BREEDING FOR RESISTANCE TO FUSARIUM ROOT ROT IN BEAN

(*PHASEOLUS VULGARIS* L.)

By

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ABSTRACT

BREEDING FOR RESISTANCE TO FUSARIUM ROOT ROT IN BEAN

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Genetic resistance to the root rot causing organism, *Fusarium solani* f.sp. *phaseoli*, has been documented in bean (*Phaseolus vulgaris*) but progress towards introgression of this resistance into the highly susceptible large-seeded market classes has been limited. Resistance to this soilborne pathogen is complex and strongly influenced by environmental factors. As an approach to reduce effects of environmental variation and aid in the identification of physiological resistance, a simple, rapid, and inexpensive perlite-based greenhouse screen was developed. The procedure allowed for the evaluation of lateral roots, correlated well with field ratings and permitted evaluation of large populations. Correlations between root rot scores of two genotypic groups measured in field and greenhouse experiments were as high as 0.99. This protocol was subsequently used to facilitate the genetic characterization of root rot resistance in bean using two recombinant inbred populations derived from resistant X susceptible crosses ('Montcalm'/FR266 and 'Seafarer'/N203). Heritability estimates calculated on an entry mean basis ranged from 0.48 to 0.71 for Montcalm/FR266 and from 0.09 to 0.66 for Seafarer/N203 based on field and greenhouse experiments. Using greenhouse data from MF, DNA from five resistant and five susceptible genotypes was pooled to create two contrasting bulks for identification of marker associations. Forty-six RAPD markers

identified by this method were significant in one-way analyses of variance using root rot data from greenhouse and field experiments. The amount of phenotypic variation explained by these markers ranged from 0.04 to 0.14. Those markers that were significantly associated with greenhouse ratings tended not to be associated with field ratings and vice versa. Mapmaker was used to determine linkage groups among 35 markers. Three of seven linkage blocks identified could be positioned on the integrated *P. vulgaris* linkage map. Based on these placements, several significantly associated markers mapped to genomic locations that corresponded to *P. vulgaris* pathogenesis-related proteins. Two RAPD markers, $P7_{700}$ and $G3_{2000}$ were identified to be consistently and significantly associated with greenhouse and field ratings, respectively, and could be utilized for marker assisted selection. Multiple regression analyses using these two markers explained from 7 to 13 % of the phenotypic variation for root rot.

To my friends and family.

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LIST OF ABBREVIATIONS

SW	100 seed weight
ANOVA	Analysis of variance
AVG	average
BCMV	Bean common mosaic virus
bp	base pair
C	Carbon
C:N	Carbon to Nitrogen ratio
CBB	Common bacterial blight
CFU	Colony forming units
cM	Centimorgan
CV	Coefficient of variation
DAP	Days after planting
DNA	Deoxyribosenucleic acid
DRK	Dark red kidney
DTF	Days to flower
f.sp.	Forma speciales
h^2	Heritability estimate
HR	Horizontal resistance
IF	Isles/FR266 population
LG	Linkage group
LOD	Likelihood of the odds ratio
LRK	Light red kidney
LSD	Least significant difference
MF	Montcalm/FR266 population
MFGH1	Montcalm/FR266 greenhouse evaluation 1
MFGH2	Montcalm/FR266 greenhouse evaluation 2
MFGH3	Montcalm/FR266 greenhouse evaluation 3
MI	Michigan
MN	Minnesota
N	Nitrogen
PCR	Polymerase chain reaction
PI	Presque Isle County, MI
PM	Perham County, MN
PR	Pathogenesis-related protein
PvPR1	*Phaseolus vulgaris* pathogenesis-related protein 1
PvPR2	*Phaseolus vulgaris* pathogenesis-related protein 2
QTL	Quantitative trait loci
r	Correlation coefficient
R^2, r^2	Coefficient of determination
RAPD	Randomly amplified polymorphic DNA
RIL	Recombinant inbred line
RRR1	Field root rot rating 1

RRR2	Field root rot rating 2
SN	Seafarer/N203 population
SNGH1	Seafarer/N203 greenhouse evaluation 1
SNGH2	Seafarer/N203 greenhouse evaluation 2
VR	Vertical resistance

LITERATURE REVIEW

RESEARCH JUSTIFICATION

Dry bean (*Phaseolus vulgaris* L.) is a major cash crop for growers in the mid and northern regions of Michigan with an annual farm gate value of $100-150 million and an additional $20 million in revenues for handling, processing and marketing (Christenson and Kelly, 1992). The strength of the industry lies in the diversity of market classes, production of both tablestock and seed crop beans and the ability to niche certain seed types to specific ecogeographic regions of the state. Over the last quarter century, northern Michigan has successfully raised dark red kidney beans for certified seed production (Copeland, 1995). Climate, isolation and management have all helped to maintain a high level of productivity. Seed from this region is planted commercially in central Wisconsin and Minnesota but problems with root rots in all three states are forcing farmers to limit their planting of dark red kidney bean cultivars from Michigan. The lack of resistance to the major root rot causing pathogen, *Fusarium solani* (Mart) Sacc. f.sp. *phaseoli* (Burk.) Snyd. & Hans. in the larger bean seed types has reduced productivity and limited the market potential of Michigan dark red kidney cultivars. Since chemical control methods are expensive and inconsistent, resistant cultivars offer growers the only effective opportunity to maintain and expand kidney bean production both in Michigan and elsewhere. Without adequate levels of resistance to this persistent soil pathogen, Michigan could, potentially, loose the kidney bean seed industry just as it lost the pea (*Pisum sativum* L.) industry to similar problems with root pathogens some fifty years ago.

Genetic resistance, identified in tropically adapted lines, needs to be characterized and incorporated into locally adapted kidney bean cultivars. Moreover, since disease ratings require destructive evaluation and are often confounded by climatic factors, indirect selection using linked markers would improve the efficiency of the breeding program. Although root rot is a special problem of kidney beans grown in northern Michigan, it reduces the yield potential of all bean types grown in Michigan and is particularly severe in wet and cool seasons. Understanding the inheritance of resistance and using indirect screening methods would permit the more rapid incorporation of resistance into a number of other commercial seed types such as navy beans. Without adequate resistance to root rot, the dark red kidney bean seed industry will continue to shrink, leaving growers in northern Michigan with few alternative crops to plant for revenue. The loss of a vital commodity, in which local growers have been effective global competitors for the last four decades, is a major concern.

DISEASE COMPLEX

Root rot in common bean is comprised of a complex of pathogens including *Fusarium solani* f.sp. *phaseoli, Rhizoctonia solani* Kuhn, *Pythium* spp., *Thielaviopsis basicola* (Berk. & Br.) Ferr. and *Aphanomyces euteiches* f.sp. *phaseoli* Drechs. *Fusarium oxysporum* Schlecht. is also a predominant component of the rhizosphere and is a serious problem in the inter mountain regions of the US, but in Midwestern production areas it is often considered to be non-pathogenic or of minor importance (Sippell and Hall, 1982b). Depending on environmental conditions and bean production region, any one of these pathogens in any combination can be of significant consequence (Reeleder and Hagedorn,

1981). Root rot of snap beans is particularly severe in the central sands of Wisconsin where continuous cropping of bean is common. Reports of the predominant root rot inciting fungus in this production area have changed over time. Yang and Hagedorn (1966) first classified *F.s.* f.sp. *phaseoli* as the major root rot pathogen in Wisconsin followed by *Pythium* spp. and *R. solani*. By 1975, Hoch et al. concluded that *Pythium* species were the predominant causal organism of root and hypocotyl rot of bean. *Pythium* species were most frequently isolated from bean hypocotyls and roots and disease symptoms on infected plants resembled those of *Pythium* infection. While *F.s.* f.sp. *phaseoli* and *R. solani* were also isolated, they were not considered major pathogens. Root rot in the central sands of Wisconsin is now considered to be predominantly caused by *Pythium* spp. and *A. euteiches* f.sp. *phaseoli* and breeding programs currently address developing resistance to these two pathogens (Rand et al., 1984). These results do not preclude *F.s.* f.sp. *phaseoli* as a major pathogen in Wisconsin in areas other than the central sands. Seed decay, pre-emergence damping-off and root rot caused by *P. ultimum* Trow. also substantially reduce yields of snap bean in New York State (Pieczarka and Abawi, 1978).

Pythium spp.

Pythium symptoms first occur 16 to 20 days after planting as water soaked lesions on hypocotyls that later become drier, tan to reddish-brown and sunken. In advanced cases hypocotyls appear papery and reddish brown and can resemble *F.s.* f.sp. *phaseoli* infection. Below-ground hypocotyl and fibrous roots can be completely destroyed in more severe infections. *Pythium* infection appears to be more severe at higher soil water

potentials than *F.s.* f.sp. *phaseoli* infection which is considered to be a dry root rot (Hoch et al., 1975). Stunting of seedlings caused by *Pythium* infection reduces total plant weight and decreases number of pods per plant which ultimately affects yield (Sippell and Hall, 1982b). In this same study, *F.s.* f.sp. *phaseoli* did not affect plant size but reduced seed weight per pod. Sippell and Hall (1982a) suggest that reductions in different yield components caused by these two pathogens reflect differing times of infection. While symptoms of both diseases can occur early in plant development, negative effects on yield are observed earlier for *P. ultimum* infection than *F.s.* f.sp. *phaseoli* infection. Stunting of plants caused by *P. ultimum* occurs before flowering which reduces total biomass and ultimately reduces number of pods per plant. In contrast, *F.s.* f.sp. *phaseoli* exerts negative effects on plant growth and development post-bloom resulting in reduced seed weight per pod.

Rhizoctonia solani

Another often cited member of the root rot complex is *R. solani. Rhizoctonia solani* is the causal organism of web blight (foliar disease) and contributes to root rot in bean. The fungus, by delaying emergence, ultimately delays maturity and reduces marketable yields (van Bruggen et al., 1986). Disease development occurs mainly under moderate to high moisture conditions and moderate temperatures. While root rot is most severe at 16 to 22° C for *F.s.* f.sp. *phaseoli* and between 14 to 21° C for *Pythium*, maximum infection by *R. solani* occurs between 21 and 27°C (Sippell and Hall, 1982a; van Brugen et al., 1986). *Rhizoctonia solani* and resulting root rot and web blight are major problems for bean growing regions of Latin America and Africa, but it is of minor

importance compared to *Pythium* and *F.s.* f.sp. *phaseoli* in the North American continent (Abawi and Pastor Corrales, 1990).

Thielaviopsis basicola and *Aphanomyces euteiches*

Other root pathogens of relative importance are *T. basicola* and *A. euteiches. Thielaviopsis basicola* is the causal organism of black root rot which has a wide host range including alfalfa, cotton, tobacco and sweet potato (Abawi and Pastor Corrales, 1990). Disease can be controlled in bean, however, using several fungicides as seed or soil treatments. High levels of resistance are also available and utilized in specific breeding programs. In contrast to *T. basicola* which can cause root rot on several hosts, *A. euteiches* consists of two formae speciales which are both pathogenic to bean, *A.e.* f.sp. *phaseoli* and *A.e.* f.sp. *pisi. Aphanomyces euteiches* f.sp. *phaseoli* is only pathogenic on bean whereas *A.e* f.sp. *pisi* infects both bean and pea. Symptoms of this root disease can resemble *Pythium* root rot infection including chlorosis, wilting, and premature defoliation. Recognition of *A. euteiches* as a serious root rot pathogen has occurred relatively recently and, as discussed above, is now believed to be the primary pathogen of bean production areas in the Wisconsin and Minnesota central sands (Rand et al., 1984).

FUSARIUM SOLANI F.SP.PHASEOLI

Some of the most devastating root pathogenic fungi include *Fusarium* spp. (Kommedahl and Windels, 1979). *Fusarium solani* f.sp. *phaseoli* is a major root rot pathogen of bean in Nebraska, Wyoming, Colorado, California, Washington, Ontario, North Dakota and Michigan (Steadman et al., 1975; Keenan et al., 1974; Smith and Houston, 1960; Burke and Silbernagel, 1965; Saettler and Anderson, 1978; Sippell and

Hall, 1982a; Rackham and Vaughn, 1959; Estevez de Jensen et al., 1998). Steadman et al. (1975) isolated *F.s.* f.sp. *phaseoli* from bean roots and hypoctols in 71% of all plants collected in western Nebraska, 90% of which were pathogenic on bean. Hall (1979) surveyed thirty-one bean fields in Ontario and identified *F.s.* f.sp. *phaseoli* in 74% of all fields sampled. Yield reductions caused by Fusarium root rot have been reported as high as 84% for pinto production in Utah, Wyoming, Colorado, and New Mexico. Likewise, Fusarium root rot is considered one of the most serious diseases of irrigated beans in the Pacific Northwest after white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) (Dryden and Van Alfen, 1984; Miller and Burke, 1986). Synergistic effects between *F.s.* f.sp. *phaseoli* and *Pythium* that increase disease severity have been demonstrated in controlled greenhouse studies (Piecarka and Abawi, 1978) but effects of *F.s.* f.sp. *phaseoli* and other root rot components are generally considered additive (Sippell and Hall, 1982a). The widespread nature and importance of *F.s.* f.sp. *phaseoli* as a predominant root rot pathogen in bean emphasizes the need for effective control against this disease which will ultimately be achieved through a combination of proper cultural management and through the development of resistant cultivars.

Symptomotology

Typical symptoms of Fusarium root rot occur 1 to 2 weeks after planting where parallel red-brown streaks appear on the hypocotyl and tap root. These lesions become more pronounced with time and, in severe cases, will converge to form larger lesions that encompass the entire underground stem and root system. The primary and lateral root tissue can die and hypocotyl tissue can deteriorate and become hollow at which stage

adventitious root growth proceeds from tissue above the site of infection. Above ground symptoms include premature defoliation, stunting, and chlorosis which leads to a reduction in number of pods and seed size (Abawi and Pastor Corrales, 1990).

Christou and Snyder (1962) present an excellent description of *F.s.* f.sp. *phaseoli* infection of bean and will be summarized as follows:

Penetration

Fusarium solani f.sp. *phaseoli* penetrates directly into healthy epidermis or proceeds through wound sites or hypocotyl stomata at soil surface level without the formation of an appresorium. Although *F. s.* f.sp. *phaseoli* is mainly an intercellular pathogen, it can invade dead or senescing cells intracellularly. Germinating spores in the vicinity of bean plant tissue possess 1 or 2 germ tubes per spore. Before penetration, germ tubes form a small thallus that attaches to the hypocotyl. Penetration through stomata proceeds directly through the stomatal opening often resulting in browning and collapse of the guard cells. Vigorous hyphal growth then proceeds in the stomatal chamber. Under optimum conditions hyphae in the stomatal chamber can re-emerge from the stomata to become sites of sporodochial formation at the soil level and sources of secondary infection. Hyphae from the thallus can also penetrate directly through the cuticle after which the fungus grows longitudinally following the long axis of epidermal cells. Hyphae will then travel through the middle lamella of epidermal cells and multiply profusely once they reach the first intercellular space of the cortical tissue. Infection proceeds intercellularly until cortical cells die or senesce.

Another source of hypocotyl or root infection occurs through wound sites. Wounding is the result of one to many injured cells that provide entrance for fungal hyphae. Once established in the epidermal middle lamella of the hypocotyl, the fungus advances into the cortical tissue as previously and subsequently described. Wound sites include the broken bases of trichomes or points of emergence of adventitious roots along the hypocotyl and often exhibit marked browning of the epidermal or cortical cells surrounding the wound. Root penetration is similar to that observed for the hypocotyl where the fungus will invade directly through the epidermis or through wound sites. Penetration of root hairs was not observed. Contrary to direct or stomatal penetration, wound penetration begins with intracellular invasion of the damaged and/or dying cells. Once established, the fungus proceeds intercellularly.

Cortical invasion

Hyphae grow rapidly through the intercellular spaces of the cortex in a parallel fashion often filling the entire intercellular space. Hyphae can also proceed laterally from the aforementioned longitudinal hyphae, invading the cortex inwardly. The perpendicular or oblique growing hyphae are very distinct exhibiting a characteristic digitate envelopment of the cortical cells. These hyphae can reorient themselves longitudinally to subsequently give rise to more inwardly-growing hyphae. Fungal invasion is stopped by the endodermis.

Longitudinally growing hyphae develop more rapidly than the inward, lateral hyphae resulting in the characteristic reddish brown streaks observed on the hypocotyl. These lesions will ultimately coalesce and can completely encircle the hypocotyl. The

reddish brown discoloration is the result of advanced infection and represents the accumulation of phenolic compounds from the host. The fungus will also invade cortical cells through rupture sites created by emerging laterals and can infect newly developed lateral roots by this route. Vascular infection by *F.s.* f.sp. *phaseoli* is believed to originate through this inlet. Infection was not observed in the pericycle but could be identified in the meristematic and procambial tissue of the root laterals which would be the source of infection observed in xylem and phloem vessels (Chatterjee, 1958).

Christou and Snyder (1962) report that the primary mode of penetration is through the stomata. Consequently, *F. solani* should be regarded as a hypocotyl pathogen as opposed to a root rot pathogen. The authors' conclusions are based on the finding that fewer infections are observed on the roots compared to the hypocotyl and root lesions are smaller. Burke and Barker (1966), however, showed that yield reductions resulted only when severe root damage occurred. The authors grew seedlings in both *F.s.* f.sp. *phaseoli* infested "islands" of soil surrounded by non-infested soil and vice versa. Yield was only reduced in the latter where lateral roots grew out of non-infested "islands" and into infested soil. In the case where the hypocotyl and taproot were in contact with infested soil and laterals were allowed to grow out of this contaminated island, no yield reductions were observed. If the root system was functional, a severely rotted hypocotyl could still sustain a productive plant. Further evidence in support of lateral importance is provided by the lack of effective root rot control by fungicides applied to hypocotyl.

After tissue death occurs and the fungus has invaded cortical and vascular tissues, chlamydospores are produced within the senescing or dead tissue. Additionally, sporodocia are produced from mycelium in the substomatal stromatic tissue and release

macroconidia that form more chlamydospores or become sources of new infection (Christou and Snyder, 1962).

The basic colony forming units (CFU) of *F.s.* f.sp. *phaseoli* is the chlamydospore which is predominantly produced from macroconidia. The canoe-shaped macroconidia of *F.s.* f.sp. *phaseoli* produce germ-tubes which form infection thalli or chlamydospores (Garrett, 1977). Chlamydospores are found in soil associated with plant tissue or humus (Nash and Snyder, 1962). Either chlamydospores or macroconidia can produce infection.

Environment

For foliar diseases, moisture is the determining factor in spore germination (Garrett, 1977). Soilborne pathogens, however, inhabit a more complicated environment and germination is influenced by other factors. Moisture in soil environments is usually not limiting. If spores of a soilborne pathogen germinated as a result of adequate moisture conditions, most would die of nutrient starvation. Thus, soilborne pathogens sense other factors and germinate under optimum conditions.

Germination

Chlamydospores of *F.s.* f.sp. *phaseoli* are uniformly distributed in the soil within the plow layer based on non-significant differences in spore counts obtained from soil core samples and are relatively immobile in the absence of the host (Nash and Snyder, 1962). Growth of *F.s.* f.sp. *phaseoli* in the presence of non-host species is negligible consisting mainly of a recycling of chlamydospores (Huber and Watson, 1970). While spore germination is observed under certain conditions in the absence of host and saprophytic growth of *F.s.* f.sp. *phaseoli* in contact with lettuce, maize, and tomato crops can result in

a doubling of the pathogen population, penetration and invasion of non-host does not occur (Garrett, 1977). It can be concluded, therefore, that a common set of environmental cues, not involved in host-pathogen recognition, stimulates spore germination. Mondel et al. (1996) concluded that there is no specific recognition of nutrient exudation of the host plant because spores germinated equally well in root extracts from bean, pea, cotton, barley and tomato. Pea, bean and cotton root extracts, however, resulted in larger chlamydospore formation suggesting a greater store of nutrients which could enhance germination capability. Spores formed on pea, bean and cotton extracts correlated with increases in virulence while greater numbers of chlamydospores were produced on oat and barley root extracts. The smallest chlamydospores were formed from *F.s.* f.sp. *phaseoli* grown in tomato extract. The major constituents of these plant exudates are carbon (C) and nitrogen (N) and most hypotheses attempt to explain stimulation of spore germination in terms of C and N and the resulting ratio (C:N).

