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GENETIC DISSECTION OF RESISTANCE TO WHITE MOLD
(SCLEROTINIA SCLEROTIORUM) IN COMMON BEAN (PHASEOLUS VULGARIS)

presented by

JUDITH MARIE KOLKMAN

has been accepted towards fulfillment
of the requirements for

DOCTOR OF PHILOSOPHY degree in Plant Breeding and
Genetics Program
Department of Crop and
Soil Sciences

James D. Kelly
Major professor

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GENETIC DISSECTION OF RESISTANCE TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*)

By

Judith Marie Kolkman

A DISSERTATION

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ABSTRACT

GENETIC DISSECTION OF RESISTANCE TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*)

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Judith Marie Kolkman

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary, is a destructive yield-limiting fungal disease of common bean (*Phaseolus vulgaris* L.). Breeding for resistance in bean offers a stable, long-term strategy to reduce yield loss to white mold in common bean. The objectives of this study were to i) determine if oxalate, a primary pathogenicity factor of *S. sclerotiorum*, could be used to indirectly screen for physiological resistance to white mold in bean, ii) study the inheritance of resistance to white mold, iii) dissect the relationship between agronomic avoidance mechanisms and physiological resistance in three populations, and iv) identify markers linked to quantitative trait loci (QTLs) conferring resistance to white mold in bean, using selective multivariate genotyping based on single and multiple phenotypic traits.

An indirect greenhouse test for physiological resistance to white mold was developed by evaluating for host resistance to oxalate (OR). Cut bean seedlings were placed in a 20 mM oxalate solution in the greenhouse, and rated based on differences in wilting response. Resistance to oxalate in 27 elite genotypes was correlated to field ratings of white mold disease severity index (DSI; r = 0.58**) and disease incidence (DI; r = 0.57**), and negatively correlated to yield (r = - 0.50**). New exotic sources of resistance to white mold were identified using the oxalate test.

Two genetic populations segregating for resistance, and one advanced line population, were evaluated for OR, DSI, DI, and agronomic avoidance traits in multiple environments. Heritability estimates were moderate for DSI (0.49) and DI (0.42), and low for OR (0.19) in a 98-entry Bunsi/Newport (BN) population; heritability estimates were higher for DSI (0.82) and DI (0.76) and moderate for OR (0.54) in a 28-entry Huron/Newport (HN) population. Resistance to oxalate was significantly correlated to DSI and DI in an advanced line population of 27 entries but not in the BN or HN populations. Different agronomic avoidance mechanisms were correlated to DSI and DI in each of the genetic populations that were not significant factors in the advanced line population.

The BN population was evaluated for markers linked to QTLs for resistance to white mold in the field and greenhouse. Markers were identified using selective multivariate genotyping comprised of DNA bulking strategies using genotypes from the extreme phenotypes for single and multiple traits. Markers linked to QTLs for resistance to *S. sclerotiorum* were identified in each of the DNA bulked screening methods, and were consistent across field environments and populations. In the BN population, the most significant marker on linkage group B2 was associated with DSI (12%) and DI (13%), while individual markers on linkage group B7 were associated with OR (9%), DSI (17%), DI (13%), and yield (37%). A unique locus for determinate growth habit in navy bean was located on B7. In the HN population, one marker on B2 was also associated with DSI (40.3%) and DI (35.4%), while one marker on B7 was associated with OR (24.3%) and yield (47.0%). Markers identified in this study will be used in marker-aided breeding for resistance to white mold in common bean.

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INTRODUCTION

White mold, caused by the ascomycete, *Sclerotinia sclerotiorum* (Lib.) De Bary, is a destructive yield-limiting fungal disease that seriously affects common bean (*Phaseolus vulgaris* L.) production in temperate regions (Steadman, 1983; Haas and Bolwyn, 1972, Purdy, 1979; Wallen and Sutton, 1967). *S. sclerotiorum* has a wide host range of over 400 plant species, including many other important crop species, such as canola *(Brassica napus* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.), soybean [*Glycine max* (L.) Merrill] and peanut (*Arachis hypogaea* L.; Boland and Hall, 1994). White mold epidemics in common bean occur during seasons of high yield potential. Total seed yield is reduced due to lower number of seeds produced per plant, reduced number of pods per plant and smaller seed size (Kerr et al., 1978; Steadman, 1979). In a recent survey of Michigan dry bean production, 64% of the 80 respondents indicated that white mold was the number one disease problem, followed by root rot (23%), bacterial blight (17%), and anthracnose (10%) (DiFonzo, 2000).

White mold infections are initiated during and after flowering, when canopy closure produces microclimate conditions that stimulate germination of the stipe and development of apothecia from soil-borne sclerotial bodies. Microclimate conditions that influence development of apothecia include 20 to 25 °C temperatures and an extended period of soil and leaf wetness (Abawi and Grogan, 1975; Boland and Hall, 1987a; Grogan and Abawi, 1975; Weiss et al., 1980a; Weiss et al., 1980b). Ascospores disperse into the plant canopy and initially require a carbon and nitrogen nutrient source, such as senescent flower blossoms, for germination. Senescent flowers have been identified as

the primary source of infection in common bean (Natti, 1971; Tu; Abawi et al., 1975; Haas and Bolwyn, 1972 Hunter et al., 1978; Cook et al., 1975). Ascospores cannot infect healthy green leaf tissue (Sutton and Deverall, 1983; Tariq and Jeffries, 1984), but will infect senescent leaf tissue (Purdy, 1958). Secondary infections involve the direct infection of mycelial growth (myceliogenic germination) from sclerotial bodies onto senescent leaf tissue (Tu, 1989a). Less than 10% of the senescent bean flowers identified on the ground, however, had been infected by mycelium produced directly from sclerotial bodies (Cook et al., 1975), indicating that secondary infections in bean is much less significant than flower blossom infections.

Developing mycelia proceed to infect the plant by exuding copious amounts of oxalic acid into the plant tissue (Maxwell and Lumsden, 1970). Oxalate chelates calcium from the pectate fraction of the xylem and associated pit vessels (Sperry and Tyree, 1988), causing the entry of air leading to xylem embolism and subsequent wilting. Oxalate was identified as a primary mode of pathogenesis for *S. sclerotiorum* (Godoy et al., 1990). Non-oxalate producing mutants of *S. sclerotiorum* were not able to infect bean leaf tissue, whereas mutant isolates that reverted to normal oxalate-production regained the ability to infect the bean leaf. The exuded oxalate provides an optimal pH for function of the polygalacturonases produced by the pathogen during the infection process (Marciano et al., 1983; Lumsden, 1976). Endo-polygalacturonase was found to be abundant within 24 hours of infection of bean leaves and was associated with the advancing margins of young lesions. It was suggested that the endo-polygalacturonase contributes to the hydrolysis of the middle lamellae of bean cells (Lumsden, 1976). In apple tissue infected with *S. sclerotiorum*, this effect was identified one to two cells in

advance of the hyphae. Pectin methylesterase was also detected early in infection in advancing hyphae. The invading mycelium may play an important role by making substrate more available for the polygalacturonases. Exo-polygalacturonase, associated with the growth of the fungus, appears at lesion margins after 48 hour, and was associated with mycelial dry weight (Lumsden, 1976).

Populations of *S. sclerotiorum* are predominately clonal. Mutation, and occasional genetic exchange and recombination provide new sources for variability (Kohli and Kohn, 1998). Sixty-four isolates of *S. sclerotiorum*, originating from 17 different dicot host species in seven different countries, were evaluated for virulence using a leaf assay, and for genetic diversity using random amplified polymorphic DNA (RAPD). Eighty-nine percent of the isolates were statistically similar in virulence, and RAPD marker data did not differentiate intraspecific virulence variation (Steadman et al., 1999). RAPD and virulence data failed to differentiate isolates from different hosts, or different geographic origin. Variation in virulence found between isolates has been contributed to oxalate secretion (Dutton and Evans, 1996). Hypovirulence found in *S. sclerotiorum* was associated with the presence of double-stranded RNAs, and due to reduced or delayed production of oxalic acid (Zhou and Boland, 1999).

Symptoms of white mold on bean plant include wilting, lesions, bleached stems, and the presence of sclerotial bodies. The fungal mycelium proceeds to spread throughout the plant and produces hardened sclerotial bodies, which are tightly packed mycelial bodies. The sclerotial bodies are returned to the soil in the crop residue and provide the inoculum source for the next season. In a three year period, sclerotial bodies that were planted at 5, 12.5, and 20 cm depths were able to produce apothecia in culture ensuring

longevity of inoculum in a three year crop rotation (Cook et al., 1975).

Agronomic management practices that result in reduced production of apothecia, less exposure to inoculum, or less development of disease, were found to contribute to reduced white mold in the field environment. A decrease in plant row width, resulting in higher plant density increased the levels of white mold in common bean (Park, 1993; Steadman et al., 1973). The relationship between high-yielding environments and reduced white mold potential is complex. Agronomic management practices that aim to maximize yield potential, typically provide optimal conditions for white mold infection. Higher yields have been associated with low to moderate levels of white mold infection. Heavy white mold pressure, however, has been found to severely reduce yield potential (Kerr et al., 1978). Optimizing yield often involves the use of overhead irrigation. Irrigation increases canopy density, thereby increasing soil surface moisture, leaf wetness, and humidity within the microclimate (Weiss et al., 1980a; Blad et al., 1978).

Microorganisms have been studied as biological agents to control sclerotial producing pathogens. *Coniothyrium minitans* Campbell is one example of a hyperparasite that has been investigated for control of sclerotinia wilt in sunflower, caused by *S. sclerotiorum* (Huang, 1992). The direct application of *C. minitans* into the soil furrow of naturally infested field sites was found to reduce the incidence of sclerotinia wilt in sunflower by 42 to 56% (Huang, 1980). In a monoculture of a susceptible crop, such as sunflower, white mold incidence was found to decrease, due to the presence of this parasitic fungus.

Protection of flowers and competition for nutrients on flowers by micro-organisms has also been studied as a method of biocontrol. In a greenhouse study,

mutant cultures of *Epicoccum purpurascens* offered improved protection bean flowers to *S. sclerotiorum* ascospore infection compared to wild type strains (Zhou and Reeleder, 1990). Ascospore germination on flowers was inhibited in bean when blossoms where sprayed with *Erwinia herbicola*. A short duration, up to 1 day, of protection is offered by the bacterial strains, and population levels were found to be dependent on environmental conditions, such as temperature. The short duration of ascospore germination inhibition, as well as the difficulties in sustaining an adequate population level at temperatures conducive to *S. sclerotiorum* infection limit the usefulness of the bacterial strains for biocontrol (Yuen et al., 1994).

Fungicide application for white mold control has met with limited success. Greenhouse studies indicate that fungicide (benomyl) application on bean leaves (not the flowers) did not protect plants from being infected by ascospores. Protection of bean from ascospore infection was shown to occur only when the flowers were sprayed with fungicide (Hunter et al., 1978). Field studies have indicated that spraying of fungicides, such as benomyl, a few days before full bloom, provided effective control of white mold. Benomyl provided control for up to 9 days past spraying on senescent and dead blossoms (Natti, 1971). Application of benomyl was more effective at decreasing white mold severity at pre-bloom than full bloom and was not effective at the post bloom stage. In another study, application of benomyl was most effective at full bloom (Morton and Hall, 1989). The most efficient control of white mold was dependent upon the number of flowers that received fungicide protection. Fungicide application is costly and must be timed in order for maximum flower blossom coverage. Application of fungicides for control of white mold is low in Michigan dry bean production. In 1999, Benlate

(benomyl) was applied to only 11.4% of 23, 000 bean acres surveyed, most likely due to a dry season. The number of acres sprayed with fungicide for control of white mold is generally low considering white mold was cited by growers as the major disease problem of dry bean (DiFonzo, 2000).

Herbicides offer a potential alternative measure for control of white mold, through potential reduction of inoculum, or suppressed disease levels. The treatment of sclerotial bodies with triazines, such as atrazine, resulted in an increase in the amount of carpogenic germination. Apothecia development, however, was either abnormal or absent (Casale and Hart, 1986; Radke and Grau, 1986). The application of the herbicide Lactofen (Cobra) was found to reduce infection levels of white mold in soybean fields with heavy disease pressure (Dann, et al., 1999). Glyceollin accumulation was identified in soybean leaves treated with lactofen, and corresponded to reduced lesion size in a leaf assay inoculated with *S. sclerotiorum* (Dann, et al., 1999). An induced resistance response was observed in soybeans treated with 2,6-dichloroisonictinic acid (INA) and benzothiadiazole (BTH). Greenhouse leaf assays, and field trials determined that susceptible genotypes had a reduced level of white mold when treated with INA or BTH (Dann et al., 1998).

The development of bean cultivars with resistance to white mold is an effective strategy in reducing yield losses to white mold. Physiological resistance to white mold has been described in several navy bean cultivars, such as Bunsi (also known as Ex Rico 23) and C-20, (Schwartz et al., 1987; Miklas et al., 1992; Tu 1985; Kelly et al., 1984). Mechanisms of physiological resistance may include several factors, including protection by cutinase (Parker and Koller, 1999), phytoalexin production (Sutton and Deverall,

1984), and resistance to oxalic acid (Tu, 1985; Tu, 1989). Bean leaves and hypocotyls treated with ascospores exhibited a hypersensitive reaction, with phaseollin and phaseollidin phytoalexins accumulating in leaf tissue, and kievitone accumulating in the hypocotyls. Treatment of bean leaves and hypocotyls with mycelium produced water-soaked lesions, with no phytoalexin production in the leaf tissue, and only kievitone production in the hypocotyls. Phaseollin and kievitone production increased when mycelium-treated hypocotyls were transferred from a 18 to 28°C environment (Sutton and Deverall, 1984).

Oxalic acid has been useful in screening for white mold resistance in several crops. An initial leaf test demonstrated that sunflower cultivars were sensitive to oxalic acid and differentiated a response that corresponded to field resistance (Noyes and Hancock, 1981). Germinating seeds on an oxalate-based medium proved to be a useful tool in selecting for resistance to *S. sclerotiorum* in alfalfa and crimson clover (*Trifolium incarnatum*; Rowe, 1993). In soybean, excised stems of 12 cultivars were placed in vials containing a 40 mM oxalic acid solution (Wegulo et al., 1998) and rated for lesion length. Correlation coefficients of field resistance among the 12 cultivars and 24 analyses of the oxalic acid stem test were highly variable. In order to increase physiological resistance to white mold in oilseed rape (*Brassica napus*), an oxalate oxidase gene isolated from barley (*Hordeum vulgare* L.) roots was introduced into oilseed rape via transformation (Thompson et al., 1995). Leaves from transgenic plants that showed oxalate oxidase activity were found to have resistance to an oxalate solution, as compared to control plant reactions.

Common bean has shown differentiation in response to oxalate. The uptake of

oxalic acid by petioles of excised primary leaves of a resistant cultivar, Bunsi was shown to be much slower than that of two susceptible cultivars, Kentwood and Seafarer (Tu, 1985). Lesion area was also shown to correspond in this fashion, in a range of oxalate concentrations from 1 mM, to 80 mM. Structural damage to the plasma membranes and chloroplasts was more severe in the susceptible 'Fleetwood', compared to the resistant cultivar, Bunsi (Tu, 1989b). Chloroplast degradation was also found in beans exposed to oxalic acid secreted by the pathogen (Tariq and Jeffries, 1985).

A change in plant architectural traits was found to influence levels of white mold in common bean (Coyne, 1980). Bunsi, an indeterminate navy bean, has an open porous canopy which has been associated with reduced levels of white mold in the field (Tu and Beversdorf, 1982; Park, 1993). Upright indeterminate navy beans escape white mold infection or spread, in comparison to determinate navy bean types, due in part to a narrower canopy, resulting in a drier microclimate underneath the canopy (Park, 1983). Alternatively, the open, porous canopy of larger-seeded determinate beans was identified as an architectural avoidance mechanism in semi-arid production regions (Coyne et al, 1974; Weiss et al., 1980a; Schwartz et al., 1987). Dense canopies generally resulted in higher white mold severity than porous canopies, due to the development of a favorable microclimate within the canopy (Blad et al., 1978; Coyne, 1980). Fewer apothecia were produced underneath the open determinate canopy of the dark red kidney bean cultivar, Charlevoix, and the upright canopy of the indeterminate small white bean, Aurora, compared to the dense canopy of several prostrate type III great northern bean cultivars (Schwartz and Steadman, 1978). Many factors influence the production of apothecia and ascospores, the infection of flowers, and the subsequent spread of disease throughout the

plant tissue. The onset of flowering and production of apothecia, however, generally occur at canopy closure under appropriate moisture conditions (Boland and Hall, 1987). The interaction between management practices, such as row width, and plant architectural traits, such as an open porous canopy, or upright architecture can affect levels of white mold in the field.

Sclerotinia stem rot has become an increasingly important disease affecting soybean production in recent years (Wrather et al., 1997). Senescent flowers are initial infection sites, and microclimate conditions, such as wet soil and wet canopy conditions at flowering, are required for infection (Grau, 1988). Certain cultivars of soybean have partial resistance to white mold, including both physiological resistance and escape mechanisms. Flowering date, plant architecture, and maturity have been identified as plant avoidance mechanisms to white mold infection in soybean (Boland and Hall, 1987; Nelson, 1991; Kim et al., 1999). Under field conditions, heritability estimates of resistance determined in an F_3-derived soybean population ranged from 0.30 to 0.71, and was 0.59 across all field environments. The moderate heritability estimate suggests that progress can be made in selecting for resistance to white mold in soybean.

S. sclerotiorum can infect the roots, stems, leaves, terminal bud, and capitulum of sunflower, causing sclerotinia wilt, stem rot, and heat rot. Sclerotinia wilt generally originates from myceliogenic germination (Huang and Hoes, 1980), whereas the infection of the head is caused by infection of ascospores. The fungus can infect the plant root system and stem at an early seedling stage, wheras, the head is susceptible to infection later in the season. Sclerotinia wilt generally has two cycles for infection. The first cycle begins with myceliogenic germination from sclerotial bodies onto seedlings via the roots

or stems near the surface. The second cycle occurs with the onset of budding and flowering (Huang and Kozub, 1990). In sunflower, two types of resistance have been suggested. The first type of resistance is defined as resistance to the penetration of *S. sclerotiorum* into the plant. The second type of resistance refers to a resistance to the extension of mycelium in the plant tissues (Castano et al., 1993). The first plant with an established root infection becomes the primary infection locus, and infection can spread from plant to plant via root contact. Factors, such as plant spacing, can inhibit the quick spread of disease from plant to plant (Huang and Hoes, 1980). Range in heritability estimates for resistance in sunflower depends upon resistance trait measured, such as leaf lesion (0.30), petiole score (0.73), stem rating (0.57), stem lesion (0.59), and disease stems (0.36) (Degener et al., 1998). The moderate heritability estimates across several disease parameters indicate that selection for resistance to white mold is possible in sunflower.

Resistance to white mold in bean is complexly-inherited (Fuller et al., 1984; Miklas and Grafton, 1992). In general, genotype x environment interaction play a major role in the expression of resistance of white mold in bean. The number of genetic and environmental factors that can influence the degree of white mold in the field can be designated into three categories: i) factors that affect the amount of inoculum produced, ii) factors that affect the exposure of the plant to inoculum, and iii) factors that determine how quickly the fungus can spread throughout the plant. Factors that affect the amount of inoculum produced include both management strategies and avoidance strategies. Wider row widths, open porous plant canopies, and upright plant architecture are three examples of factors that influence the microclimate underneath the canopy. An increase in the air

flow underneath the canopy leads to a drier microclimate, which may reduce the apothecia production. The second category, includes factors that affect the exposure of the plant to inoculum. The ascospores require a minimum amount of time of leaf wetness, conditioned by soil moisture and humidity within the plant canopy. An open, porous canopy may reduce humidity and the time for initial infection. Ascospores require senescent flowers for infection, therefore factors affecting flowering characteristics will affect exposure to inoculum. Initial primary infections are typically senescent flowers that lodge on stems (Tu, 1989a). Flowers are the initial infection sites, and methods to protect these are useful in reducing infection levels. The effectiveness of fungicides in reducing white mold depends upon the ability to efficiently protect the flowers (Natti, 1971; Morton and Hall, 1989; Hunter et al., 1978). Other factors that may reduce the exposure of the plant to inoculum include biological competition by organisms such as *Epicoccum*, for nutrients on flowers (Zhou and Reeleder, 1990). The third category influencing resistance to white mold involves factors that affect how fast the fungus spreads throughout the plant. Physiological resistance mechanisms, such as phytoalexin production (Sutton and Deverall, 1984) and resistance to oxalate (Tu, 1985), reduce the rate at which the fungus can infect the plant. Microclimate may also be a factor in this category, since, temperature and humidity may affect spread of fungus. A hot, dry environment is not conducive to fungal growth. Complete resistance to white mold in bean is not known. Instead, phytoalexin production and/or resistance to oxalate may be more effective in an environment where the spread of fungus is less rapid due to microclimate conditions that limit its virulence. Breeding for resistance to white mold must take into account the various factors which affect the production of inoculum, the

exposure of the plant to inoculum, and the resistance of a plant to fungal development once infection has established. Combining genes for resistance to white mold should include those that affect avoidance mechanisms as well as those that control physiological resistance mechanisms.

Progress in breeding for resistance in common bean is hindered by environmental conditions and factors that confound the expression and detection of physiological resistance mechanisms. In the field, the detection of physiological resistance can be masked by architectural avoidance mechanisms, such as an open canopy or upright architecture, in which the microclimate within the canopy limits production of inoculum and subsequent infection (Schwartz and Steadman, 1978; Blad et al., 1978; Park, 1993). Improvement of resistance to white mold in bean, therefore, must take into account the ability to identify and select individual genotypes with physiological resistance to white mold, and agronomically-desirable architectural avoidance mechanisms in advanced breeding trials and differentiating populations. Heritability estimates for resistance to white mold in bean were found to be lower for physiological resistance, versus field resistance. In three different populations, estimates of heritability for a lesion length stem assay were 0.27, 0.38, and 0.66, whereas the estimates for the same three populations in the field were 0.77, 0.58, and 0.70, respectively (Miklas and Grafton, 1992). Progress in selection for resistance to white mold in the field environment should be feasible with the moderate to high estimates of heritability. Lower estimates for physiological resistance, as measured in the lesion length assay, indicate that physiological resistance is most likely complexly-inherited, and significantly influenced by the environment. Higher selection intensity may be required to select for physiological resistance, particularly in

the populations with low heritability.

