Darby, W. J., Ghalioungui, P., and L. Griveth. 1977. Food: the Gift of Osiris. Academic Press. N.Y. 877pp.

Davis, D. W., Shehata, M. A., and H. L. Bissonette. 1976. Minnesota 108 pea breeding line. HortScience 11(4):434.

Eisa, N. A. and F. M. Barakat, F. M. 1978. Relative efficiency of fungicides in the control of damping-off and Stemphylium leaf spot of broadbean. Pl. Dis. Rptr. 62:114-118.

Evans, L. E., Seitzer, J. F., and W. Bushuk. 1972. Horsebeans - a protein crop for western Canada? Can. J. Pl. Sci. 52:657-659.

Freigoun, S. O. 1980. Effects of sowing dates and watering intervals on the incidence of wilt and root rot diseases in faba beans. FABIS 2:41.

Grau, C. R. and T. P. Reiling. 1977. Effect of Trifluralin and dinitramine on Aphanomyces root rot of peas. Phytopathology 67:273-276.

Haglund, W. A. 1960. Studies on the sulfur nutrition of Aphanomyces euteiches and its relationship to root rot of peas. Ph. D. Diss. Abstr. 20:962.

Hawtin G. C. and P. D. Hebblethewaite. 1982. Background and history of fababean production. In: The Faba Bean (Vicia faba L.) (P. D. Hebblethwaite, ed.) pp 3-22. Butterworths, London.

Hawtin, G. C. and M. Omar. 1981. Breeding faba beans for use as a green vegetable. FABIS 3:60-62.

Hawtin, G. C. and R. Stewart. 1979. The development, production and problems of faba beans in West Africa. FABIS 1:7-9.

Jacobsen, B. J. and H. J. Hopen. 1981. Influence of herbicides on Aphanomyces root rot of peas. Plant Disease 65:11-16.

Johnson, H. G. 1954. Investigations on the control of root rot of peas. Diss. Abstr. 14:1124.

Jones, F. R. and C. Drechsler. 1925. Root rot of peas in the United States caused by Aphanomyces euteiches. J. Agr. Res. 30:293-325.

King, T. H. and Y. S. Cho. 1962. Oospore formation of Aphanomyces euteiches in root tips of Pisum sativum as a method of evaluating resistance. U. S. Dept. Agr. Plant Dis. Rptr. 46:777-779.

King, T. H., Davis, D. W., Shehata, M. A., and F. L. Pfleger. 1981. Minnesota 494-A11 pea germplasm. HortScience 16:100.

Kotova, V. V. and N. A. Tsvetkova. 1980. Effectiveness of chemical control measures against Aphanomyces root rot of pea. Rev. of Pl. Path. 59:3471.

Kraft, J. M. 1980. Laboratory/glasshouse screening procedure for identifying resistance to soil-borne diseases of peas. In: Proceedings of the Consultants' Group Discussion on the Resistance to Soil-borne Diseases of Legumes. ICRISAT. Andhra Pradesh, India. 167 pp.

Lamari, L. 1982. Etiology of seedling blight and root rot of fababean in Mantitoba. M.Sc. Thesis. University of Manitoba.

Lamari, L., Rashid, R. Y., and C. C. Bernier. 1984. Evaluation of faba bean accessions for resistance to Aphanomyces root rot. (Abstr.) Can J. Pl. Path. 6:263.

Lazarovits, G ., Unwin, C. H., and W. B. Ward. 1980. Rapid assay for systemic fungicides against Phythophthora rot of soybeans. Plant Disease 64:163-165.

Lewis, J. A. 1973. Effect of mineral salts on Aphanomyces euteiches and Aphanomyces root rot of peas. Phytopathology 63:989-993.

Lewis, J. A. and G. C. Papavizas. 1971. Effect of sulfur-containing compounds and vapours from cabbage decompostion on A. euteiches. Phytopathology:61:208-214.

Llanos, C. M. and J. L. Lockwood. 1960. Factors affecting zoospore production by Aphanoymces euteiches. Phytopathology 50:826-830.

Lockwood, J. L. 1960a. Pea introductions with partial resistance to Aphanomyces root rot. Phytopathology 50:621-624.

Lockwood, J. L. 1960b. Lysis of mycelium of plant pathogenic fungi by natural soil. Phytopathology 50:787-789.

Lockwood J. L. and J. C. Ballard. 1960. Evaluation of pea introductions for resistance to Aphanomyces and Fusarium root rot. Mich. Agr. Expt. Sta. Quart. Bu. 42:704-713.