Upon closer analysis of spore germination stimulated by host exudate, it was observed that glucose, alone, resulted in 16 to 20% germination while amino acids triggered from 40 to 50% germination. Glucose in combination with ammonium (NH_4^+) nitrogen or potassium nitrate also triggered from 40 to 50% germination whereas zero germination was observed when N was added alone (Cook and Snyder, 1965). These results indicate that both N and C are required for spore germination (Toussoun, 1970). The minimal germination rates observed in glucose amended soil could be explained by the presence of background N in soils in which the spores were tested. It appears, therefore, that N requirements for spore germination are minor (Griffin, 1964). Low N requirements could provide *F.s.* f.sp. *phaseoli* a competitive advantage over other soil microorganisms

under N-limiting conditions. Toussoun (1970) reports that as little as 10 to 20 ppm of N is required for spore germination whereas 100 to 1000 ppm of glucose is necessary to achieve germination. It can be inferred, accordingly, that with respect to spore germination, carbon is the limiting nutrient. However, high C soil amendments such as barley straw (83:1) can reduce spore germination and Fusarium root rot (Griffin, 1964; Maier, 1959; Huber et al., 1965; Maurer and Baker, 1965). High C amendments effectively immobilize all available soil N below levels required for spore germination. This observed reduction in root rot is rapidly diminished with the addition of N in the form of ammonium or nitrate (NO_3^-) (Griffin, 1964). Griffin (1964) demonstrated that with the addition of as little as 5 ppm NH_4NO_3 the suppressive effect of barley straw could be overcome.

Stimulation and inhibition of spore germination are also strongly influenced by the microbial environment and its impact on nutrient availability. Cook and Snyder (1965) observed, in their studies on spore germination, that asparagine, added to a sandy loam soil, induced 30% germination of chlamydospores but within a short period the germ tubes and spores were disrupted by autolysis believed to be initiated by carbon starvation. Less than 4% of germinated chlamydospores survived under this treatment. In contrast, glucose amendments in the presence of background N resulted in 15% germination with no lysis. These results provide further evidence in support of the influence of carbon availability on spore germination. The following theory is presented to achieve a broader perspective. In soil, very high C:N (> 30:1) will suppress spore germination because the presence of large amounts of C effectively immobilizes any available N required to support germination. When the C:N decreases to a more moderate level (25:1), enough N is present to initiate

germination. *Fusarium solani* f.sp. *phaseoli*, however, requires much less N for germination than many of the other, more competitive microorganisms in the soil (Lindsey, 1965; Griffin, 1964). Thus, while *F.s.* f.sp. *phaseoli* is germinating under a relatively minimal amount of N, the available C is not being consumed by other soil microorganisms that are active under higher amounts of N. Saprophytic growth of *F.s.* f.sp. *phaseoli* continues or, in the presence of host tissue, infection can ensue. At lower C:N (8:1), enough N is available to support active growth of more competitive soil microorganisms which subsequently consume available carbon, depleting this nutrient and enhancing autolysis of *F.s.* f.sp. *phaseoli* spores. The C:N content of soil appears to effect spore germination inasmuch as it influences soil microbiota and that *F.s.* f.sp. *phaseoli* persistence is a reflection of this process.

While the nutrient stimulation of spore germination has been thoroughly addressed, recent research by Ruan et al. (1995) has identified a second stimulation mechanism of spore germination. The presence of the flavanone, naringenin, and the flavone, apigenin, have been identified in the rhizosphere of legumes and have been shown to induce *nod* gene expression of many *Rhizobia* species. *nod* gene expression is involved in the initiation of the symbiotic plant-bacteria interactions required for nodulation and N_2-fixation. *Fusarium solani* f.sp. *phaseoli* spore germination appears to be stimulated by the isoflavone, genistein, and flavanone, naringenin, which are both inducers of *nod* genes in bean. Beans appear to exude adequate concentrations of these secondary metabolites to significantly enhance spore germination. The pterocarpan phytoalexins, medicarpin and maackiain from chickpea (*Cicer arietinum* L.) also stimulated spore germination of *F.s.* f.sp. *phaseoli*. Different formae speciales of *F. solani* exhibit different stimulatory

responses to different sets of flavonoids suggesting a mechanism for host recognition. Spore germination of the pea pathogen *F.s.* f.sp. *pisi* (Jones) Snyd & Hans was stimulated by pisatin, medicarpin, and maackiain; phytoalexins for which *F.s.* f.sp. *pisi* possesses a corresponding degradation enzyme. The bean phytoalexin, phaseollin, to which *F.s.* f.sp. *pisi* is sensitive did not stimulate spore germination of this fungus. These pathogens may have developed an ability to not only tolerate these toxins but to use them to their advantage for host recognition.

Penetration

Although spore germination is the initial step necessary for successful infection and disease development, the degree of germination above a certain threshold has little influence on the severity of Fusarium root rot in bean. After examining chlamydospore behavior in soil, Baker and Nash (1965) concluded that spore lysis does not significantly reduce root rot symptoms in bean. The deciding factor contributing to both disease incidence and severity is the ability of the fungus to penetrate and colonize host tissue (Toussoun, 1970). It appears, moreover, that the optimal nutrient requirements for fungal penetration are opposite those that enhance spore germination. Many researchers agree that abundant soil nitrogen enhances root rot severity especially when present as NH_4^+ (Weinke, 1962; Huber and Watson, 1970; Huber et al., 1965; Snyder et al., 1959; Maurer and Baker, 1964; Griffin, 1964; Toussoun and Patrick, 1963; Toussoun, 1970). Weinke (1962) observed that ammonium N amendments increased root rot severity but had little effect on the size of the pathogen population. The pathogen appears to become more aggressive in the presence of high levels of exogenous N as evidenced by an increase in

the number and size of hypocotyl lesions and an increase in thallus development. Using a double pot experiment where roots of individual bean plants were grown in both infested and non-infested soil, applications of N to the roots in non-infested soil did not enhance root rot severity in roots exposed to inoculum. The addition of N, however, to infested soils resulted in a marked increase in root rot severity suggesting that exposure to soil N, not plant N, is the source of the more aggressive pathogen behavior. Toussoun (1970) reported that maximum spore germination was achieved in soil solutions of glucose but rapid penetration of host tissue required an abundant source of N. In another study, phytotoxic compounds released through the decomposition of crop residue were shown to increase root rot severity (Toussoun and Patrick, 1963). Ether and aqueous extracts from several crop species were applied to hypocotyls of bean seedlings which were subsequently inoculated with *F.s.* f.sp. *phaseoli*. The application of these phytotoxic compounds elicited the release of nitrogenous compounds probably through the degradation of plant tissue and this source of N may have stimulated pathogenicity.

The effect of N on root rot severity is further complicated by the observation that different forms of N have different effects. It was first observed that N amendments could nullify the beneficial effects of carbonaceous residues and that this was particularly apparent when using ammonium inputs (Huber et al., 1965). Maurer and Baker (1965) demonstrated that although the addition of nutrient amendments to soil in controlled experiments reduced root rot symptoms, disease severity was greatest when cellulose and $(NH_4)_2SO_4$ was added as opposed to cellulose and KNO_3. The process of biological oxidation of NH_4^+ to nitrate NO_3^- is called nitrification and occurs readily in the soil. Without NH_4^+ fertilization, soil N is either tied up in microbial metabolism or present as

NO_3^- (Huber et al., 1965). Both NH_4^+ and NO_3^- are biologically available to plants but it appears that the presence of abundant NH_4^+ increases the aggressiveness of *F.s.* f.sp. *phaseoli* on bean. Although the form of nitrogen present in the soil appears to play a role in pathogen aggressiveness, this observation could be a secondary result of acidic soil conditions. When NH_4^+ is not limiting, nitrification is dependent on pH and tends to increase under less acidic conditions (Paavolainen and Smolander, 1998). Therefore acidic soil conditions tend to favor the presence of NH_4^+. Furthermore, a decrease in *F.s.* f.sp. *phaseoli* virulence has been observed with increasing soil pH (Mondal and Hyakumachi, 1998). Oyarzun et al., (1998) also reported that variables which reflect acidic soil conditions tended to increase the soil receptivity of *F.s.* f.sp. *pisi.* Enhancing the nitrification capacity of the soil by increasing soil pH or by other means poses to be an effective control against Fusarium root rot by converting the root rot enhancing form of N, NH_4^+, to the relatively less active form of NO_3^- without eliminating the beneficial effects of ammonium fertilizer on bean growth. Alternatively, in areas where Fusarium root rot is a problem, it may behoove the grower to seek alternative sources of N. Crop residues that inhibit nitrification have been shown to increase Fusarium root rot severity when NH_4^+ fertilizer is also applied. Likewise, the addition of N-serve, a product that inhibits nitrification, with NH_4^+ amendments was also demonstrated to increase disease severity (Maurer and Baker, 1965; Huber et al., 1965). Clearly, there is compelling evidence supporting a connection between Fusarium root rot severity, nitrification and the abundance of NH_4^+. It has been suggested that C:N ratios of various crop residues may influence disease severity inasmuch as they influence nitrification.

The enhanced aggressiveness of *F.s.* f.sp. *phaseoli* in the presence of abundant NH_4^+ is believed to be due to changes in soil microorganism activity as evidenced by the lack of observable differences in pathogenicity when different N sources were used in experiments conducted under sterile laboratory conditions (Huber et al., 1965; Toussoun, 1970). The soil microorganism in question is believed to be an unclassified bacteria that is often found in intimate contact with *F.s.* f.sp. *phaseoli* spores (Huber et al., 1965). This bacterium was identified in roughly 12% of the soil samples assayed and appeared to induce fungal cell necrosis. In laboratory culture, the presence of the bacteria associated with spores was observed to prevent pathogenicity without affecting fungal population size. When the bacteria was absent, pathogenicity was restored (Huber and Watson, 1970). In the absence of *F.s.* f.sp. *phaseoli,* this particular bacteria utilized only ammonium N. In contact with the fungus, however the bacteria was capable of using nitrate. This finding may suggest that the increase in pathogenicity observed in the presence of NH_4^+ may be due, in part, to the lack of association between the fungus and bacteria. Alternatively, the bacteria may only be associated with the fungus when pathogenicity is reduced and that a diminishment in pathogenicity reflects the predominant form of available N in the soil.

Pathogenicity of *F.s.* f.sp. *phaseoli* and disease severity is further determined at the host level and involves tolerance to plant defense mechanisms and host resistance. *De novo* phytoalexin synthesis is a frequently cited defense mechanism of bean. *Fusarium solani* f.sp. *phaseoli* can metabolize many bean phytoalexins including kievitone, phaseollin, phaseollidin and phaseollinisoflavan and this detoxification is believed to be related to the pathogenicity ability of *F.s.* f.sp. *phaseoli* on bean (Li et al., 1995; Choi et

al., 1987). Keivitone detoxification by the hydratase enzyme, keivitone hydratase, has been correlated with *F.s.* f.sp. *phaseoli* virulence on bean (Li et al., 1995). Isolates pathogenic to bean always possess keivitone hydratase activity. The converse however is not true. Many isolates of *F. solani* not pathogenic or classified in a different forma speciales also possess keivitone hydratase activity. Cloning of the *khs* gene and transformation into non-pathogenic fungus resulted in the ability of the non-pathogenic fungi to hydrate keivitone hydratase but did not convert the fungus to a pathogen of bean. Additionally, keivitone hydratase enzyme activity was found to be independent and separate from phaseollin hydratase activity. These results suggest that although keivitone hydratase is required for *F.s.* f.sp. *phaseoli* virulence in bean, it is not sufficient to establish pathogenicity (Choi et al., 1987). Furthermore, Morris and Smith (1978) reported that although keivitone was strongly induced by biotic stresses caused by both *R. solani* and *Pythium* spp. (Morris and Smith, 1978; Li et al., 1995), a corresponding level of induction was not observed for *F.s.* f.sp. *phaseoli*. Other phytoalexins like phaseollin, however, did accumulate when beans were exposed to *F.s.* f.sp. *phaseoli*. These results could not be explained by detoxification of keivitone but might reflect either the inability of the fungus to induce keivitone biosynthesis or the ability of the fungus to repress keivitone biosynthesis. It would be interesting to observe whether keivitone enhanced spore germination of *F.s.* f.sp. *phaseoli* and to determine if keivitone was, in any way, involved in host-pathogen recognition.

In a related study, Mohr et al. (1998) observed a rapid induction of other defense-related enzymes after bean roots were inoculated with *F.s.* f.sp. *phaseoli*. Bean chitinase, β-1,3-glucanase and phenylalanine ammonia-lyase activity increased four-fold in response to *F.s.* f.sp. *phaseoli* infection. These results were further confirmed at the transcript level.

In contrast, no corresponding accumulation of enzyme or product was isolated from bean roots inoculated with *F.s.* f.sp. *pisi* nor with the mychorrizal fungus *Glomus mosseae*. Inoculation of pea pods with *F.s.* f.sp. *phaseoli* has been demonstrated to induce class I chitinase activity, however, results from pod infection may not acurately reflect root infection by *F.s.* f.sp. *phaseoli (*Mauch et al., 1984). Apparently, defense-related genes including those involved in phytoalexin biosythenthesis are induced as a result of the compatible interaction between bean and *F.s.* f.sp. *phaseoli. Glomus mosseae* does not elicit nor does it suppress the host defense response suggesting that its interaction with the host is quite different from that of the pathogen. Clearly, bean roots recognize *F.s.* f.sp. *phaseoli* and mount a concerted defense response against the fungus which is not similarly observed in the presence of non-pathogenic *F.s.* f.sp. *pisi. Fusarium solani* f.sp. *phaseoli* possesses the capacity, in some form, to tolerate these antifungal products. Lange et al. (1996) observed that class I and class IV chitinases are proteolytically cleaved during infection of bean roots by *F.s.* f.sp. *phaseoli.* Detoxification of several plant defense products appears to be an effective strategy employed by the fungus to circumvent host resistance but is probably not the only factor involved in pathogenicity.

Inoculum Density

Fusarium root rot severity may be influenced by initial *F.s.* f.sp. *phaseoli* population density but, as has been implied in previous sections, is certainly not the deciding factor in determining disease severity. Hall (1996) provides an excellent review of *F.s.* f.sp. *phaseoli* population dynamics and its effect on root rot in bean and concludes that practices which reduce *F.s.* f.sp. *phaseoli* population densities should be a component

of any integrated effort towards reduced root rot. Population densities of *F.s.* f.sp. *phaseoli* range from 0 to 5,500 colony forming units (CFU) g^{-1} of soil but in controlled experiments only 5 CFU g^{-1} of soil were required to infect 80% of plants. Field studies also confirm that low population densities can result in moderate root rot infection. Twenty to 40% of hypocotyl rot was achieved from population densities of 26 CFU g^{-1} (Dryden and Van Alfen, 1984). Sippel and Hall (1982a) reported only an 8-fold increase in root rot severity with a 1,000 fold increase in population density. Furthermore, reports of high infection rates at low inoculum densities suggest that fungal population reductions may not be an effective means of control (Hall, 1996). Conversely, reports of reduced root rot in the presence of large pathogen populations support the conclusion that population density is not an important factor in determining Fusarium root rot severity (Huber et al., 1965).

Abawi and Cobb (1984), on the other hand, demonstrated significant correlations between inoculum density and root weight ($r = -0.54$) and hypocotyl ($r = 0.67$) and root disease severity ($r = 0.65$) but not with yield reductions in small field plots. In contrast, Maloy and Burkholder (1959) found no significant relationship between *F.s.* f.sp. *phaseoli* density and disease severity although a trend towards this hypothesis was observed. *Fusarium solani.* f.sp. *phaseoli* is considered a minor pathogen when plants are grown under optimal conditions and it is only when environmental conditions conducive to disease occur that Fusarium root rot significantly reduces yield (Burke and Miller, 1983). It was not surprising, therefore, that *F.s.* f.sp. *phaseoli* populations as high as 4,000 CFU g^{-1} did not result in significantly reduced yields under non-stress environments (Abawi and Cobb, 1984). Studies, however, assessing *F.s.* f.sp. *phaseoli* population densities as a

factor of hectare-years in Ontario concluded that the average density per county was positively correlated ($r^2 = 0.98$) with the intensity of bean production but the author did not address the effect of population densities on root rot severity (Hall, 1996). In crop rotation studies on fungal population dynamics, Nash and Snyder (1962) reported a decline in pathogen population from 1,497 CFU g^{-1} to 373 CFU g^{-1} in the same year. Furthermore, population densities fluctuated from 310 to 1,165 CFU g^{-1} measured in another field monocropped to bean (Hall and Phillips, 1992). Variations in population density suggest that conclusions based on fungal population densities can serve to identify infested fields and indicate disease potential but will not be an effective predictor of root rot severity nor will management practices directed towards decreasing pathogen populations necessarily reduce disease severity (McFadden et al., 1989).

Seed treatments and other chemical controls to reduce soil inoculum or prevent disease have proven ineffective against Fusarium root rot (Silbernagel, 1990). Fungicides like Vorlex and methyl bromide have been effective as fumigants to control root rot severity but this method provides only temporary mitigation, is environmentally unsound and not cost-effective (Abawi and Edds, 1981). Soil fumigation can disrupt the microbial balance of the soil eliminating beneficial microorganisms as well as pathogens. Additionally, increased restrictions on pesticide use will preclude future fungicide fumigation as an effective control of root rot in bean. Chemical seed treatments have been effective for the control of damping-off caused by *R. solani* and *Pythium* but are ineffective as a control of Fusarium root rot (Miller et al., 1979). A recent report by Vogeli-Lange et al. (1995) demonstrated a developmental control of *F.s.* f.sp. *phaseoli* root rot susceptibility in bean. The authors suggested that emerging radicles lack a root

surface component required for the attachment of *F.s.* f.sp. *phaseoli* spores thereby excluding infection. Seedlings infected with the pathogen at stage four compared to stage ten had significantly fewer spores attached to the root as observed by low-temperature scanning electron microscopy. These results were paralleled with the pea/*F.s.* f.sp. *pisi* host/pathogen system and a correlation between spore adhesion and fungal virulence has also been demonstrated in the cucurbit pathogen *F.s.* f.sp. *cucurbitae.* The increasing susceptibility of bean with age to Fusarium spore adhesion may explain why seed treatments are ineffective.

Organic soil amendments

Crop rotation is one management practice that was believed to influence root rot severity by reducing pathogen populations. *Fusarium solani* f.sp. *phaseoli* fungal populations increase with successive plantings of bean and can decrease in certain non-host crops. After fifteen years of monocropping to bean, *F.s.* f.sp. *phaseoli* was as high as 560 CFU g-1 (Hall and Phillips, 1992). In crop rotation studies using repeated bean, corn-soybean-corn, wheat-soybean-wheat, repeated red clover and fallow, Hall (1996) demonstrated that population densities in fields under non-bean rotations declined to 60 to 70% of the repeated bean sequence but could not be eliminated entirely. *Fusarium solani* f.sp. *phaseoli* was not found in non-cultivated soils nor in soils never cropped to bean but can be introduced to new fields from seed dust or infested debris or through waterborne or airborne soil (Hall, 1996). Population density will increase with active growth of bean crops but may decrease in the absence of bean. However, a reduction in pathogen population does not necessarily translate to a reduction in root rot severity (Huber et al.,

1965; Maloy and Burkholder, 1959; Lindsey, 1965; Huber and Watson, 1970; Oyarzun et al., 1998). This does not suggest, however, that a reduction in root rot due to crop rotation does not occur but that the observed decrease in severity may be due to factors other than a decline in pathogen population.

Organic soil amendments have the potential of influencing disease in several ways. Crop residues alter the relative C and N content as well as the form of N available in the soil. The activity of soil microorganisms also fluctuates depending on many factors especially nutrient availability and can be enhanced or diminished by different crop residue. As has been discussed previously, C, N and microbiota play an important role in *F.s.* f.sp. *phaseoli* growth, development and pathogenicity. Beyond impact on fungal growth and development, crop residue can influence the health and vigor of the host plant through the modification of soil structure, release of phytotoxins, and nutrient availability. The combination of all of these factors ultimately influences the incidence and severity of disease.

Several studies have been conducted to determine the impact of specific crop residues on Fusarium root rot in bean. Several authors have concluded that disease severity increases with decreasing C:N (Baker, 1970; Griffin, 1964; Maurer and Baker, 1965; Snyder et al., 1959; Hall, 1996). In one study, Fusarium root rot was reduced with the addition of barley straw (C:N 83:1), wheat straw (80:1), corn stover (60:1) and pine shavings (400:1), and increased by soybean meal and alfalfa (7:1 and 21:1, respectively; Snyder et al., 1959). When green barley straw was used, or when ammonium nitrate was added with mature barley straw, root rot severity on bean hypocotyls increased. In a similar study, Maier (1959) reported that sorghum, soybean, corn, and barley reduced root

rot severity; corn and cotton had no effect; and lettuce, alfalfa and tomato increased disease. Green barley, in this case, had no effect. The beneficial effect of residues with high C:N was nullified, however, with the addition of ammonium nitrate as was also found in other studies (Griffin, 1964; Maurer and Baker, 1965).