Selection of complexly-inherited traits, such as resistance to white mold, may be facilitated by the identification of single factors linked to or associated with complex traits, but unaffected by environmental variation. The association between markers and quantitatively-inherited traits was first reported between seed coat patterns/color and seed size of beans. Linkage between bean seed color and size allowed for the indirect selection of seed size (Sax, 1923). In breeding for disease resistance, markers allow the plant breeder to select for disease resistance traits without having to handle highly variable and virulent pathogens (Kelly, 1995). The increased utilization of molecular markers linked to economically important traits of interest in plants has allowed for the indirect identification and selection of quantitative trait loci (QTL) (Beckmann and Soller, 1983; Darvasi and Soller, 1994; Tanksley et al., 1989).

Markers linked to QTLs conferring resistance to white mold have been identified in both soybean and sunflower. In soybean, three QTLs were associated with disease severity index (DSI), accounting for 8, 9, and 10 % of the phenotypic variability for DSI across environments. Two of the QTLs were also associated with plant avoidance mechanisms, such as plant height, lodging, and date of flowering (Kim and Diers, 2000). In sunflower, four QTLs were associated with leaf resistance and two QTLs were associated with capitulum resistance to *S. sclerotiorum*. One of the QTLs was associated with both leaf and capitulum resistance. The QTLs accounted for up to 60% of the leaf resistance, and up to 38% of the capitulum resistance. Agronomic traits, such as seed weight and oil content were found to have overlapping regions with QTL regions. Apical branching pattern was suggested as exhibiting the best resistance to infection of the

capitulum (Mestries et al., 1998). The association between days to flowering and resistance to *S. sclerotiorum* in sunflower was found to be dependent upon the population (Castano et al., 1993). Plant avoidance mechanisms may play an important role in resistance to *S. sclerotiorum* in sunflower.

Marker-assisted selection (MAS) has been advocated as a new tool for plant breeders to select indirectly for economically important traits (Tanksley et al., 1989). Markers that are linked to traits of interest offer a unique advantage for selection purposes. Molecular markers are single Mendelian loci, that are not influenced by environmental conditions. Complexly-inherited traits are typically difficult to evaluate due to environmental variation. Markers linked to QTLs controlling complexly-inherited traits would allow for screening without having to conduct extensive and laborious testing associated with such traits (Staub and Serquen, 1996).

Markers can be utilized for both foreground and background selection using MAS. In foreground selection (Melchinger, 1990), flanking markers near the donor QTL need to be selected in order to introgress a particular QTL into breeding population. Marker-assisted selection can also be used for background selection in backcross breeding (Visscher et al., 1996). Selection for the adapted genome reduces the amount of extraneous donor genome on non-carrier chromosomes thereby limiting the amount of donor genome surrounding the target QTL. Foreground and/or background selection using MAS may be useful for introgression of QTLs from more unadapted or wild material via Advanced Backcross QTL analysis (Tanksley and Nelson, 1996), or Inbred Backcross Line Development (Bliss, 1993; Butruille et al., 1999).

The efficiency of selection based on markers is dependent upon several factors,

such as heritability, selection intensity, selection generation, linkage distance, population size, economics of MAS, robustness of the marker and QTL (Hospital et al., 1992; Moreau et al., 2000; Paterson et al., 1991). Traits with low heritability are the most efficient traits to select for utilizing MAS, since low heritability is associated with difficulty in phenotyping. Stringent selection pressure must be used in MAS for traits of low heritability, since the QTLs are linked to traits with high environmental variation (Knapp, 1998). Marker-assisted selection is most efficient in early generations, since the marker can be detected in the generations where phenotypic selection is hindered by a lack of replication and small number of experimental units (Lande and Thompson, 1990; Stromberg et al., 1994). Flanking marker are desirable, and should be tightly linked to the QTL, in order to reduce recombination in the region between the marker and the QTL (Frisch et al., 1999). The identification of markers linked to QTLs is dependent upon the initial population size. When large populations are used, minor QTLs will be more easily defined. Marker-assisted selection involves extensive effort, in DNA extraction, genotyping and phenotyping the population. Efficiency in MAS will increase, however, after one cycle of selection (Moreau et al., 2000), since the initial start-up cost of marker identification is high. The cost of using markers for screening populations will therefore, be reduced with each cycle of selection. The efficiency of a marker also increases with the robustness of the marker across genotypes, generations and environments (Paterson et al., 1991).

An approach to increase the efficiency of identifying markers linked to QTLs involves the use of selective genotyping (Lander and Botstein, 1989) and bulked segregant analysis (Michelmore et al., 1991). A limited number (10 - 14%) of extreme

phenotypes are pooled and screened for the presence of polymorphic bands. Primers identified to have polymorphic bands in the two DNA bulks, are then tested on the entire population. This approach is particularly useful if a saturated linkage map has not been developed for the population. The genotypes included in the DNA bulks are important, since only a small percentage of the population is used for identification of markers. Biases in marker evaluation may occur when only few genotypes are utilized in marker identification (Wang and Paterson, 1994). Repetitiveness of screening the DNA bulks twice, sequentially leads to cost inefficiencies. Computer simulation models indicate that potential biases in the identification of markers based on extreme values of a single phenotypes may be limited, and suggest that multiple correlated traits may more efficiently identify useful markers (Ronin et al., 1998). In a recent study of nematode resistance in citrus, two sets of DNA bulks were used to identify important polymorphic markers. Markers were first identified in a set of DNA bulks with 6 genotypes each, and confirmed in a second set with 15 genotypes each (Ling et al., 2000).

The utility of markers linked to QTLs for economically important traits, such as resistance to white mold, lies in the actual value of MAS. Marker-assisted selection allows for the identification and selection of traits in genotypes without having to employ effort into phenotyping a large number of individuals. The strength of the marker is dependent upon the phenotypic data. In the case of many quantitatively-inherited traits, accurate phenotypic data requires extensive testing, over multiple variable environments. The ability to detect useful markers is limited to the individual genotypes in the bulks, since only a small percentage of the population is included in the screening process. Utility of markers is dependent upon robustness across multiple environments and

populations. Replacing laborious screening of quantitatively-inherited traits with MAS would have several advantages in a breeding program. Few examples exist at present of the utilization of MAS for quantitative trait improvement, despite the obvious benefits to be gained from using MAS in breeding programs. Resistance to white mold is an excellent example of the potential for MAS, since screening for resistance in the field is difficult. Physiological resistance to white mold in complexly-inherited and also difficult to evaluate in greenhouse or field environments. In breeding programs, the detection of the resistance to white mold in heavily influenced by environmental variation from season to season which hinders the normal selection procedures for important quantitative traits, and increases the importance of MAS (Tanksley et al., 1989). In order to detect markers linked to useful QTLs for resistance to white mold, great care must be taken to collect accurate phenotypic data. Marker-assisted selection must be based on a data set that is uncompromised in quality and reproducibility. Breeding for resistance to white mold in bean would be greatly enhanced with the discovery and use of stable markers that would allow for selection of physiological resistance without confounding environmental factors.

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CHAPTER I

AN INDIRECT TEST USING OXALATE TO DETERMINE PHYSIOLOGICAL RESISTANCE TO WHITE MOLD IN COMMON BEAN

INTRODUCTION

White mold (*S. sclerotiorum*) is a destructive fungal disease that can infect over 400 plant species, including many important crop species, such as common bean, sunflower, alfalfa, soybean, rape and peanut (Boland and Hall, 1994). In common bean, total seed and pod yield are reduced due to lower number of seeds produced per plant, reduced number of pods per plant, and smaller seed size (Kerr et al., 1978). Progress in breeding for resistance is hindered by environmental conditions and plant avoidance mechanisms that confound the expression and detection of physiological resistance mechanisms in the field. Methods to detect physiological resistance to white mold in common bean include the limited-term inoculation method (Hunter et al., 1981), excised-stem inoculation technique (Miklas et al., 1992a), leaf-agar plug assay (Steadman et al., 1997), straw test (Petzoldt and Dickson, 1996), and growing callus on medium containing pathogen filtrate (Miklas et al., 1992b). Most of these tests utilize a limited number of genotypes and depend upon fungal mycelium in screening procedures. Variability in virulence among isolates (Maxwell and Lumsden, 1970; Morrall et al., 1971; Miklas et al., 1992a; Pratt and Rowe, 1995) and potential pathogen sensitivity to high temperatures (Abawi and Grogan, 1975; Boland and Hall, 1987) limit greenhouse screening methods utilizing the pathogen.

White mold mycelium exude copious amounts of oxalate during infection of plant

tissue (Maxwell and Lumsden, 1970). Using non-oxalate producing mutants, oxalate was identified as the primary pathogenicity factor for *S. sclerotiorum* (Godoy et al., 1990). A low pH environment (pH 4.0) created by exuded oxalate is optimal for function of the polygalacturonase produced by the pathogen (Marciano et al., 1983). Differentiation for resistance to oxalate has been identified in a leaf test in sunflower (Noyes and Hancock, 1981), a germination test in alfalfa and crimson clover (*Trifolium incarnatum* L.) (Rowe, 1993), an excised stem test in soybean (Wegulo et al., 1998), and a leaf test in transgenic rape (Thompson et al., 1995). Common bean has also shown genotypic differentiation in response to oxalate. The uptake of oxalic acid by petioles of excised primary leaves of the resistant cultivar Bunsi (also known as Ex Rico 23) was shown to be slower than in two susceptible cultivars, Kentwood and Seafarer (Tu, 1985). Cultivars that were susceptible to white mold exhibited more severe structural damage to the plasma membranes and chloroplasts than resistant cultivars when exposed to an oxalate solution (Tu, 1989).

Breeding for resistance to white mold in common bean is limited by the lack of a simple, consistent screening method to quickly evaluate a broad array of genotypes for physiological resistance to white mold. An indirect screening method that bypasses the need for the plant to flower, would be valuable in screening unadapted, photoperiod-sensitive germplasm for new sources of resistance to white mold. An indirect screening method that eliminates the use of the pathogen would also reduce variability often associated with greenhouse tests. The objective of this study was to develop an indirect greenhouse test for physiological resistance to white mold in common bean, using oxalate, a primary pathogenicity factor of *S. sclerotiorum*.

MATERIALS AND METHODS

High-yielding common bean genotypes were evaluated for resistance to oxalate in three greenhouse tests. Thirty (Test 1) or 36 (Tests 2 and 3) genotypes were evaluated, including cultivars and breeding lines from the navy, black, pink, pinto, great northern, cranberry, and kidney commercial classes, new sources of resistance from breeding programs across North America and the Caribbean, as well as genotypes entered in the National Sclerotinia White Mold Nursery. Twenty seven genotypes were common across all three tests. In the greenhouse oxalate test, each entry (genotype) was planted in three 15 cm diameter pots containing Baccto High Porosity Planting Mix, with 9 seeds per pot, and grown under greenhouse conditions with ambient temperature and a 16 hr daylength. Twenty-day old seedlings (2^{nd} trifoliate emerging) were cut at the base of the stem at night to avoid wilting due to the potential of high transpiration rates during daylight hours. A foam stopper was placed around the base of the seedling, and placed in a perforated foam board in a 78 L plastic container (67 cm length, 47 cm width, 21 cm depth). Each container held 11 L of a 20 mM oxalic acid solution that had been adjusted to a pH of 4.0 with NaOH. The perforated foam board was positioned above the solution, which kept the seedlings upright while the cut stem was immersed in the solution. Four (Test 2 and 3) or five (Test 1) seedlings (samples) were used for each genotype in each of the three containers (replications) of the Randomized Complete Block Design (RCBD). In each experiment, a separate control replication (single container) consisted of two (Tests 2 and 3) or three (Test 1) seedlings per genotype being placed in a 11 L solution of distilled water that had been adjusted to a pH of 4.0 with HCl. The three replications and one control replication were placed in a greenhouse chamber covered in clear

polyethylene to minimize wilting as a result of exposure to direct light.

A fourth greenhouse test was designed to evaluate resistance to oxalate in wild, landrace, and exotic, cultivated materials, including thirty-two accessions from the *Phaseolus* core collection that were identified to have physiological resistance to white mold, as determined via the straw test or leaf agar plug assay (K.F. Grafton, and J.R. Steadman, personal communication). New sources of unadapted and adapted genotypes were also incorporated in this test, and included genotypes originating from Mexico, Peru, Colombia, and California. Two pots with nine seeds per pot, were planted for each entry, and grown in greenhouse conditions. The oxalate test was initiated 20 days after planting, and was similar in methodology to the previously mentioned tests. The oxalate test was designed as an RCBD, with four replications, and three seedlings (samples) per genotype in each replication. No control replication was utilized in this experiment.

The seedlings were rated for wilting symptoms after 12 to 15 h of exposure to the oxalate solution (approximately 6 to 9 h of daylight). A 1 to 6 scale was used to measure wilting, where 1 = no wilting symptoms visible, 2 = 1 leaf with wilting symptoms (the two unifoliate leaves were rated together as one leaf, and the 3 leaflets of a trifoliate leaf were rated together as one leaf), 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem (total plant) collapsing. Wilting symptoms ranged from curled leaf tip, to total loss of turgidity in the entire leaf.

Genotypes in greenhouse Tests 1, 2, and 3, were evaluated for comparison with reaction to white mold resistance in the field. The field experiments were grown at the Montcalm Research Farm, Entrican, MI, in 1996 (Test 1), 1997 (Test 2), and 1998 (Test 3). Planting was delayed to the second week in June in all three field experiments to

favor disease development. A 0.5 m row spacing was used for the four row plots, with 6 m row length, where the outer two rows were planted with a white mold susceptible uniform border ('Midland'), and the inner two rows were planted with the experimental genotypes. The soil type at the Montcalm Research Farm sites is a combination of Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Standard agronomic practices for tillage, fertilization, and herbicide were applied to ensure good crop growth and development. Plots were irrigated during initial flowering with 13 mm of water at approximately three day intervals, depending upon rainfall, in order to promote uniform disease pressure across the field. The field experiments were irrigated with an overhead sprinkler system five times in 1996, three times in 1997, and six times in 1998. Uniform infection of white mold in dry bean at the Montcalm Research Farm was identified in previous field studies. Plots were rated for disease severity and disease incidence (DI) (Steadman 1997; Kolkman and Kelly, 1998; Steadman et al., 1998) using a 'quarter scale' (Hall and Phillips, 1996), shortly before harvest, when the majority of plants had reached physiological maturity. Thirty plants per plot were each given a rating from 0 to 4, where 0 = no disease present, 1 = 1 to 25% of the plant with white mold symptoms, 2 = 26 to 50% of the plant with white mold symptoms, 3 = 51 to 75% of the plant with white mold symptoms, and 4 = 76 to 100% of the plant with white mold symptoms. A Disease Severity Index (DSI) was calculated for each plot on a percentage basis, using the following formula:

$$\text{DSI} = \frac{\Sigma\,(\text{rating of each plant})}{4 \times (\text{number of plants rated})} \times 100$$

Disease incidence was calculated as the number of plants out of the thirty individuals with white mold infection, based as a percentage. Plots were harvested after disease rating.

All greenhouse experiments were analyzed as RCBDs, using PROC GLM (SAS, 1995). The three field experiments were analyzed separately using PROC LATTICE (SAS, 1995). The 1996 field experiment was analyzed as a rectangular lattice, and the 1997 and 1998 field experiments were each analyzed as a partially balanced triple lattice. The 27 common genotypes were analyzed across all three tests (greenhouse) and years (field) as a RCBD, using PROC GLM (SAS, 1995). Environments were considered as a random effect, and genotypes as a fixed effect.

RESULTS AND DISCUSSION

Significant genotypic differences were identified in the response to a 20 mM oxalate solution (Tables 1.1 and 1.2). Preliminary experiments using a subset of genotypes and a 10, 20, 40, 80, and 100 mM range of oxalate concentrations over time, indicated that the 20 mM oxalate concentration was suitable for bean. Wilting symptoms in the control container in each experiment were negligible and not significant, indicating the importance of oxalate in the appearance of wilting symptoms. The temperature in the three oxalate tests ranged from 24 to 40 °C (Test 1), 21.5 to 26 °C (Test 2), and 22.5 to 26.5 °C (Test 3), and 23.5 to 25 °C (Test 4). Significant differences between tests (Tables 1.1 and 1.2) indicate the influence of the environment in affecting the estimate of resistance to oxalate, and the importance of including known resistant and susceptible control cultivars in each experiment. Significant correlations between the oxalate test

Table 1.1. Mean squares of the greenhouse oxalate test scores, and the field ratings for disease severity index and disease incidence, for 27 bean genotypes across three environments.

		Mean Squares		
		Greenhouse	Field	
Source	df†	Oxalate Test	Disease Severity Index	Disease Incidence
Genotype	26	2.3399****	1512.9****	2566.2****
Environment	2	35.7039****	8817.5****	5426.0****
Genotype x Environment	52	0.4713****	418.7***	734.4**
Rep (Environment)	6	0.3012 ns	175.9 ns	273.5 ns
Error	156	0.1817	206.1	403.5

, *, **** Significant at $P < 0.01$, 0.001 and 0.0001 levels, respectively; ns = non significant.

ratings and field disease ratings were identified in each experiment Tests 1, 2, and 3 (Table 1.3). The highest correlation between the oxalate test and the DSI (r=0.58; P=0.0015) and DI (r=0.57; P=0.0019) ratings in the field (Table 1.4) was observed with the 27 genotypes across three years.

The oxalate test results confirm resistance found in several common bean sources (Tables 1.2 and 1.4). Bunsi, has been identified as resistant in both greenhouse tests (Tu, 1985; Tu, 1989; Miklas et al., 1992a; Miklas et al., 1992b), and field trials (Tu and Beversdorf, 1982; Schwartz et al., 1987; Miklas et al., 1992a). Bunsi-derived cultivars, such as Stinger, Crestwood, I92919, N90618, and ND88-106 were resistant to both white mold in the greenhouse oxalate test and in the field. 'C-20' (Kelly et al., 1984) and C-20 - derived cultivars, such as Huron (Kelly et al., 1994), represent another source of navy bean with physiological resistance (Miklas et al., 1992a) and field resistance. Low oxalate test ratings verified the presence of physiological resistance to white mold in Huron (Table 1.2). I9365-3, I9365-14, I9365-5-pk, I9365-19, and 92BG-7, released as sources of white mold resistance (Miklas et al., 1998), also showed resistance to oxalate.

Resistance to oxalate varied in the unadapted photoperiod-sensitive wild and landrace accessions that were previously identified through two the straw test and leaf agar plug assay (Table 1.5). Accessions from both the Mexico core collection, such as PI 318695, and from the Central and South American core collection, such as PI 399169, PI 313609, and PI 313598, were resistant to oxalate. Variability in the detection of physiological resistance across screening methods, is indicative of the variability that can be found within an accession, as well as the variability for phenotyping an accurate measure of resistance based on the differing tests. Other new sources of resistance to oxalate were identified in this test. Two black bean lines from Mexico, Tacana, and V8025, and two genotypes from CIAT, DOR 364 (Beebe et al., 1998) and Sea 5, were shown to have resistance to oxalate similar to that of the most resistant check, Huron. Chaucha Chuga, a cultivar from Peru (Beebe et al., 1998), was also resistant to oxalate. An Australian navy bean, CH428-4D, with reported resistance to white mold in the field (Redden and Tatnell, 2000), was also resistant to oxalate. G122, a cranberry bean (Shonnard and Gepts, 1994; Miklas et al., 2000), was included in all four greenhouse tests. In the first three tests, G122 had low to moderate levels of susceptibility for resistance to oxalate (Table 1.2). In the fourth test, G122 had low to moderate levels of resistance to oxalate (Table 1.5). This genotype was previously identified as having resistance to white mold in the field (Kmiecik and Nienhuis, 1998), and most likely has moderate levels of physiological resistance to white mold, that is highly influenced by environmental conditions.

Table 1.2. Ratings of resistance to oxalate in the greenhouse oxalate test for three individual tests and the combined test scores for 27 common bean cultivars across the three tests.

	Greenhouse Oxalate Tests[†]			
Genotypes[‡]	Test 1	Test 2	Test 3	Combined
Midland	4.00[†]	2.75	3.50	3.42
Isles	3.20	3.42	3.17	3.27
Othello	3.73	2.83	3.00	3.20
Frontier	3.93	2.17	2.83	2.98
Newport	3.80	1.50	3.58	2.97
Mackinac	3.53	2.50	2.58	2.87
Weihing	2.47	2.33	3.58	2.81
I9365-31	3.53	2.00	2.67	2.74
N94080	3.40	1.58	2.75	2.58
G122	3.13	1.92	2.33	2.47
Mayflower	3.27	1.42	2.58	2.43
Raven	2.93	1.50	2.33	2.27
N90618	2.67	1.25	2.75	2.23
OAC Laser	3.07	1.33	2.25	2.23
92BG-7	3.07	1.17	1.83	2.03
Stinger	2.53	1.08	2.42	2.02
Crestwood	2.93	1.08	2.00	2.01
I9365-19	2.60	1.00	2.42	2.01
Bunsi	2.67	1.00	2.33	2.00
T39	2.60	1.50	1.83	1.99
Vista	3.00	1.50	1.42	1.98
I9365-5-pk	2.60	1.17	2.17	1.98
ND88-106	2.00	1.33	2.50	1.96
Huron	2.20	1.25	2.08	1.85
I9365-14	2.13	1.17	2.00	1.77
I9365-3	2.60	1.25	1.33	1.73
I92919	2.67	1.08	1.33	1.70
Mean[§]	2.99	1.72	2.46	2.35
LSD (0.05)	0.69	0.57	0.76	0.40
CV (%)	14.1	20.3	18.9	18.1

[†] Scale from 1 to 6, where 1 = no wilting (similar to control), 2 = 1 leaf wilting, 3 = 2 leaves wilting, 4 = 3 or more leaves wilting, 5 = petioles wilting, 6 = total plant collapse.

[‡] 27 common genotypes across the three tests

[§] Mean values for 30 genotypes in test 1, 36 genotypes in tests 2 and 3, and 27 common genotypes in the combined analysis.

Table 1.3. Pearson correlation coefficients of oxalate test ratings to the disease severity index, disease incidence, and yield for common bean genotypes tested in three greenhouse oxalate tests and corresponding field tests at the Montcalm Research Farm.