Marx, G. A., Schroeder, W. T., Provvidenti, R., and W. Mishanec. 1972. A genetic study of tolerance in peas to Aphanomyces euteiches. J. Am. Soc. Hort. Sci. 97:619-621.

McKeen, C. D. 1952. Aphanomyces cladogamus Drechs., a cause of damping-off in pepper and certain other vegetables. Can. J. Bot. 30:701-709.

Mitchell, J. E. and D. J. Hagedorn. 1969. Effect of sodium P-(Dimethylamino)Benzenediazosulfonate on activity of Aphanomyces euteiches in soil. Pl. Dis. Rptr. 53(9):697-701.

Mitchell, D. J. and J. E. Mitchell. 1973. Oxygen and carbon dioxide concentration effects on the growth and reproduction of Aphanomyces euteiches and certain other soil-borne plant pathogens. Phytopathology 63:1053-1059.

Morrison, R. H. 1972. Factors affecting the production of zoospores by Aphanomyces euteiches and their behavior towards roots of Pisum sativum Ph.D. Diss. Abstr. 1340B.

Papavizas, G. C. 1967. Comparison of treatments suggested for control of Aphanomyces root rot of peas. Pl. Dis. Rptr. 51(2):125-129.

Papvizas, G. C. and W. A. Ayers. 1974. Aphanomyces species and their root diseases in peas and sugar beets. U. S. D. A. Tech. Bull. 1485. 158 pp.

Papvizas, G. C. and J. A. Lewis. 1971. Effects of amendments and fungicides on Aphanomyces root rot of peas. Phytopathology 61:215-220.

Platford R. G. and C. C. Bernier. 1973. Diseases of faba beans in Manitoba Proc. Man. Agron. Conf. pp 92-93.

Pfender W. A. and D. J. Hagedorn. 1982. Apanomyces euteiches f. sp. phaseoli, a casual agent of bean root rot and hypocotyl rot. Phytopathology 72:306-310.

Rowlinson C. J. and J. Colhoun. 1970. Chemical treatment of cereal seed in relation to plant vigor and control of soil fungi. Ann. Appl. Biol. 65:459-472.

Royle. D. J. and C. J. Hickman. 1964. Analysis of factors governing in vitro accumulation of zoospores of Pythium aphanodermatum on roots. Can. J. Bot. 10:151-161.

Salt, G. A. 1980. Diseases of grain legumes. Rothamsted Report for 1979. Pt. 1. 175.

Salt, G. A. 1982. Factors affecting resistance to root rot and wilt diseases. In: Proceedings of the International Conference for Improvement of Fababeans. Cairo. March 1981. ICARDA. Aleppo. Syria.

Salt, G. A. 1983. Root diseases of Vicia faba L. In: The Faba Bean (Vicia faba L.). (P. D. Hebblethwaite, ed.) pp 150-268. Butterworths, London.

Salt, G. A. and Smalley, J. L. 1979. Root diseases of field beans (Vicia faba). Rothamsted Report for 1973. Pt. 1:141-142.

Scharen, A. L. 1960. Germination of oospores of Aphanomyces euteiches embedded in plant debris. Phytopathology 50:274-277.

Schultz,. H. 1950. Studies on the role of Pythium species as agents of Lupin foot-rot. Rev. Appl. Mycol. 30:418.

Scott, W. W. 1961. A monograph of the genus Aphanomyces Virginia Agr. Exp. Sta. Tech. Bull. 151.

Shehata. M .A., Davis, D. W., and H. L. Bissonnette. 1976. A new approach for breeding peas resistant to common root rot caused by Aphanomyces euteiches Drechs. J. Amer. Soc. Hort. Sci. 101:257-261.

Shehata, M. A., Davis, D. W., and F. L. Pfleger. 1983. Breeding for resistance to Aphanomyces euteiches root rot and Rhizoctonia solani stem rot in peas. J. Amer. Sco. Hort. Sci. 108(6):1080-1085.

Sherwood, R. T. 1958. Aphanomyces root rot of garden peas. Phd. Thesis. University of Wisconsin. 119pp.

Sherwood, R. T. and D. J. Hagedorn. 1961. Effect of oxygen tension on growth of A. euteiches Phytopathology 51:492:493.

Sherwood, R. T. and D. J. Hagedorn. 1962. Studies on the biology of Aphanomyces euteiches. Phytopathology 52:150-154.

Sideris, C. P. 1931. Pathological and histological studies on Pythiaceous root rots of various agricultural plants. Phytopath. Z. 3:137-161.

Slinkard, A. E. and J. Buchan. 1980. The potential for special crop production in Western Canada. In: Canadian Wheat Board, Prairie Production Symposium. October 29-31, Saskatoon. Canadian Wheat Board. Saskatoon.