Observations that the beneficial effects of barley are nullified with the addition of as little as 5 ppm ammonium nitrate, that the form of N (NH_4^+ versus NO_3^-) influences root rot severity and that reductions in disease can be achieved with residues of varying C:N, suggest that an inverse relationship between C:N and disease severity is not an adequate explanation (Huber et al., 1965). A more comprehensive hypothesis proposed by Huber et al. (1965) and Huber and Watson (1970) considers the effect of specific crop residues on nitrification. Alfalfa, soybean, pea and corn residue, when added to plates containing Stephenson's medium at a 2 ton $acre^{-1}$ equivalent, were demonstrated to enhance nitrification while wheat, oats, and cellulose had no effect, and barley and glucose inhibited nitrification. These results seem to coincide with previous field reports of crop residue influence on Fusarium root rot. The dichotomy with barley can then be explained by the capacity of barley to affect nitrification. In the absence of ammonium fertilizer, barley effectively immobilizes all available N thereby preventing infection. In contrast, when NH_4^+ is present, barley inhibits biological oxidation and NH_4^+ remains available to exert its effect on pathogen aggressiveness. Further evidence in support of this hypothesis is demonstrated by the observation that N-serve added to non-amended soils significantly increases root rot severity (Maurer and Baker, 1965). Although it is not always the case, as is seen with barley, there is a general tendency for residues with high C:N to increase nitrification. It can be concluded, therefore, that C:N is an important component

influencing disease severity inasmuch as it influences nitrification (Huber and Watson, 1970). Considering the above conclusions, soil amendments which enhance nitrification may be an effective means in controlling Fusarium root rot of bean.

PHASEOLUS VULGARIS

Environment

Environmental factors which impact the host plant also contribute to disease occurrence and severity. Root rot caused by *F.s.* f.sp. *phaseoli* is generally not economically significant unless compounded by environmental stresses that restrict root growth (Burke and Miller, 1983; Allmaras et al., 1988). Root growth can be adversely affected by many environmental conditions including low soil temperatures, soil compaction, low soil fertility, drought, flooding resulting in anoxia, and plant competition. Low soil temperatures that do not favor healthy and rapid bean root growth aggravate Fusarium root rot incidence whereas warmer soil temperatures can reduce this effect (Burke, 1980). Planting later in the season when soil temperatures are higher can help reduce Fusarium root rot.

Soil Compaction and Anoxia

The effects of sub-optimal temperatures on disease are compounded by other root restricting environmental factors such as soil compaction and oxygen stress (Smucker and Erickson, 1987; Miller and Burke, 1985b; Allmaras et al., 1988). Soil compaction due to subsurface tillage pans and tillage methods to incorporate herbicides is a major contributor to root-limiting and disease predisposing environments. Soil compaction restricts root

growth to the plow layer where roots are in contact with 99% of soil inoculum. Furthermore, diseased roots infected with *F.s.* f.sp. *phaseoli* are less capable of penetrating compact soil (Dryden and Van Alfen, 1984; Miller and Burke, 1985b). In greenhouse experiments, Miller and Burke (1985b) demonstrated that plants can compensate for subsurface tillage pans by producing more root mass above the compaction layer but in Fusarium infested soils, this compensating ability is substantially reduced for root rot susceptible cultivars. In field studies, deep tillage significantly reduced root rot severity and increased plant dry weight and yield in Fusarium infested soils (Tan and Tu, 1995). An increase in dry weight and yield was also observed in non-infested soil but increases were more dramatic in the infested soils.

In addition to physical obstruction, soil compaction also negatively restricts root growth and development by reducing soil aeration resulting in anoxia. This type of stress can also be achieved by excessive moisture. The most Fusarium resistant cultivars succumbed to infection under severe oxygen stress in greenhouse experiments (Smucker and Erikson, 1988; Miller and Burke, 1985a). Plant injury in the form of retarded growth incurred under severe oxygen stress in soils infested with of *F.s.* f.sp. *phaseoli* was found to be irreversible whereas *Pythium, R. solani* and *T. basicola* infected roots recovered from periods of poor soil aeration (Burke and Miller, 1983). Stress under excess moisture environments can be so severe as to predispose plants to infection by the pea pathogen (*F.s.* f.sp. *pisi;* Miller et al., 1980). Cochrane et al. (1963) demonstrated that ethanol can serve as the sole carbon source in the initiation of *F.s.* f.sp. *phaseoli* spore germination. Under anoxic conditions plant survival relies heavily on ethanol fermentation as an energy source. Ethanol fermentation is defined as the metabolic process which converts pyruvate

to ethanol to form ATP. If *F.s.* f.sp. *phaseoli* can utilize ethanol as a carbon source, this ability may provide the fungus with a competitive advantage over other soil microorganisms which cannot survive under oxygen stress conditions. A greater number of spores of *F.s.* f.sp. *pisi* were reported to germinate in the rhizosphere of stressed pea roots and anaerobic conditions were observed to increase root rot of pea by this pathogen by 400% (Smucker and Erikson, 1987). Clearly, implementing cultural practices that reduce oxygen stress will help reduce root rot damage in *F.s.* f.sp. *phaseoli* infested soils.

Tillage

While excessive moisture can have a devastating effect on root rot severity in bean, periods of intermittent drought also exacerbate the disease. Adequate irrigation planned to avoid periods of excessive or insufficient moisture may be a means to reduce plant stress and help decrease Fusarium root rot in infested fields. Subsoiling between bean rows before planting helps reduce the negative impact of *F.s.* f.sp. *phaseoli* on plant yield by eliminating root growth restrictions due to soil compaction, increasing water holding capacity of the soil and improving drainage (Burke and Miller, 1983). Burke and Miller (1983) demonstrated that subsoiling to a depth of 45 to 50 cm increased yield of bean in both infested and non-infested fields. Positive subsoiling effects, however, are negated once tillage is reapplied to the field after planting.

Organic soil amendments

Another cultural management practice that involves improving soil structure is crop rotation. As was previously mentioned, crop rotation can help reduce inoculum density, but more likely reduces Fusarium root rot by enhancing soil properties. Crop

sequences that involve alfalfa or small grains add large amounts of root and plant residue to the soil which can improve water holding capacity and reduce soil compaction. Soil channels developed from the deep rooting system of a previous crop of alfalfa allow access to deeper soil levels where excess moisture may be present or where *Fusarium solani* f.sp. *phaseoli* is not prevalent (Burke and Miller, 1983). Soil channels produced from a previous alfalfa crop may, however, serve to increase root rot. Roots grow and water flows along the path of least resistance. As a result, bean roots may commonly advance through channels left by decaying roots of the previous crop concentrating bean roots within this region (Rasse and Smucker, 1999). Inoculum potential of *F.s.* f.sp. *phaseoli* increased by saprophytic growth on decaying alfalfa will also be present in these channels and could potentially concentrate soil inoculum in the area of bean root growth. This may offer an explanation why some studies have reported conflicting results as to the effect of previous alfalfa crops on root rot severity.

In addition to soil structure modification, some crop species may influence disease severity through the production of phytotoxic substances evolved during decomposition which can injure bean roots. It has been previously discussed that bean hypocotyls exposed to various phytotoxic plant extracts are more susceptible to infection (Toussoun and Patrick, 1963). Ether and aqueous extracts of rye, barley, broccoli and broad beans increased Fusarium root rot lesions when applied to hypocotyls before or with *F.s.* f.sp. *phaseoli* inoculation. Since disease severity was increased in spite of being inoculated after the toxic extracts had been removed, the authors concluded that wounding of plant tissue and its subsequent impact on Fusarium infection was important. Nitrogenous compounds exuded from wounded plant tissue were implicated in enhancing fungal pathogenicity. It

was also observed that extract products obtained from material decomposing under cold, wet and anaerobic conditions were more toxic (Burke and Miller, 1983). Whether this toxicity enhances Fusarium root rot by providing a point of entry for the fungus, increasing pathogen aggressiveness, increasing host plant susceptibility or any of these factors in combination remains to be seen. If nitrogenous compounds exuded from damaged bean roots do, in fact, stimulate pathogenesis, this could help explain why stressed or damaged roots are predisposed to Fusarium root rot. Under drought, soil compaction and water stressed conditions, roots become weakened and finer roots and root hairs begin to decompose. This decomposition may provide the nitrogen needed to aggravate pathogenicity of *F.s.* f.sp. *phaseoli.*

Resistance

Germplasm Sources

Traditional sources of genetic resistance for Fusarium root rot have been limited to *P. coccineus* germplasm, and the *P. vulgaris* introduction PI 203958 (N203; Baggett and Frazier, 1959; Wallace and Wilkinson, 1966). NY-2114-12, a small-seeded, tan colored breeding line developed by Wallace and Wilkinson (1965) is the best known source of resistance derived from *P. coccineus* and is often reported as possessing better resistance than N203 (Hassan et al., 1971; Beebe et al., 1981). Smith and Houston (1960) tested 12,000 bean accessions and identified seven that they considered Fusarium resistant. Each of these seven genotypes was crossed to ten commercial bean cultivars and only three of the 70 combinations resulted in progeny with superior root rot resistance. Of these three, two involved crosses with N203. After five years of selection from six different Fusarium

root rot resistance sources, Dickson (1973) identified five resistant genotypes all of which traced back to N203. It appears that N203 is often used as a source of genetic resistance to Fusarium root rot because it possesses good combining ability for resistance. Furthermore, although N203 is photoperiod sensitive, it is not as sensitive as many tropical genotypes and can mature in temperate regions in most years. A higher proportion of lines with acceptable maturity are available from crosses with N203 contributing to its success as a parent.

Root rot resistance to several pathogens including *F.s.* f.sp. *phaseoli*, *Rhizoctonia solani* and *Phythium* spp. was evaluated for bean germplasm of Latin American origin (Beebe et al., 1981). Based on a scale from 1 to 9 (1 = Resistant; 9 = susceptible), *F.s.* f.sp. *phaseoli* ratings greater than 5.0 were not observed indicating a higher level of resistance than was expected. A list of other reportedly Fusarium root rot resistant germplasm is available in Abawi and Pastor Corrales (1990). In general, Fusarium root rot resistance has been identified in genotypes of Middle American origin. However, breeding efforts over the years have resulted in the release of several large-seeded breeding lines resistant to root rot. Wisconsin root rot resistant (RRR) lines 36, 46, 83, 77 were released in the late 1970s but were not widely adopted as parental germplasm (Hagedorn and Rand, 1978; 1979). The Fusarium resistant snap bean breeding line, FR266, appears to have high levels of Fusarium root rot resistance and a large vigorous root system (Silbernagel, 1987). N203 was used five times in the background of this breeding line suggesting that resistance can be transferred from small-seeded to large-seeded genotypes.

Inheritance

Several genetic studies have been conducted to characterize the resistance present in N203 (Smith and Houston, 1960; Bravo et al., 1969; Hassan et al., 1971). Based on F_2, F_3 and backcross (BC) data, Smith and Houston (1960) concluded that resistance derived from both N203 and PI 165435 was controlled by one dominant and one recessive gene as evidenced by a 13:3 ratio in the F_2 generation of several populations. This report, however, may have been misleading since progress towards improved resistance in large-seeded bean has been limited (Bravo et al., 1969). A study using crosses between 'Redkote' light red kidney with five breeding lines and N203 estimated that from three to seven genes were involved in resistance. Conclusions were based on parental, F_1 and F_2 data collected from field experiments. In three of the crosses, the mean of F_1 and F_2 progeny was skewed towards the resistant side of the mid-parent value while the mean of progeny from the other three crosses occurred near the resistant parent. In only two of the six populations did the mean and distribution of the F_2 progeny differ substantially from that of the F_1. None of the progeny from any of the six populations exhibited root rot susceptibility similar to Redkote. These results are in contrast to Smith and Houston's (1960) study that reported all F_1 as susceptible and only a few F_2 progeny as resistant. These contrasting results can be explained by the subjectivity involved in classifying root rot scores into one of two categories: resistant or susceptible. Smith and Houston (1960) reported that very few progeny scored less than the resistant parent and it is assumed, therefore, that the rest were considered susceptible. Care must be taken when attempting to compartmentalize disease scores into two classes for traits exhibiting a more quantitative nature since misleading conclusions can result.

Hassan et al. (1971), in another study, attempted to elucidate the nature of genetic resistance to *F.s.* f.sp. *phaseoli* in bean. Two resistant parents, N203 and NY-2114-12, were compared by crossing each to the other and to Redkote light red kidney. Data from this study were collected from F_1, F_2 and BC progeny both in the field and greenhouse. Narrow sense heritability esitmates (h^2) based on parent offspring regression for the Redkote/NY-2114-12 population were reported as 44.3 % in greenhouse experiments and ranged from 33.6 to 78.6 % for field studies involving several different scoring dates. Eight genes were estimated to be involved in resistance for this population. Heritabilities for the Redkote/N203 population ranged from 25.9 to 84.8 % for greenhouse and field experiments. Four genes were reported to be involved in the resistance derived from N203. No segregation was observed for progeny from the NY-2114-12/N203 cross indicating that some of the resistance genes present in N203 may be similar to those originating from *P. coccineus.* The authors concluded that Fusarium root rot resistance should be considered a complex trait for which quantitative breeding approaches would be the most appropriate improvement strategy.

CONCLUSION

A multitude of factors combine to result in severe Fusarium root rot in bean. The reader is referred to the classic disease triangle which describes disease in terms of interactions between pathogen, host and environment. For root rot caused by *F.s.* f.sp. *phaseoli,* the environment and its impact on both the host and pathogen appear to be the predominant determinant of disease severity. As a consequence, quantification of disease, evaluation of host resistance and consistent methods for control are difficult to achieve.

Figure 1 illustrates the different environmental aspects that affect the host and pathogen. It also lists aspects inherent to *F.s.* f.sp. *phaseoli* and *P. vulgaris* that interact with environmental conditions to cause disease. Saprophytic ability, spore germination, penetration, and infection are all factors that can influence the aggresiveness of the pathogen. These attributes are constant components of the fungus and can only be reduced or enhanced through manipulation of the environment or host. Disease control, therefore is obtained by altering environmental factors like soil C:N, nitrification and microbial activity which effect changes in germination, penetration, infection, etc. of the pathogen. Alternatively, aspects of host response can be manipulated to achieve greater control of root rot severity by influencing fungal pathogenicity. Cultural management practices that can help eliminate stress environments, such as irrigation, subsoiling, crop rotation, etc. have and will continue to be important for crop improvement and disease control. Some of these practices, however, may not be feasible in certain growing areas or may be uneconomical. Furthermore, environmental conditions are unpredictable making it difficult to establish consistent principals. Genetic improvement of traits inherent to host genotype including seed type, disease resistance, stress resistance, growth habit and root morphology can potentially provide a durable, consistent and effective means of control against root rot. Improved genotypes and cultivars combined with established root rot-reducing cultural practices affecting both host and pathogen should become the predominant method for combating Fusarium root rot in bean.

The ubiquitous presence of Fusarium root rot in bean production areas, its potential for substantial negative impact on yield and lack of adequate cultural control emphasizes the need to develop genetic resistance to Fusarium root rot in bean. Root rot

resistance combined with stress tolerance will ultimately improve the performance of bean in *F.s.* f.sp. *phaseoli* infested areas (Burke and Miller, 1983). Resistance has been identified in *P. vulgaris* and related species (*P. coccineus*) but has been difficult to transfer into adapted large-seeded germplasm. Conclusive evidence elucidating the genetic control of Fusarium root rot resistance has remained elusive in part because contemporary genetic research has neglected to emphasize this area in bean. The proceeding chapters of this research will address the inheritance of Fusarium root rot resistance using the traditional source of resistance, PI 203958. The development of a controlled greenhouse screen and the identification of molecular markers associated with Fusarium root rot resistance in common bean is discussed.

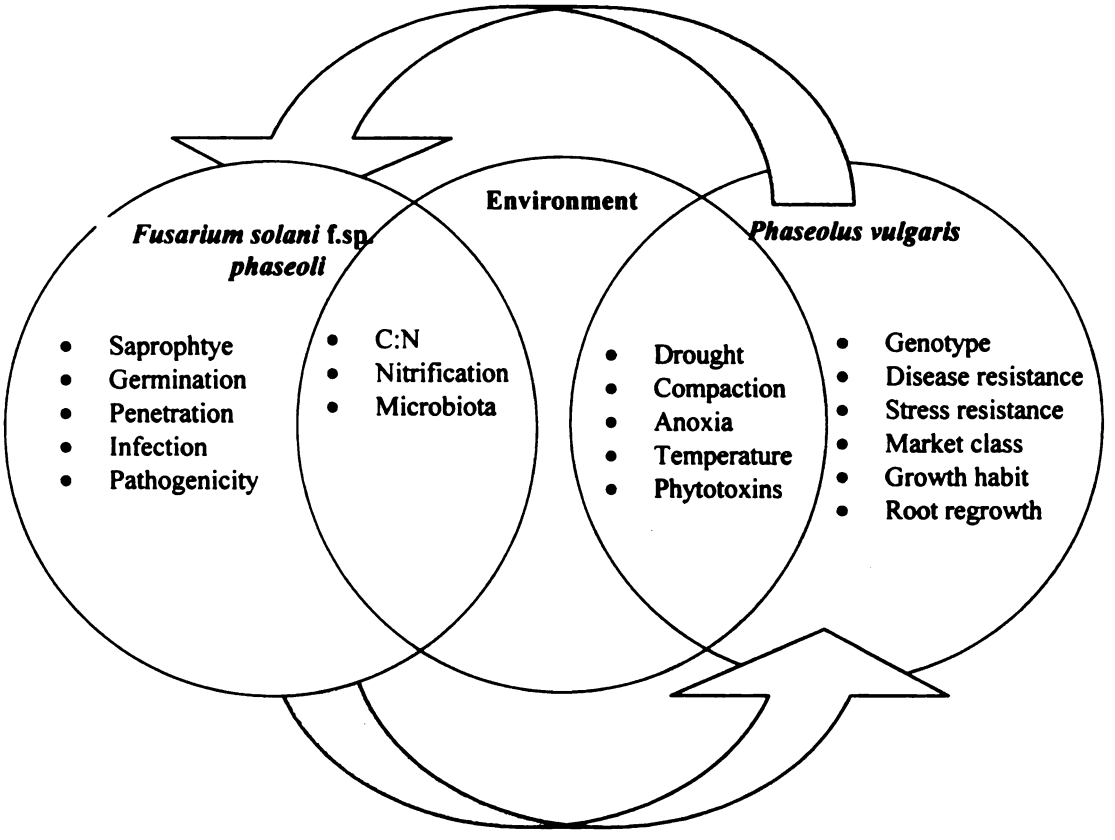

Figure 1. Interaction between environment, fungal pathogen and host. Environmental aspects which fall into the *F.s.* f.sp. *phaseoli* circle have been shown to alter characteristics of the pathogen. Environmental aspects which fall into the *Phaseolus vulgaris* circle influence plant growth and disease susceptibility.

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CHAPTER 1

A GREENHOUSE SCREENING PROTOCOL FOR THE IMPROVEMENT OF FUSARIUM ROOT ROT IN BEAN (*PHASEOLUS VULGARIS* L.)

ABSTRACT

Root rot caused by *Fusarium solani* f.sp. *phaseoli* is a devastating disease of bean for which successful control has been elusive. Resistance to this pathogen is considered quantitative and strongly influenced by environmental factors. To reduce environmental variation and facilitate selection in earlier generations, an accurate, consistent, and non-destructive greenhouse screen is desired for the evaluation of root rot resistance in bean. In this chapter, we propose a protocol which involves the germination of seedlings in perlite, inoculation of roots and hypocotyl 10 d after planting and evaluation within four weeks. The accuracy of this greenhouse screen was confirmed by demonstrating strong correlation between greenhouse and field ratings. Two genotypic groups which included 24 and 21 genotypes, respectively, were evaluated in the greenhouse and these ratings were significantly and positively correlated with field ratings. Correlation coefficients were as high as 0.99. Hundreds of genotypes can be evaluated within one month for relatively minimal costs and labor. Furthermore, once roots have been rated and dipped in benomyl, plants can be transplanted to pots for production of seed. This simple, rapid and inexpensive protocol reduces environmental variation inherent to field ratings thereby more accurately representing physiological resistance while maintaining a close association with observed field ratings.