Field Ratings	Oxalate Test Ratings			
	Test 1‡ (30 genotypes)	Test 2 (36 genotypes)	Test 3 (36 genotypes)	Common§ (27 genotypes)
Disease Severity Index¶	0.534**	0.315†	0.457**	0.580**
Disease Incidence#	0.482**	0.318*	0.394**	0.571**
Yield	-0.358*	-0.413**	-0.245 ns	-0.505**

*, **, *** Significant at the $P < 0.05$, 0.01, and 0.001 levels, respectively; ns = non significant

† Significant at $P < 0.10$ level

‡ Ratings from oxalate tests 1, 2, and 3 were correlated to field ratings from the 1996, 1997, and 1998 field environments, respectively

§ Means of 27 common genotypes from three oxalate testes were correlated to the combined field ratings over years.

¶ Disease Severity Index $= \dfrac{\Sigma \text{ (rating of each plant)}}{4 \times \text{(number of plants rated)}} \times 100$

Disease Incidence = percentage of 30 plants with white mold infection.

Resistance to oxalate is a resistance mechanism that may work singly, or more likely in a combination with a number of plant avoidance mechanisms or alternative physiological mechanisms to provide consistent levels of resistance to white mold in the field. Mechanisms can provide plant avoidance to white mold, in which the plant escapes the initial infection of the pathogen. Favorable conditions for the formation of apothecia, the corresponding onset of flowering for inoculation via ascospores, and appropriate temperatures following infection are critical components of the epidemiology of *S. sclerotiorum* (Boland and Hall, 1987). Plant avoidance mechanisms, such as early flowering or maturity, or an open porous canopy may limit the initial inoculation and subsequent infection of white mold. Physiological resistance mechanisms may not be restricted to resistance to oxalic acid. Alternative resistance mechanisms at the cellular

Table 1.4. Field ratings of white mold disease severity and incidence for the 27 bean cultivars in three individual field tests and combined field tests across the three tests (years), at the Montcalm Research Farm in 1996, 1997, and 1998.

Genotypes[†]	Test 1 (1996)		Test 2 (1997)		Test 3 (1998)		Combined (1996-98)	
	DSI[‡]	DI[§]	DSI	DI	DSI	DI	DSI	DI
Midland	60.0	70.0	53.3	73.3	39.1	73.2	51.4	74.8
Othello	79.3	87.8	50.6	66.7	12.4	19.6	47.5	58.5
Newport	45.8	60.0	61.4	83.3	25.6	59.7	43.4	65.9
N94080	50.8	72.2	41.9	53.3	9.8	34.8	34.4	53.3
I9365-19	46.7	62.2	42.2	60.0	9.8	28.3	33.9	51.8
Weihing	25.0	34.5	64.4	77.8	11.2	29.4	33.7	47.4
Mackinac	50.8	75.5	25.6	41.1	22.8	62.3	32.5	58.9
I9365-3	36.7	56.7	43.9	62.2	13.0	35.0	31.1	51.1
Raven	30.8	41.1	37.2	50.0	22.1	50.2	30.0	47.0
Frontier	36.7	53.3	45.0	70.0	6.2	17.5	29.4	48.1
T39	21.7	33.3	45.0	57.8	13.9	43.5	27.9	46.3
Vista	18.3	34.4	49.2	66.7	5.0	15.4	23.2	36.7
I9365-14	21.7	47.8	41.7	55.6	3.9	14.0	22.4	38.9
Stinger	9.2	16.7	31.9	47.8	14.1	35.2	18.9	34.8
Crestwood	6.7	13.3	37.2	44.4	7.4	19.6	18.8	29.6
Isles	4.2	6.7	30.8	50.0	14.9	42.8	16.5	32.6
I9365-31	15.8	38.9	27.2	48.9	5.9	17.6	15.6	33.7
G122	10.0	22.2	21.7	32.2	12.2	34.2	14.9	31.1
I9365-5-pk	18.3	34.5	18.3	36.7	5.9	16.6	14.4	30.0
Bunsi	8.3	14.4	30.3	48.9	2.8	8.8	13.4	23.0
Mayflower	14.2	22.2	12.8	21.1	14.3	38.6	13.2	26.7
92BG-7	1.7	6.6	23.1	36.7	13.9	36.6	12.8	27.0
Huron	17.5	36.7	7.8	21.1	10.6	31.4	11.3	28.5
N90618	9.2	17.8	11.4	20.0	6.5	18.9	8.4	17.8
ND88-106	0.0	2.2	18.1	25.6	5.8	17.3	7.4	13.0
I92919	0.0	2.2	7.2	16.7	5.5	16.8	3.9	11.1
OAC Laser	1.7	3.3	1.1	2.2	3.9	14.7	3.1	7.8
Mean[¶]	24.3	36.7	32.4	47.1	12.2	30.7	22.7	38.0
LSD (0.05)	26.3	31.0	24.7	29.4	18.4	31.6	13.4	18.7
CV (%)	67.7	53.7	47.9	40.6	96.0	67.3	63.2	52.9

[†] 27 common genotypes across the three tests

[‡] Disease Severity Index $= \dfrac{\Sigma\,(\text{rating of each plant})}{4 \times (\text{number of plants rated})} \times 100$

[§] Disease Incidence = percentage of 30 plants with white mold infection.

[¶] Mean values for 30 genotypes in test 1, and 36 genotypes in tests 2 and 3

Table 1.5. Ratings of resistance to oxalate in the greenhouse oxalate test for selected wild, landrace and cultivated *Phaseolus vulgaris* germplasm.

Genotype	origin	core[†]	seed class[‡]	seed size[¶]	oxalate score[§]	st. dev.	no. of plants tested[$]
PI 263596	Mexico	Mex.	unknown	38.0	3.7	0.47	12
PI 313348	Mexico	Mex.	landrace	22.0	3.6	0.74	12
PI 311974	Mexico	Mex.	landrace	13.0	3.5	0.51	9
PI 313671	Ecuador	CASA	cultivated	42.0	3.4	0.32	12
PI 415913	Ecuador	CASA	uncert. impr. st.	56.0	3.3	0.47	12
PI 201354	Mexico	Mex.	unknown	47.0	3.3	0.82	12
PI 316024	Peru		cultivated (nuna)	30.0	3.3	0.19	9
T3147-2[£]	Mich/Mex		breeding line	27.5	3.3	0.42	12
PI 415936	Ecuador	CASA	uncert. impr. st.	50.0	3.2	0.51	9
PI 282016	Colombia	CASA	cultivar	74.0	3.1	0.57	12
PI 417782	Mexico	Mex.	wild	5.2	3.1	.	3
PI 313425	Mexico	Mex.	landrace	24.0	3.1	0.74	12
Othello	Washington		cultivar	43.8	2.9	0.57	12
PI 415906	Ecuador	CASA	uncert. impr. st.	58.0	2.9	0.63	12
PI 415886	Ecuador	CASA	landrace	56.0	2.8	1.00	12
PI 325653[#]	Mexico	Mex.	landrace	23.0	2.8	0.43	12
PI 201010	Guatemala	CASA	wild	9.0	2.8	1.11	12
Newport	Michigan		cultivar	21.2	2.8	0.32	12
PI 312018[#]	Mexico	Mex.	landrace	26.0	2.7	0.67	12
PI 313254	Mexico	Mex.	landrace	19.0	2.7	1.12	12
PI 310515	Honduras	CASA	cultivated	24.0	2.7	0.38	12
PI 309837	Costa Rica	CASA	landrace	26.0	2.6	1.13	12
PI 311843	Guatemala	CASA	landrace	32.0	2.5	1.07	9
PI 311794	El Salvador	CASA	landrace	18.0	2.5	0.43	12
PTMex80	Mexico		cultivated	27.0	2.5	0.33	12
PI 313850	Peru	CASA	cultivated	46.0	2.3	0.47	12
PI 310865	Nicaragua	CASA	cultivated	21.0	2.3	0.90	12
PI 417721	Mexico	Mex.	landrace	24.0	2.3	0.72	12
T3008-1[£]	Mich/Mex		breeding line	26.0	2.3	0.84	12
PI 325685	Mexico	Mex.	landrace	3.6	2.3	0.50	12
PI 319683	Mexico	Mex.	landrace	34.0	2.2	1.02	9
PI 325691	Mexico	Mex.	landrace	3.6	2.2	0.00	4
PI 189016	Guatemala	CASA	unknown	30.0	1.9	0.42	12
PI 318695	Mexico	Mex.	wild	3.5	1.9	0.57	12
CH428-4D	Australia		breeding line	22.0	1.8	0.88	12

G122	India		breeding line	48.2	1.8	0.88	12
PI 313598	Colombia	CASA	cultivated	60.0	1.8	0.88	12
PI 313609	Colombia	CASA	cultivated	73.0	1.8	0.17	12
Chaucha Chuga	Peru		cultivated	38.8	1.6	0.50	12
V8025	Mexico		cultivar	25.0	1.5	0.19	12
DOR 364	CIAT		cultivar	22.0	1.4	0.32	12
PI 399169	Nicaragua	CASA	uncert. impr. st.	24.0	1.3	0.19	12
Sea 5	CIAT		breeding line	26.0	1.2	0.33	12
Tacana	Mexico		cultivar	25.8	1.2	0.33	12
Huron	Michigan		cultivar	23.9	1.2	0.19	12
mean					2.4		
CV, %					25		

[†] Mexico and CASA (Central and South American) core collections
[‡] seed class: uncert. impr. st. = uncertain improvement status
[¶] seed size (g 100seed^{-1}) obtained from NPGS/GRIN database; data on seed size for cultivars obtained from Kelly et al., 1999.
[§] oxalate score determined using a scale from 1 to 6, where 1 = no wilting (similar to control), 2 = 1 leaf wilting, 3 = 2 leaves wilting, 4 = 3 or more leaves wilting, 5 = petioles wilting, 6 = total plant collapse.
[$] Number of plants tested per genotype over the entire 4 replications of the experiment
[£] Breeding lines developed for tolerance to drought (Schneider et al., 1997)
[#] PI 325653 = flesh colored seed only; PI 312018 = black colored seed only; PI 189016 = red colored seed only

level, such as phytoalexins (Sutton and Deverall, 1984), may be important to white mold resistance in the field.

Any genotype that escapes infection in the field can significantly skew the correlation between the greenhouse oxalate test ratings and field disease ratings. OAC Laser, an upright navy bean cultivar with a porous canopy, does not have high levels of resistance to oxalate in the greenhouse tests, yet is very resistant to white mold infection in the field (Tables 1.2 and 1.4). Plant avoidance mechanisms and moderate to low levels of resistance to oxalate in OAC Laser most likely work in combination to provide excellent resistance in the field. Two early-flowering cultivars, Isles and Othello, can have low incidence of white mold in the field, but exhibit high oxalate ratings (Tables 1.2

and 1.4). The high oxalate test ratings indicate that both Isles and Othello have little physiological resistance to oxalate. Alternatively, less adapted germplasm, such as I9365-19 and I9365-3 (Miklas et al., 1998), were identified to be resistant to oxalate, yet had high disease ratings in the field (Table 1.4). I9365-19 and I9365-3 represent useful sources of physiological resistance for introgression into adapted germplasm. Unadapted germplasm has been identified to carry putative physiological resistance using the straw test (Miklas et al., 1999). Resistance to oxalate was identified in unadapted germplasm, including wild, landrace and exotic cultivated material (Table 1.5). The unadapted genotypes of varying seed size and origin offer a useful source of physiological resistance to white mold for both large- and small-seeded market classes. New sources of resistance are important in breeding strategies for improved disease resistance. Few large- or small-seeded genotypes have been identified that have physiological resistance to white mold. New sources of resistance allow for the potential to improve resistance in susceptible market classes, and pyramid genes for resistance in market classes, such as the navy bean, in order to create more stable resistant cultivars. The success of the oxalate test confirms the segregation of responses of resistant and susceptible common bean cultivars to oxalate (Tu, 1985; Tu, 1989) and pathogen filtrate (Miklas et al., 1992b). The oxalate test indirectly identifies genotypes that have physiological resistance to white mold via oxalate resistance, bypassing the need for field testing where the detection of physiological resistance is confounded by plant avoidance mechanisms.

A highly significant negative correlation (r=-0.50; P=0.0072) between the oxalate test ratings and yield for the 27 cultivars across three field environments implies the association between resistance to oxalate and high yield under white mold pressure

(Table 1.3). The lack of a significant correlation between the oxalate ratings and yield in the 1998 field trial may be indicative of the lower yield potential during the growing season.

The oxalate test is useful for determining physiological resistance in the greenhouse. Photoperiod-sensitive unadapted germplasm can be tested for physiological resistance, since plants are tested at the seedling stage (2nd trifoliate emerging) and are therefore, not influenced by flowering (reproductive) traits. A large number of lines can be evaluated in a relatively short time period. Inoculation of the cut seedlings into a common solution of oxalate reduces variability that may be observed when utilizing agar plugs of *S. sclerotiorum*. The inherent variability within a single isolate (Maxwell and Lumsden, 1970), or isolate variability from test to test is reduced (Miklas et al., 1992a). The time between inoculation of seedlings and rating of the response to oxalate is very short (12 to 15 h after inoculation) reducing the potential variability in environmental conditions that exist in a greenhouse over a longer period of time. The rating scale in the oxalate test was designed to effectively quantify the degree of damage to a genotype using a quick visual estimate. Extreme high temperatures can limit the ability to screen effectively using the fungus (Abawi and Grogan, 1975; Boland and Hall, 1987). In the oxalate test, temperatures up to 40 °C were encountered that did not adversely affect the correlation between greenhouse and field results. The differential response of common bean genotypes exposed to an oxalate solution has a highly significant correlation to corresponding white mold field ratings for DSI and DI, and a highly significant negative correlation to yield (Table 1.3). Screening genotypes for resistance to oxalate, a primary pathogenicity factor for *S. sclerotiorum*, is an efficient indirect method to test for

physiological resistance to white mold in common bean, and an effective method to identify new and unique sources of physiological resistance in wild and unadapted bean germplasm.

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CHAPTER 2

RELATIONSHIP OF AGRONOMIC TRAITS AND RESISTANCE TO WHITE MOLD IN THREE BEAN POPULATIONS

INTRODUCTION

White mold, caused by *S. sclerotiorum*, is a destructive yield-limiting fungal disease that seriously affects common bean production in temperate regions (Steadman, 1983; Haas and Bolwyn, 1972; Kerr et al., 1978; Purdy, 1979; Wallen and Sutton, 1967). White mold infections in bean are initiated during flowering, coinciding with canopy closure and microclimate conditions that stimulate the development of apothecia from soil-borne sclerotial bodies (Boland and Hall, 1987). Ascospores dispersed into plant canopy require a nutrient source, such as flowers, for germination (Abawi et al., 1975; Haas and Bolwyn, 1972; Hunter et al., 1978). Oxalate was identified as a primary pathogenicity factor for *S. sclerotiorum* (Godoy et al., 1990). Developing mycelium proceed to infect the plant by exuding copious amounts of oxalate into the plant tissue, creating optimal conditions for the function of polygalacturonases from the fungus that break down plant cell walls (Maxwell and Lumsden, 1970; Marciano et al., 1983). Symptoms of white mold on bean plant include wilting, lesions, bleached stems, and presence of sclerotial bodies in infected tissue.

Resistance to white mold in common bean is complexly-inherited (Fuller et al., 1984; Miklas and Grafton, 1992). Physiological resistance to white mold has been described in several navy bean cultivars, such as Bunsi and C-20, (Schwartz et al., 1987; Miklas et al., 1992; Tu and Beversdorf, 1982; Kelly et al., 1984). Mechanisms of

physiological resistance may involve several factors, including phytoalexin production (Sutton and Deverall, 1984), and resistance to tissue damage from oxalic acid (Tu, 1985). Resistance to oxalate (OR) was found to be significantly correlated to resistance to white mold in the field in a group of elite high-yielding bean genotypes (Kolkman and Kelly, 2000).

Agronomic management practices that result in reduced production of apothecia or exposure to inoculum were found to contribute to reduced white mold infections in the field environment. A decrease in plant row width, resulting in higher plant density will increase the levels of white mold (Park, 1993; Steadman et al., 1973). Elevating the canopy of a prostrate highly-susceptible indeterminate great northern bean reduced white mold infection, and increased yield (Fuller et al., 1984). Plant architectural traits that influence levels of white mold were also identified in several cultivars. Upright indeterminate navy beans escape white mold infection or spread, in comparison to determinate navy bean types, most likely due to a narrower canopy, resulting in a drier microclimate underneath the canopy (Park, 1983). Bunsi, an indeterminate navy bean, has an open porous canopy, which has been associated with reduced levels of white mold in the field (Tu and Beversdorf, 1982; Park, 1993). Alternatively, the open, porous canopy of larger-seeded determinate beans was identified as an architectural avoidance mechanism (Coyne et al., 1974; Weiss et al., 1977; Schwartz et al., 1987). Dense canopies generally resulted in higher white mold severity than porous canopies, due to the development of a favorable microclimate within the canopy (Blad et al., 1978; Coyne, 1980). Fewer apothecia were produced underneath the open canopy of the determinate dark red kidney bean cultivar, Charlevoix, and the upright canopy of the indeterminate

small white bean, Aurora, compared to the dense canopy of several prostrate type III great northern bean cultivars (Schwartz and Steadman, 1978). Many factors influence the production of apothecia and ascospores, the initial infection, and subsequent spread of disease throughout the plant tissue. The onset of flowering and production of apothecia, however, generally occurs at canopy closure under appropriate moisture conditions (Boland and Hall, 1987). The interaction between management practices, such as row width and plant density, and plant architectural traits, such as an open porous canopy, or upright architecture can affect levels of white mold in the field.

Progress in breeding for resistance in common bean is hindered by environmental conditions and factors that confound the expression and detection of physiological resistance mechanisms. The ability to identify and select individual genotypes with physiological resistance to white mold, and agronomically-desirable, architectural avoidance mechanisms, in advanced breeding lines and differentiating populations is essential in breeding for resistance to white mold. The objective of this study was to determine the relationship between specific agronomic traits, physiological resistance, measured indirectly as OR, and resistance to white mold in the field in three contrasting populations.

MATERIALS AND METHODS

Populations:

The first population was an assembly of advanced-line genotypes consisting of resistant and susceptible elite lines, advanced breeding material, and cultivars. The advanced line population was evaluated in three years and three greenhouse tests for OR

(Kolkman and Kelly, 2000). The three field tests consisted of 30 (Test 1) and 36 (Tests 2 and 3) genotypes, including cultivars and breeding lines from the navy, black, pink, pinto, great northern, cranberry, and kidney commercial classes, new sources of resistance from breeding programs across North America and the Caribbean, as well as genotypes entered in the National Bean White Mold Nursery. Twenty-seven genotypes were common across all three tests. The 27 genotypes were typically high-yielding cultivars, or advanced breeding lines previously selected for useful agronomic attributes (Kolkman and Kelly, 2000).

The second population was an 98 line F_3-derived population derived from a biparental cross between Bunsi, an indeterminate (Type II) resistant cultivar with an open porous canopy, and Newport, a determinate (Type I) susceptible cultivar (Kelly et al., 1995; Kolkman and Kelly, 2000). Bunsi has been identified to have physiological resistance to white mold, OR, and plant avoidance due to an open porous canopy (Tu and Beversdorf, 1982; Schwartz et al., 1978; Miklas et al., 1992; Tu, 1985; Kolkman and Kelly, 2000). Ninety-eight F_2 lines were advanced in the greenhouse to the F_3 generation using single seed descent. Seed from individual F_3 plants was bulked, and advanced in a greenhouse. Seed harvested from three $F_{3:4}$ plants was bulked and $F_{3:5}$ plants were increased in a winter nursery in Puerto Rico. No selection for agronomic traits was made during generation advancement.

The third population was a 28 $F_{5:6}$ recombinant inbred line (RIL) population, developed by single seed descent from a biparental cross between the resistant indeterminate (Type II) cultivar, Huron, and the susceptible determinate (Type I) cultivar, Newport (Kelly et al., 1994). Huron is a C-20 - derived cultivar, and has both

physiological resistance to white mold, as determined through OR, and resistance to white mold in the field (Kolkman and Kelly, 2000).

Physiological Resistance:

Physiological resistance in all three populations was determined indirectly, by screening the populations for OR (Kolkman and Kelly, 2000). The advanced line population was evaluated in three separate tests representing genotypes tested in field trial at the Montcalm Research Farm, Entrican, MI in 1996 (MRF96; Test 1), 1997 (MRF97; Test 2), and 1998 (MRF98; Test 3). Thirty (Test 1) and 36 (Tests 2 and 3; Kolkman and Kelly, 2000). Four (Test 2 and 3) or five (Test 1) seedlings (samples) were used in each of the three replications of the randomized complete block design (RCBD). The Bunsi/Newport (BN) population was evaluated for OR in an RCBD using four replications over time, using four samples per entry in each of four replications over time. The Huron/Newport (HN) population was evaluated three times for OR, using five samples per entry for each of three replications in an RCBD. Twenty-day old seedlings (2^{nd} trifoliate emerging) were cut at the base of the stem, placed in a 20 mM oxalate solution (pH = 4.0), and rated for wilting symptoms, using a 1 to 6 scale used to measure wilting (see Chapter 1 for details).

Field Experiments:

All three populations were evaluated for resistance to white mold in the field. The advanced line population were grown in the field trials MRF96, MRF97, and MRF98. The BN $F_{3:6}$ population was grown in the field in MRF97, and Sanilac Cooperator Farm

(SCF97), and $F_{3:7}$ lines were grown in MRF98. Only 88 of the 98 $F_{3:6}$ lines in the BN population were grown in SCF97, due to limited seed availability. The HN RIL population was grown in MRF96, MRF97, MRF98, as well as in SCF97. Planting was delayed to the second week in June in all field experiments to favor disease development. In the Montcalm Research Farm experiments, plots were 6 m in row length, with a 0.5 m row spacing in the Montcalm Research Farm experiments. At the SCF97 field site, all plots were 3 m in row length, and 0.76 m in row width. The inner two rows of each four row plot were planted with the experimental line, while the outer two rows were planted with a highly susceptible cultivar, Midland, used as a uniform border. Standard agronomic practices for tillage, fertilization, and herbicide were applied to ensure good crop growth and development at both field sites. In MRF, plots were irrigated during initial flowering with 13 mm of water at approximately three day intervals, depending upon rainfall, in order to promote uniform disease pressure across the field. The field experiments were irrigated with an overhead sprinkler system five times in 1996, three times in 1997, and six times in 1998. Uniform infection of white mold of dry bean grown at the MRF, was identified in previous field studies. The field site at SCF97 was located in a cooperators field, and selected based on past history of heavy white mold infection in previous years.