Smith, P. G. and J. C. Walker. 1941. Certain environal and nutritional factors affecting Aphanomyces euteiches root rot of garden pea. J. of Agri. Res. 63(1):1-20.

Sneb, B., Humble, S. J., and J. L. Lockwood. 1977. Parasitism of oospores of P. megasperma and A. euteiches in soil by Oomycetes, Chytridiomycetes, Hyphomycetes, Actinomycetes, and bacteria. Phytopathology 67:622-628.

Sparrow F. K. 1960. Aquatic Phycomycetes. University of Michigan Press. pp438.

Sundheim, L. 1972. Physiologic specialization in Aphanomyces euteiches. Physiol. Pl. Path. 2:301-306.

Tannahill. R. 1973. Food in History. Stein and Day, New York. pp350.

Tao, Z. X. 1981. Faba bean production in China. Fabis 3:24-25.

Temp, M. and D. J. Hagedorn. 1964. Some effect of cropping on disease indices of Aphanomyces euteiches. (Abstr.) Phytopathology 54:910.

Temp, M. and D. J. Hagedorn. 1967. Influence of cropping practices on Aphanomyces root rot potential of Wisconsin pea fields. Phytopathology 57:667-669.

Walker J. C. and F. L. Musbach. 1939. Effects of moisture, fertility and fertilizer placement on root rot of canning peas in Wisconsin. J. Agr. Res. 59(8):579-590.

Watson, A. M. 1981. Faba bean Production in Egypt: A study of the economic and intrastructural context. ICARDA/IFAD Nile Valley Project, Consultancy Report. pp107.

Yang, C. Y. 1970. Enzyme-induced germination of Aphanomyces oospores. (Abstr.) Phytopathology 60:1320.

Yokosawa, R., and S. Kuninaga. 1977. Longevity and infectivity of Aphanomyces euteiches and Aphanomyces cochlioides zoospores in soil. Annals of the Phytopathological Society of Japan 43(5):501-507.

Zerlik, G. M. 1979. A late yellowing and foot rot of peas. In: Soil-borne Plant Pathogens. (B. Schippers and W. Gams, eds.) pp 337-342. Ap. Press. London.

APPENDIX 1. Mean root dry weight of uninoculated faba bean cultivars and mass selected populations (MSP).

Days After Planting					
28			35		
Cv/MSP*	Mean Wt. (gm)**		Cv/MSP	Mean Wt. (gm)	
Aladin	0.90	a***	Aladin	1.31	a
I12	0.85	a b	I12	1.21	a
2N19	0.82	a b	2N19	1.15	a
2N134	0.82	a b	2N94	1.12	a
2N37	0.77	a b	2N114	1.01	a
2N94	0.70	a b	2N37	0.97	a
2N114	0.69	a b	2N134	0.95	a
Pi222128	0.57	b	Pi222128	0.59	a

*Cv = 2 susceptible cultivars, MSP= 6 MSP with partial resistance.

**20 seedlings of each cultivar and MSP were used.

*** numbers followed by the same letter are not signifcantly different at p=0.05 (Duncan's Multiple Range Test).

APPENDIX 2. Mean dry weight of uninoculated faba bean cultivars and mass selected populations (MSP).

Days After Planting					
28			35		
Cv/MSP*	Mean Wt. (gm)**		Cv/MSP	Mean Wt. (gm)	
I12	1.47	a***	2N37	3.02	a
Aladin	1.44	a	2N94	2.75	ab
2N37	1.39	ab	2N114	2.51	ab
2N134	1.37	ab	2N134	2.33	bc
2N19	1.32	ab	2N19	2.23	bc
2N94	1.29	ab	Aladin	1.87	cd
2N114	1.20	ab	I12	1.83	cd
Pi222128	0.99	b	Pi222128	1.51	d

* Cv = 2 susceptible cultivars and MSP = 6 MSP with partial resistance.

** 20 seedlings of each cultivar and MSP were used.

*** numbers followed by the same letter are not significantly different at p=0.05 (Duncan's Multiple Range Test).

APPENDIX 3. Procedure followed for the production of zoospores by Aphanomyce euteiches (Lamari, 1982).

1: Inoculate 2 % water ager plate with mycelium of A. euteiches.

2: Flood the agar with maltose (3 %) peptone (1 %) broth.

3: Incubate at room temperature (22 +/- 2 C) for 4-5 days.

4: Remove broth and wash with tap water twice.

5: Cover the mycelium with tap water for 2 hours.

6: Replace tap water with distilled water and check after 6 hours.