INTRODUCTION

Fusarium root rot is caused by the soilborne fungal pathogen *Fusarium solani* f.s. *phaseoli* (Burk.) Snyd. & Hans. and has been reported to reduce yield of bean (*Phaseolus vulgaris* L.) in California, Colorado, Wisconsin, Washington, Nebraska, North Dakota, New York, Minnesota, and Michigan (Smith and Houston, 1960; Burke and Silbernagel, 1965; Keenan et al., 1974; Steadman et al., 1975; Saettler and Anderson, 1978; Sippell and Hall, 1982; Estevez de Jensen et al., 1998). The pathogen invades underground roots and stems directly through the epidermis, stomates and wounds. Infection results in characteristic red streaks along the base of the hypocotyl and discoloration and deterioration of the main taproot and laterals. Severely diseased roots cannot sustain plant growth and development resulting in visible symptoms such as chlorosis, defoliation and stunting of the above ground plant (Abawi and Pastor Corrales, 1990). Fusarium root rot is a particular problem in dark red kidney beans due, in part, to the lack of any degree of genetic tolerance to this pathogen. Compounding this problem, drought conditions aggravated by the sandy soils on which this market class is produced inhibit root health and vigor which may ultimately lead to increased disease susceptibility.

Environmental factors like drought stress play a large role in Fusarium root rot severity and can influence disease both at the pathogen and host levels. Soil factors such as carbon and nitrogen content of the soil affect fungal growth, development and pathogenicity by altering spore germination, penetration, and invasion (Christou and Snyder, 1962; Huber et al., 1965; Toussoun, 1970). Soil nutrient availability also exerts an indirect effect on fungal development through its impact on microbial activity (Huber et

al., 1965). Cultural management practices that do not favor pathogenicity can be an effective means of controlling disease but approaches which also involve enhancing host defenses will be more consistent and comprehensive. Host defense responses can be enhanced by eliminating stressful environmental conditions which affect root health and vigor. A robust root system with active defense mechanisms is more likely to avoid, tolerate or resist pathogen infection. Although root health and vigor are often determined by environmental conditions, they are also a function of genotype. By genetically improving disease resistance or stress tolerance, in combination with optimized cultural practices, yield reductions in bean caused by Fusarium root rot will be minimized.

Resistance to *F.s.* f.sp. *phaseoli* is considered quantitative due to environmental variation and polygenic inheritance. While genetic variation for root rot resistance exists and root rot resistant *P. vulgaris* genotypes have been documented, progress towards improving root rot resistance has been limited. Polygenic inheritance compounded by strong environmental effects has made it difficult to achieve a complete understanding of physiological disease resistance mechanisms under field conditions (Smith and Houston, 1960; Wallace and Wilkinson, 1965; Bravo et al., 1969; Hassan et al., 1971; Boomstra et al., 1977; Beebe et al., 1981; Silbernagel, 1990; Tu and Park, 1993). Furthermore, evaluating roots, in general, is difficult and not amenable to large-scale population analysis. It was our objective therefore, to develop an efficient, controlled greenhouse screen to evaluate genotypes for Fusarium root rot resistance while eliminating environmental variation inherent to field experiments. The screening method presented will ultimately assist us in the identification and evaluation of physiological resistance mechanisms present in bean.

Many studies examining Fusarium root rot resistance in bean have utilized greenhouse screening methods with variable results (Baggett and Frazier, 1959; Wallace and Wilkinson, 1965; Baggett et al., 1965; Hassan et al., 1971; Boomstra et al., 1977; Beebe et al., 1981; Tu and Park, 1993). In most cases, an association between greenhouse and field data was not established because a corresponding field trial including similar genotypes to those used in the greenhouse screen was not conducted. Wallace and Wilkinson (1965) proposed an elaborate greenhouse screening method whereby six inch pots, placed in wooden frames, heated by sub-surface coils and filled with field soil were used to evaluate seedlings. From six to 15 seeds were planted into collars and covered with sand to which liquid inoculum was added. Collars were subsequently removed upon evaluation to expose the infected hypocotyl. Field tests were also conducted using the same genotypes but greenhouse results did not appear to correspond well with field ratings (Wallace and Wilkinson, 1965). Furthermore, ratings based on hypocotyl infection may not be an accurate indication of lateral root damage which is reported to be the predominant determinant of yield reduction caused by *F.s.* f.sp. *phaseoli* (Burke and Barker, 1966). The technique proposed by Wallace and Wilkinson (1965) was relatively cumbersome such that only 27 to 58 pots could be measured in a single test. The number of individual plants in 58 pots is sufficient to rate a large F_2 population. For rating replicated trials of 100 or more recombinant inbred lines, however, this method does not appear feasible (Hassan et al., 1971).

Another technique developed by Boomstra et al. (1977) attempted to compare two different greenhouse screens with field ratings recognizing that to be effective, data from any greenhouse evaluation must reflect observed field root rot severity. The first screen

involved a nutrient culture technique where seedlings were germinated in perlite, removed, dipped in inoculum and transferred to a hydroponic nutrient solution. The second greenhouse evaluation was a variation on Wallace and Wilkinson's (1965) pot test. Ratings from the nutrient culture technique which were based on hypocotyl and foliar symptoms did not appear to correspond well with field ratings nor pot test results which were based on hypocotyl and root damage. The nutrient culture technique was, however, an improvement over Wallace and Wilkinson's (1965) pot test since many individuals could be evaluated using minimal resources.

Other methods involve evaluating seedlings grown in infested soils obtained from documented *F.s.* f.sp. *phaseoli* infested fields (Park and Tu, 1994). For evaluating resistance to the root rot complex in general, this technique is adequate. However, separating and confirming reactions to individual components of the root rot complex, such as *F.s.* f.sp. *phaseoli,* may be difficult. Evaluation of tap and lateral roots is further complicated by this method because soil particles adhere tightly to the root mass preventing clear observations. To prevent this problem, sand was adopted as the soil medium of choice in two studies (Beebe et al., 1981; Baggett, 1969). Beebe et al. (1981) sowed seeds into flats containing a sand and inoculum mixture. The authors scored root rot resistance based on hypocotyl and root lesions separately and reported a non-significant correlation between these two ratings of 0.47. Type of soil media, form and concentration of inoculum, hypocotyl versus root ratings, number of ratings, labor and cost are all considerations that must enter into the development of a useful greenhouse screening method. It was our objective to choose the best aspects of all these methods to create a greenhouse screen that was inexpensive and relatively simple to allow evaluation

of lateral roots that correlated well with field ratings and would permit the evaluation of large populations.

MATERIALS AND METHODS

Fungal Isolation Procedure

F. solani was isolated from Michigan dry bean production fields in Presque Isles, Gratiot, and Saginaw counties. Roots infected with *F.s.* f.s. *phaseoli* sampled from these fields were stored at 4° C until isolation. Stem and root sections were bisected longitudinally and then cut into 0.5 cm pieces. Plant segments from each plant were wrapped in cheesecloth and soaked under running deionized water for 24 h. Stem sections were then surface sterilized in 95% ethanol, soaked in 10% Clorox for five minutes, and dried on a paper towel moistened with 10% Clorox. Sections were placed in a petri dish containing water agar (15 g agar in 1 L of water) and left under continuous light. Four to five days later, mycelia emanating from plant sections was removed at the growing point and transferred to PDA media containing 100 mg L^{-1} ampicillin. These isolates were grown under continuous light for four to five days and then replated on more PDA plus ampicillin. Isolates were stored at 4° C in sterilized soil. Ten ml of distilled water were added to spore cultures after four to five days of growth and mycelia and conidia were scraped into solution. Two ml of this solution was then added to a screw cap, 20 ml, test tube with 3 ml of sterilized potting soil. The test tubes were set under continuous light for four to five days and then transferred to the refrigerator. One ml of this inoculum was grown out on PDA plus ampicillin when desired. Isolates were characterized by conidial shape, blue spore color on PDA and pathogenicity on bean. Continued culture of the

fungus and verification of pathogenicity was performed by inoculating several plants with the specific isolate and reisolating the fungus using the above procedure.

Greenhouse Screen

Several greenhouse screening tests were attempted and the following protocol was adopted. Using perlite-filled 72-well greenhouse flats, a single seed was germinated in each well, with 3 to 6 seedlings per variety per replication. The perlite was saturated with half strength Hoagland's solution at planting and flats were fertilized once every week thereafter with the same. Ten days after planting, 10 ml of 2 X 10^5 spore suspension of *F.s.* f.s. *phaseoli* macroconidia was applied over the base of hypocotyl using a 4 L hand pump sprayer. Inoculum was prepared by scraping PDA plates of *F.s.* f.s. *phaseoli* macroconidia into a distilled water solution which was then quantified by a hemocytometer and adjusted to the proper spore concentration. The isolate Hawks 2b was used for all inoculations (Table 13). Fourteen d after inoculation, seedlings were removed from flats, cleaned of excess Perlite and rated on a scale from 1 to 7 (Table 1; Figure 2). When seed was desired from inoculated seedlings, roots were dipped in a fungicidal solution of benomyl and transplanted to normal potting soil.

The aforementioned greenhouse screen was used to evaluate two sets of genotypes. The first experiment (GH96) included 24 cultivars and advanced breeding lines some of which have been previously reported as possessing good root rot resistance. The 24 genotypes were replicated four times and three plants were sowed per replication (Table 2). The second greenhouse evaluation (GH97) consisted of 21 of the most popular

Table 1. Description of disease rating scale used for all root rot experiments.

Score	Phenotypic Description
1	healthy root system with no discoloration of root or hypocotyl tissue and no reduction in root mass;
2	localized reddening at base of hypocotyl with majority of root mass size and appearance healthy;
3	increase in intensity and size of localized root/hypocotyl lesion and some discoloration but no reduction in size of root mass;
4	increasing intensity of discoloration in hypocotyl lesion with lesions becoming extended and very little reduction in root mass with some brown discoloration;
5	increasingly discolored and extended hypocotyl lesions. Roots on these plants were brown and had reduced root mass;
6	hypocotyl lesions encircled stem and become more extended, very little root mass was present and was highly discolored; and
7	pithy or hollow hypocotyl with very extended lesions. Root was limited in abundance and functionally dead.

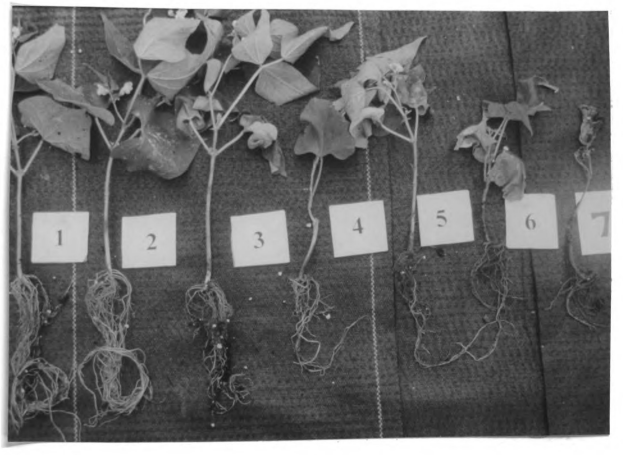

Figure 2. Visual representations of bean roots at corresponding disease severity ratings described in Table 1.

Michigan-grown bean cultivars and advanced breeding lines. Six of the seven market classes (except cranberry) were represented in this experiment (Table 3). Greenhouse evaluation was similar to GH96 and consisted of four replications with three plants per replication.

Field Trials

Field trials were conducted on a Grace very fine sandy loam soil (coarse-silty, mixed, frigid, Typic, Hapludulfs) in Presque Isles County, MI where previous *F. solani* f.sp. *phaseoli* infections have been reported and where beans have been planted without sufficient rotation. The first field trial was conducted in the summer of 1996 and included the same twenty-four cultivars and advanced breeding lines described in GH96 (Table 2). Pre-plant incorporated Eptam herbicide treatment was applied. Two-row plots, 7.6 m long were hand planted on June 17, 1996 and replicated four times. Between-row spacing was 0.76 m. Two weeks after planting, plots were thinned to 13 plants m^{-1}. Plots were arranged in a randomized complete block design with four replications. Twice during the growing season at pod fill (51 days after planting (DAP) and 70 DAP), five random plants from each plot were carefully removed from soil using a shovel and rated for Fusarium root rot symptoms using the root rating scale described in Table 1 and Figure 2. Yield was recorded on a 6.6 m section of each plot for a total harvestable area of 10.00 m^2. Seed moisture samples were also taken for each plot and yields were adjusted to 18% moisture (Table 2). Field root rot evaluations were averaged over the two ratings for each genotype to provide an average score.

Table 2. Seed type, greenhouse rating (GH96), first (RR1) and second (RR2) field ratings, average root rot rating of the two field evaluations (AVG), seed yield, and 100 seed weight (SW) for 24 genotypes grown in Presque Isle County, MI in 1996.

			Field				
Genotype	Seed Type†	GH96	RR1‡	RR2	AVG	Yield (kg ha^{-1})§	SW (g)
B95219	Black	1.9	2.3	2.1	2.2	774	19.3
N203	Black	2.0	1.4	1.2	1.3	-	-
Newport	Navy	2.2	2.8	2.8	2.8	1002	17.1
Huron	Navy	2.3	1.9	1.7	1.8	1573	24.6
T39	Black	2.4	2.0	1.7	1.8	920	19.3
FR266	Snap	2.5	1.2	1.5	1.3	-	-
A300	Cream	2.5	1.7	1.7	1.7	823	19.2
N94080	Navy	2.5	2.3	2.1	2.2	1137	18.8
NW590	Pinto	2.6	2.4	2.3	2.4	699	28.7
Seafarer	Navy	2.6	2.4	2.4	2.4	1075	19.9
Sierra	Pinto	2.7	1.9	2.1	2.0	827	32.1
Mackinac	Navy	2.8	2.7	2.9	2.8	955	20.2
Roza	Pink	2.9	2.8	2.3	2.5	1165	31.7
UI-114	Pinto	3.0	2.1	2.0	2.0	635	37.7
NW 63	Small Red	3.0	2.1	2.6	2.3	1153	31.3
K93654	LRK	3.0	3.0	4.8	3.9	1605	59.8
Viva	Pink	3.1	3.0	1.5	2.3	1134	28.0
Chinook	LRK	3.4	2.8	4.3	3.5	1492	53.4
Redhawk	DRK	3.6	4.6	5.8	5.2	1807	52.5
K94515	LRK	3.9	4.0	5.1	4.6	1321	55.6
K94803	WK	4.1	3.7	5.4	4.5	1407	52.5
Chinook 2000	LRK	4.2	3.0	4.3	3.6	1502	54.1
Montcalm	DRK	4.5	3.1	4.7	3.9	1529	55.9
Isles	DRK	4.8	4.7	5.4	5.0	1428	62.6
Mean		3.0	2.6	3.0	2.8	1182	36.4
LSD (0.05)		0.9	1.0	0.9		446	3.5
CV (%)		17.0	27.4	20.3		26.3	6.7

†LRK, DRK and WK signify light red kidney, dark red kidney and white kidney seed types, respectively.
‡ RR1 and RR2 are the root rot ratings taken 51 and 70 days after planting, respectively.
§ Yield data is unavailable for the genotypes marked with a "-" due to problems with maturity.

Table 3. Seed type, greenhouse rating (GH97), first (RR1) and second (RR2) field ratings, average root rot rating of the two field evaluations (AVG), seed yield, and 100 seed weight (SW) for 21 genotypes grown in Presque Isle County, MI in 1997.

			Field				
Genotype	Seed Type†	GH97	RR1‡	RR2	AVG	Yield -- kg ha^{-1} --	SW --- g ---
Avanti	Navy	2.4	2.0	2.8	2.4	2410	19.0
Newport	Navy	2.4	2.3	2.5	2.4	1849	19.3
T39	Black	2.4	2.6	2.4	2.5	953	18.0
A300	Cream	2.5	2.5	2.4	2.5	2267	20.1
B95204	Black	2.5	2.9	2.2	2.5	1739	18.0
Mackinac	Navy	2.6	2.0	3.2	2.6	1740	19.1
N94080	Navy	2.6	2.7	2.6	2.6	1529	18.2
Mayflower	Navy	2.8	3.2	2.4	2.8	1091	19.5
Vista	Navy	2.9	2.7	3.0	2.9	1378	14.8
Alpine	GN	3.2	2.9	3.5	3.2	997	29.5
Huron	Navy	3.3	2.8	3.7	3.3	1635	20.5
Kodiak	Pinto	3.4	3.7	3.2	3.4	997	30.2
Matterhorn	GN	3.7	3.1	4.3	3.7	791	30.1
K93629	LRK	4.1	3.8	4.5	4.2	740	55.8
Aztec	Pinto	4.1	3.6	4.7	4.2	1153	33.7
Redhawk	DRK	4.4	4.1	4.6	4.4	1565	55.1
Chinook 2000	LRK	4.5	3.8	5.1	4.5	1717	54.4
Isles	DRK	4.5	4.1	4.9	4.5	638	61.8
K93613	LRK	4.7	4.0	5.3	4.7	1797	63.7
Chinook	LRK	5.0	4.5	5.5	5.0	493	47.0
Montcalm	DRK	5.1	4.6	5.5	5.1	1279	52.0
Mean		3.5	3.2	3.7	3.5	1369	33.2
LSD (0.05)		0.8	1.3	1.1		873	2.2
CV (%)		14.5	24.3	17.9		30.5	3.2

†LRK and DRK signify light red kidney and dark red kidney seed types, respectively.
‡RR1 and RR2 are the root rot ratings taken 52 and 86 days after planting, respectively.

A second field trial consisting of the same 21 cultivars and advanced breeding lines described for GH97 was conducted in 1997 on the same field in Presque Isle County, Michigan. No herbicide treatment was applied. A randomized complete block design experiment with three replications was planted on June 13, 1997 with plot area and design similar to the 1996 trial. Plots were rated twice during pod fill, 52 DAP and 86 DAP, and the scoring procedure was identical to the 1996 experiment.

Statistical Analysis

Analyses of variance for all experiments were performed using SAS's procedure GLM (SAS Institute, 1994). Pearson rank correlation coefficients between all ratings and yield data were calculated using SAS's PROC CORR.

RESULTS

Significant genetic variation for root rot ratings was observed for all greenhouse and field experiments conducted. Significant genetic variation was also observed for yield and 100 seed weight (SW) for both field trials. Root rot ratings for GH96 ranged from 1.9 to 4.8 with a mean of 3.0 (Table 2). The black bean breeding line B95219 had the lowest root rot rating followed by N203, the traditional source of root rot resistance (Wallace and Wilkinson, 1966). FR266, which derives its resistance from N203, was the lowest rated large-seeded genotype with a score of 2.4 (Silbernagel, 1987). Black beans tended to be the most resistant followed by navy beans. Pinto and pink seed types scored moderately from 2.6 to 3.1 and the most susceptible seed types were the large-seeded dark red, light red and white kidneys. 'Isles' had the highest root rot rating of 4.8 followed by the highly susceptible dark red kidney, Montcalm.

Field trial root rot ratings confirmed these tendencies and demonstrated a positive and significant correlation with greenhouse ratings (r = 0.73; Table 4). Field ratings ranged from 1.3 to 5.2 with a mean of 2.8 (Table 2). The dark and light red kidney genotypes tended to score the highest for root rot but also tended to be the highest yielding. Redhawk, a dark red kidney cultivar released from Michigan State University, was the highest yielding genotype but also received the highest root rot score followed by Isles. N203 and FR266 were the most highly resistant genotypes in this trial. B95219, which was the most resistant genotype in GH96 was the most susceptible black bean genotype in the field trials. However, black bean genotypes remained the most resistant followed by pinto and navy seed types. Dark red kidney genotypes scored, on average, from 3.9 to 5.2 and light red kidneys scored from 3.5 to 4.6. Mean root rot scores based on field evaluations were 2.6 and 3.0 for the first (RR1) and second (RR2) evaluations, respectively, and ratings were positively and significantly correlated (r = 0.87***; Table 4).

The coefficients of variation (CV) were moderate to high for yield and field root rot ratings while the CV was lower for the greenhouse evaluation. Yields for the variety trial conducted in 1996 ranged from 910 to 2,592 kg ha^{-1} with a mean of 1,702 kg ha^{-1} (Table 2). Yield data were difficult to interpret for this trial because many genotypes lacked adequate adaptation to this northern environment. Yield was positively and significantly correlated with field and greenhouse root rot ratings and was significantly correlated with seed size. The highest yielding genotypes were the regionally-adapted large-seeded kidney types. The tendency for certain market classes to cluster for root rot

Table 4. Pearson rank correlation coefficients for yield, 100 seed weight (SW), first (RRR1) and second field (RRR2) root rot ratings, averaged field root rot rating (AVG), and greenhouse (GH) evaluations for each group of genotypes, GH96 and GH97. GH96 values are presented in normal print in the upper right-hand diagonal whereas the GH97 values are printed in bold in the lower left-hand diagonal.