Disease Severity and Incidence:

Plots were rated for disease severity index (DSI) and disease incidence (DI) (Steadman 1997; Kolkman and Kelly, 2000; Steadman et al., 1998) using a 'quarter scale' (Hall and Phillips, 1996), approximately one month prior to maturity (early season

rating), and shortly before harvest (final season rating), when the majority of plants had reached physiological maturity. The change in DSI and DI was calculated as the difference between the early and final disease ratings. Thirty plants per plot were each given a rating from 0 to 4, where 0 = no disease present, 1 = 1 to 25% of the plant with white mold symptoms, 2 = 26 to 50% of the plant with white mold symptoms, 3 = 51 to 75% of the plant with white mold symptoms, and 4 = 76 to 100% of the plant with white mold symptoms. Disease Severity Index was calculated for each plot on a percentage basis, using the following formula:

$$DSI = \frac{\Sigma \text{ (rating of each plant)}}{4 \times \text{(number of plants rated)}} \times 100 \quad .$$

Disease incidence was calculated as the number of plants out of the thirty individuals with white mold infection, based as a percentage.

Agronomic Traits:

Genotypes in all three populations, were evaluated for the following agronomic traits: growth habit, days to flowering, mid-season canopy height, mid-season canopy width, architecture, days to maturity, lodging, yield, and seed size. Growth habit was determined during the growing season, as either indeterminate (Type II or III) or determinate (Type I). Days to flowering were characterized by the number of days following planting, when 50% of the plants in a plot have at least one open flower. At mid-season (post main flower flush) canopy height and width measurements were

averaged on each individual plot, from six measurements per plot (three measurements per row) in all experiments except for the MRF96 trials, where 10 measurements per plot were taken. Plots were evaluated for architecture at maturity, using a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate. Days to maturity were calculated as the number of days following planting, until 90% of the pods were physiologically mature and drying down. Lodging was determined at maturity, based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging. All plots were harvested at maturity after the late season ratings were taken. Plots were individually pulled, and threshed in a Hege combine. Seed weight was recorded and reported at a moisture content of 18%. Seed size was determined as the weight of 100 seeds adjusted to 18% moisture content.

Statistical Analysis:

All greenhouse experiments were analyzed as RCBDs, using PROC GLM (SAS, 1995). In the advanced line populations, the MRF96 experiment was analyzed as a rectangular lattice, and the MRF97 and MRF98 field experiments were each analyzed as a partially balanced triple lattice using PROC LATTICE (SAS, 1995). The 27 common genotypes were analyzed across all three tests (greenhouse) and years (field) as a RCBD, using PROC GLM (SAS, 1995), with environments considered as a random effect, and genotypes as a fixed effect. The BN and HN populations were evaluated in individual greenhouse tests and field environments as RCBDs, using PROC GLM (SAS, 1995). Both genetic populations were analyzed across the three years as a RCBD, using PROC GLM, with both genotypes and environments considered as random effects. Estimates of

heritability for all traits were calculated on a plot basis, where $h^2 = (\sigma_g^2)/[\sigma^2/(re) + \sigma_{ge}^2/e + \sigma_g^2]$ (Hallauer and Miranda, 1988). Growth habit in the genetic populations was either determinate or indeterminate, and a chi-square goodness of fit test was used to test for a normal segregation ratio. Pearson correlation coefficients (r) were calculated by PROC CORR (SAS, 1995).

RESULTS

Significant genotypic variation for OR, DSI, DI, and agronomic traits across environments was identified in all three populations (Tables 2.1, 2.2, and 2.3). Mean values and range in DSI and DI indicated that adequate disease pressure was attained in each environment (Tables 2.4, 2.5, 2.6 and 2.7), except for the MRF98 environment, in which an early DSI and DI ratings were not taken due to the short season. Parents of the genetic populations differed for OR, DSI, DI, and all agronomic traits, except lodging. Significant variation for lodging was identified in the progeny of both BN and HN populations, even though the parental genotypes were not different. Other architectural traits may have been segregating in both populations to sufficiently affect transgressive segregation for lodging. Lodged plants during the growing season may affect the microclimate to favor white mold development.

The correlations between OR, DSI, DI and agronomic traits were markedly different in the three populations across environments (Table 2.8). Disease severity was significantly correlated to DI ($P<0.0001$) in each population, and in each environment. In the advanced line population, OR was significantly correlated to DSI ($r=0.58$; $P<0.01$)

Table 2.1. Analysis of variance for resistance and agronomic traits in the advanced line population, for 27 common genotypes tested across three greenhouse assays and three field environments (1996-1998).

Source	Mean squares			
Resistance Traits:	Genotype (G)	Environment (E)	G x E	Replication (Environment)
df	26	2	252	6
Greenhouse:				
OR†	2.3****	35.7****	0.5****	0.3
Field:				
Final DSI†	1512.9****	8817.5****	418.7***	175.9
Final DI†	2566.2****	5426.0**	734.4**	273.5
Early DSI	243.1***	2.4	68.9*	37.1
Early DI	1479.4****	891.8	244.9	102.2
Agronomic Traits:				
Days to Flowering	86.1****	519.7****	2.5****	3.6**
Canopy Height	87.7****	10387.8****	25.4****	7.6
Canopy Width	57.1***	1536.7****	20.2****	41.5****
Architecture	8.0****	0.2	0.5****	0.2
Lodging	7.4****	15.3*	0.8****	1.8****
Days to Maturity	98.3****	12992.2****	25.1****	34.8****
Seed Size	1070.9****	39.5	18.9****	2.7
Yield	76.8***	156.8	27.1****	88.9****

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† OR = resistance to oxalate; DSI = disease severity index; DI = disease incidence

and DI (r=0.57; P<0.01), whereas in both the BN and HN populations, OR was not significantly correlated to DSI or DI.

The only agronomic avoidance trait significantly associated with DSI (r=-0.54; P<0.01) and DI (r=-0.51; P<0.01) in the advanced population was days to maturity. An increase in days to maturity was associated with low DSI (r =-0.51; P<0.01) and DI (r=-0.43; P<0.01) ratings. Days to maturity, however, was the only agronomic avoidance mechanism that was not associated with DSI or DI in the BN population. Fewer days to

Table 2.2. Analysis of variance for resistance and agronomic traits in the Bunsi/Newport population, tested across three greenhouse assays and three field environments (1997-1998).

Source	Mean squares			
Resistance Traits:	Genotype (G)	Environment (E)	G x E	Replication (Environment)
df	97	2	184	6
Greenhouse:				
OR‡	37.6†	-	-	40.4****
Field:				
Final DSI‡	1580.5****	12803.7**	838.1****	828.4*
Final DI‡	2069.5***	37766.5****	1196.5****	1278.5*
Early DSI	394.8**	252.4	232.1****	185.8
Early DI	1832.2***	69591.5***	865.2****	1441.7**
Agronomic Traits:				
Days to Flowering	47.5****	8.9	4.6****	5.9**
Canopy Height	135.1****	260.8	15.0****	190.3****
Canopy Width	85.4****	21774.7****	30.2****	230.3****
Architecture	1.0****	8.4**	0.3****	0.8***
Lodging	3.5****	16.2	0.8**	4.2****
Days to Maturity	89.2****	56614.3****	22.8*	80.1***
Seed Size	33.8****	399.1****	3.3****	9.0****
Yield	107.8****	3728.7****	44.5****	83.6****

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ OR = resistance to oxalate; DSI = disease severity index; DI = disease incidence

flowering, a more upright architecture, shorter and narrower canopy at mid-season, and a lower lodging score were all significantly associated with lower DSI and DI scores in the BN population (Table 2.8). Alternatively, shorter canopy height at mid-season was the only agronomic avoidance mechanism to be significantly associated with DSI (r=0.59; P<0.001) or DI (r=0.73; P<0.0001) in the HN population. Correlations between disease resistance and agronomic traits varied between individual environments, yet general trends remained similar (Tables 2.9, 2.10, and 2.11). In individual environments,

Table 2.3. Analysis of variance for resistance and agronomic traits in the Huron/Newport population, tested across three greenhouse assays and four field environments (1996-1998).

Source	Mean squares			
Resistance Traits:	Genotype (G)	Environment (E)	G x E	Replication (Environment)
df	27	3	81	8
Greenhouse:				
OR‡	1.2**	27.2****	0.6****	0.5*
Field:				
Final DSI‡	1362.8****	16878.7***	24.7	732.8**
Final DI‡	2128.2****	47810.3****	510.7	1357.7**
Early DSI	371.6***	3409.1**	130.0	283.4*
Early DI	1535.7**	28965.7**	626.1**	1734.7***
Agronomic Traits:				
Days to Flowering	58.3****	641.5****	3.6****	2.7*
Canopy Height	62.6****	7492.7****	21.3****	60.7****
Canopy Width	64.0**	5717.1****	27.2***	197.7****
Architecture	2.7****	11.9****	0.5****	0.3*
Lodging	2.5***	23.6**	0.7*	1.7***
Days to Maturity	166.3****	6900.7****	8.1	21.7**
Seed Size	57.3****	217.3***	3.6****	12.5****
Yield	76.0**	1396.7****	36.3****	59.7****

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ OR = resistance to oxalate; DSI = disease severity index; DI = disease incidence

OR was only significantly associated with DSI and DI in the advanced line population. The BN population had a large number of agronomic avoidance mechanisms, such as days to flowering, canopy width, and lodging, that were correlated to DSI and DI. The HN population had only few agronomic avoidance traits that were significantly associated with DSI and DI, such as canopy height. Overall, striking variability in correlations between agronomic avoidance mechanisms existed between the advanced population, and both the BN and HN populations.

Table 2.4. Means and ranges for resistance and agronomic traits for the advanced line population tested across individual and combined environments.

	MRF96[†]	MRF97[†]	MRF98[†]	Combined Environments
Resistance Traits:	mean (range)[‡]	mean (range)	mean (range)	mean (range)
OR[¶]	3.0 (2.0-4.0)	1.7 (1.0-3.4)	2.5 (1.3-3.6)	2.4 (1.7-3.4)
Final DSI[¶] (%)	24.3 (0.0-79.3)	32.4 (1.1-70.3)	12.2 (1.7-40.8)	22.7 (3.1-51.4)
Final DI[¶] (%)	36.7 (2.2-87.8)	47.1 (2.2-86.7)	30.7 (5.6-81.1)	38.0 (7.8-74.8)
Early DSI (%)	6.8 (0.0-43.1)	6.4 (0.8-16.7)	--	6.5 (0.7-28.3)
Early DI (%)	20.0 (0.0-67.8)	23.8 (3.3-56.7)	--	21.3 (2.8-57.2)
Agonomic Traits:				
Days to flowering	40.3 (32.0-45.3)	44.9 (38.7-49.3)	42.9 (36.7-47.3)	42.7 (36.6-47.1)
Canopy height (cm)	34.1 (27.4-40.3)	55.6 (46.6-62.6)	51.9 (40.3-60.8)	46.8 (40.4-51.2)
Canopy width (cm)	35.1 (30.5-39.7)	42.7 (36.7-52.2)	42.1 (32.9-47.7)	40.3 (34.8-44.4)
Architecture[§]	2.7 (1.0-5.0)	2.6 (1.0-4.3)	2.6 (1.0-5.0)	2.7 (1.0-4.2)
Lodging[£]	2.2 (1.0-5.0)	2.9 (1.0-4.7)	2.3 (1.0-4.7)	2.5 (1.2-4.6)
Days to maturity	98.9 (87.3-108.7)	112.1 (102.3-119.3)	86.9 (80.0-99.3)	99.4 (89.9-105.7)
Seed size (g 100seed^{-1})	25.7 (16.9-64.9)	27.4 (16.3-79.6)	25.1 (17.8-58.4)	25.6 (17.0-67.6)
Yield (kg·ha^{-1})	3122 (2235-4077)	3380 (2167-4436)	3099 (2010-3785)	3212 (2325-3661)

[†] MRF96 = Montcalm Research Farm, 1996, MRF97 = Montcalm Research Farm, 1997; MRF98 = Montcalm Research Farm, 1998

[‡] Mean values for 30 genotypes in MRF96 (Test 1), 36 genotypes in MRF97 (Test 2) and MRF98 (Test 3), and 27 combined gentoypes in the combined analysis.

[¶] OR = resistance to oxalate, where 1 = no wilting symptoms, 2 = 1 leaf with wilting symptoms, 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem collapsing; DSI = disease severity index; DI = disease incidence

[§] Architecture based on a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate

[£] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

Yield was significantly associated with DSI and DI in both the advanced population and BN population, but not in the HN population (Table 2.8). In the BN population, large seed size was also significantly associated with low DSI (r=-0.44; P<0.0001) and DI (r=-0.43; P<0.0001). Seed size in the BN population may be both a strong component of yield, and vary as a result of white mold infection (Kerr et al., 1978). Physiological resistance, measured as OR, was significantly associated with yield

Table 2.5. Means and ranges for resistance and agronomic traits for the Bunsi/Newport population tested across three individual environments.

Resistance Traits:	MRF97[†] mean (range)	SCF97[†] mean (range)	MRF98[†] mean (range)
Final DSI[‡] (%)	35.5 (3.9-85.0)	46.5 (0.8-86.1)	32.4 (1.4-76.1)
Final DI[‡](%)	47.6 (7.8-95.6)	71.1 (2.2-100.0)	52.0 (5.6-97.8)
Early DSI (%)	18.9 (2.2-59.4)	20.6 (1.1-45.8)	--
Early DI (%)	38.2 (5.6-93.3)	62.0 (3.3-98.9)	--
Agronomic Traits:			
Days to flowering	42.7 (36.7-49.5)	42.7 (37.0-48.7)	42.4 (38.3-47.0)
Canopy height (cm)	49.2 (35.0-58.2)	50.9 (37.5-59.6)	49.2 (33.8-58.1)
Canopy width (cm)	43.4 (34.1-51.5)	59.0 (43.2-70.4)	42.9 (31.8-49.6)
Architecture[¶]	2.7 (1.0-3.0)	2.9 (2.0-3.3)	2.5 (1.3-3.7)
Lodging[§]	2.7 (1.7-5.0)	3.3 (1.3-5.0)	3.0 (1.0-5.0)
Days to maturity	111.6 (100.0-121.3)	110.9 (97.0-117.7)	86.8 (83.0-95.7)
Seed size (g 100seed^{-1})	20.9 (17.1-26.4)	22.9 (17.0-29.0)	20.7 (16.7-25.3)
Yield (kg ha^{-1})	2740 (1696-4009)	3481 (1348-4705)	2785 (1853-3717)

[†] MRF97 = Montcalm Research Farm, 1997, SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998

[‡] DSI = disease severity index; DI = disease incidence

[¶] Architecture based on a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate

[§] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

within and across environments for both the advanced line (r=-0.51; P<0.01) and BN population (r=-0.31; P>0.01; Tables 2.8, 2.9, 2.10, and 2.11). Yield was not associated with OR for the HN population in the individual environments.

Growth habit ranged from determinate (Type I), to indeterminate (Type II), and indeterminate prostrate (Type III) were observed in the advanced line population. Growth habit segregated between determinate (Type I) and indeterminate (Type II) growth habit in the BN population [χ^2 = 1.98 (5:3); 0.15<P<0.20], and the HN population [χ^2 = 0.57 (1:1); 0.40<P<0.50]. The indeterminate growth habit was significantly associated with low OR ratings in the advanced line population (r=-0.56; P<0.01), the

Table 2.6. Means and ranges for resistance and agronomic traits for the Huron/Newport population tested across individual environments.

Resistance Traits:	MRF96[†] mean (range)	MRF97[†] mean (range)	SCF97[†] mean (range)	MRF98[†] mean (range)
OR[‡]	2.9 (1.7-3.7)	1.8 (1.1-3.2)	2.4 (1.5-3.5)	--
Final DSI[‡] (%)	23.0 (1.7-54.4)	20.4 (1.1-50.3)	48.4 (7.8-69.7)	17.6 (3.1-40.0)
Final DI[‡] (%)	36.1 (3.3-81.1)	32.7 (2.2-71.1)	82.2 (21.1-96.7)	34.8 (5.6-68.9)
Early DSI (%)	11.1 (1.4-33.0)	13.5 (0.3-35.8)	23.2 (6.1-46.7)	--
Early DI (%)	32.7 (5.6-92.2)	26.1 (1.1-56.7)	61.0 (14.4-90.0)	--
Agronomic Traits:				
Days to flowering	37.2 (31.0-41.7)	43.4 (37.7-47.3)	42.7 (37.3-52.0)	41.2 (36.7-48.3)
Canopy height (cm)	32.4 (28.1-36.9)	52.9 (43.4-56.6)	52.1 (42.1-56.7)	46.8 (37.7-53.3)
Canopy width (cm)	32.5 (28.3-36.4)	40.9 (36.4-44.9)	52.0 (44.8-63.7)	37.7 (29.3-44.7)
Architecture[¶]	2.6 (1.0-3.0)	2.5 (1.0-3.3)	2.8 (2.0-3.7)	1.9 (1.0-3.0)
Lodging[§]	2.1 (1.3-3.7)	2.7 (1.7-4.3)	3.3 (2.0-4.7)	2.3 (1.0-3.3)
Days to maturity	95.8 (88.3-105.3)	104.0 (96.0-113.3)	106.6 (98.0-115.0)	86.5 (80.0-97.0)
Seed size ($g\ 100seed^{-1}$)	20.6 (16.9-25.4)	20.8 (16.3-25.2)	24.0 (19.4-28.4)	22.3 (17.7-27.3)
Yield ($kg\ ha^{-1}$)	2852 (2123-3661)	2869 (1827-3440)	3706 (1943-4301)	2684 (1999-3421)

[†] MRF96 = Montcalm Research Farm, 1996, MRF97 = Montcalm Research Farm, 1997, SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998

[‡] OR = resistance to oxalate averaged over three tests, where 1 = no wilting symptoms, 2 = 1 leaf with wilting symptoms, 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem collapsing; Greenhouse Tests 1, 2, and 3.

[¶] Architecture based on a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate

[§] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

BN population (r=-0.36; P<0.001), and the HN population (r=-0.50; P<0.01). Growth habit was not associated with DSI or DI in the advanced line population, most likely due to the presence of susceptible indeterminate prostrate Type III cultivars, and determinate large-seeded cultivars that escaped infection. The determinate growth habit was significantly associated with high DSI (r=-0.20; P<0.05) in the BN population. Growth habit was not associated with DSI or DI for the HN population in either the individual or combined environments. Architectural avoidance mechanisms, such as canopy height

Table 2.7. Parental and progeny means, progeny ranges, and estimates of heritability (h^2) for resistance and agronomic traits in the Bunsi/Newport and Huron/Newport populations tested across combined environments.

	Bunsi/Newport Population				Huron/Newport Population			
	Parental means		Progeny		Parental means		Progeny	
Resistance Traits	Bunsi	Newport	mean (range)	h^2	Huron	Newport	mean (range)	h^2
OR[†]	1.2	2.1	1.9 (1.1-2.7)	0.19	2.0	3.3	2.4 (1.6-2.9)	0.54
Final DSI[†] (%)	28.7	68.7	37.8 (4.4-68.6)	0.47	19.8	50.7	27.3 (5.6-50.1)	0.82
Final DI [†] (%)	35.3	80.2	56.4 (8.5-88.5)	0.42	35.3	72.9	46.4 (12.2-73.9)	0.76
Early DSI (%)	11.4	32.4	19.7 (2.2-41.1)	0.60	12.3	34.1	15.9 (5.5-30.7)	0.65
Early DI (%)	35.6	62.2	49.5 (5.6-96.1)	0.61	34.8	71.3	39.9 (11.5-75.2)	0.59
Agronomic Traits								
Days to flowering	41.6	43.1	42.6 (37.7-48.4)	0.90	40.5	41.3	41.1 (35.7-47.3)	0.98
Canopy height (cm)	48.0	52.5	49.7 (35.7-57.8)	0.89	46.0	47.4	46.1 (38.2-50.1)	0.66
Canopy width (cm)	49.0	43.5	48.0 (37.0-55.1)	0.65	39.6	39.3	40.8 (36.2-45.3)	0.58
Architecture[‡]	3.0	2.6	2.7 (1.5-3.3)	0.73	1.9	2.7	2.4 (1.4-3.1)	0.33
Lodging[¶]	2.9	2.9	3.0 (1.3-4.4)	0.78	2.7	2.7	2.6 (1.7-3.7)	0.71
Days to maturity	105	100	102.7 (91.0-110.6)	0.74	98	97	98.2 (90.8-107.7)	0.95
Seed size (g 100seed^{-1})	22.9	19.1	21.5 (17.5-26.9)	0.90	23.8	19.1	21.9 (18.0-25.8)	0.94
Yield (kg ha^{-1})	3531	2288	2987 (1988-3818)	0.59	3256	2268	3028 (2542-3488)	0.52

[†] OR = resistance to oxalate averaged over three tests, where 1 = no wilting symptoms, 2 = 1 leaf with wilting symptoms, 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem collapsing; DSI = disease severity index; DI = disease incidence

[‡] Architecture based on a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate

[¶] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging

Table 2.8. A comparison of Pearson correlation coefficient (r) for resistance to oxalate, disease severity index, and disease incidence to resistance and agronomic traits between the 27 common genotypes in the advanced line population, and the BN and HN populations across combined environments.

	Advanced Line Population			Bunsi/Newport Population			Huron/Newport Population		
Resistance Traits:	OR‡	DSI‡	DI‡	OR	DSI	DI	OR	DSI	DI
OR	-	0.58**	0.57**	-	0.18†	0.14	-	-0.14	-0.31
Final DSI	0.58**	-	0.97****	0.18†	-	0.97****	-0.14	-	0.95****
Final DI	0.57**	0.97****	-	0.14	0.97****	-	-0.31	0.95****	-
Early DSI	0.64***	0.83****	0.76****	0.13	0.81****	0.79****	-0.28	0.81****	0.81****
Early DI	0.65***	0.87****	0.85****	0.05	0.79****	0.80****	-0.46*	0.83*	0.89****
Agronomic Traits:									
Growth habit	-0.56**	-0.29	-0.32	-0.36***	-0.20*	-0.18†	-0.50**	-0.10	0.08
Days to flowering	-0.39*	-0.07	0.02	0.02	0.51****	0.52****	-0.27	0.29	0.36†
Canopy height	-0.23	-0.24	-0.19	-0.20†	0.22*	0.25*	-0.43*	0.59***	0.73****
Canopy width	-0.24	-0.15	-0.14	-0.09	0.52****	0.59****	0.09	0.16	0.22
Architecture	0.06	0.16	0.12	0.18*	0.20*	0.23**	0.54**	0.00	-0.12
Lodging	-0.07	0.22	0.21	0.10	0.33***	0.34***	-0.11	-0.05	0.03
Days to maturity	-0.54**	-0.51**	-0.43*	-0.29**	0.11	0.16	-0.14	0.19	0.26
Seed size	0.49**	0.02	0.01	-0.07	-0.44****	-0.43****	0.08	-0.19	-0.16
Yield	-0.51**	-0.63***	-0.66***	-0.31**	-0.49****	-0.44****	-0.34†	-0.28	-0.12

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ OR = resistance to oxalate; DSI = disease severity index; DI = disease incidence

Table 2.9. Pearson correlation coefficient (r) for resistance to oxalate, disease severity index, disease incidence, and agronomic traits in the individual environments for the advanced line population.

	MRF96‡			MRF97‡			MRF98‡		
Resistance Traits	OR¶	DSI¶	DI¶	OR	DSI	DI	OR	DSI	DI
OR	-	0.48**	0.41*	-	0.31†	0.32†	-	0.46**	0.39*
Final DSI	0.48**	-	0.97****	0.31†	-	0.98****	0.46**	-	0.92****
Final DI	0.41*	0.97****	-	0.32†	0.98****	-	0.39*	0.92****	-
Early DSI	0.47**	0.83****	0.77****	0.46**	0.82****	0.80****	-	-	-
Early DI	0.41*	0.86****	0.87****	0.47**	0.83****	0.82****	-	-	-
Agronomic Traits									
Growth habit	-0.06	-0.05	-0.01	-0.51**	-0.05	-0.07	-0.44**	-0.34*	-0.41*
Days to flowering	-0.27	-0.10	0.03	-0.42**	0.04	0.11	-0.38	-0.26	-0.11
Architecture	-0.22	0.18	0.17	0.21	0.44**	0.42**	0.10	0.07	-0.06
Canopy height	-0.04	0.35†	0.42*	-0.16	-0.17	-0.16	-0.44**	-0.41*	-0.26
Canopy width	-0.32†	0.12	0.23	0.05	0.26	0.26	-0.27	-0.01	-0.04
Lodging	-0.18	0.24	0.29	0.23	0.62****	0.61****	-0.13	0.05	0.04
Days to maturity	-0.41*	-0.39*	-0.32†	-0.18	-0.65****	-0.61****	-0.18	-0.29†	-0.20
Seed size	0.10	-0.02	-0.06	0.73****	0.04	0.05	0.38*	0.06	-0.03
Yield	-0.35†	-0.52**	-0.43*	-0.41*	-0.36*	-0.38*	-0.28†	-0.31†	-0.31†

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ MRF96 = Montcalm Research Farm, 1996, MRF97 = Montcalm Research Farm, 1997; MRF98 = Montcalm Research Farm, 1998

¶ OR = resistance to oxalate (each greenhouse test corresponding to an individual field test); DSI = disease severity index; DI = disease incidence

Table 2.10. Pearson correlation coefficient (r) for resistance to oxalate, disease severity index, disease incidence, and agronomic traits in the individual environments for the Bunsi/Newport population.

	MRF97‡			SCF97‡			MRF98‡		
Resistance Traits:	OR¶	DSI¶	DI¶	OR	DSI	DI	OR	DSI	DI
OR	-	0.18†	0.15	-	-0.01	-0.12	-	0.22*	0.23*
Final DSI	0.18†	-	0.98****	-0.01	-	0.95****	0.22*	-	0.96****
Final DI	0.15	0.98****	-	-0.12	0.95****	-	0.23*	0.96****	-
Early DSI	0.21*	0.89****	0.85****	-0.02	0.90****	0.86****	-	-	-
Early DI	0.18†	0.91****	0.92****	-0.06	0.92****	0.95****	-	-	-
Agronomic Traits:									
Growth habit	-0.34***	-0.29**	-0.29**	-0.42****	-0.02	0.06	-0.33***	-0.08	-0.10
Days to flowering	0.03	0.41****	0.43****	0.05	0.71****	0.69****	-0.04	-0.03	0.01
Architecture	0.14	0.30**	0.35***	0.21*	0.33**	0.30**	0.15	0.14	0.17
Canopy height	-0.14	0.33***	0.31**	-0.22*	0.24*	0.28**	-0.20*	0.10	0.14
Canopy width	-0.05	0.49****	0.53****	-0.15	0.66****	0.72****	-0.03	0.40****	0.45****
Lodging	0.19†	0.39****	0.43****	0.08	0.23*	0.22*	0.05	0.32**	0.28**
Days to maturity	-0.31**	-0.02	-0.00	-0.20†	0.58****	0.68****	-0.17†	-0.05	-0.05
Seed size	-0.02	-0.41****	-0.44****	-0.06	-0.49****	-0.45****	-0.14	-0.31**	-0.30**
Yield	-0.36***	-0.42****	-0.42****	-0.12	-0.70****	-0.63****	-0.29**	-0.51****	-0.42****

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ MRF97 = Montcalm Research Farm, 1997, SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998

¶ OR = resistance to oxalate; DSI = disease severity index; DI = disease incidence

Table 2.11. Pearson correlation coefficient (r) for resistance to oxalate, disease severity index, disease incidence, and agronomic traits for individual environments in the Huron/Newport population.

	MRF96‡			MRF97‡			SCF97‡			MRF98‡		
Resistance Traits:	OR¶	DSI¶	DI¶	OR	DSI	DI	OR	DSI	DI	OR	DSI	DI
OR	-	-0.23	-0.38 *	-	-0.22	-0.19	-	0.00	-0.07	-	-0.10	-0.10
Final DSI	-0.23	-	0.96 ****	-0.22	-	0.96 ****	0.00	-	0.88 ****	-0.10	-	0.95 ****
Final DI	-0.38 *	0.96 ****	-	-0.19	0.96 ****	-	-0.07	0.88 ****	-	-0.10	0.95 ****	-
Early DSI	-0.28	0.75 ****	0.75 ****	-0.13	0.87 ****	0.82 ****	-0.19	0.77 ****	0.64 ***	-	-	-
Early DI	-0.50 **	0.83 ****	0.89 ****	-0.24	0.91 ****	0.69 ****	-0.21	0.83 ****	0.82 ****	-	-	-
Agronomic Traits:												
Growth habit	-0.53 **	-0.14	0.06	-0.34 †	-0.24	-0.09	-0.45 *	0.12	0.24	-0.45 *	-0.29	-0.31
Days to flowering	-0.42 *	0.10	0.17	-0.31	0.31	0.34 †	-0.08	0.46 *	0.51 **	-0.18	0.07	0.05
Canopy height	-0.39 *	0.44 *	0.48 **	-.05	0.26	0.27	-0.24	0.42 *	0.52 **	-0.26	0.00	0.03
Canopy width	-0.45 *	0.35 †	0.42 *	-0.15	0.34 †	0.34 †	0.30	0.32 †	0.49 **	-0.22	-0.02	-0.05
Architecture	0.37 †	0.06	-0.06	0.47 **	0.19	0.18	0.53 **	0.22	0.17	0.33	0.27	0.22
Lodging	-0.26	0.06	0.12	0.02	0.37	0.39 *	0.08	0.22	0.36 †	-0.13	-0.02	-0.14
Days to maturity	-0.19	-0.08	0.00	-0.15	0.06	0.20	-0.08	0.46 *	0.58 **	0.00	-0.25	-0.26
Seed size	0.13	-0.22	-0.22	0.14	-0.36 †	-0.28	0.02	-0.25	-0.16	0.12	-0.62 ***	-0.67 ****
Yield	-0.25	-0.36 †	-0.23	-0.26	-0.07	0.05	-0.17	-0.34 †	-0.24	-0.48	-0.35 †	-0.32 †

*, **, ***, **** significant at $P < 0.05$, 0.01, 0.001 and 0.0001 levels, respectively

† significant at $P < 0.10$

‡ MRF96 = Montcalm Research Farm, 1996; MRF97 = Montcalm Research Farm 1997; SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998

[1] OR = resistance to oxalate averaged across three greenhouse tests; DSI = disease severity index; DI = disease incidence

and width most likely affected the correlations between field disease ratings in the population and growth habit.

In all environments, the correlation between DSI and DI was highly significant (Tables 2.8, 2.9, 2.10, and 2.11). Genotypic variation for the change in DSI between early and final ratings were significant for all three populations. Significant genotypic variation in the change in DI was only identified in the advanced line population. The only significant association between change from early to final DSI and DI, and OR was observed in the advanced line population across environments (DSI; r=0.42; P<0.05), at the MRF96 environment (DSI; r=0.40; P<0.05).

Heritability estimates for resistance to white mold in the BN population were 0.47 for DSI, and 0.42 for DI (Table 2.7). Estimates of heritability in the HN population for DSI (0.82) and DI (0.76) were higher than those for the BN population. Heritability estimates for OR in the BN population (0.19) and HN population (0.54) were both lower than the corresponding heritability estimates for DSI and DI.

DISCUSSION

The relationship between resistance and agronomic traits in three different populations of common bean was investigated in this study. The first population was a group of advanced cultivars and germplasm of various market classes and geographic origin. Physiological resistance, as determined through OR, was significantly correlated to DSI and DI, while agronomic traits, such as days to flowering, canopy height and width, were not important factors relating to disease levels in the field. The BN

population, was comprised of F_3-derived lines with no prior selection for high yield potential. In this population, a weak association existed between OR and DSI in the field across environments (r=0.18; P<0.10). There was no association between OR and DSI or DI in the field in the HN population. Similar results have been found using an alternative measure of physiological resistance to white mold in bean. The length of fungal lesions on stems was significantly correlated to white mold resistance in the field, in a small group of relatively elite germplasm (Miklas et al., 1992). The correlation between the lesion length and white mold resistance in the field varied, however, in a segregating genetic populations (Miklas and Grafton, 1992). Genetic correlation between lesion length and disease resistance in the field was not significant in a cross between Bunsi and a susceptible determinate navy bean, D76125. In two other populations with a resistant determinate (Type I) snap bean, NY5262, and differing susceptible indeterminate (Type III) pinto bean parents, genetic correlations varied between significant and non-significant correlations. Architectural avoidance mechanisms were cited as reasons for variability in correlations between a measure of physiological resistance (lesion length), and resistance to white mold in the field (Miklas and Grafton, 1992). The BN and HN populations both shared the common susceptible parent, Newport. The variation in correlations between agronomic and disease-related traits indicate that the choice of resistant parent is very important in determining potential avoidance mechanisms in a segregating genetic population. The heritability estimate for OR was much higher in the HN population, than in the BN population. The oxalate test rates for a wilting response, that can be influenced by the environmental conditions during the test. The overall mean of OR in the parents and progeny of the BN population was lower than the tests for the HN population or the

advanced line population. The environmental conditions may have influenced wilting response, and the corresponding heritability estimate. The HN population was at a more advanced stage of homozygosity than the BN population. In addition, the oxalate test was repeated three times, with three replications per test in the HN population, whereas the BN population was tested with four replications over time.

The methodology in rating physiological resistance can also be an important source of variability of correlations between physiological resistance and field ratings. In soybean, three unique tests for physiological resistance on 18 cultivars resulted in varying associations within tests, as well as to the field evaluations (Kim et al., 2000). One of the methodologies involved placing mycelial plugs on soybean cotyledons. Two mycelial plug tests were conducted on the same genotypes, and the results between tests were not significantly correlated to each other. Choice of screening method for determining physiological resistance is an important factor in evaluating advanced line populations as well as segregating populations.

Agronomic traits that could contribute to disease avoidance in the field environment, such as days to flowering and canopy width, were significantly associated with the presence of disease in the field in the BN population. Days to maturity, which was a significant factor in the field disease ratings in the advanced line population, was not associated with the presence of disease in the field for the BN population. In soybean, agronomic traits, such as flowering date, plant height, lodging and maturity, have been shown to play a significant role in disease levels in the field in a segregating soybean population (Kim and Diers, 2000). Bunsi and Newport are both well adapted to the Michigan environment. Segregation among progeny for architectural traits and

phenological traits, such as days to flowering, was large and can greatly affect the detection of resistance versus avoidance mechanisms. Greater architectural similarity between Huron and Newport, resulted in less segregation for architectural traits, and less architectural avoidance in the HN population. The determinate growth habit in larger-seeded determinate genotypes was identified with an open porous canopy, and a component of architectural avoidance in previous studies under the semi-arid conditions of intermountain states (Coyne, 1980). In the BN and HN navy bean populations, the indeterminate growth habit had physiological resistance to white mold, whereas the determinate growth habit was identified with low OR ratings. Determinate navy bean cultivars such as Newport and Midland, grown in the Midwest region, were very susceptible to white mold, as seen in the advanced line population (Kolkman and Kelly, 2000).

Heritability estimates varied for traits across populations. In the BN population, estimates of heritability for the agronomic traits that were significantly correlated to DSI and DI were generally much higher than those for the OR, DSI and DI. Estimates of heritability for days to flowering was very high, at 0.90, and moderate to high for canopy width at 0.65. Heritability estimates for resistance to white mold in the field can be misleading, if traits with high heritability, such as days to flowering, play a major avoidance role in the field. If selection in the BN population was based solely on disease ratings, gain for disease resistance may increase, but such selection may be inadvertently identifying genotypes with undesirable agronomic avoidance traits that reduce yield. In the HN population, DSI and DI were only significantly associated with canopy height (h^2 = 0.66) across environments. The estimate of heritability for DSI in this population was

higher than in the BN population, possibly due less environmental variation in architectural traits, less variability in days to flowering, and less avoidance mechanisms. Agronomic avoidance traits, such as a more upright architecture in parents, may have reduced overall variability in resistance to white mold. Plant breeders making selections for low DSI or DI cannot rely solely on ratings at the end of the season. The breeder must know if the population is segregating for agronomically undesirable avoidance mechanisms that have a negative effect on yield.

The study of complexly-inherited traits is highly dependent upon the physical environment in which the phenotype is measured, and the genetic composition of the population. Low to moderate levels of white mold infection have been associated with higher yields, whereas high levels of white mold infection can result in severe yield loss (Kerr et al., 1978). The SCF97 environment had a very severe level of white mold infection compared to the other environments, and the mean yield was higher than in other environments. Genotypes in the SCF97 environment produced both the highest yield, as well as the lowest yield in the BN and HN populations (Tables 2.5 and 2.6). A number of important architectural and phenological avoidance traits, such as days to flowering, can be of variable importance in differing genetic populations. In the advanced line population, OR was important in DSI and DI ratings, whereas agronomic avoidance factors other than days to maturity, were not pertinent in the expression of resistance. The number of lines that had undesirable agronomic avoidance mechanisms were few in the advanced line population were few. Architectural traits also varied greatly in this population, from lines with an open porous canopy, an upright architecture, dense canopies and prostrate plant types. These genotypes, however, were generally

high-yielding, and trials in similar environments resulted in higher overall yields, than that found in either of the two genetic populations. Agronomic avoidance mechanisms may mask physiological resistance in the genetic populations with no prior selection for yield, creating difficulty in selection of superior genotypes.

In genetic populations, an indirect screen for physiological resistance becomes very important, even if the results are not correlated with field data. Ratings for DSI and DI at physiological maturity provided sufficient information regarding disease resistance in the field. There was no significant correlation between OR and the change in DI, which may indicate that OR plays a more important role in the development and spread of disease, and a less effect on infection late in the season.

Combining physiological resistance with desirable agronomic avoidance mechanisms, such as an open porous canopy, or upright architecture, is a valuable strategy in improving levels of resistance to white mold across environments. Heritability estimates for DSI and DI were moderate in the BN population, and moderate to high in the HN population, which suggest that progress can be made in breeding for resistance to white mold. The genotypes studied in the advanced line population, generally had fewer agronomic avoidance traits, compared to the BN and HN genetic populations. Agronomic avoidance mechanisms played a large role in resistance in the BN population. The HN population also had significant architectural mechanisms associated with DSI and DI, different from those identified in the BN population. Generally, navy bean genotypes with a determinate growth habit were found to be very susceptible to oxalate, and such cultivars were generally very susceptible to white mold in the field. The choice of parents can be an important factor in dictating how much

variability in DSI and DI is attributable to agronomic avoidance traits versus physiological resistance. Care must be taken in selecting lines from segregating populations that do not possess undesirable agronomic avoidance traits that could contribute to lower yield. Early generation selection against highly heritable, undesirable agronomic avoidance traits, such as early flowering, may be a useful approach in minimizing the selection of less desirable genotypes. Understanding the type of variability present in genetic populations is critical if progress is to be made in breeding for resistance to white mold.

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CHAPTER 3

MOLECULAR MARKER DISSECTION OF QTLS CONFERRING RESISTANCE TO WHITE MOLD AND GROWTH HABIT IN TWO NAVY BEAN POPULATIONS

INTRODUCTION

White mold, caused by *S. sclerotiorum*, is a devastating fungal disease that can infect over 400 plant species (Boland and Hall, 1994). In common bean, white mold causes a reduction in yield, due to a decrease in pods per plant, seed size, and seed quality (Kerr et al., 1978; Steadman, 1979). Under appropriate moisture conditions, typically found during canopy cover and flowering, apothecia germinate from sclerotial bodies, producing ascospores that disperse into the plant canopy (Boland and Hall, 1987). The ascospores germinate on senescent flowers, and the subsequent developing mycelium invade the plant tissue. Oxalate, a primary pathogenicity factor of *S. sclerotiorum* (Godoy et al., 1990), is exuded into the plant tissue, followed by the release of polygalacturonases (Marciano et al., 1983).

Resistance to white mold in common bean is complexly-inherited (Fuller et al, 1984; Miklas and Grafton, 1992). Physiological resistance has been described in certain genotypes, based on different greenhouse assays (Pedzoldt and Dickson, 1998; Miklas et al., 1992a; Miklas et al., 1992b; Steadman, 1998; Hunter et al., 1981; Kolkman and Kelly, 2000). Progress in breeding for resistance to white mold has been hindered by the limited expression and detection of physiological resistance in the field environment. Avoidance mechanisms, such as an open porous canopy, can play a major role in the development of disease in the field throughout the season (Fuller et al., 1984; Steadman

et al., 1973). Agronomically undesirable avoidance mechanisms, such as early flowering, may reduce white mold infection levels, but place a major restraint on the ability of the breeders to select for high-yielding genotypes. Few genotypes have been identified as resistant in both field and greenhouse assays. Bunsi (Ex Rico 23), an indeterminate navy bean, has both physiological resistance to white mold and an open porous canopy that deters white mold development (Tu and Beversdorf, 1982; Tu, 1985; Kolkman & Kelly, 2000). A second navy bean, C-20 (Kelly et al., 1984), and C-20 - derived lines, such as Huron (Kelly et al., 1994), have also been identified as having resistance to white mold in both field and greenhouse tests (Miklas et al., 1992a; Kolkman and Kelly, 2000)

Marker-assisted selection (MAS) allows for the identification and selection of superior genotypes without having to employ undue effort in phenotyping large numbers of individuals. The difficulty in detection of desirable phenotypes, due to factors such as environmental variation, hinders normal selection procedures for important quantitative traits, and increases the importance of MAS (Tanksley et al., 1989). Molecular markers linked to both qualitative and quantitative traits of economic importance, including disease resistance, have been identified in common bean (Kelly and Miklas, 1998).

Selective genotyping (Lander and Botstein, 1989) and bulked segregant analysis (BSA) (Michelmore et al., 1991) have been utilized to efficiently screen large numbers of polymorphic markers, without having to genotype entire populations. Selective genotyping involves the identification of a subset, usually 10 - 14% of the genotypes, that possess extreme phenotypes of the population. A small percentage of the total genotypes that exhibit extreme phenotypic values for the trait of interest are grouped together, and either analyzed as individuals, or through BSA, where the DNA of the similar phenotypes

are pooled. Selective genotyping and BSA has been used successfully in the identification of QTLs for quantitatively-inherited traits. In a computer simulation study, the ability to detect markers linked to QTLs for the trait of interest was improved if alternate DNA bulks were used for traits that were correlated to the main trait of interest (Ronin et al., 1998). Selective genotyping may be restrictive, when a saturated linkage map is not available. A small number of individuals are used for both linkage map construction and QTL estimation, which may result in a bias of the genetic variation that is present in the population (Wang and Paterson, 1994; Martinez, 1996).

A population was developed from a biparental cross between two navy bean genotypes Bunsi and Newport, differing in resistance to white mold. An initial study of the Bunsi-derived (BN) population indicated that certain agronomic avoidance traits, such as days to flowering, may have confounded the expression or detection of a significant correlation between physiological resistance, measured as resistance to oxalate (OR) and white mold disease levels in the field (Chapter 2). DNA bulks comprised solely of a small number of lines in the extreme phenotypes, may not adequately represent resistant genotypes in the population. DNA pooling strategies based on *a priori* knowledge about the population should help resolve useful markers linked to QTLs, and discern the location of QTL regions (Wang and Paterson, 1994). Genotyping multiple traits that are related to the trait of interest have been shown to be effective in identifying QTLs that may not be detected through screening extreme phenotypes (Ronin et al., 1998). The first objective of this study, was to identify markers linked to QTLs conferring resistance to white mold in common bean. The second objective of this study was to determine if selective multivariate genotyping (SMG), using more than one phenotypic trait in the

pooling of DNA bulks, is more efficient than creating DNA bulks from single traits in the identification of markers linked to QTLs for resistance to white mold.

MATERIALS & METHODS

Plant Material and Marker evaluation:

An F_3-derived BN mapping population was generated from a cross between two navy bean genotypes, Bunsi and Newport, that varied in resistance to white mold. Bunsi is an elite cultivar with an indeterminate (Type II) growth habit. Bunsi possesses both physiological resistance and a porous canopy for avoidance to white mold (Tu and Beversdorf, 1985; Schwartz et al., 1987; Miklas et al., 1992; Kolkman and Kelly, 2000). Newport is a susceptible navy bean cultivar with a determinate (Type I) growth habit (Kelly et al., 1995; Kolkman and Kelly, 2000). Ninety-eight F_2 lines were advanced in the greenhouse to the F_3 generation using single seed descent. Seed of individual F_3 plants was bulked, and advanced in a greenhouse. Seed harvested from three $F_{3:4}$ plants were bulked and $F_{3:5}$ plants were increased in a winter nursery in Puerto Rico. Bulked $F_{3:6}$ lines were grown in field trials at the Montcalm Research Farm and Sanilac Cooperator Farm in 1997. $F_{3:7}$ lines were grown in a Montcalm Research Farm field trial in 1998. No selection for agronomic traits was made during generation advancement.