	Yield	SW	RRR1	RRR2	AVG	GH
Yield		0.69***	0.63***	0.74***	0.72***	0.61**
SW	**-0.33**		0.72***	0.89***	0.85***	0.87***
RRR1	**-0.38**	**0.86****		0.87***	0.95***	0.77***
RRR2	**-0.54****	**0.87*****	**0.84*****		0.98***	0.83***
AVG	**-0.47***	**0.91*****	**0.94*****	**0.97*****		0.73***
GH	**-0.46***	**0.91*****	**0.94****	**0.97*****	**0.99****	

*, **; *** Significance at the P < 0.05. 0.01, 0.001 levels, respectively.

ratings was reflected in a significant and positive correlation (r = 0.85***) between SW and average root rot score (Table 4).

Average ratings for GH97 ranged from 2.4 to 5.1 with a mean of 3.5. Field ratings mirrored greenhouse ratings almost exactly (r = 0.99***; Table 4) so subsequent results can be applied to either field or greenhouse experiments. The dark red kidney cultivar Montcalm, demonstrated the highest greenhouse rating whereas 'Avanti' navy, 'Newport' navy and 'T39' black exhibited the highest levels of resistance with scores of 2.4 (Table 3). Navy genotypes clustered as the most resistant market class along with black bean cultivars 'T39' and B95204 in contrast to the highly susceptible kidney cultivars. 'Huron' was the only navy bean that scored above a 3.0 for this trial. Medium seeded, race Durango genotypes scored from 3.2 to 4.1 and kidneys scored from 4.1 to 5.1. The average score for field evaluations was 3.5 similar to the mean of GH97 (3.5). The CV for

GH97 was comparable to that observed in GH96. Coefficients of variation for both greenhouse experiments ranged from 3 to 10% less than CVs for field ratings (Table 2 and 3). Repeatability was confirmed based on GH97 as a significant and positive correlation between repeated greenhouse experiments (data not shown).

Yield for the 1997 field trial ranged from 493 to 2,410 kg ha^{-1} with a mean of 1,369 (Table 3). Average yields were calculated on two replications only because of extreme weed pressure in the third replication. Weeds were controlled manually but were difficult to contain since no herbicide was applied. Avanti was the top yielding cultivar as well as one of the most resistant genotypes of this trial. Contrary to the positive correlation between yield and GH96 root rot ratings, yield was negatively correlated with average root rot score in 1997 (Table 4). 100 SW was significantly and positively correlated with field root rot scores and GH97 (Table 4).

Pearson rank correlation coefficients (r) for root rot ratings of 11 common genotypes grown in both GH96 and GH97 were also calculated. An r value of 0.83** resulted between average 1996 and 1997 field scores. GH97 was correlated with 1996 field scores (0.77**) and GH96 was positively correlated with 1997 field ratings (0.92***). GH96 and GH97 scores were also positively correlated (r = 0.86**).

DISCUSSION

Our objectives were to develop a greenhouse screen that was inexpensive and relatively simple to allow evaluation of lateral roots that correlated well with field ratings and would permit the evaluation of large populations. The current perlite-based greenhouse screen was inexpensive and required little maintenance. From 12 to 24

gentoypes can be grown in one 54x27x6 cm flat. Minimal requirements for greenhouse space ensure that large populations can be evaluated with limited resources. Time and labor constraints are also minimized since the time from planting to evaluation takes four weeks and only daily watering and weekly fertilization is necessary. Using perlite, an inexpensive soil medium, roots could be cleared of adhering particles with relative ease so that ratings were not based on hypocotyl symptoms alone. Hypocotyl ratings are not adequate indicators of root rot damage as evidenced by Burke and Barker's (1966) observation that lateral root damage was the more important contributor to yield reductions. The authors further demonstrated using infested and non-infested islands of soil surrounding the hypocotyl and taproot, that severely diseased hypocotyls could sustain adequate plant growth and development. The greenhouse screening presented in this paper provides a means to evaluate both hypocotyl and lateral root damage. Figure 3 shows typical root symptoms of a severely infected Montcalm seedling in contrast to a non-inoculated control. The reddish discoloration typical of *F.s.* f.s. *phaseoli* root infection is striking and easily scorable using a visual rating system.

Positive and highly significant correlations between greenhouse and field ratings provide further evidence supporting the advocacy of this screening technique. Correlations between field root rot ratings and greenhouse scores were strongly positive for both trials and were as high as 0.99 between GH97 and corresponding field ratings (Table 4). Interrelationships between 11 genotypes common to both trials also demonstrated a significant and positive correlation. The positive and significant relationship achieved between the proposed greenhouse screening method and replicated field ratings prove that

a controlled experiment can reflect field tolerance while reducing environmental variation and reducing resource limitations.

Reductions in environmental variation using this greenhouse screen are confirmed by a decrease in CVs between field and greenhouse evaluations. The CVs for GH96 and GH97 were from 3 – 10% less than the CVs from corresponding field evaluations (Table 2 and 3). Coefficients of variation are a means to compare the variability of two experiments with different means and express the standard deviation as a percentage of the mean. A decrease in CV can reflect an increase in mean value, a decrease in the standard deviation or both. A comparison of mean scores between field and greenhouse ratings were similar for both groups of genotypes indicating that the reduction in CV reflects a decrease in the variability present for greenhouse trials thereby meeting our main objective to develop a greenhouse screening method which could reduce the influence of environmental factors.

Considering the aforementioned criteria for an inexpensive, accurate, consistent, simple and rapid greenhouse screen to evaluate bean resistance to *F.s.* f.sp. *phaseoli,* the causal organism of root rot in Michigan, the proposed protocol accurately addresses these issues. Furthermore, once rated, plants can be dipped in benomyl fungicide solution and transplanted. The non-destructive nature of this protocol allows for the testing of early generation material for which advanced generation seed is desired. Simple inoculation and plant culture procedures, positive and significant correlation for greenhouse and field evaluations, minimal space and resource requirements combine to make this an effective procedure to further the improvement of root rot resistance in bean.

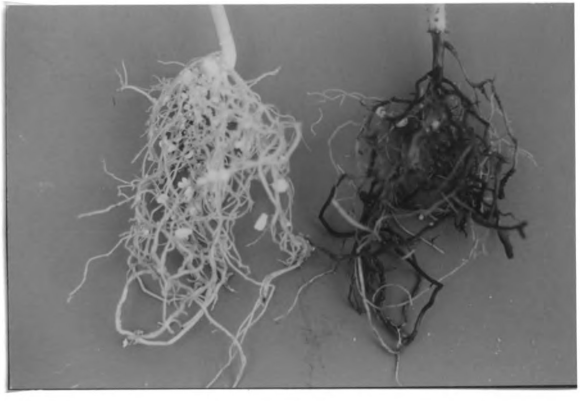

Figure 3. Fusarium root rot infection of 'Montcalm' dark red kidney (right) compared to a non-inoculated control (left).

Economic damage from any disease is ultimately manifested by its effect on yield. However, correlation between field and root rot using the genotypes described in this study exemplifies the potential problems which can arise when measuring disease severity based on yield potential. For the genotypes in GH96, yield was significantly and positively correlated with field and greenhouse root rot ratings which means that an increase in disease severity corresponds to an increase in yield (Table 4). This counterintuitive result can be explained by the lack of adaptation for many of the more root rot resistant genotypes. Cultivars UI-114, 'Viva', 'Roza' are reported as being root rot resistant according to Abawi and Pastor Corrales (1990) but were not adapted to northern Michigan and consequently did not mature nor achieve optimum yield. Cultivars NW 590, NW 63 and 'Sierra', all of which scored, on average, below 3.0, also lacked adequate adaptation to this Northern latitude (>45°N). In contrast, the large-seeded genotypes were developed specifically for Michigan kidney production areas and were highly adapted to the Presque Isle County, MI environment. These same large-seeded genotypes possess high levels of susceptibility and all scored, on average, 3.0 or greater for root rot infection (Table 2). It is not surprising, therefore, to observe a significant positive correlation between root rot resistance and yield for these genotypes and any further conclusions based on these correlations must be made with caution.

Contrary to the positive correlation observed between root rot ratings and yield for the GH96 genotypes, yield and root rot ratings for GH97 genotypes were significantly and negatively correlated (Table 4). These results are not surprising considering differences in the genotypes tested and the environmental conditions encountered in each year. One of the objectives of GH97 was to evaluate levels of root rot resistance in advanced breeding

lines and popular cultivars developed for Michigan. All of the genotypes in this experiment were adapted to Michigan and would, therefore, be expected to perform well in Presque Isle County, MI. Conditions at planting, however, were considerably drier than optimum (Figure 7) and problems with emergence resulted, especially for some of the large-seeded genotypes. Many of the large-seeded genotypes like Isles and Chinook struggled to germinate under the dry conditions present in 1997 thereby delaying maturity. Many green pods and stems were observed at harvest. Although yield data reflect problems with seasonal conditions, root rot ratings confirm levels of resistance reported in the 1996 experiment for those lines which were duplicated in 1997. Furthermore, significant correlation between field root rot ratings and greenhouse evaluations verify that resistance to root rot can be visually measured but, depending on environmental conditions will not be good indicators of yield reductions caused by this disease. To more accurately define yield reductions caused by Fusarium root rot, control experiments in non-infested soils should be conducted simultaneously with Fusarium-infested treatments in the same location. This will, however, be difficult to achieve with this ubiquitous pathogen.

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CHAPTER 2

ANALYSIS OF RESISTANCE TO FUSARIUM ROOT ROT IN BEAN AND THE IDENTIFICATION OF ASSOCIATED QTL

ABSTRACT

Using recombinant inbred populations, genetic analysis of the nature of resistance to the bean root rot pathogen, *Fusarium solani* f.sp. *phaseoli* (Burk.) Snyd. & Hans, was conducted and RAPD markers associated with QTL controlling this trait were identified. Several markers demonstrated significant associations with root rot resistance determined from both greenhouse and field evaluations. Markers associated with field root rot ratings tended not to be associated with greenhouse and vice versa except for the RAPD P_{700} which was significantly associated with greenhouse and field data. Markers identified in this study did not explain more than 15% of the phenotypic variation for root rot resistance but a combination of 4 markers explained 29% of the phenotypic variation for field root rot ratings. Based on these results, it was concluded that genetic resistance to *F.s.* f.sp. *phaseoli* derived from PI 203958 (N203) is polygenically controlled and strongly influenced by environmental factors. Supporting this conclusion were moderate heritability estimates (h^2) that ranged from 0.48 to 0.71 for one population and from 0.09 to 0.66 for another population. We identified two regions of the genome associated with root rot resistance that also corresponded to locations of *Phaseolus vulgaris* pathogenesis-related proteins. It appears that mechanisms associated with host defense responses may be involved in Fusarium root rot resistance and selection directed towards enhancing these

traits may play a role in the future improvement of root rot resistance caused by *F.s.* f.sp. *phaseoli* in bean.

INTRODUCTION

Root rot caused by *F.s.* f.sp. *phaseoli* negatively affects all large-seeded bean production areas of the US and is often considered one of the most serious diseases of irrigated beans in the Pacific Northwest after white mold (Dryden and Van Alfen, 1984; Miller and Burke, 1986). The disease is particularly severe on large-seeded genotypes due to a lack of genetic resistance in these market classes. Montcalm, a popular dark red kidney cultivar in Michigan, Minnesota and Wisconsin exemplifies the high degree of susceptibility inherent to this market class. An overemphasis on quality traits in both dark red kidney and snap market classes and consequent reduction in genetic variability may have contributed to the lack of resistance in these seed and pod types. Small-seeded genotypes, although not immune to Fusarium root rot, do not appear as susceptible as the larger seed types. The large-seeded (Andean) are distinguished from small-seeded (Middle American) by morphological, biochemical and molecular characteristics (Gepts, 1988; Haley et al., 1994). Genetic aspects like these, largely unknown, and unique to Andean genotypes may enhance sensitivity to this pathogen. Likewise, soil types and environmental conditions specific to large-seeded production areas can increase disease severity.

Several attempts have been made to elucidate the genetic control of resistance to *F.s.* f.sp. *phaseoli* but results from these studies have failed to rapidly advance improvement of Fusarium root rot resistance, especially in large seed and pod types

(Smith and Houston, 1960; Wallace and Wilkinson, 1965; Hassan et al., 1971). Conclusions based on these studies may be misleading for several reasons one of which concerns the population structure employed. The studies of Smith and Houston (1960), Wallace and Wilkinson (1965) and Hassan et al. (1971) were based on methods and populations appropriate for a more qualitative type of inheritance. Evidence supporting quantitative control of Fusarium root rot include the lack of immunity to *F.s.* f.sp. *phaseoli* in *P. vulgaris* and observable differences in levels of susceptibility which suggest polygenic control (Baggett et al., 1965). Furthermore, *F.s.* f.sp. *phaseoli* disease incidence and severity are strongly influenced by the environment. These two observations define Fusarium root rot resistance as a quantitative trait. Conclusions from previous genetic studies were based on unreplicated F_1, F_2 and BC population structures. Scoring of individual plants in unreplicated experiments can be problematic for traits strongly influenced by environmental factors and an average score for a particular genotype is preferred.

While the need to control environmental variation through replicated field trials is important for analyzing Fusarium root rot resistance, the actual scoring method is equally significant. Evaluation for root rot in the Hassan et al. (1971) study was based on hypocotyl reaction in both field and greenhouse studies (rating systems in the other two studies were not documented). As has been previously discussed (Literature Review), hypocotyl ratings are not indicative of root damage which has been implicated as a more accurate indicator of yield reductions caused by *F.s.* f.sp. *phaseoli* (Burke and Barker, 1966). A method for evaluation that includes assessment of lateral root infection will provide more relevant results.

The third limitation of the aforementioned genetic studies involves the utilization of wide, inter gene pool crosses. While recombination between Andean and Middle American gene pools occurs readily, hybrid lethality can result (Koinange and Gepts, 1992). Skewed segregation as a result of this phenomenon is common and may lead to misinterpretation. More recent development of root rot resistant, Andean breeding lines provide an opportunity to study inheritance patterns within the same gene pool.

Molecular markers may prove valuable as another tool that can be employed to facilitate genetic analysis of root rot resistance in bean. RAPD markers associated with quantitative trait loci (QTL) controlling resistance to common bacterial blight (CBB; *Xanthomonas campestris* pv. *phaseolicola* (Smith) Dye*)* and white mold (*Sclerotinia sclerotiorum*) in bean have been identified (Miklas et al. 1996; Miklas et al., 1999; Park et al., 1999). Marker assisted selection used to indirectly select for resistant genotypes could facilitate improvement of disease resistance for traits like Fusarium root rot resistance where field selection is laborious and demanding. While the practical application of marker assisted selection for quantitative traits has yet to be realized, many studies recognize its potential to facilitate the improvement of quantitative traits (Pilet et al., 1998; Mangin et al., 1999; Schechert et al., 1999; Miklas et al., 1998; Faris et al., 1999; Lubberstedt et al., 1998). QTL-marker associations may also provide a basis for a greater understanding of quantitative disease resistance through the identification of loci that influence resistance to more than one disease. The opportunity for cloning QTL controlling complex disease resistance, and identification of syntenic areas in other species may result from this type of research.

Objectives

The objective of this study was to i) define the inheritance of root rot resistance in bean using recombinant inbred populations with replicated field and greenhouse trials; and ii) identify significant QTL-marker associations which could be used to facilitate indirect selection of Fusarium root rot resistance.

MATERIALS AND METHODS

Germplasm and Population Development.

Five parental genotypes were used in this study. Montcalm dark red kidney, 'Isles' dark red kidney and Seafarer navy cultivars were utilized as susceptible parents whereas FR266 and PI 203958 (N203) obtained from the USDA Germplasm Bank (Prosser, WA) were used as resistant parents. Montcalm and Isles are determinate, dark red kidney cultivars developed at Michigan State University and adapted to kidney bean production areas in Michigan. Both are full season cultivars and highly susceptible to root rot caused by *F.s.* f.sp. *phaseoli*. Seafarer, a determinate, early season navy bean cultivar developed at Michigan State University is also reported as being susceptible to Fusarium root rot. N203 is a small, viny, photoperiod sensitive black bean from Mexico identified by plant collector, explorer, Oliver Norvell, as resistant to Fusarium root rot (Wallace and Wilkinson, 1966). FR266 is a Fusarium resistant snap bean breeding line possessing a large root system, white seed, and snap bean-like pods (Silbernagel, 1987). It tends to flower earlier but mature later than other cultivars in the study.

Three populations were developed using these five parents in intra gene pool, resistant by susceptible crosses. Montcalm and Isles were crossed to FR266 to create MF

and IF recombinant inbred Andean populations, respectively, and Seafarer was crossed to N203 to develop SN Middle American recombinant inbred population. Progeny from MF, SN, and IF were advanced by single seed descent to the F_4 generation where seed was bulked and advanced to create 79, 93 and 68 F_4-derived recombinant inbred lines (RILs), respectively. Seed from individual F_2 plants in SN was bulked to create 105 $F_{2:4}$ RILs which were used in field experiments conducted in 1997. An individual plant from each $F_{2:4}$ RIL was selected and advanced to create an F_4-derived RIL population.

Root Rot Evaluation

Field

Field trials were conducted in both Presque Isle County, MI (PI) and Perham County, MN (PM) in 1997 and 1998. Selected fields were previously identified as infested with *F.s.* f.sp. *phaseoli*. All field experiments were planted in a randomized complete block design with three to four replications. A combination of pre-emergence herbicide and mechanical and hand cultivation was used to control weeds. Seed was treated with a combination fungicide and insecticide containing thiophanate methyl, captan and lorsban. Agronomic practices were applied to insure good crop growth and development. Single-row plots consisting of 10 seeds each were 1.2 m long and 0.76 m wide. During mid and late pod-fill, five plants per plot were uprooted with a shovel taking care not to disturb the main portion of the root system. Roots were cleaned of debris and rated on a scale from 1 to 7 (Table 1; Figure 2).

One hundred and two SN $F_{2:4}$ RILs, seven checks and both parents were planted in PI on June, 13, 1997 and replicated four times. Ratings were taken 52 and 86 DAP.

Ninety-four SN $F_{2:4}$ RILs were planted in three replications on June 1, 1997 in PM and rated 53 and 71 d after planting. An experiment consisting of 92 SN $F_{4:6}$ RILs, two parents and four checks replicated three times was also planted on June 4, 1998 in PI using the same experimental design and plants were rated 91 DAP. Only one rating was conducted for this experiment due to problems with emergence.

Field trials for MF were planted on June 4, 1998 in PI and on May 21, 1998 in PM. Eighty $F_{4:6}$ RILs from MF, two parents and 10 checks, replicated four times, were planted in PI and plots were rated 78 DAP. Seventy-eight MF $F_{4:6}$ RILs, two parents and four checks, replicated three times were planted in PM and plots were rated 74 DAP. Only one rating, recorded during late pod-fill, was taken for these 1998 MF experiments due to problems with plant emergence.

In addition to root rot evaluation experiments, all RIL for both MF and SN populations were increased in four- and two-row unreplicated plots, respectively, in Ingham County, MI in 1998. Data for days to flower (DTF) and days to maturity were recorded based on this experiment.

Greenhouse

The MF, SN and IF populations were evaluated for root rot resistance in the greenhouse using the perlite screen described in the previous chapter. Two SN greenhouse evaluations were conducted using and $F_{4:5}$ (SNGH1) and $F_{4:7}$ (SNGH2) SN recombinant inbred populations. The genotypes were the same as those reported for the 1997 and 1998 field trials, respectively. Three replications with three plants per genotypic entry were planted and scored. Three MF greenhouse screenings were conducted using $F_{4:5}$ (MFGH1)

and $F_{4:7}$ (MFGH2, MFGH3) MF recombinant inbred populations using four replications and three plants per genotypic entry. Genotypes included in these screenings were the same as those used in the 1998 PI trial. A single unreplicated greenhouse screen consisting of six plants each of 68 $F_{4:5}$ IF recombinant inbred population, parents and six checks was also conducted. *Fusarium solani* f.sp. *phaseoli* isolates used for each greenhouse evaluation of SN, MF and IF and corresponding county from which the isolates were collected are given in Table 5.

Statistical Analysis

All experiments conducted in the field and greenhouse involving MF and SN were analyzed as one-way analyses of variance (ANOVA) using the PROC GLM and PROC MIXED programs of SAS (SAS Institute, 1994). Root rot ratings from the five plants scored from each plot were averaged to give a plot mean which was subsequently used for ANOVAs. Mean scores, averaged over all plants rated for the 1997 SN experiments conducted in PI and PM, were used in a combined analysis over locations. Due to uneven plant numbers per plot, analysis of MF and SN experiments conducted in PI and PM in 1998 included a subsampling error.

Heritability estimates were calculated on an entry mean basis for all greenhouse and field trials according to Hallauer and Miranda (1981) except for the IF population which was an unreplicated greenhouse screen. Pearson rank correlation coefficients were calculated using SAS PROC CORR to compare means of root rot ratings for RILs within MF for all greenhouse and field evaluations conducted for this population (SAS Institute, 1994).

Table 5. *Fusarium solani* f.sp. *phaseoli* isolates and location of origin used to inoculate greenhouse evaluations for three recombinant inbred populations, Seafarer/N203 (SN), Montcalm/FR266 (MF) and Isles/FR266 (IF). Two greenhouse screens were conducted using SN (SNGH1 and SNGH2) and three were performed using MF (MFGH1, MFGH2, MFGH3).

Evaluation	Isolate	Origin
SNGH1	Huron 2a	Huron County, MI
SNGH2	Hawks 2b	Presue Isle County, MI
MFGH1	F.s.12	Presque Isle County, MI
MFGH2	Huron 2a	Huron County, MI
MFGH3	Hawks 2b	Presque Isle County, MI
IF	Hawks 2b	Presque Isle County, MI

Marker analysis

DNA Extraction

DNA was extracted from SN and IF F_2 individuals using a mini-extraction protocol described by Schneider et al. (1997). DNA was extracted from tissue sampled from 6 plants of each $F_{4:6}$ MF RIL and all parents using a larger DNA extraction method (Kisha et al. 1998). Each of the F_2 individuals sampled for DNA extraction in these two populations was the progenitor of a corresponding RIL.

RAPD Protocol

RAPDs were generated using the protocol outlined by Schneider et al. (1997) with slight modification. PCR products and repeatability was enhanced by doubling the amount of enzyme used in each reaction. RAPDs greater than 1200 bp were amplified using GIBCO BRL® brand Taq polymerase (Life Technologies, MD) and RAPDs less than 1200 bp were amplified by AmpliTaq® DNA polymerase, Stoffel Fragment (Perkin Elmer, CT). Seven hundred random 10-mer primers (Operon Technology, CA) were used to amplify random regions of the genome. DNA was amplified using a Perkin Elmer Cetus DNA Thermal Cycler 480 (Perkin Elmer, Cetus, Norwalk, CT) using the following cycles: 3 cycles of 1 min. at 94 °C, 1 min. at 35 °C, and 2 min. at 72 °C, 34 cycles of 1 min. at 94 °C, 1 min. at 40 °C, and 2 min. at 72 °C (final step extended by 1 sec for each of the 34 cycles), and a final extension cycle of 5 min. at 72 °C (Haley et al., 1994a). Approximately 20 µl of amplified DNA from each sample was run on a 1.4% agarose gel containing ethidium bromide (0.5 µg ml^{-1}), 40 mM Tris-acetate, and 1 mM EDTA. DNA was viewed under ultraviolet light and photographed for permanent record.

While populations were being developed, primers were screened against Seafarer, N203, Montcalm and FR266 parents to identify polymorphisms which would subsequently be utilized in the population analyses. A method of selective genotyping proposed by Lander and Botstein (1989) and utilized by Miklas et al. (1996) was employed to facilitate the identification of markers associated with QTL controlling Fusarium root rot resistance in MF. DNA from the five lowest scoring or most resistant RIL of MF based on data from MFGH2 was pooled to create a resistant bulk (R). Likewise, DNA from the 5 highest

scoring or most susceptible RIL was pooled to create a susceptible bulk (S). RAPD markers polymorphic between R and S bulks were subsequently screened against the individuals. If the RAPD continued to segregate according to R and S classifications of the individuals used to make the bulks, the primer was screened against the entire population. RAPDs reported on an integrated *P. vulgaris* linkage map were also used for screening MF although many were not polymorphic between Montcalm and FR266 and, thus, uninformative (Freyre et al., 1998).

Significant associations between root rot resistance and marker genotype were determined by one-way ANOVAs using SAS's PROC GLM (SAS Institute, 1994). Marker-QTL associations were considered significant at $P < 0.05$. Multiple regression analyses using combinations of significant markers were also performed using PROC GLM. RAPDs which did not exhibit a 1:1 segregation were disregarded. Linkage relationships among these same markers were determined using MAPMAKER/EXP group command with minimum LOD of 4.0 and maximum distance of 50 cM (Lincoln et al., 1987).

RESULTS

Root rot evaluations

Significant variation for root rot scores was observed in all tests of MF (Table 6). Results for SN were more variable. Significant genetic variation for root rot ratings was observed for one of the two greenhouse experiments (SNGH1) and the 1998 PI trial (Table 6). Genetic variation for the 1997 field trial combined over two locations, PI and

Table 6. Analyses of variance for root rot ratings for the recombinant inbred populations, Seafarer/N203 (SN) and Montcalm/FR266 (MF). Four experiments are presented for each population. Two greenhouse experiments (SNGH1 and SNGH2) for SN and three for MF (MFGH1, MFGH2 and MFGH3). A combined analysis over two locations in Presque Isle County, MI (PI) and Perham County, MN (PM) is presented for both populations. The SN population was evaluated in 1997 and the MF in 1998. An additional PI experiment was conducted for SN in 1998 and is presented.

	MF			SN		
Source	df	MS	F Test	df	MS	F-Test
	----MFGH1----			----SNGH1----		
Rep‡	1	1.99		2	1.23	
Genotype	1	1.59	2.14***	92	0.41	1.96**
Error	83	0.74		174	0.21	
	----MFGH2----			----SNGH2----		
Rep	3	1.25		2	2.88	
Genotype	78	0.97	2.11***	93	0.36	1.22
Error	225	0.46		179	0.30	
	----MFGH3----			----1998 SN PI†----		
Rep	3	9.30		2	0.07	
Genotype	77	0.57	1.97***	92	2.58	2.60***
Rep*Genotype				170	0.99	2.42***
Error	228	0.30		862	0.41	
	----1998 MF Combined----			----1997 SN Combined----		
Loc.	1	916.05		1	212.45	
Rep within Loc	5	27.85			4.41	
Rep	3	25.32		5	0.43	
Genotype	79	4.53	3.23***	101	0.47	0.92
Loc*Genotype				91	0.21	2.22***
Rep*Genotype	227	1.40	2.10***			
Error	1894	0.67		481	0.5	

*, **; *** Significance at the P < 0.05. 0.01, 0.001 levels, respectively.

†All 1998 field trials were analyzed using a subsampling term for individuals scores per plot.

‡Rep = replication; Loc = location

Table 7. Root rot ratings of the top and bottom five Montcalm/FR266 (MF) recombinant inbred lines based on the second MF greenhouse evaluation (MFGH2). Rankings of these genotypes in all other evaluations including two greenhouse screenings (MFGH1 and MFGH3), two field trials conducted in Presque Isle County (1998 PI), MI and Perham County, MN (1998 PM) and the combined analysis over these two locations are given in parentheses. Scores for check genotypes evaluated for each experiment are also given for comparison.

Genotype	MFGH2		MFGH1		MFGH3		1998 PI		1998 PM		Combined	
MF33	2.8	(1)	1.9	(1)	3.0	(9)	4.1	(43)	5.1	(19)	4.5	(23)
MF8	3.0	(2)	2	(2)	2.6	(2)	4.4	(62)	4.9	(11)	4.7	(41)
MF21	3.0	(3)	2.3	(8)	2.9	(6)	4.2	(47)	6.2	(65)	5.3	(68)
MF17	3.0	(4)	2.3	(9)	3.1	(10)	3.7	(25)	5.2	(27)	4.6	(28)
MF26	3.0	(5)	2.3	(10)	3.5	(47)	2.4	(1)	5.4	(37)	3.9	(8)
MF4	4.6	(74)	3.1	(57)	4.0	(48)	4.3	(59)	6.1	(36)	5.1	(50)
MF20	4.6	(75)	3.1	(55)	3.5	(72)	4.4	(52)	5.3	(63)	4.9	(60)
MF13	4.7	(76)	3.3	(75)	3.3	(30)	4.6	(71)	6.4	(73)	5.5	(74)
MF9	5.3	(77)	4.0	(78)	4.5	(77)	4.2	(51)	6.5	(74)	5.3	(72)
MF77	4.6	(84)	2.8	(38)	3.3	(27)	4.4	(63)	6.5	(75)	5.6	(77)
FR266	2.5		2.1		2.4		3.7		4.5		4.2	
Montcalm	5.3		4.2		5.2		4.4		6.2		5.4	
N203	2.5		-		2.6		-		-		-	
Seafarer	3.1		-		3.3		2.8		-		3.5	
Redhawk	5.3		-		5.3		4.8		6.0		5.3	
Isles	-		4.2		5.1		4.9		-		5.5	
Foxfire	-		-		-		-		5.4		4.7	
K95757	4.3		-		4.6		4.6		-		5.3	
K93613	4.5		-		4.6		4.7		-		5.4	
Tendergreen Imp	3.2		-		3.1		4.4		-		5.2	
WI RRR36	3.2		-		3.2		-		2.7		2.0	
G122	4.0		3.9		3.6		3.8		5.0		4.1	
Taylor Hort.	-		-		-		3.9		-		4.7	
Mean	3.8		3.4		3.5		4.2		5.4		4.7	
LSD (" = 0.05)	1.0		1.7		0.8		1.0		1.1		0.7	
CV (%)	18.5		25.6		15.9		14.6		17.4		16.6	

Table 8. Root rot ratings of the top and bottom five Seafarer/N203 (SN) recombinant inbred lines based on the first SN greenhouse evaluation (SNGH1). Rankings of these genotypes in all other evaluations including a second greenhouse screenings (SNGH2), two field trials conducted in Presque Isle County (1997 PI), MI and Perham County, MN (1997 PM) in 1997, the combined analysis over these two locations and another field trial conducted in 1998 in Presque Isle County, MI (1998 PI) are given in parentheses. Scores for check genotypes evaluated for each experiment are also given for comparison.

Genotype	SNGH1		SNGH2		1997 PI		1997 PM		Combined		1998 PI	
SN03-220	1.1	(1)	2.9	(64)	1.5	(14)	2.8	(42)	2.1	(25)	2.4	(38)
SN08-240	1.3	(2)	2.8	(58)	1.3	(1)	2.9	(54)	2.1	(22)	2.7	(51)
SN09-249	1.5	(3)	2.8	(47)	1.5	(16)	5.1	(82)	3.3	(90)	1.8	(5)
SN28-314	1.7	(4)	2.3	(16)	1.7	(12)	2.9	(55)	2.3	(15)	1.9	(55)
SN33-330	1.7	(5)	2.3	(65)	-		-		-		2.0	(56)
SN15-270	2.9	(89)	2.8	(54)	2.0	(78)	2.4	(9)	2.2	(29)	3.4	(89)
SN07-235	3.0	(90)	3.0	(75)	1.6	(27)	2.4	(6)	2.0	(5)	2.7	(49)
SN08-241	3.1	(91)	-		1.6	(23)	3.0	(56)	2.3	(48)	-	
SN09-247	3.2	(92)	2.6	(36)	1.8	(66)	2.5	(19)	2.2	(30)	3.0	(72)
SN29-312	3.3	(93)	2.9	(66)	1.7	(44)	2.5	(14)	2.1	(21)	2.4	(36)
N203	1.6		2.1		1.9		2.1		1.7		1.5	
Seafarer	2.4		3.6		3.0		4.1		3.0		4.0	
Mackinac	-		2.9		-		-		-		4.1	
Newport	-		3.4		-		-		-		3.1	
A300	2.9		-		3.5		-		3.5		-	
T39	2.6		3.6		2.9		-		2.9		3.0	
FR266	2.0		-		-		-		2.7		-	
WI RRR36	2.7		2.8		1.7		-		2.6		-	
Montcalm	5.3		-		5.6		5.2		5.4		-	
Isles	5.4		-		6.4		4.9		6.4		-	
Redhawk	3.8		-		6.3		-		6.3		-	
Foxfire	-		-		-		3.7		3.7		-	
Mean	2.3		2.7		2.9		2.7		2.4		2.7	
LSD (" = 0.05)	0.8		0.8		0.9		1.0		0.6		1.0	
CV (%)	20.3		20.3		21.0		23.8		20.2		23.8	

PM was not significant for SN nor was genetic variation significant for SNGH2. Location by genotype interactions were significant for the combined analyses of variance over two locations in both populations. Means, LSDs and CVs are presented in Tables 7 and 8 for all experiments conducted in both populations. In general, the mean score of MF was higher than the mean of SN, and MF had a wider distribution of scores than SN (Figure 4). The CVs remained relatively constant for each population and ranged from 14.9 to 25.6 % for MF (Table 7) and from 20.0 to 24.5 % for SN (Table 8). Analysis of MFGH1 resulted in a higher than expected CV than the rest of the MF tests because it comprised only two replications due to limitations on seed and was not chosen to establish ranked order.

Narrow sense h^2 was consistent and significantly different from zero for MF. Heritability estimates ranged from 0.48 to 0.71 suggesting a moderate to high heritability for root rot resistance in this population (Table 9). In five of the six experiments presented for MF, h^2 remained at approximately 50%. The one exception was the 1998 PI trial which resulted in h^2 of 0.71. The h^2 for SN varied with experiment and ranged from 0.09 to 0.66 (Table 9). As expected, h^2 significantly different from zero occurred only in those experiments which demonstrated significant genetic variation. Moderate to low heritability for this population was suggested by these results.

Representative frequency distributions are illustrated in Figure 4. Continuous variation was observed over the range of root rot scores for both populations but the maximum score for SN rarely exceeded a score of 4.0 although susceptible check genotypes scored as high as 6.0 (Table 8). Seafarer, the susceptible parent for this

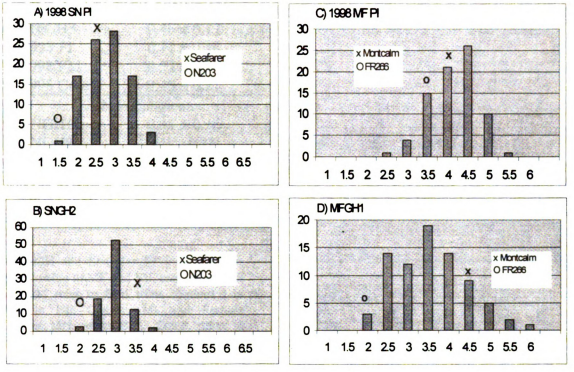

Figure 4. Representative distributions of root rot scores for Seafarer/N203 (SN) and Montcalm/FR266 populations (MF). A) Distribution of root rot scores for the 1998 SN experiment conducted in Presque Isle County, MI; B) Distribution of root rot scores for the second SN greenhouse evaluation (SNGH2); C) Distribution of root rot scores for the 1998 MF experiment conducted in PI; D) Distribution of root rot scores for the first MF greenhouse evaluation (MFGH1).

Table 9. Heritability estimates (h^2) and standard errors of Fusarium root rot ratings for the Montcalm/FR266 (MF) and Seafarer/N203 (SN) populations. Six greenhouse and field experiments were analyzed for each population.

Experiment†	h^2 and SE
MF Population	
MFGH1	0.53 ± 0.17
MFGH2	0.49 ± 0.17
MFGH3	0.48 ± 0.17
1998 PI	0.71 ± 0.18
1998 PM	0.52 ± 0.18
1998 Comb.	0.48 ± 0.21
SN Population	
SNGH1	0.48 ± 0.16
SNGH2	0.17 ±0.17
1997 PI	0.09 ± 0.16
1997 PM	0.22 ± 0.16
1997 Comb.	0.20 ± 0.17
1998 PI	0.66 ± 0.16

† MFGH and SNGH refer to greenhouse experiments in MF and SN, respectively; PI and PM refer to field trials conducted in Presque Isle County, MI and Perham County, MN, respectively; Comb. refers to the combined analysis over both PI and PM locations.

population, generally did not score above a 3.0. While this is a significantly higher score than that observed for N203, the range of values for SN was not large enough to achieve consistent genetic variation especially for experiments involving the $F_{2:3}$ lines. For purposes of this study, SN was not subsequently used for genetic analysis. MF distributions were much broader as evidenced by the significant genetic variation observed in all experiments involving this population (Table 6, Table 8 and Figure 4). FR266 scored from 2.0 to 4.5 whereas Montcalm scored from 1 to 2 points more than FR266 in all experiments. Transgressive segregation towards susceptibility was observed.

Correlations between greenhouse and field evaluations were positive and significant for all comparisons except between MFGH2 and 1998 PI and between MFGH2 and 1998 combined analysis (Table 10). Greenhouse trials were positively and significantly correlated with each other and correlation coefficients (r) ranged from 0.40 to 0.67. Data from individual field locations were also positively and significantly correlated with an r value of 0.41. Significant negative correlations between field ratings and days to flower (DTF) were observed as well as between MFGH1 and DTF. Data collected on days to maturity was not reported because severe drought conditions during the 1998 growing season prevented accurate determination of this measurement.

Marker evaluation

Of the 330 primers analyzed against the parents, 50% demonstrated at least one polymorphic band between both sets of parents. A total of 680 primers which included polymorphic primers identified in the preliminary parental screen were analyzed against

Table 10. Pearson rank correlation coefficients between means of Fusarium root rot ratings for 6 greenhouse and field experiments conducted using the Montcalm/FR266 population and days to flower (DTF).

	MFGH1	MFGH2	MFGH3	1998 PI	1998 PM	1998 Comb
DTF‡	-0.27*	-0.13	-0.11	-0.40**	-0.53***	-0.53***
MFGH1		0.40**	0.67***	0.20†	0.25*	0.22*
MFGH2			0.63***	0.12	0.19†	0.16
MFGH3				0.31**	0.19†	0.25*
1998 PI					0.41***	0.76***
1998 PM						0.85***

†,*, **; *** Significance at the P < 0.10, 0.05. 0.01, 0.001 levels, respectively.

‡ MFGH refers to greenhouse experiments in MF; PI and PM referred to field trials conducted in Presque Isle County, MI and Perham County, MN, respectively. Comb. refers to the combined analysis over both PI and PM locations.

Table 11. Linkage groups (LG) generated by MAPMAKER/EXP from 35 RAPDs screened against the Montcalm/FR266 population. Markers not presented were independent.

LG	RAPDs	Distance (cM)
1	$D3_{600}$* (B6)	11.2
	$P7_{1550}$	
2	$P7_{700}$* (B2)	19.4
	$P10_{1600}$	9.0
	$G6_{1100}$* (B2)	
3	$I18_{1800}$	21.6
	$I18_{1700}$* (B3)	
4	$AG2_{800}$	14.7
	$G17_{900}$	
5	$G3_{800}$	16.0
	$G3_{2000}$	1.4
	$P9_{1550}$	
6	$Y11_{600}$	1.4
	$O12_{800}$	
7	$S8_{500}$* (B3)	7.6
	$V12_{1100}$	

*Previously mapped RAPDs reported on the bean integrated map (Freyre et al., 1998). The number in parentheses corresponds to the linkage group on which these RAPDs reside.

R and S bulks in MF and 46 RAPDs polymorphic between bulks were analyzed against the entire population. Eleven were discarded due to skewed segregation ratios. The remaining 35 were analyzed by MAPMAKER/EXP to determine linkage relationships. Seven linkage groups resulted in none with more than three markers (Table 11). Five of the 35 RAPDs analyzed corresponded to markers previously reported on the *P. vulgaris* integrated linkage map (Freyre et al., 1998). Twenty-five of the markers identified and screened against MF significantly associated with at least one of the 6 experiments conducted on this population. Coefficients of determination (r^2) which reflect the amount of variation

explained by a given marker were low and ranged from 5 - 15% (Table 12). The three markers in linkage group (LG) 2 appeared to associate consistently with greenhouse ratings. RAPD $P7_{700}$ was also significantly associated with 1998 PM and the combined analysis. In this study, $P7_{700}$ and $G6_{1200}$ were 28.4 cM apart which confirms the linkage relationship between these two markers on linkage group B2 (Figure 5; Freyre et al. 1998). Located in the vicinity of these two markers is the locus for the pathogenicity-related protein, PvPR2, perhaps implicating a role for this protein in Fusarium root rot resistance. The alleles associated with lower root rot scores for all three of these markers originated from the FR266 parent.