A second recombinant inbred line (RIL) population of 28 individuals was developed using single seed descent from a cross between Huron and Newport. Huron is an C-20 - derived cultivar, with an upright plant type and indeterminate growth habit (Kelly et al., 1994). Huron has both physiological resistance to white mold, via the greenhouse oxalate test, and resistance to white mold in the field (Kolkman and Kelly,

2000).

Plant tissue was harvested from parental genotypes and approximately 10 $F_{3:7}$ greenhouse grown plants for each F_3 - derived family from the BN population. DNA was harvested from approximately 10 greenhouse $F_{5:7}$ plants from the Huron/Newport (HN) population. DNA was extracted from the plant tissue using a mini-prep procedure (Edwards et al, 1991; Haley et al., 1994b). Parental genotypes of the BN population were screened for the presence of polymorphic bands with the Polymerase Chain Reaction (PCR) and approximately 600 Operon random 10-mer primers (Williams et al., 1990), using Gibco Taq DNA polymerase (Miklas et al., 1993; Haley et al., 1994a). Approximately 100 primers were found to be polymorphic between the parental genotypes, Bunsi and Newport. The parents were also screened twice with 111 RAPD primers from the integrated bean linkage map (Freyre et al., 1998), once with Gibco Taq DNA polymerase, and once with Stoffel fragment Taq DNA polymerase. Polymerase Chain Reaction was conducted in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc) programmed for 3 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; 34 cycles of min at 94 °C, 1 min at 40 °C, and 2 min at 72 °C with the final step extended by 1 s for each of the 34 cycles, and a final extension cycle of 5 min at 72 °C (Haley et al., 1994a). RAPD markers are identified by the name of the Operon primer, followed by the size of the polymorphic fragment.

The parental genotypes and DNA bulks were also screened for polymorphic bands using eight AFLP primer pair combinations (Vos et al., 1995). Primer combinations that produced bands that segregated between the parental genotypes and DNA bulks were screened on the entire population. Polyacrylamide gel electrophorese (PAGE) was used

to separate AFLP fragments. Fragments were visualized using a silver staining procedure, according to the directions of a commercial silver staining kit (Promega), with the addition that both the fix/stop and developing solution were partially frozen. Gels were scored for band polymorphism, estimating band size in reference to a 10 and a 25 base pair DNA ladder. Gels were transferred to chromatography paper (Barrett and Kidwell, 1998). The first three letters of the AFLP marker names indicate the *Eco* RI +3 (+ANN) selective nucleotide, while the second three letters indicate the *Mse* I + 3 (+CNN) selective nucleotides used in this study. The number following the six letter enzyme/primer combination represents the size of the polymorphic fragment generated by the specific marker. RAPD and AFLP marker protocols for the HN population were similar to those described for the BN population.

Traits:

Physiological resistance in both populations was determined indirectly, by screening the populations for OR (Kolkman and Kelly, 2000). The BN population was evaluated for OR in an RCBD using four replications. The HN population was evaluated twice for OR with three replications in a RCBD. Briefly, twenty-day old seedlings (2^{nd} trifoliate emerging) were cut at the base of the stem and placed in a 20 mM oxalate solution (pH 4.0). The seedlings were rated for wilting symptoms using a 1 to 6 scale (see Chapters 1 and 2 for details).

Both populations were evaluated for resistance to white mold in the field across several environments in Michigan. The BN population was grown at the Montcalm Research Farm in 1997(MRF97) and 1998 (MRF98), and Sanilac Cooperator Farm in

1997 (SCF97). The HN RILs were grown in MRF97, MRF98, and SCF97, as well as in MRF in 1996 (MRF96; see Chapter 2). Plots were rated for disease severity index (DSI) and disease incidence (DI) using a 'quarter scale' (Hall and Phillips, 1996), shortly before harvest, when the majority of plants had reached physiological maturity (see Chapter 2 for details). The DSI was calculated for each plot on a percentage basis, using the following formula:

$$DSI = \frac{\Sigma \text{ (rating of each plant)}}{4 \times \text{(number of plants rated)}} \times 100 \quad .$$

Disease incidence was calculated as the number of plants out of the thirty individuals with white mold infection, based as a percentage. Genotypes were also evaluated for a number of agronomic traits, including: growth habit, days to flowering, mid-season canopy height, mid-season canopy width, architecture, days to maturity, lodging, yield, and seed size (see Chapter 2).

Selective Multivariate Genotyping:

Selective genotyping, using both single traits and multiple traits, was used to create DNA bulks, and identify significant markers for the BN population. Three sets of resistant and susceptible DNA bulks were established for extreme phenotypes of DSI, DI, and OR (see Chapter 2). The multivariate bulks were comprised of lines that were either resistant and high-yielding, or susceptible and low-yielding, within a fixed flowering range from 40 - 45 days to flowering (Table 3.1). The 4 sets of DNA bulks were

screened with the polymorphic primers, in order to identify markers linked to the resistance phenotype. Primers that were polymorphic in the bulks were then tested for polymorphism in the population.

Table 3.1. DNA pooling strategies based on single or multiple traits

DNA pool	DNA pool phenotype
Disease Severity Index:	
S1	resistant: low DSI†, based on field data
S2	susceptible: high DSI, based on field data
Disease Incidence:	
I1	resistant: low DI†, based on field data
I2	susceptible: high DI, based on field data
Resistance to Oxalate:	
O1	resistant: low OR† score, based on greenhouse data
O2	susceptible: high OR score, based on greenhouse data
Multivariate Analysis:	
M1	resistant: high yielding, low DSI, between 40-45 days to flowering
M2	susceptible: low yielding, high DSI, between 40-45 days to flowering

† DSI = disease severity index; DI = disease incidence; OR = resistance to oxalate

Markers were scored for the presence or absence of the RAPD or AFLP band. Chi-square tests indicated whether the markers were segregating in 5:3 ratio (for F_3-derived lines) or a 1:1 ratio (in the instance where the DNA collected for each family was a representative sample of a RIL). Significant markers were identified via analysis of variance and correlation analysis (SAS, 1995) to indicate linkage between markers, as well as the confirmation of linkage to resistance or agronomic traits. Linkage and linkage

order of markers were determined with MAPMAKER/EXP (Lander et al., 1987), using the Kosambi mapping function (Kosambi, 1944), a minimum LOD score of 3.0 and a maximum recombination frequency of 0.30.

Statistical Analysis:

All greenhouse experiments were analyzed as RCBDs, using PROC GLM (SAS, 1995). Greenhouse and field experiments for the BN and HN populations were evaluated individually as RCBDs, using PROC GLM (SAS, 1995). Both populations were analyzed across field environments, and greenhouse tests, as a RCBD, using PROC GLM, with both genotypes and environments considered as random effects. Resistance and agronomic traits that were significantly ($P<0.01$) associated with DSI and DI in the BN populaton, were initially tested for analysis of variance, and Pearson correlation coefficient (SAS, 1995).

Traits significantly associated with DSI and DI across environments in the BN population ($P<0.01$) were mapped onto the constructed linkage groups using interval mapping via QTL Cartographer software program (Basten et al. 1994; Basten et al., 1999). Threshold LOD scores (95%) for individual traits were determined through a permutation test, with 1000 permutations (Churchill and Doerge, 1994). Significant markers that were most closely associated to regions with major QTLs, were confirmed also via analysis of variance and Pearson correlation coefficient, and tested for significance in combined and individual environments. The most significant marker(s) in each linkage group for OR, DSI, DI, and yield was tested in the HN population. Analysis of variance and correlation analysis were used to detect significance between the marker

and OR, DSI, DI, and yield. Effect of single markers was determined using a students t-test (SAS, 1995). The effect of multiple markers on phenotypic expression of traits by markers on more than one linkage group was analyzed using multiple regression (SAS, 1995).

RESULTS & DISCUSSION

Marker and QTL associations:

Markers were identified using each DNA bulking method in SMG. The markers identified in this study were robust across environments, and were also generally robust across resistant genotypes. In total, 38 polymorphic markers were evaluated in the BN population. Linkage map construction, using MAPMAKER/EXP placed 23 markers on four main linkage groups (Figure 3.1), for a total of comprised 220 cM. Markers that were detected via SMG were included within the four linkage groups (Table 3.2), except for one unlinked marker, aaccaa195. Marker segregation ratios were tested against both a 5:3 ratio or 1:1 ratio, since small sample size used for DNA extraction may have limited the ability to adequately sample a segregating F_3-derived line in the BN population (Tables A7 and A8). Mapping of F_2 or F_3 populations with dominant markers is not ideal, since heterozygous individuals cannot be identified. Mapping RIL or doubled-haploid (DH) populations with dominant markers is much more efficient, since heterozygous genotypes are minimal to non-existent in the population (Knapp et al., 1995). In BSA, however, dominant markers are more informative in F_2 populations than backcross populations, since recombination frequency is higher and results in closer linkage to the QTL (Mackay and Caligari, 2000).

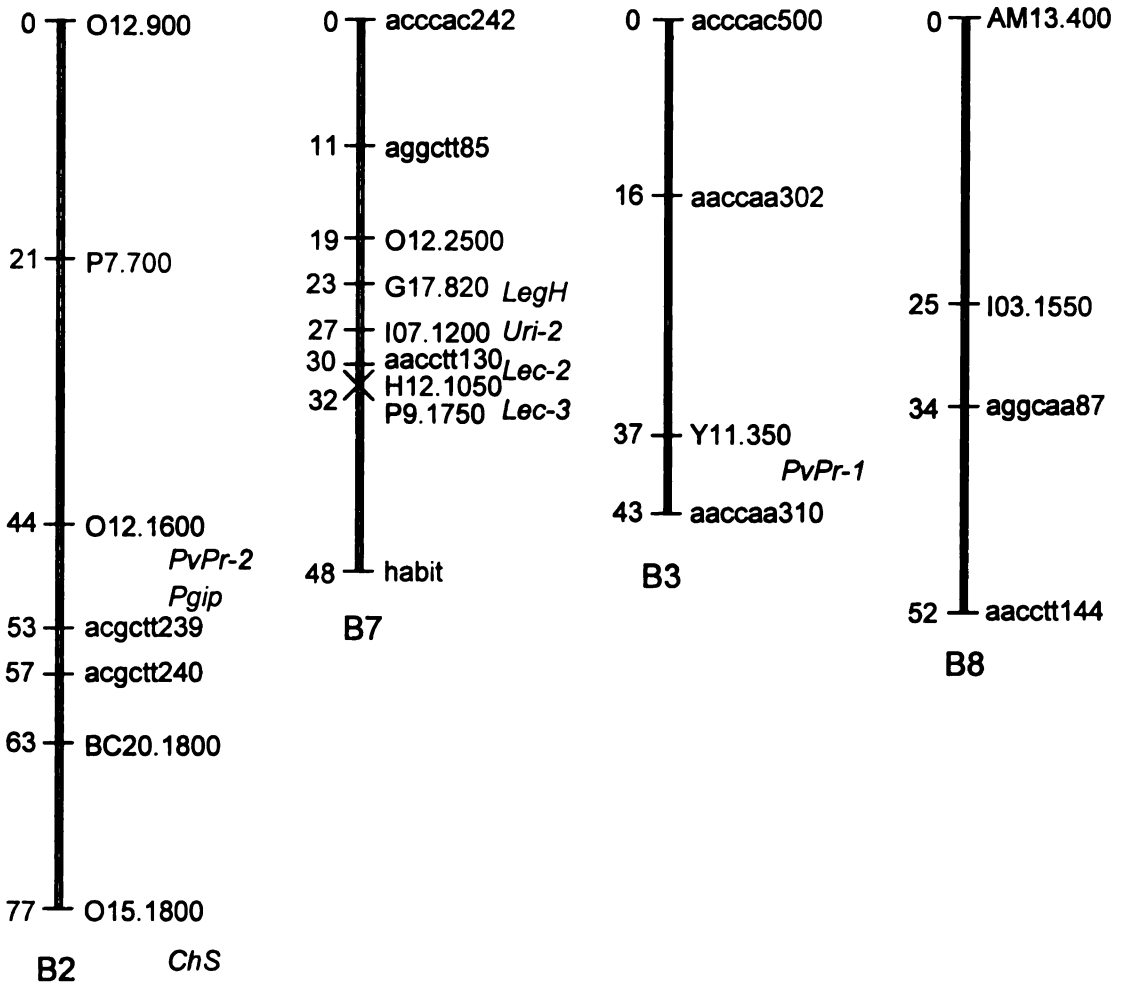

Figure 3.1. Linkage map with 13 RAPD markers, 10 AFLP markers and one phenological marker, constructed with Mapmaker/Exp, from 98 F_3 - derived lines from the Bunsi/Newport population. Distances are in Kosambi cM units and are listed on the left-hand side of the linkage groups. Locations of known genes are listed in italics. Linkage groups B2, B7, B3, and B8, and gene location correspond to the integrated linkage map (Freyre et al., 1998)

Table 3.2. RAPD and AFLP markers, linkage phase (C=coupling; R=repulsion), and corresponding linkage group designation, identified through selective multivariate genotyping with 4 sets of resistant and susceptible DNA bulks: Multivariate (M), Disease Severity Index (S), Disease Incidence (I), and Resistance to Oxalate (O) ('-' = no polymorphic bulk)

Linkage Group B2[†]			Linkage Group B3			Linkage Group B7			Linkage Group B8		
Marker	Phase	Bulk	Marker	Phase	Bulk	Marker	Phase	Bulk	Marker	Phase	Bulk
O15.1800	C	M, S, I	aaccaa310	C	-	acccac242	R	M	AM13.400	C	M, I, O
BC20.1800	R	M, S, I	Y11.350	C	O	aggctt85	R	M, I, O	I03.1550	C	I
acgctt240	C	I	aaccaa302	C	O	O12.2500	R	M, I, O	aggcaa87	R	-
acgctt239	R	-	acccac500	C	O	G17.820	C	M, I, O	aacctt144	R	-
O12.1600	R	-				I07.1200	C	M, O			
P7.700	R	-				aacctt130	C	M, O			
O12.900	C	-				H12.1050	C	M, I, O			
						P9.1750	C	M, S, I, O			
						habit[‡]	C	-			

Unlinked Locus		Bulk
aaccaa195	R	I

[†] linkage groups B2, B3, B7, and B8 correspond to the integrated linkage map (Freyre et al., 1998)
‡ the indeterminate (Type II) growth habit is associated with the resistance phenotype

The first linkage group had four markers that were anchored in a similar orientation to Linkage Group B2 of the integrated linkage map (Freyre et al., 1998). The second linkage group had two anchor markers in common with Linkage Group B7 of the integrated linkage map. The third and fourth linkage groups each had one marker in common with Linkage Group B3 and B8 of the integrated linkage map, respectfully. More markers are needed to anchor these two linkage groups to the integrated linkage map. One of 13 markers not identified with a linkage group was associated with OR.

Genotype and genotype by environment interactions were significant for all resistance and agronomic traits in the BN population (see Chapter 2), and the two parental genotypes varied significantly for all traits measured, except lodging. Segregation of growth habit was tested to fit a 5:3 ratio using chi-square goodness of fit test, ($\chi^2 = 1.98$; $0.15<P<0.20$). Disease pressure appeared adequate for each test environment in the BN population. Parental genotypes segregated for OR, DSI, DI, and yield. Environmental variation was significant for DSI, DI and agronomic traits. Although data in individual environments were not always normally distributed, data was not transformed to correct for skewness (Doerge et al., 1997). Combined environments were generally normally distributed (Figure 3.2).

Markers associated with QTLs for resistance and agronomic traits were found to be relatively robust across environments. Markers were also stable between DSI and DI measurements, which is expected since the phenotypic correlation between DSI and DI was very high. One major QTL for DSI and DI was identified on linkage group B2, and was located near RAPD markers BC20.1800 and O15.1800 (Figure 3.3). BC20.1800, the closest marker associated with this QTL, accounted for 11.6 % of the phenotypic

variability for DSI, and 12.6 % of the phenotypic variability for DI (Table 3.3) across the three environments. This marker was significant in individual MRF97 and MRF98 environments, accounting for the most variability in the MRF97 environment for both DSI (R^2 = 13.0%), and DI (R^2 = 13.2%). BC20.1800 was not significantly associated to DSI or DI within the SCF97 environment, however, possibly due to the extreme high disease pressure encountered in that particular environment (see Chapter 2).

The region around O15.1800 and BC20.1800 on the integrated linkage map contains several identified genes that may be important in disease resistance. A *P. vulgaris* pathogenesis-related gene, *PvPR-2* (Walter et al., 1990), a polygalacturonase-inhibiting protein, *Pgip* (Toubert et al., 1992), are located in this region of Linkage Group B2. Fungal defense-related genes may be triggered as a general resistance response to *S. sclerotinia* infection. *Pgip* is particularly noteworthy, since polygalacturonase is generated by the pathogen during white mold infection. Chalcone synthase, (*ChS*; Ryder el at., 1987), located near O15.1800, is an isoflavonoid-derived phytoalexin involved in host defense, and may be important in general resistance to *S. sclerotiorum*. The resistance found in this linkage group was most likely due to physiological resistance associated with a generalized host defense response, but not associated with OR, which requires some tolerance to the actual chemical. Marker P7.700 was previously identified to be linked to resistance to Fusarium root rot in bean (Schneider and Kelly, 2000). In the B2 linkage group, there were significant QTLs for lodging (acgctt239; R^2 = 7.4%), days to maturity (O12.1600; R^2 = 13.5%), and seed size (acgctt240; R^2 = 6.9%) across environments (Table 3.3) that were identified in the analysis of variance, but not in interval mapping. Disease severity index and DI most likely influenced seed size on B2,

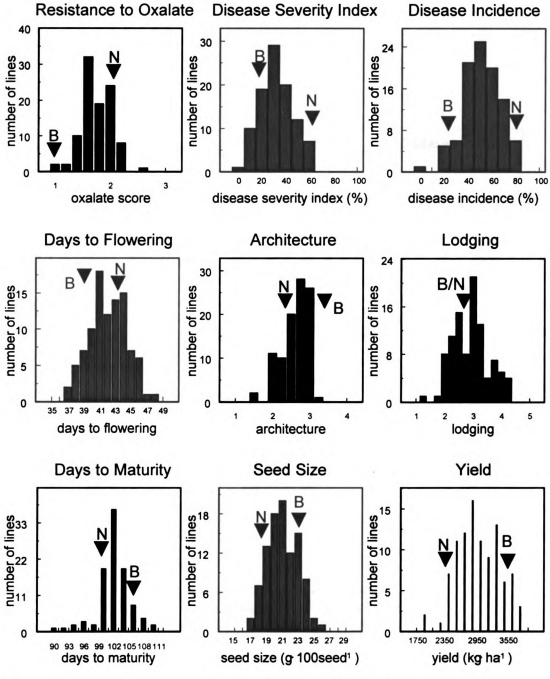

Figure 3.2. Phenotypic distribution for 98 lines of the Bunsi/Newport population evaluated for resistance to oxalate, disease severity index, disease incidence, and agronomic traits that were associated with resistance traits (see Chapter 2) including: days to flowering, architecture, lodging, days to maturity, seed size, and yield. Location of mean values for Bunsi (B) and Newport (N) are located at arrows.

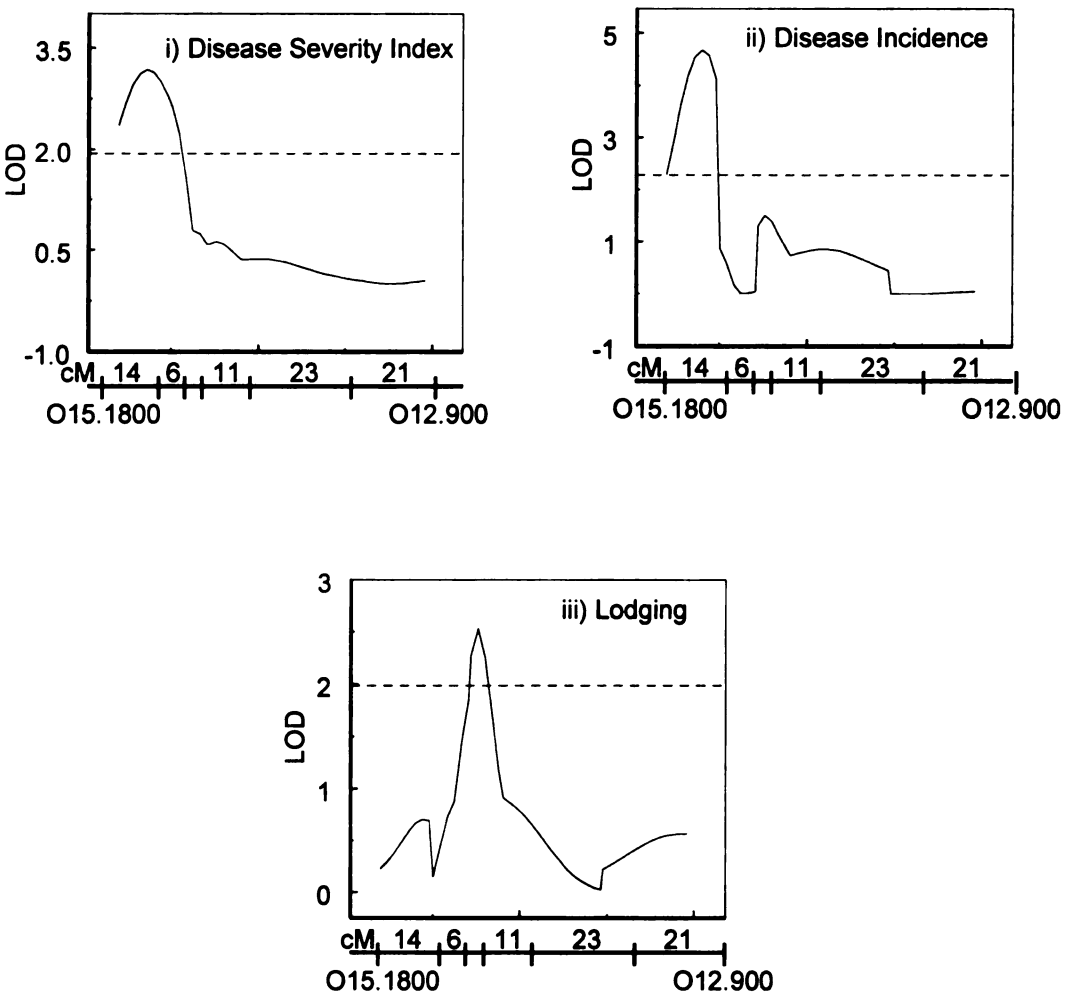

Figure 3.3. LOD scores for interval mapping analysis of i) disease severity index, ii) disease incidence, and iii) lodging on Linkage Group B2 in the Bunsi/Newport population. LOD scores above the threshold level (---) indicate experiment-wise error rate of 0.05, determined using 1000 permutations. The x-axis corresponds to centimorgan distance and marker intervals located on Figure 3.1.

Table 3.3. Summary of markers identified on linkage group B2 in the BN population, found to be most closely linked to the QTL for significant resistance and agronomic traits including phenotypic variability associated with the marker in combined ($P<0.01$) and individual environments, effect of the presence of the marker across environments, and SMG/DNA bulking strategy used to identify marker.

	Linkage group B2				
Trait	marker	Environment[‡]	R^2 (%)	Effect	Bulk[¶]
DSI[§] (%)	BC20.1800	Across Environments	11.6	9.3	M, S, I
		MRF97	13.0		
		SCF97	ns		
		MRF98	9.9		
DI (%)	BC20.1800	Across Environments	12.6	11.1	M, S, I
		MRF97	13.2		
		SCF97	ns		
		MRF98	10.5		
Lodging[$]	acgctt239	Across Environments	7.4	0.3	-
		MRF97	5.0		
		SCF97	3.7[†]		
		MRF98	4.7*		
Days to Maturity	O12.1600	Across Environments	13.5	2.4	-
		MRF97	10.5		
		SCF97	6.1*		
		MRF98	19.1		
Seed Size (g 100seed^{-1})	acgctt240	Across Environments	6.9	1.0	I
		MRF97	6.9		
		SCF97	4.5*		
		MRF98	6.2		

* significant at $P<0.05$

[†] significant at $P<0.10$; ns = non significant

[‡] MRF97 = Montcalm Research Farm, 1997; SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998; Across Environments = average across MRF97, SCF97, and MRF98

[¶] DNA bulks, where M = multivariate, S = disease severity index, I = disease incidence, and O = Oxalate; '-' = no bulk; see Table 3.1

[§] DSI = disease severity index; DI = disease incidence

[$] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

since seed size has been shown to decrease with higher levels of disease infection (Kerr et al., 1978). Seed size must not be reduced dramatically by disease, however, since yield is not a significant factor in this linkage group.

The largest linkage group identified in this study was associated to the B7 (Freyre et al., 1998), by anchor markers H12.1050 and I07.1200 (Figure 3.1). Markers in this linkage group were significantly associated with OR, DSI, DI, days to flowering, lodging, seed size and yield (Figure 3.4; Tables 3.4 and 3.5). G17.820 was associated with 8.8 % of the phenotypic variability for OR in the greenhouse. Two of the most important markers, aggctt85 and aacctt130, contributed up to 16.8 % and 15.8 % of the phenotypic variability for DSI, respectively, and 13.3% and 12.7% of the phenotypic variability for DI, respectively. The same two markers generated the greatest significance in MRF97, but were not associated with either DSI or DI in SCF97 (Table 3.5). AFLP marker aacctt130 was significantly associated to days to flowering (R^2 = 13.9%) across environments, particularly in SCF97 (R^2 = 18.3%). The same two markers, were significantly associated with days to maturity across environments in the analysis of variance.

A potential QTL region for architectural traits was also located via analysis of variance on B7. Marker I07.1200 was associated with architecture (R^2 = 9.1%) and lodging (R^2 = 9.1%), across environments (Table 3.5). In a previous unrelated study involving a population derived from a cross of Type II x Type III genotypes, H12.1050 was associated with plant uprightness, and branch density (Jung et al., 1996). The association with architectural traits is confirmed in this study, since I07.1200 is linked to H12.1050 marker on B7. I07.1200 was significantly associated with seed size across

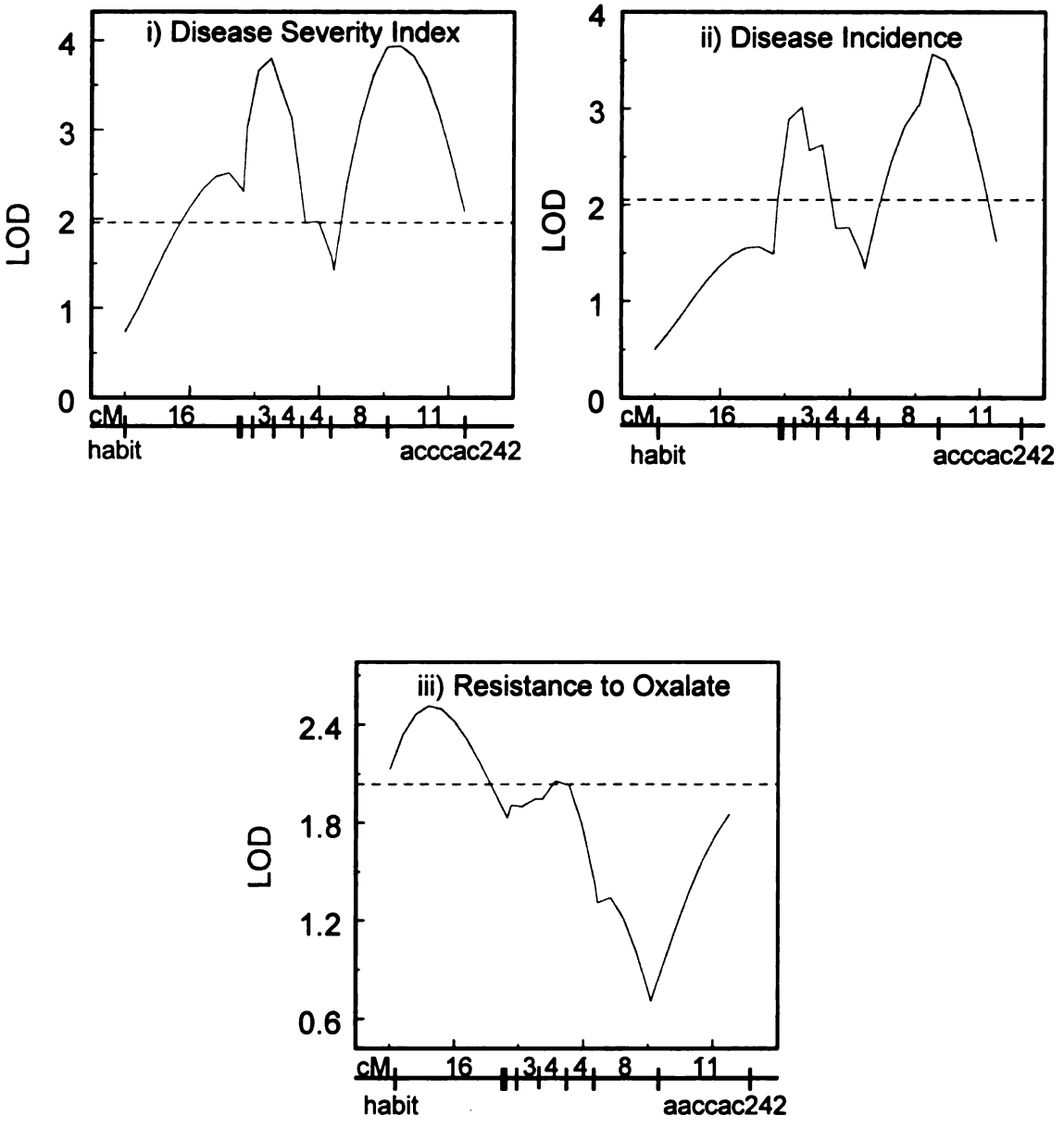

Figure 3.4. LOD scores for interval mapping analysis of i) disease severity index, ii) disease incidence, and iii) resistance to oxalate on Linkage Group B7 in the Bunsi/Newport population. LOD scores above the threshold level (---) indicate experiment-wise error rate of 0.05, determined using 1000 permutations. The x-axis corresponds to centimorgan distance and marker intervals located on Figure 3.1.

Table 3.4. Summary of markers identified in the BN population, to be most closely linked to the QTL for resistance to oxalate (OR), including phenotypic variability ($P<0.01$) associated with the marker, effect of the presence of the marker, and SMG/DNA bulking strategy used to identify the marker.

	Putative Linkage group B3				
Trait	Marker	Environment	R^2 (%)	Effect	Bulk[†]
OR[‡]	aaccaa302	Greenhouse	11.4	0.19	O
			Unlinked Loci		
			R^2 (%)	Effect	Bulk
	aaccaa195	Greenhouse	8.8	0.17	I
			Linkage Group B7		
			R^2 (%)	Effect	Bulk
	G17.820	Greenhouse	8.8	0.17	M, I, O
	habit[¶]		8.0	0.16	-

[†] DNA bulks, where M = multivariate, S = severity, I = incidence, and O = Oxalate; see Table 3.1
[‡] OR = resistance to oxalate where 1 = no wilting symptoms, 2 = 1 leaf with wilting symptoms, 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem collapsing
[¶] the indeterminate (Type II) growth habit is associated with resistance to oxalate

environments (R^2 = 19.5%), and within individual environments MRF97 (R^2 = 19.8%), SCF97 (R^2 = 23.4%), and MRF98 (R^2 = 11.4%). Previously, seed size was thought to have been affected by white mold, since plants with heavy disease pressure have a smaller seed size than those in the absence of disease (Kerr et al., 1978). Seed size is a complex factor that has an affiliation with this particular linkage group for another reason. Seed size is also affected by the presence of seed proteins. For example, the 'T' phaseolin seed protein, located on another region of Linkage group B7, has been associated with larger-seeded Andean genotypes, such as G122 (Johnson et al., 1996), whereas the S phaseolin is associated with smaller-seeded genotypes. Other seed proteins, such as lectins and uridenes with no known effect on seed size have also

Table 3.5. Summary of markers identified on linkage group B7 in the BN population, to be most closely linked to the QTL for significant resistance and agronomic traits, including phenotypic variability associated with the marker in combined (P<0.01) and individual environments, the effect of the presence of the markers on the phenotype, and the SMG/DNA bulking strategy used to identify marker.

		Linkage group B7			
Trait	Marker	Environment[‡]	R^2 (%)	Effect	Bulk[¶]
DSI[§] (%)	aggctt85	Across Environments	16.8	11.3	M, I, O
		MRF97	15.6		
		SCF97	ns		
		MRF98	6.4 *		
	aacctt130	Across Environments	15.8	10.9	M, O
		MRF97	18.9		
		SCF97	ns		
		MRF98	4.3 *		
DI (%)	aggctt85	Across Environments	13.3	11.5	M, I, O
		MRF97	13.6		
		SCF97	ns		
		MRF98	6.1 *		
	aacctt130	Across Environments	12.7	11.1	M, O
		MRF97	17.2		
		SCF97	ns		
		MRF98	3.6 *		
Days to Flowering	aacctt130	Across Environments	13.9	1.7	M, O
		MRF97	17.4		
		SCF97	18.3		
		MRF98	3.8 †		
Architecture[$]	I07.1200	Across Environments	9.1	0.2	M, O
		MRF97	11.1		
		SCF97	3.6 †		
		MRF98	4.2 *		
Lodging[£]	I07.1200	Across Environments	9.1	0.4	M, O
		MRF97	12.0		
		SCF97	5.2 *		
		MRF98	6.0 *		
Days to Maturity	aacctt130	Across Environments	6.0 *	1.6	M, O
		MRF97	6.4		

		SCF97	5.4*		
		MRF98	4.7*		
	aggctt85	Across Environments	3.6[†]	1.2	M, I ,O
		MRF97	8.1		
		SCF97	3.4[†]		
		MRF98	5.9		
Seed Size (g 100seed^{-1})	I07.1200	Across Environments	19.5	1.7	M, O
		MRF97	19.8		
		SCF97	23.4		
		MRF98	11.4		
	habit[#]	Across Environments	18.3	1.9	-
		MRF97	19.0		
		SCF97	9.2		
		MRF98	20.6		
Yield (kg ha^{-1})	I07.1200	Across Environments	36.5	477	M, O
		MRF97	42.5		
		SCF97	9.8		
		MRF98	25.8		
	aacctt130	Across Environments	34.4	462	M, O
		MRF97	45.6		
		SCF97	5.3		
		MRF98	28.3		
	G17.820	Across Environments	27.2	408	M, I, O
		MRF97	33.7		
		SCF97	5.1		
		MRF98	17.3		
	aggctt85	Across Environments	24.9	396	M, I, O
		MRF97	26.1		
		SCF97	6.9		
		MRF98	13.5		

* significant at $P<0.05$

[†] significant at $P<0.10$; ns = non significant

[‡] MRF97 = Montcalm Research Farm, 1997; SCF97 = Sanilac Cooperator Farm, 1997; MRF98 = Montcalm Research Farm, 1998; Across Environments = average across MRF97, SCF97, and MRF98

[¶] DNA bulks, where M = multivariate, S = severity, I = incidence, and O = Oxalate; see Table 3.1

[§] DSI = disease severity index; DI = disease incidence

[$] Architecture based on a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate

[£] Lodging based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

[#] the indeterminate (Type II) growth habit is associated with seed size

been located at the base of linkage group B7 (Freyre et al., 1998).

The bottom of linkage group B7, appears to be the region associated with yield in the BN population, and is supported by the very high LOD scores on the linkage map (Figure 3.5). Most of the markers significantly associated with yield within and across environments in this linkage group included: I07.1200 (R^2 = 36.5%), aacctt130 (R^2 = 34.4%), G17.820 (R^2 = 27.2%), and aggctt85 (R^2 = 24.9%). The most significant marker for yield, I07.1200, was also the most significant marker for seed size. The markers were most responsive in the MRF97 environment, accounting for 26.1% (aggctt85) up to 45.6% (aacctt130) of the phenotypic variability for yield. Interactions between these markers and the MRF98 environment, which was a lower yielding environment, were still very significant, with associations ranging from 13.5% (aggctt85) to 28.3% (aacctt130) of the phenotypic variability for yield. The associations between the markers and yield in the SCF97 environment were significant, but much less than the MRF97 and MRF98 environments, ranging from 5.1% (G17.820) to 9.8% (I07.1200). Among individual environments, I07.1200 had the most significant association for seed size in the SCF97 environment, accounting for 23.4% of the phenotypic variability. The SCF97 environment, despite having such heavy disease pressure, was by far, the highest yielding environment, even though the marker associations with yield were lower.

The significance of the marker associations with yield and disease resistance in this linkage group indicates that there must be important physiological components in this region. The seed lectins (phytohaemogluttanins), uridenes, and the leghaemoglobbin genes located near this region may be very important indirect contributors to yield. Seed lectins have been studied for their potential role in plant defense (Shewry and Lucas,

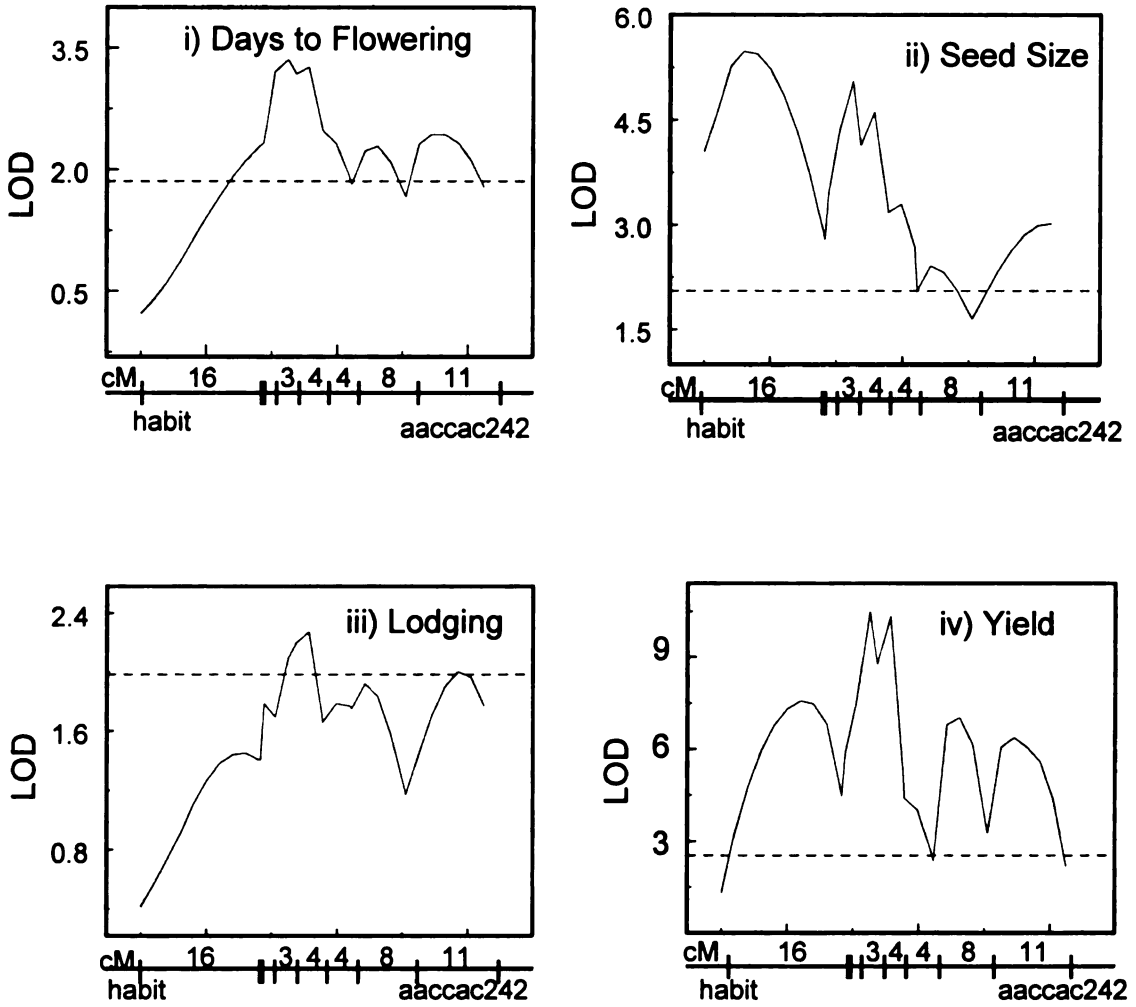

Figure 3.5. LOD scores for interval mapping analysis of i) days to flowering, ii) seed size, iii) lodging and iv) yield on Linkage Group B7 in the Bunsi/Newport population. LOD scores above the threshold level (---) indicate experiment-wise error rate of 0.05, determined using 1000 permutations. The x-axis corresponds to centimorgan distance and marker intervals located on Figure 3.1.

1997). Lectins isolated from red kidney bean seeds were found to reduce germ tube elongation in *Botryodiplodia theobromae* (Brambl and Gade, 1985). Resistance to oxalate, DSI, DI, which are yield components, as well as the associations with architecture, days to flowering, days to maturity, and lodging, indicate that the resistance identified in this linkage group may be the result of a constitutive structural component of the plant.

The third linkage group detected in this study was identified in the Oxalate bulks and was associated only with OR (Figure 3.1). The RAPD marker, Y11.350, corresponds to linkage group B3 of the integrated linkage map (Freyre et al., 1998). The most closely linked marker to a QTL for OR was the AFLP marker, aaccaa302 (R^2 = 11.4 %), which is closely linked to Y11.350 (Figure 3.6; Table 3.4). Flanking Y11.350 on the integrated linkage map, is another pathogenesis-related protein, *PvPR-1* (Walter et al., 1990). Greater marker density is needed to confirm if this is an important region for resistance to white mold. Significant markers linked to QTLs for resistance to Fusarium root rot in bean were previously detected near the *PvPr-1* gene on B3 in both greenhouse and field tests (Schneider and Kelly, 2000). The identified markers linked to both resistance to Fusarium root rot and resistance to white mold indicates that this region may contain general defense-related genes.

The fourth linkage group in this study contains I03.1550, which corresponded to Linkage Group B8 of the integrated linkage map (Freyre et al., 1998; Figure 3.1). This linkage group was identified using the multivariate, DI and OR bulks. The marker was present in the susceptible multivariate, DI, and OR DNA bulk for AM13.400, and in the susceptible DI bulk for I03.1550. In the two examples, however, the band was present

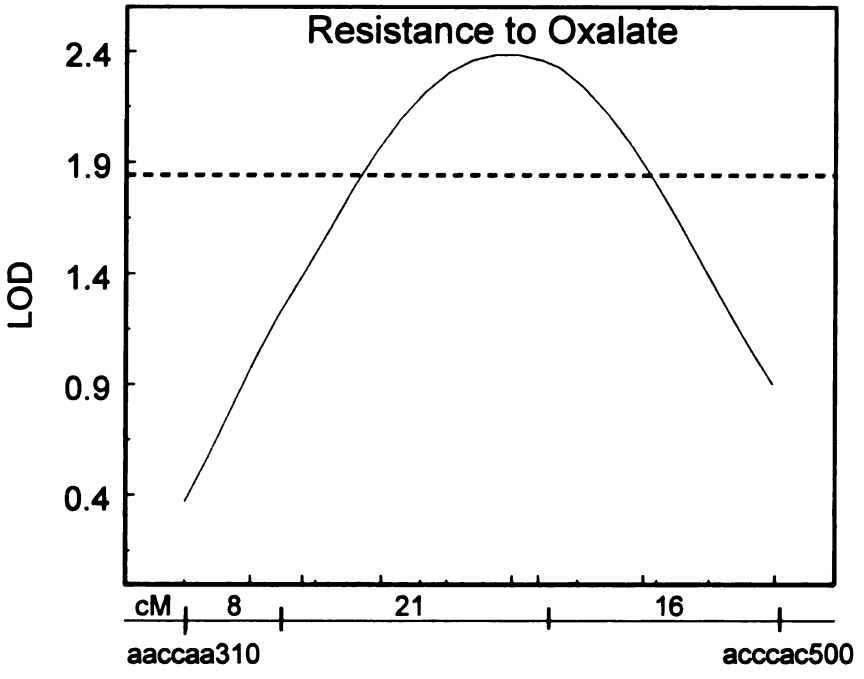

Figure 3.6. LOD scores for interval mapping analysis of resistance to oxalate on Linkage Group B3 (tentative) in the Bunsi/Newport population. LOD scores above the threshold level (---) indicate experiment-wise error rate of 0.05, determined using 1000 permutations. The x-axis corresponds to centimorgan distance and marker intervals located on Figure 3.1.

in the resistant Bunsi parent. In this scenario, the presence of the band was associated with a shorter plant phenotype. No resistance or yield traits were associated with this linkage group, although markers were associated with canopy height and architecture.