A different set of markers, those from LG5, were consistently associated with field root rot ratings in MF and $G3_{2000}$ explained as much as 13 % of the variation for field root rot resistance (Table 12). No corresponding area representing these markers was found on the *P. vulgaris* linkage map. Alleles associated with reduced root rot scores for the markers in LG5 originated from Montcalm and one of the RAPDs, $G3_{800}$ was also associated with DTF. Generally, RAPDs were either significantly associated with greenhouse ratings or with field ratings and those associated with field ratings were also associated with DTF. This is expected considering the correlation observed between field ratings and DTF. $P7_{700}$ was an exception to this generalization since it was significantly associated with both greenhouse and field ratings and not with DTF (Table 12). $I18_{1700}$ on LG3 is located on chromosome B3 of the *P. vulgaris* map suggesting a position for both $I18_{1700}$ and $I18_{1800}$ on this chromosome (Figure 6). $I18_{1800}$ was positively and significantly associated with greenhouse and field ratings and DTF. $S8_{500}$ was also reported to be on

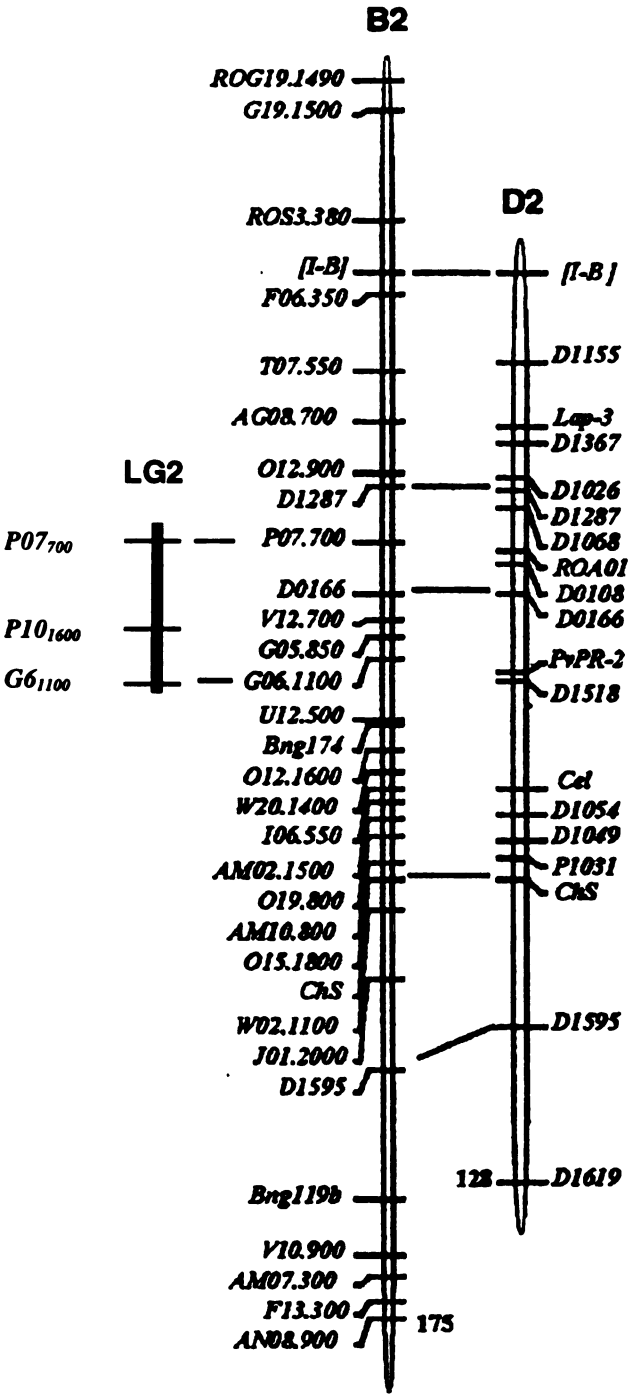

Figure 5. Comparative linkage maps of linkage group (LG) 2 and its corresponding position on linkage group B2 from the integrated bean linkage map and linkage group D2 from the Davis map. B2 and D2 are reproduced from Freyre et al. (1998). All markers on LG2 were signficantly associated with root rot resistance (Table 10).

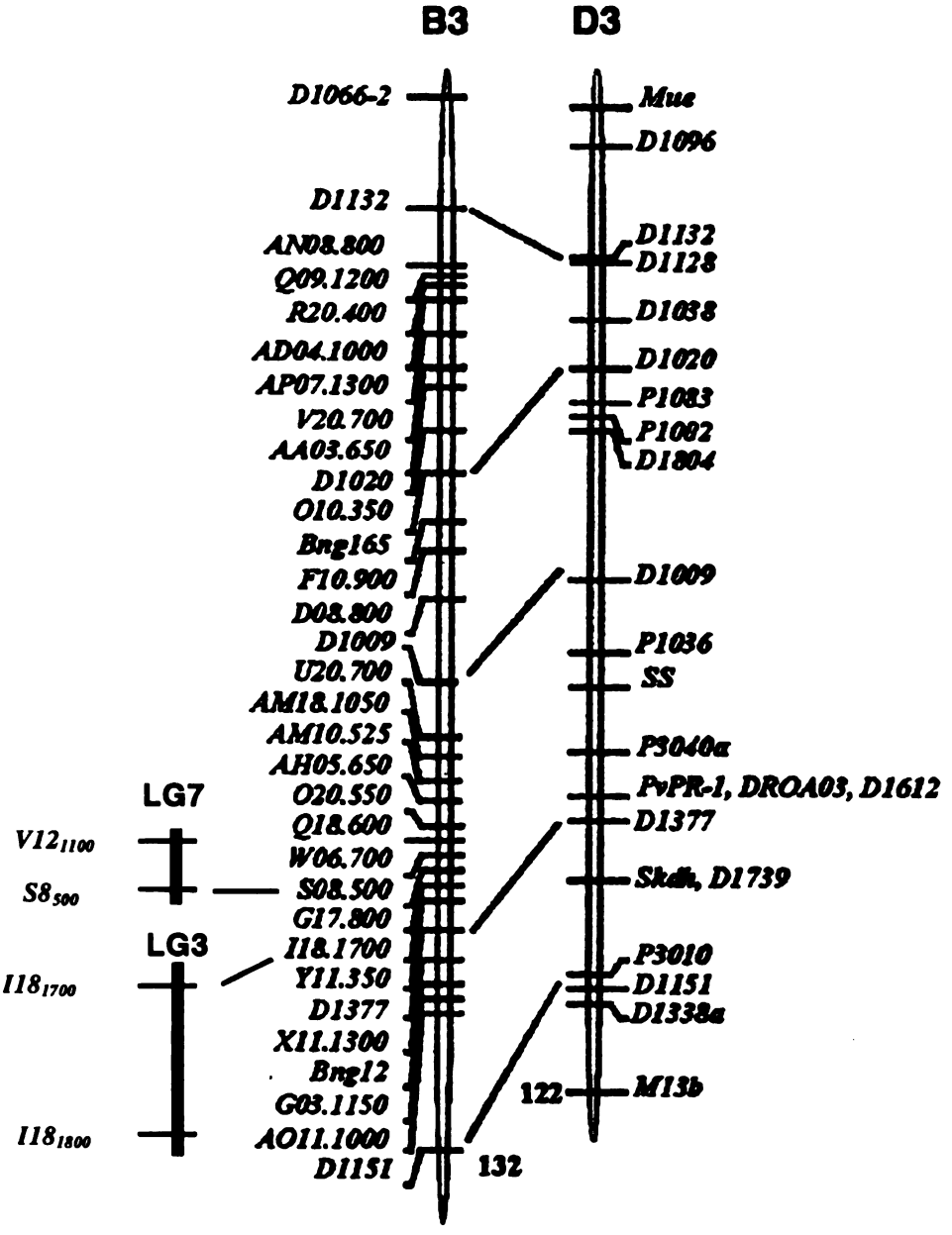

Figure 6. Comparative linkage maps of linkage groups (LG) 7 and 3 and their corresponding positions on linkage group B3 from the integrated linkage map and linkage group D3 from the Davis map. B3 and D3 are reproduced from Freyre et al. (1998). Markers $OPS8_{500}$ and $OPI18_{1800}$ were both significantly associated with root rot resistance (Table 10).

Table 12. Coefficients of determination (r^2) from one-way analyses of variance for a select group of RAPDs significantly associated with mean root rot scores of 79 Montcalm/FR266 F_4-derived RILs collected from three greenhouse experiments (MFGH1, MFGH2, MFGH3), two field trials located in Presque Isle County, MI (PI) and Perham County, MN (PM), and the combined analysis over locations. The linkage group (LG) is also given and corresponds to those linkage arrangements presented in Table 11. The r^2 for RAPDs significantly associated with days to flower (DTF) is also presented.

RAPD	LG	MFGH1	MFGH2	MFGH3	PI	PM	Comb	DTF
$P10_{1600}$	2	0.14***‡		0.09***				
$G6_{1100}$	2	0.11***	0.05*	0.04				
$P7_{700}$	2	0.10***	0.07*	0.10***		0.06*	0.05*	
$G3_{2000}$	6				0.11***	0.06*	0.13***	
$G3_{800}$	6				0.06*		0.05*	0.07*
$P9_{1550}$	6				0.11***	0.05*	0.13***	
$AG2_{800}$	5		0.05*					0.07**
$G17_{900}$	5					0.05*	0.07*	0.11***
$I18_{1800}$	3	0.04	0.07*	0.06*		0.04		0.06*
$S8_{500}$	7		0.05		0.07*	0.03	0.06*	0.08*

*, **; *** Significance at the P < 0.05. 0.01, 0.001 levels, respectively.

‡ Only r^2 values from significant associations are presented

chromosome B3 linked to $I18_{1700}$ (Freyre et al., 1998). Although these two markers were not linked in the present study, $S8_{500}$ was significantly associated with greenhouse and field ratings suggesting that this region of chromosome B3 may also influence Fusarium root rot resistance. In close linkage with $S8_{500}$ and $I18_{1700}$ on the integrated *P. vulgaris* map (Figure 6) is PvPR1 implicating another PR protein acting in response to Fusarium root rot. $S8_{500}$ was also significantly associated with DTF explaining 8% of the phenotypic variation for root rot resistance.

Multiple regression analyses using combinations of significant markers and their interactions revealed that epistatic interactions were not significant. Up to 14% and 29% of the phenotypic variation for greenhouse and field ratings, respectively, was explained by a set of markers including $G3_{2000}$, $G17_{900}$, $U12_{1500}$ and $P7_{700}$. Interestingly, this same set of markers explained 24% of the variation for DTF. When $U12_{1500}$ and $G17_{900}$ were eliminated because both were strongly associated with DTF, the remaining two markers $G3_{2000}$ and $P7_{700}$ accounted for 7 to 13% of the phenotypic variation for root rot ratings in both field and greenhouse with no significant association observed with DTF.

Primers G3, P7 and G6 were screened against the 68 F_2 individuals from IF. RAPDS $G6_{300}$ and $G3_{800}$ were linked at 7.6 cM. $G6_{300}$ was not a polymorphic loci in MF. $G3_{2000}$ was unscorable in IF, $G6_{1100}$ and $G3_{800}$ were not associated with $F_{4:5}$ root rot scores whereas $P7_{700}$ was significantly associated ($P < 0.05$) with root rot ratings for this population confirming previously reported associations for this marker.

DISCUSSION

The predominant constraint to realizing maximum yield potential in bean is disease (Kelly et al., 1998). Bean is a host to many bacterial, viral and fungal pathogens and is often used as a model system for studying host/pathogen interactions (Lamb et al., 1989). Genetic resistance to anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.), bean common mosaic virus and rust (*Uromyces appendiculatus* (Pers.:Pers.) Unger) are classic bean examples of Flor's (1956) gene-for-gene disease interaction model (Christ and Groth, 1982; Kelly and Miklas, 1998). All three of these diseases involve monogenic, vertical (VR) resistance for which the knowledge base is highly advanced. Germplasm sources are well characterized, the incorporation of single-gene resistance is common practice for disease control, tightly-linked molecular markers have been identified, gene-pyramiding for more durable resistance is being conducted, and cloning of resistance genes is underway (Rivkin et al., 1999; Creusot et al., 1999). The classification of genetic resistance into discrete qualitative units for many other complex bean diseases has not, however, been as forthcoming.

Vertical resistance is often criticized because of its numerous failures in the face of pathogen co-evolution (Parlevliet, 1977). A single disease resistance gene which confers immunity imposes an intense selection pressure on the pathogen in favor of novel avirulence genes which are not recognized by the host. Failure of monogenic resistance during a given growing season can have disastrous consequences. Complex horizontal resistance (HR), on the other hand, appears to be more durable. Vanderplank (1963) described HR as independent of pathogen genotype or pathotype in contrast to VR as resistance. Resistance of this type is usually characterized by a continuous distribution and

polygenic control. Horizontal resistance is generally manifested as a reduction in disease as opposed to complete immunity which can be the result of host resistance to infection, delay of infection, reduced development of pathogen after infection or reduced reproductive capacity of the pathogen (Simmonds, 1991).

Horizontal resistance is regarded as more stable than VR because the likelihood that a pathogen can defeat several resistance genes at once is minimal. Furthermore, the lack of immunity allows for pathogen reproduction in the absence of strong selective pressure (Simmonds, 1991). In practice, VR has remained a preferred form of resistance, when available, due to its relative genetic simplicity and ease of use but most breeders recognize the need for more stable, HR especially for diseases for which no VR has been documented.

The inability to classify root rot scores from either greenhouse or field trials into discrete categories suggests that root rot resistance in bean is under polygenic control and resistance should be treated as a quantitative trait (Figure 4). Consistently moderate h^2 over greenhouse and field experiments support the idea that improvement of genetic resistance to *F.s.* f.sp. *phaseoli* can be achieved but progress may be slow (Table 9). However, since HR is more durable than qualitative resistance, improving resistance to *F.s.* f.sp. *phaseoli* could, perhaps, provide protection against more than one pathogen if a general strengthening of host defense responses is involved (Simmonds, 1991).

The disease reaction of a Fusarium resistant genotype mirrors that of a susceptible genotype in symptomotology except that disease progress in the resistant genotype is substantially reduced. An incompatible reaction involving the hypersensitive response is not observed for resistant genotypes inoculated with this pathogen. Under severe disease

pressure, resistance can be overcome as evidenced by root rot ratings greater than 4.0 for FR266 (Table 7). Susceptible genotypes, however, scored consistently more than the resistant parents supporting the conclusion that rate of progress of disease is substantially reduced in the resistant genotypes and that environmental factors play a large role in disease severity (Table 7; Figure 4).

Fusarium solani f.sp. *phaseoli* infection elicits host defense responses which include the induction of phytoalexin biosynthesis, chitinase, ß,1-3 glucanase, PR proteins, phenylalanine ammonia lyase and the release of phenolic compounds (Morris and Smith, 1978; Mohr et al., 1998). The fungus, however, appears to tolerate these defense products and is known to possess enzymes for the detoxification of the phytoalexins, phaseollin and keivitone (Choi et al., 1987; Li et al., 1995). Although *F.s.* f.sp. *phaseoli* can metabolize keivitone, the fungus does not appear to induce the production of this particular phytoalexin or, alternatively, it may repress the biosynthesis of keivitone as a means for enhanced pathogenesis (Morris and Smith, 1978). Furthermore, it was demonstrated that the flavanone, naringenin, and the isoflavanone, genistein, and phytoalexins, medicarpin and maackiain, from chickpea (*Cicer arietinum* L.) stimulate spore germination of *F.s.* f.sp. *phaseoli*. Studies examining bean defense responses to *F.s.* f.sp. *phaseoli*, generally concern the response of cultivars highly susceptible to *F.s.* f.sp. *phaseoli* but do not document the responses of resistant genotypes. Clearly the interaction between host defenses and pathogen infection is complicated. Differences in the expression of genes involved in plant defense response may exist between genotypes and could possibly correspond to differing levels of resistance to *F.s.* f.sp. *phaseoli*.

In the current study, RAPD markers associated with Fusarium root rot resistance in bean were identified using a method of selective genotyping. Several regions and independent markers were identified which significantly associated with field and greenhouse root rot ratings but none explained over 15% of the phenotypic variation for this trait (Table 12). In multiple regression analyses, only 29% of the phenotypic variation could be accounted for by a subset of four markers. QTL analysis for resistance to CBB and bean golden mosaic virus (BGMV) revealed four and five QTL for BGMV and CBB, respectively (Miklas et al., 1996). A single QTL identified as associated with CBB in this study explained up to 60% of the variation for this trait while two QTL associated with BGMV accounted for as much as 60% of the variation for this resistance. In another study involving quantitative resistance to the soilborne pathogen, *Macrophomina phaseolina* (Tassi) Goidanich, the causal agent of ashy stem blight which infects bean roots and stems, Miklas et al (1999) identified RAPD markers associated with this trait that, individually, could account for no more than 19% of the phenotypic variation for resistance. Only three of the eight RAPDs significantly associated in the *Macrophomina* study were consistently significant across two locations and multiple regression analyses using four selected markers explained from 28 to 47 % of the phenotypic variation in this population. Soilborne pathogens appear to have a complex life cycle which is strongly influenced by environmental factors. The large amount of environmental variation observed for diseases caused by soilborne pathogens makes quantification difficult and could explain why QTL associated with these traits are so difficult to resolve.

In the current study, markers which were significantly associated with field root rot ratings were not significantly associated with greenhouse root rot ratings and vice versa.

Furthermore, those RAPDs associated with field ratings tended to be associated with DTF (Table 12). These results suggest independent mechanisms involved in resistance to Fusarium root rot between natural and artificial inoculations and that natural infections may reflect differences in maturity. Data from greenhouse experiments was generally significantly and positively associated with field trials for MF except for the non-significant but positive correlation between MFGH2 and 1998 PI trial (Table 10). In the previous chapter, the perlite greenhouse screen was successfully used to discriminate between resistant and susceptible individuals and should be indicative of physiological resistance. Data from repeated greenhouse screenings of MF were positively correlated among experiments and reflect segregation of physiological resistance in this population. The observation that the same QTL associated with greenhouse ratings were not associated with field experiments and vice versa is not surprising. Genetic variation for physiological resistance present in MF may have been masked by strong environmental factors present in field trials such that QTL associated in greenhouse trials could not be resolved from field data. Furthermore, environmental effects influencing disease development present in field trials were absent in greenhouse trials and QTL associated with these factors were, therefore, not identified.

An example of an environmental factor present in field trials and not in greenhouse is DTF. Greenhouse plants were rated for disease severity before flowering but field trial ratings were conducted post-bloom. The significant negative relationship between DTF and field root rot ratings could also correspond to differences in developmental stages at the time of rating (Table 8). All RILs were rated at the same time regardless of development. Thus, those genotypes which were more mature also had more disease. This

association between maturity and disease has been observed for other pathogens (Miklas et al., 1996; Pilet, 1998; Schechert et al., 1999) and has been reported previously for *F.s.* f.sp. *phaseoli* (Abawi, 1989). A negative association between root rot resistance and DTF is not unexpected, however, especially when considering genotypes with determinate growth habit. In these genotypes, vegetative growth ceases at flowering and the plant partitions its resources to the developing seed resulting in less available energy for secondary processes like host defense. Furthermore, subsequent root decay may tend to aggravate the pathogen through the release of nitrogenous compounds which can stimulate aggressiveness of the fungus (Toussoun and Patrick, 1970). Considering the negative correlation between DTF and root rot resistance, breeders intending to improve Fusarium root rot resistance would benefit from selection methods like greenhouse screens and marker assisted selection that avoid concomitantly increasing DTF and associated late maturity.

Two linkage groups (LG2 and LG5) for which all markers were consistently associated with either greenhouse or field ratings were identified. LG2 containing $P7_{700}$, $P10_{1600}$ and $G6_{1100}$ were all significantly associated with root rot in at least one of the greenhouse trials conducted and P_{700} was also significantly associated with root rot ratings in the 1998 PM trial and combined analysis (Table 12). Markers on LG5 were all positively and significantly associated with field root rot ratings but not greenhouse ratings. P_{700} and $G6_{1100}$ from LG2 are located on chromosome B2 (Figure 5) and span a region that encompasses the PvPR2 locus suggesting a role for this PR protein in Fusarium root rot resistance. PvPR2 and its counterpart PvPR1 are low molecular weight acidic proteins induced during fungal elicitation (Walter et al., 1990). These bean PR

proteins share similarities with PR proteins in pea, potato and parsley. The role of PvPR proteins is further confirmed by the significant association observed between S_{800} from LG7 and $I18_{1800}$ of LG3 which map to chromosome B3 in the region of PvPR1 (Figure 6). Differences in PvPR gene arrangements were detected between anthracnose resistant and susceptible genotypes indicating that polymorphism between PvPR as well as other defense response-related genes may contribute to our understanding of HR (Walter et al., 1990). QTL associated with resistance to the late blight fungus (*Phytophthora infestans* (Mont.) de Bary) of potato (*Solanum tuberosum* L.)) have also been reported to associate with two potato PR proteins (Gephardt et al., 1991). To capitalize on the assumption that defense proteins may be associated with quantitative resistance, a method of candidate gene analysis, whereby genes known to be involved in host defense response are used as markers to identify potential QTL associated with disease resistance, has been applied in other crops and could be employed for Fusarium root rot resistance in bean (Goldman et al., 1993; Causse et al., 1995; Byrne et al., 1996; Faris et al., 1999).

RAPDs $G3_{2000}$ and $P7_{700}$ were used in a multiple regression analyses and demonstrated significant associations with all greenhouse and field trials explaining between 7 to 19% of the genetic variation for root rot scores in MF. Selection based on these markers will be useful but not sufficient to improve Fusarium root rot resistance. Neither of these markers were significantly associated with DTF. Analysis of IF F_2 DNA and root rot data based on corresponding $F_{4:5}$ RIL indicated that P_{700} was significantly associated with greenhouse root rot scores in this population confirming the association observed in MF. $G6_{1100}$, however, was not significantly associated with root rot ratings. $G3_{2000}$ was unscorable in this population and $G3_{800}$ was not significantly associated root

rot ratings. This is not surprising since $G3_{800}$ was only associated with field ratings and not with greenhouse ratings. The IF population was constructed as a means to verify marker associations in a population other than the one in which the marker was identified. DNA from F_2 individuals of IF was used to confirm the effectiveness of early generation selection based on these markers. The observation that the P_{700} allele associated with improved root rot resistance was also present in N203 and not in Seafarer, further supports the usefulness of this marker. These results indicate that P_{700} will be a useful marker for early generation selection for root rot resistance when using FR266 as a resistance source.

One-way analyses of variance using $P7_{700}$ as marker genotype for analysis against SN root rot data failed to identify significant associations. The lack of genetic variation and adequate score distribution in this population prevented the identification of significant differences between marker genotype. These results however do not preclude the usefulness of this marker for selection in populations derived from N203 provided that polymorphism between the two parents of a given population is present. The insignificant associations for $P7_{700}$ in SN can be attributed to higher levels of resistance in Seafarer than was expected which ultimately contributed to the lack of adquate distribution of root rot scores in this population.

Fusarium root rot in bean involves a complex interaction between host, pathogen and environment. We have concluded that resistance to this disease should be considered quantitative and approaches which can reduce environmental variation, like greenhouse screening and marker assisted selection, may accelerate progress towards the incorporation of enhanced physiological resistance. The consistently moderate h^2 which

was observed for root rot resistance in MF indicates that progress made by conventional selection will be limited (Table 9). Heritability estimates reflect the proportion of the total phenotypic variation of a population that is inherited. An h^2 of approximately 0.50, like that observed for MF, suggests that even for greenhouse evaluations a large proportion of the phenotypic variation is due to environment. It might be expected, however, that h^2 derived from greenhouse experimental data should be substantially higher than field h^2 for a given population since the objective for developing greenhouse evaluations was to reduce environmental variation. But, in addition to reducing environmental variation, greenhouse screening also reduced the total amount of genetic variation by eliminating the contribution made by other traits like DTF and stress tolerance to the observed levels of field resistance.

Marker assisted selection using P_{700} and $G3_{2000}$ combined with later generation conventional selection has the potential to enhance progress towards incorporating Fusarium root rot resistance in dark red kidney bean genotypes. Specific applications for the use of markers in improving Fusarium root rot resistance in the dark red kidney market class are presented. The introgression of Fusarium root rot resistance into large-seeded kidney germplasm will necessarily involve the utilization of resistance sources from other market classes. As such, agronomically acceptable dark red kidney genotypes possessing root rot resistance will not be achieved through single-cross breeding approaches. Systems involving recurrent selection and backcross breeding are the preferred alternatives for the improvement of this trait. Utilizing markers and greenhouse seedling evaluations to facilitate selection provides an opportunity for the breeder to make educated decisions about which progeny should be intermated or backcrossed before crosses are performed.

This additional information can save the breeder valuable time and resources. Ideally, selection for progeny to be involved in subsequent backcrosses should be selected prior to flowering to ensure that superior genotypes are included. In the case of quantitative traits like Fusarium root rot resistance, phenotypic selection, performed on a single plant basis, is not reliable. In the absence of accurate selection, backcross progeny are essentially selected at random which may substantially delay progress. The RAPD markers associated with root rot resistance identified in this study provide a means to genotypically select for Fusarium resistant progeny that could then be utilized in subsequent backcrosses. The added information afforded by these markers increases the likelihood that a desirable dark red kidney genotype possessing root rot resistance can be achieved. Resistance levels of inbred backcross progeny selected based on marker genotype can then be confirmed using greenhouse evaluations before the line is advanced to costly and laborious field trials.

Alternatively, RAPD markers associated with QTL controlling Fusarium root rot resistance can be used to select progeny possessing complementary QTL for intermating. A genotype possessing the $P7_{700}$ allele can be recombined with another genotype possessing the $G3_{2000}$ allele with the ultimate goal of combining these two resistance QTL into a single genotype that will possess higher levels of resistance than either genotype alone. The markers identified in this chapter that were associated with Fusarium root rot resistance, individually, explained only a small amount of variation for this trait. The usefulness of marker assisted selection involving these markers as a substitute for conventional selection approaches is, therefore, questionable. However, these markers can be utilized to enhance conventional breeding approaches by providing information that the

breeder can use to make educated decisions and choices of putatively linked resistance loci.

One of the initial intentions for this study was to classify the resistance present in N203 using an intra gene pool cross. The SN population was abandoned because consistent significant genetic variation for root rot resistance was not observed. It appears that Seafarer is not as susceptible as was previously thought and that small-seeded genotypes, as a whole, are not as susceptible as the large-seeded kidney beans. An overemphasis on the improvement of quality traits in the kidney and snap market classes may be responsible for the absence of genetic resistance to *F.s.* f.sp. *phaseoli.* These factors are compounded compounded by the observation that genetic diversity, in general, is lacking within the cultivated Andean germplasm (Becerra Velasquez and Gepts, 1994; Sonnante et al., 1994). Alternatively, differences in host defense responses between gene pools that have not been documented may provide an explanation for the high degree of susceptibility to Fusarium root rot observed in large-seeded germplasm.

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APPENDIX A

APPENDIX A

GREENHOUSE PROTOCOL

Table 13. A comparison of mean root rot scores of control treatment (inoculated with deionized water) and 5 different isolates isolated from Presque Isle County, MI (F.s. 12, Hawks 2a, 2b and 6d) and Huron County, MI (Huron 2a) inoculated on to three bean genotypes possessing differing levels of resistance.

Isolate	Root Rot Score
Montcalm (S)	
Control	1.7
F.s. 12	4.6
Hawks 2a	3.5
Hawks 2b	5.2
Hawks 6d	4.6
Huron 2a	4.4
N203 (R)	
Control	1.5
F.s. 12	2.1
Hawks 2a	1.4
Hawks 2b	1.5
Hawks 6d	1.9
Huron 2a	1.9
Seafarer (I)	
Control	1.1
F.s. 12	3.2
Hawks 2a	1.8
Hawks 2b	2.6
Hawks 6d	2.7
Huron 2a	2.9
Mean	2.7
LSD (0.05)	0.3
CV (%)	13.2

†S, susceptible; R, resistant; I, intermediate.

Table 14. Analysis of variance including degrees of freedom (DF), sums of squares (SS) and error mean square (EMS) for the 24 entries planted in the 1996 Presque Isle County, MI root rot trial.

Source	DF	SS	EMS	F Value
		---- Yield ----		
Rep	3	302700.545	100900.182	
Genotype	21	9147338.847	435587.564	4.51***
Error	61	5889042.832	96541.686	
		---- 100 Seed Weight ----		
Rep	3	45.219	15.073	
Genotype	21	21754.575	1035.93	174.24***
Error	61	362.675	5.945	
	---- Root Rot Rating 51 Days After Planting----			
Rep	3	1.751	0.584	
Genotype	23	75.122	3.266	6.29***
Error	69	35.816	0.519	
	---- Root Rot Rating 70 Days After Planting----			
Rep	3	0.090	0.030	
Genotype	26	201.348	8.754	23.45***
Error	69	25.756	0.373	
		---- Greenhouse Screen ----		
Rep	2	3.221	1.610	
Genotype	23	41.827	1.801	6.88***
Error	45	11.895	0.264	

*, **; *** Significance at the $P < 0.05$. 0.01, 0.001 levels, respectively.

Table 15. Analysis of variance including degrees of freedom (DF), sums of squares (SS) and error mean square (EMS) for the twenty-four entries planted in the 1997 Presque Isle County, MI root rot trial.

Source	DF	SS	EMS	F Value
		---- Yield ----†		
Rep	1	1432642.942	1432642.942	
Genotype	20	5185207.627	259260.381	3.11**
Error	20	1667050.924	83352.546	
		---- 100 Seed Weight ----		
Rep	1	3.149	3.149	
Genotype	20	11805.969	590.298	512.72***
Error	20	23.023	1.151	
		---- Root Rot Rating 52 Days After Planting----		
Rep	2	17.548	8.774	
Genotype	20	36.950	1.848	2.98**
Error	62	24.825	0.621	
		---- Root Rot Rating 86 Days After Planting----		
Rep	2	2.561	1.281	
Genotype	20	81.811	4.091	9.14***
Error	62	17.892	0.447	
		---- Greenhouse Screen 1----		
Rep	2	8.263	4.132	
Genotype	20	53.977	2.699	10.48***
Error	40	10.303	0.258	
		---- Greenhouse Screen 2----		
Rep	2	10.730	5.365	
Genotype	20	17.153	0.858	2.56**
Error	40	41.279	0.335	

*, **; *** Significance at the $P < 0.05$. 0.01, 0.001 levels, respectively.

†The third replication's measurements for yield and 100 seed weight were abandoned due to extreme weed pressure.

APPENDIX B

APPENDIX B

GENETICS AND MARKER ANALYSIS

Table 16. Analyses of variance including degrees of freedom (DF), sums of squares (SS), error mean square (EMS), F-value, experimental mean, LSD ($\alpha < 0.05$) and coefficient of variation (CV) for indivdiual field ratings performed on the Montcalm/FR266 (MF) population in Presque Isle County, MI and Perham County, MN in 1998. The analyses of variance combined over locations and calculated for all greenhouse experiments are presented in Table 6.

Source	DF	SS	EMS	F Value
	Perham County, MN, 1998			
Rep	2	0.719	0.360	
Genotype	77	244.060	3.170	1.92***
Rep*Genotype	144	237.951	1.652	1.87***
Error	693	613.857	0.886	
Mean, CV, LSD	5.44	17.3	1.1	
	Presque Isle County, MI, 1998			
Rep	3	127.233	42.410	
Genotype	78	273.419	3.506	2.97***
Rep*Genotype	201	237.242	1.180	3.07***
Error	1030	395.916	0.384	
Mean, CV, LSD	4.16	14.9	1.0	

Table 17. Analyses of variance including degrees of freedom (DF), sums of squares (SS), error mean square (EMS), F-value, experimental mean, LSD ($\alpha < 0.05$) and coefficient of variation (CV) for each of the two ratings (RR1 and RR2) performed on the Seafarer/N203 (SN) population in Presque Isle County, MI and Perham County, MN in 1997. The analyses of variance combined over locations and calculated for all greenhouse experiments are presented in Table 6.

Source	DF	SS	EMS	F Value
	---- Presque Isle County, MI (RR1) ----†			
Rep	3	16.150	5.383	
Genotype	100	37.319	0.373	1.10
Error	298	101.405	0.340	
Mean, CV, LSD	1.8	33.2	0.5	0.8
	---- Presque Isle County, MI (RR2) ----†			
Rep	3	3.650	1.217	
Genotype	100	20.140	0.201	0.81
Error	298	73.221	0.247	
Mean, CV, LSD	1.7	29.9	0.7	
	---- Perham County, MN (RR1) ----			
Rep	2	24.518	12.259	
Genotype	93	63.479	0.683	1.29
Error	186	98.149	0.528	
Mean, CV, LSD	3.1	23.2	1.2	
	----1997 Perham County, MN (RR2) ----			
Rep	2	10.020	5.010	
Genotype	93	126.459	1.360	3.26***
Error	186	77.472	0.417	
Mean, CV, LSD	2.6	24.6	1.0	

*, **; *** Significance at the $P < 0.05$. 0.01, 0.001 levels, respectively.

Table 18. Probability, coefficient of deterimination (r^2), number of individuals (n), mean of the group of individuals lacking the RAPD (X1) and mean of the group of individuals possessing the RAPD band (X2) for all significant associations determined using one-way analyses of variance against root rot data collected from three greenhouse evaluations (MFGH1, MFGH2, MFGH3), two field trials conducted in Presque Isle County, MI (PI98) and Perham County, MN (PM98), the combined analysis over these two locations (C98), days to flower (DTF) and days to maturity (MAT).

RAPD		MFGH1	MFGH2	MFGH3	PI98	PM98	C98	DTF	MAT
$AG2_{800}$†	P<			0.0662				0.0159	
	r^2			0.4674				0.0790	
	n			0.0467				73	
	X1			3.00				40.7	
	X2			3.15				42.4	
AX11	P<	0.0124		0.0087					
	r^2	0.0837		0.0929					
	n	74		73					
	X1	3.74		3.00					
	X2	3.82		3.23					
$BC16_{700}$	P<	0.0138	0.0117						
	r^2	0.0791	0.0828						
	n	75	75						
	X1	3.8	4.0						
	X2	3.3	3.7						
$BC16_{800}$	P<							0.0455	
	r^2							0.0537	
	n							74	
	X1							41	
	X2							42	
$BE4_{600}$	P<			0.0526				0.0476	
	r^2			0.0533				0.0557	
	n			70				70	
	X1			3.00				42	
	X2			3.200				41	

Table 15. (cont'd).

RAPD		MFGH1	MFGH2	MFGH3	PI98	PM98	C98	DTF	MAT
$G17_{900}$	P<					0.0474	0.0243	0.0030	
	r^2					0.0514	0.0650	0.1102	
	n					77	78	78	
	X1					5.57	4.88	40.4	
	X2					5.33	4.61	42.5	
$G19_{1800}$	P<			0.0143		0.0320	0.0894	0.0089	
	r^2			0.0763		0.0598	0.0375	0.0901	
	n			78		77	78	75	
	X1			40.7		5.59	4.83		
	X2			42.4		5.31	4.64		
$G3_{2000}$	P<				0.0026	0.0260	0.0010		0.0162
	r^2				0.1128	0.0644	0.1342		0.074
	n				78	77	78		78
	X1				3.75	5.30	4.55		110.8
	X2				4.12	5.59	4.93		105.8
$G3_{800}$	P<				0.0337		0.0410	0.0197	0.0509
	r^2				0.0580		0.0538	0.0695	0.0492
	n				78		78	78	78
	X1				3.81		4.63	42.3	110.2
	X2				4.08		4.87	40.7	106.1
$G5_{1100}$	P<				0.0863				
	r^2				0.0392				
	n				76				
	X1				4.07				
	X2				3.85				
$G5_{450}$	P<					0.0878	0.0252	0.0543	
	r^2					0.0389	0.0651	0.0485	
	n					76	77	42.2	
	X1					5.34	4.61	40.8	
	X2					5.57	4.87		

Table 15. (cont'd).

RAPD		MFGH1	MFGH2	MFGH3	PI98	PM98	C98	DTF	MAT
$G5_{900}$	P<				0.0069	0.0676	0.0061		
	r^2				0.0932	0.0444	0.9590		
	n				77	76	76		
	X1				4.11	5.57	4.89		
	X2				3.76	5.33	4.58		
$G6_{1200}$	P<	0.0138	0.0033	0.0496					
	r^2	0.0771	0.1081	0.0504					
	n	78	78	77					
	X1	3.16	3.61	3.01					
	X2	3.65	3.94	3.18					
$G8_{1400}$	P<		0.0125						
	r^2		0.1118						
	n		76						
	X1		3.65						
	X2		3.94						
$I18_{1700}$	P<		0.0761						
	r^2		0.04134						
	n		77						
	X1		3.68						
	X2		3.88						
$I18_{1800}$	P<	0.0609	0.0178	0.0423		0.0823		0.0352	
	r^2	0.0461	0.0726	0.0547		0.0408		0.0586	
	n	77	77	76		75		76	
	X1	3.24	3.66	3.01		5.35		42.2	
	X2	3.62	3.93	3.2		5.59		40.7	
$O12_{1800}$	P<					0.0892		0.0811	
	r^2					0.0390		0.0405	
	n					75		76	
	X1					5.54		41.0	
	X2					5.31		42.3	

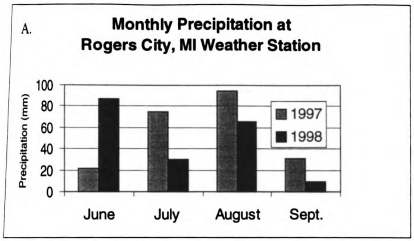
Table 15. (cont'd).

RAPD		MFGH1	MFGH2	MFGH3	PI98	PM98	C98	DTF	MAT
$O12_{800}$	P<			0.0722				0.0020	
	r^2			0.0425				0.1200	
	n			77				77	
	X1			3.00				42.8	
	X2			3.17				40.6	
$P10_{1600}$	P<	0.0210	0.0005						
	r^2	0.0673	0.1469						
	n	79	79						
	X1	3.21	3.63						
	X2	3.68	4.02						
$P7_{700}$	P<	0.0378	0.0057	0.0171		0.0402	0.0569		
	r^2	0.0555	0.0962	0.0735		0.0556	0.0475		
	n	78	78	77		76	77		
	X1	3.20	3.65	3.00		5.33	4.63		
	X2	3.62	3.96	3.21		5.60	4.86		
$P9_{1550}$	P<				0.0032	0.0528	0.0018		0.0484
	r^2				0.1113	0.0504	0.1247		0.0516
	n				76	75	76		76
	X1				3.73	5.31	4.55		110.4
	X2				4.10	5.56	4.90		106.2
$S8_{500}$	P<		0.0634		0.0177	0.0910	0.0251	0.0146	
	r^2		0.0446		0.0727	0.0381	0.0651	0.0770	
	n		78		77	76	77		
	X1		3.68		3.78	5.33	4.60	42.4	
	X2		3.88		4.08	5.57	4.86	40.8	
$T1_{1700}$	P<		0.0908		0.0053		0.0623	0.0246	0.0452
	r^2		0.0367		0.0979		0.0450	0.0647	0.0518
	n		79		78		78	78	78
	X1		3.90		4.15		4.87	40.6	105.7
	X2		3.71		3.80		4.65	42.1	110.0

Table 15. (cont'd).

RAPD		MFGH1	MFGH2	MFGH3	PI98	PM98	C98	DTF	MAT
$U12_{1500}$	P<				0.0231	0.0461	0.0233	0.0017	
	r^2				0.0660	0.520	0.0659	0.1223	
	n				78	77	78	78	
	X1				4.06	5.56	4.84	40.6	
	X2				3.77	5.29	4.58	42.8	
$Y11_{350}$	P<				0.0922				
	r^2				77				
	n				0.374				
	X1				77				
	X2				4.06				
					3.85				
$Y11_{600}$	P<							0.0006	
	r^2							.01467	
	n							77	
	X1							42.9	
	X2							40.6	
$Z4_{550}$	P<				0.0007		0.0056	0.0101	
	r^2				0.1424		0.0978	0.0850	
	n				77		77	77	
	X1				4.14		4.89	40.7	
	X2				3.71		4.57	42.4	

† RAPD markers which were analyzed against the MF population but did not result in significant associations were the following: $AD17_{1100}$, $AT3_{900}$*, $D3_{600}$, $E19_{900}$*, $G17_{1300}$, $G19_{1500}$*, $G5_{500}$*, $G5_{600}$*, $I18_{500}$, $O12_{1800}$, $P7_{350}$, $P9_{450}$*, $P9_{800}$*, $S18_{450}$, $S18_{700}$, $U12_{1600}$*, $V12_{1100}$, $V12_{400}$*, $V12_{700}$*, $X11_{900}$*. Those RAPDs marked with a "*" were discarded because of skewed segregation ratios.



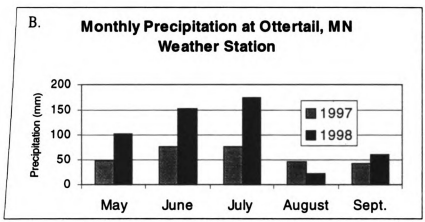


Figure 7. Monthly precipitation totals for the weather stations in A. Presque Isle County, MI and B. Perham County, MN for 1997 and 1998.