An unlinked loci, aaccaa195, was also identified in the DI bulk, and was found to be highly associated with OR (R^2 = 8.8%) (Table 3.4). This marker was not found to be associated with any other agronomic trait, nor was associated with any known inkage group.

Overall, the three markers G17.820, aaccaa302, and aaccaa195, accounted for up to 26 % of the phenotypic variability found for OR in the BN population. Resistance to oxalate appears to be complexly-inherited, and greatly influenced by the environment, which is consistent with the low estimate of heritability for OR in the BN population (Chapter 2). Combining selection for BC20.1800 and aggctt85, or BC20.1800 and aacctt130 resulted in phenotypic associations for DSI of 27%. A similar coefficient of determination was identified with these markers and DI. Combining BC20.1800, aggctt85 and aactt130 produced 30% of the phenotypic variability for DSI, and 27% of the phenotypic variability for DI. The markers alone, and in combination are significantly associated with major QTLs for resistance to white mold.

Selective multivariate genotyping was an efficient method for detecting markers for resistance to white mold in bean. Markers located on B2 were identified using the multivariate, DSI, and DI DNA bulks (Table 3.2), and not the OR bulks. The candidate QTL loci in the B2 region include genes involved in defense response mechanisms, which would not have been ascertained in a test for resistance to oxalate. Selective

multivariate genotyping was also effective in identifying all of the molecular markers on B7. Only one of the eight molecular markers in this linkage group was detected in all 4 sets of DNA bulks. Four of the markers were identified with the multivariate, DI, and OR bulks, while two of the markers, I07.1200, and aacctt130, were identified using the multivariate and OR bulks only. In B7, creating DNA bulks using the extreme phenotypes from DSI and DI, OR, as well as the multiple traits related to yield and resistance within a fixed flowering range, was an effective method in determining markers and QTLs for resistance to white mold. Seven of the eight molecular markers in B7 were detected using the OR bulks even though OR was not significantly associated to DSI or DI. As well, all eight of the markers were identified using the multivariate bulks. Genotyping a chosen set of individuals with specific phenotypes, based on *a prior* knowledge about the population, was an efficient method to detect markers that were linked to the resistance phenotype.

Determinate vs. Indeterminate Growth Habit:

In the BN population, growth habit was treated as a phenotypic marker, since the progeny were either determinate (Type I) or indeterminate (Type II). The placement of this phenotypic marker on linkage group B7 was therefore an indirect result of the mapping of markers related to resistance to white mold. Growth habit was, unexpectedly, significantly associated with markers on linkage group B7. The growth habit marker was detected on one end of the linkage group, corresponding to bottom of linkage group B7. The gene for determinate growth habit, *fin*, had previously been mapped onto linkage group B1 (Freyre et al., 1998). The 15 RAPD markers on linkage group B1 of the

integrated linkage map were not polymorphic between the Bunsi and Newport parents, suggesting that the determinate growth habit in the BN population may reside at a different location in the genome. Differing origins of the determinate growth habit in populations previously used to map this phenotype, and the mapping population used in this study validates the likelihood of the contrasting results. Wide crosses, using the Andean gene pool as the source for the determinate growth habit, were previously used for mapping the *fin* gene (Freyre et al., 1998). The determinate growth habit is a prominent trait in Andean germplasm (Singh et al., 1991; Koinange et al., 1996). Alternatively, the determinate growth habit in genotypes of the Middle American gene pool exists mainly in cultivated materials of the navy bean market class (Kelly, 2000). Few examples exist of the determinate growth habit in more exotic, Middle American germplasm. Early attempts to introgress the determinate growth habit from Andean germplasm into navy bean germplasm through breeding proved to be unsuccessful. The determinate growth habit was, therefore, first introduced into the navy beans using mutagenesis. The first determinate navy bean cultivar, Sanilac, released in 1956, was the most widely used source of the determinate growth habit in navy bean breeding programs worldwide (Kelly, 2000). It is reasonable, therefore, that the determinate growth habit trait in the navy bean germplasm is controlled by a different locus than that of the *fin* gene in Andean germplasm. The determinate growth habit, or terminal inflorescence is controlled by more than one loci in other plant species, such as *Arabidopsis* (Ratcliffe et al., 1998), *Brassica* (Mimida et al., 1999) and *Pisum* (Singer et al., 1999). In *Pisum sativum* L., up to six independent mutants characterize various aspects of inflorescence architecture. This is the first report of the determinate growth habit trait in navy bean

germplasm being associated with a completely unique locus, unlinked to the *fin* locus.

Unique phenotypic differences exist between the determinate growth habit of navy beans, and genotypes of Andean origin. Determinate navy bean cultivars, such as Midland and Newport, are generally very susceptible to white mold (Kolkman and Kelly, 2000). Seafarer, a similar determinate navy bean, was found to have a highly concentrated flowering period characterized by a very short flowering duration, resulting in the production of a main flush of flowers, many of which do not set pods. A higher pod abscission rate was identified in determinate navy bean genotypes in comparison to indeterminate navy and black bean cultivars, and resulted in lower yield in the determinate navy bean genotypes (Izquierdo and Hosfield, 1983). Alternatively, the determinate growth habit in larger-seeded genotypes, such as Montcalm, is characterized by a significantly lower number of flowers, compared to the determinate navy bean, Seafarer (Subhadrabandhu et al., 1978). The determinate growth habit has been recognized as a resistance phenotype in larger-seeded bean genotypes (Fuller et al., 1984; Miklas et al., 2000; Steadman et al., 1973). Isles, a determinate kidney bean, was found to avoid infection to white mold, compared to highly susceptible determinate navy bean cultivars, such as Newport and Midland (Kolkman and Kelly, 2000). The determinate phenotype at the *fin* locus was associated with resistance to white mold, in an A55/G122 population (Miklas et al., 2000). A QTL for canopy porosity was also located near the *fin* locus in the G122, indicating that the resistance phenotype was due to an avoidance mechanism. Excessive flower production in the determinate navy bean cultivars, may lead to an increase in potential inoculation sites, and be an overall component of the susceptibility to white mold in the determinate navy bean. Empirical knowledge from

breeders working in this germplasm group indicates that determinate navy bean genotypes are very susceptible to white mold. In the BN population, growth habit was also associated with yield, since average yield of the indeterminate genotypes was 364 $kg \cdot ha^{-1}$ higher than the determinate genotypes, across three environments. The open porous canopy of determinate genotypes of Andean origin has been characterized as an avoidance mechanism in resistance to white mold in the field in semi-arid production areas (Coyne, 1980) and a characteristic that may limit yield.

Confirmation of Markers in HN Population:

The most significant markers identified in each linkage group for OR, DSI, DI or yield, were tested on the HN population (Table 3.6). On linkage group B2, BC20.1800 segregated in a 1:1 segregation ratio ($\chi^2 = 0.57$; $0.40<P<0.50$) between the parental and progeny genotypes. BC20.1800 was a significant marker in this population for DSI accounting for 40% of the phenotypic variability for DSI and 35% of the phenotypic variability for DI across environments. In the MRF96 environment, associations between BC20.1800 and DSI ($R^2 = 60.9$ %) and DI ($R^2 = 53.2$ %) indicated the presence of a major QTL for resistance in this population. BC20.1800 was only significantly associated with DSI ($P<0.05$), representing up to 20.2 % of the variability in the SCF97 environment and was not significantly associated with DI in SCF97. Heavy disease pressure in the SCF97 environment, may have limited the expression of the QTL located near BC20.1800. The small population size of the HN population may also have been a limitation for evaluating the marker association in the SCF97 environment.

Markers on linkage group B2 have previously been associated with QTLs for

disease resistance in bean. Markers linked to QTL for common bacterial blight (CBB) resistance were located near the *PvPr-2* and *ChS* genes (Nodari et al., 1993; Jung et al., 1996). Markers linked to QTLs for resistance to both CBB and web blight were identified on linkage group B2, located near *ChS* and *PvPr-2* in another population (Jung et al., 1996). Plant uprightness, an avoidance mechanism for web blight, was also mapped to this region of the genome (Jung et al., 1996). Marker P7.700 was also associated with a QTL for resistance to Fusarium root rot on B2 (Schneider and Kelly, 2000). Linkage group B2 may have a cluster of defense-related genes that have broad application across pathogens.

All markers associated with linkage group B7 were tested on the HN population, since yield was such a predominantly significant factor within the entire linkage group in the BN population. Six of the markers did not have the favorable allele of the resistance phenotype. Marker H12.1050 was polymorphic between the parental genotypes, but the band corresponding with the marker, was not present in any of the 28 progeny. Only one marker, G17.820, segregated between the parental and progeny genotypes in the HN population, and had a normal 1:1 segregation ratio ($\chi^2 = 0.14$; $0.70<P<0.80$). G17.820 was associated with OR ($R^2 = 24.3\%$), in addition to yield across environments ($R^2 = 47.0$ %), and within the MRF96 environment ($R^2 = 34.5\%$). It was not associated with yield in the SCF97 environment, and was not correlated with DSI or DI in either environment. As in the BN population, G17.820 was associated with growth habit. Growth habit was, however, not significantly associated with DSI, DI, or yield in the HN population. Although the indeterminate phenotype may be associated with genes for resistance to white mold, selection for the indeterminate phenotype alone would be insufficient in

selecting for resistance. Resistance identified in the Huron genotype may be, in part, due to the QTL associated with BC20.1800, as well as G17.820. Huron shares two markers for resistance in common with Bunsi. Huron may have other QTLs conferring resistance to white mold. A larger population will be needed to test for the presence and effects of additional QTLs.

Quantitative trait loci for resistance to white mold has previously been identified on linkage group B7. The *Phs* locus, located on B7, was found to be associated with up to 36% of the variability for physiological resistance in an A55/G122 population, as determined using a straw test (Miklas et al., 2000). Days to maturity was also found to be associated with the *Phs* locus in the A55/G122 population, where later maturing lines had less disease (Miklas et al., 2000). Both physiological resistance and avoidance mechanisms QTLs were located on B7 in a PC-50/XAN-159 population (Park et al., 2000a; Park et al., 2000b). A single RAPD marker, J09.950, was associated with partial field resistance, plant height, partial physiological resistance, determined via the straw test, seed weight, and resistance to CBB in the PC-50/XAN-159 population. RAPD marker, H12.1050, was previously associated with plant uprightness and branch density, contributing more evidence of plant avoidance mechanisms located on B7 (Jung et al., 1996). Significant markers for both physiological resistance and plant avoidance to white mold reside on linkage group B7.

Resistance genes have been found to be tightly linked. Certain resistance genes, such as genes for resistance to downy mildew in lettuce (*Lactuca serriola* L.) are located in clusters on the genome (Maisonneuve et al., 1994). Mechanisms of resistance genes, such as protein kinases (PK), nucleotide binding sites (NBS), and leucine-rich repeats

have also been found to be conserved resistance genes across species (Kanazin et al., 1996; Salmeron et al., 1999). Similarity in function and location of resistance genes suggest a common evolutionary origin for resistance to pathogens in plants (Maisonneuve et al., 1994; Kanazin et al., 1996; Salmeron et al., 1996; Geffroy et al., 1999). Microclusters of disease-resistance related sequences were identified in bean when analyzed with primers based on a conserved NBS found in several plant disease resistance genes (Rivkin et al., 1999). One of the identified clones from this analysis mapped close to a gene for rust resistance in bean. Candidate gene analysis using known defense-related genes may be a useful approach towards identifying genes associated with resistance to white mold in bean. In sunflower, a candidate gene approach was used to identify genes for resistance to white mold (Gentzbittel et al., 1998). Homology cloning of NBS-like genes, and serine-threonine PK-like genes, was used to detect genes for resistance to downy mildew and white mold. The NBS-like loci were associated with resistance to downy mildew, and 25 cM away from a PK-like loci that was associated with resistance to white mold (Gentzbittel et al., 1998). The presence of QTLs for physiological resistance to white mold in bean on similar linkage groups in several populations is suggestive of a similar mode of action for resistance (Miklas et al., 2000; Park et al., 2000a). The same QTL regions for resistance to both Fusarium root rot and white mold, located on B2 and B3, indicate that similar defense-related genes may effective against both pathogens.

The markers identified in the BN population are useful tools in understanding resistance to white mold in bean, and for future marker-aided breeding for resistance to white mold. QTLs for resistance to white mold identified in Linkage Groups B2 and B7

were significant across environments, and in certain cases, were confirmed in a second population. High-yielding environments are very conducive to heavy white mold pressure. The high LOD scores and coefficients of determination between markers on Linkage Group B7 and yield under white mold pressure, indicates that breeding for high yield and resistance to white mold is achievable. The indeterminate navy bean has been

Table 3.6. Phenotypic variability ($P<0.01$) for OR, DSI, DI and yield associated with markers in HN population in combined and individual environments.

Marker	Putative Linkage Group	HN population marker- trait analysis		
		Trait	Environment	R^2 (%)
BC20.1800	B2	DSI[†]	Across Environments	40.3
			MRF96	60.9
			MRF97	16.4*
			SCF97	20.2*
			MRF98	13.2[†]
		DI	Across Environments	35.4
			MRF96	53.2
			MRF97	ns
			SCF97	ns
			MRF98	21.0
G17.820	B7	OR	Greenhouse	24.3
		Yield	Across Environments	47.0
			MRF96	34.5
			MRF97	38.4
			SCF97	ns
			MRF98	42.5

*, [†] = significant at $P<0.05$ and 0.10, respectively; ns = non significant
[†] DSI = disease severity incidence; DI = disease incidence; OR = resistance to oxalate

shown to be higher-yielding, with greater yield stability than the determinate navy bean (Kelly et al., 1987). QTL analysis of yield under heavy white mold stress confirmed the significant variation in yield that was observed in the BN population. Combining the

resistant cultivars, Bunsi and Huron, may offer an improved phenotype, if Huron was to carry additional QTLs for resistance that were not identified in the BN population. The markers identified in this study indicate that improving resistance by MAS in a Huron/Bunsi hybridization may not be an effective strategy. Phenotypically, Bunsi has an open porous canopy, while Huron has a more upright plant architecture and tight canopy. Phenotypic selection for a taller, more upright plant architecture, with an open canopy may be an efficient strategy for improving resistance to white mold. OAC Laser, an indeterminate navy bean, is an example of a navy bean with an upright plant architecture and open porous canopy that has low DSI and DI scores in white mold environments (Kolkman and Kelly, 2000). Relationships between architectural and seed size traits have been previously associated with elongation factors, such as internode length, structural factors relating to sturdiness, branch angle and number, and reproductive factors relating to pod distribution traits (Acquaah et al., 1992). Previous convention has held that physiological resistance and avoidance mechanisms, such as plant architecture are separate unlinked entities. Markers on B7 contrast that theory, since QTLs for OR, days to flowering, days to maturity and architecture were identified on the same linkage group. The QTL for OR identified on linkage group B7 may be an indirectly constitutive defense mechanism that is a pleiotropic effect of genes for architecture, that improve plant structure and rigidity in bean.

QTL Mapping:

The identification of significant QTLs for complexly-inherited traits is highly dependent upon the environment in which the trait is measured. In many instances,

microclimate changes can significantly alter the expression of the trait. A change in microclimate can play a major role in the expression and detection of resistance to white mold in bean. Experiments with populations that are used to study such complexly-inherited traits, must take into account not only the physical environment that the genotypes are exposed to, but also the phenotypic variability within the population, that may affect the microclimate. In this study, populations were developed from narrow crosses between elite navy bean lines that were adapted to the Michigan environment. A genetically narrow population is ideal for the study of QTLs of minor effect. If a wide cross is utilized, QTLs will be identified, but may be associated with broad differences in germplasm groups, rather than minor differences associated with complexly-inherited traits. The BN population varied for growth habit and plant architecture. More minor QTLs may be identified in a population derived from a cross between resistant and susceptible parents that have a similar architecture and growth habit. The importance of evaluating QTLs in a genetic background that can adequately identify the genetic potential of QTL regions is the main focus of the Inbred Backcross Method (Bliss, 1993), or the Advanced Backcross QTL Method (Tanksley and Nelson, 1996). The latter has been successfully used to introgress QTLs from diverse germplasm, including wild species, into an adaptive background (Bernacchi et al., 1998) An appropriate genetic population should be the first priority in studying QTLs, whereas the second priority then becomes identification of linkage groups, and linkage map construction.

The construction of genetic linkage maps, however, are dependent upon maximum recombination, assuming adequate coverage of the genome. Genetic maps are constructed between market classes, gene pools, and species, depending upon genetic

similarity within the species of interest. In common bean, an integrated linkage map was created to bring together three previously mapped populations consisting of morphological markers, isozymes, RFLP markers, and RAPD markers (Freyre et al., 1998). The integrated linkage map was very useful in determining the location of QTLs in the BN population. Genes identified on previous maps that are in the region of the QTLs identified in the BN population become candidate genes for further study of possible Mendelian factors controlling the QTLs for resistance to white mold in common bean. Such genes include the *PvPR-2*, *Pgip*, and *ChS* on linkage group B2, the seed lectins on linkage group B7, and the *PvPr-1* gene on B3.

The importance of testing for multiple traits that are correlated to the trait of interest was suggested as an important method in the identification of markers linked to QTLs (Ronin et al., 1998). Selective multivariate genotyping, using single and multiple traits to design resistant and susceptible DNA bulks was an efficient strategy for the identification of markers linked to QTLs in the BN population. In the absence of an adequate screen for physiological resistance, the multivariate DNA bulk offers an opportunity to select for a specific set of genotypes with desirable characteristics. This is an important factor in the study of resistance to white mold in common bean, since various factors, such as days to flowering may affect the number of lines that avoid infection, rather than resist infection. The QTLs identified in this study were found in regions of the genome most likely to have either general plant defense mechanisms, such as *PvPr-1*, *PvPR-2*, and *Pgip*, or seed protein factors, which may also have antifungal activity, as well as an influence on the architectural phenotype. Studies in other species have shown that general mechanisms of plant defense may play an important role in

resistance to white mold. In this study, the most important QTLs in the BN population were found in regions of the genome that were associated with phenological and agronomic traits, such as days to flowering, days to maturity, plant architecture and yield. In soybean, QTLs were found in regions of the genome associated with agronomic traits such as lodging, and plant height and flowering date (R1; Kim and Diers, 2000). QTLs for branching pattern, seed weight, oil content and flowering date were also found to be associated with resistance to white mold in sunflower (Mestries et al., 1998). A structural component of plant architecture may play an important role in the constitutive resistance to white mold in crop species. Other general constitutive pathogenesis-related defense mechanisms may also be important for resistance to white mold in common bean.

Markers were identified that were linked to QTLs for resistance to white mold, in two main linkage groups of common bean. The markers were relatively stable across environments, with the exception of being less significant in one environment with extreme disease pressure. The association of the BC20.1800 marker to resistance to white mold in linkage group B2 was confirmed in a second RIL population. One marker on linkage group B7 was associated with OR and yield in the second RIL population. One of the linkage groups accounted for major variability for yield under white mold pressure. Markers in the two linkage groups offer unique insight into potential breeding strategies, such as QTL pyramiding, for the improvement of resistance to white mold in common bean.

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Table A7. Chi-square goodness of fit test for segregation of RAPD and AFLP markers on Linkage Groups B2 and B7.

Linkage Group B2					Linkage Group B7				
	Segregation Ratio					Segregation Ratio			
Marker	observed	expected	χ^2	*P*-value	Marker	observed	expected	χ^2	*P*-value
O12.900	58:36	5:3	0.03	0.90<*P*<1.00	acccac242	60:38	5:3	0.07	0.80<*P*<0.90
		1:1	5.15	*P*<0.03			1:1	4.94	*P*<0.03
P7.700	52:41	5:3	1.72	0.20<*P*<0.25	aggctt85	55:43	5:3	1.70	0.15<*P*<0.20
		1:1	1.30	0.20<*P*<0.30			1:1	1.47	0.20<*P*<0.30
O12.1600	58:40	5:3	0.46	0.50<*P*<0.60	O12.2500	56:42	5:3	1.20	0.30<*P*<0.40
		1:1	3.31	*P*<0.08			1:1	2.00	0. 15<*P*<0.20
acgctt239	54:44	5:3	2.29	0.10<*P*<0.15	G17.820	45:49	5:3	1.70	0.15<*P*<0.20
		1:1	1.02	0.30<*P*<0.40			1:1	0.17	0.70<*P*<0.80
acgctt240	52:46	5:3	3.73	0.05<*P*<0.08	I07.1200	46:49	5:3	8.03	*P*<0.003
		1:1	0.37	0.60<*P*<0.70			1:1	0.17	0.70<*P*<.80
BC20.1800	50:48	5:3	5.51	*P*<0.02	aacctt130	52:46	5:3	3.73	*P*<0.08
		1:1	0.04	0.90<*P*<1.00			1:1	0.37	0.60<*P*<0.70
O15.1800	48:40	5:3	2.38	0.10<*P*<0.15	H12.1050	54:43	5:3	1.93	0.15<*P*<0.20
		1:1	0.73	0.40<*P*<0.50			1:1	1.25	*P*=0.30
					P9.1750	56:42	5:3	1.20	0.30<*P*<0.35
							1:1	2.00	0.15<*P*<0.20

Table A8. Chi-square goodness of fit test for segregation of RAPD and AFLP markers on Putative Linkage Groups B3 and B8.

Linkage Group B3					Linkage Group B8				
	Segregation Ratio					Segregation Ratio			
Marker	observed	expected	χ^2	*P*-value	Marker	observed	expected	χ^2	*P*-value
aaccaa310	45:51	5:3	10.00	$P<0.002$	AM13.400	49:37	5:3	1.12	$0.30<P<0.40$
		1:1	0.38	$0.60<P<0.70$			1:1	1.67	$0.20<P<0.30$
Y11.350	57:40	5:3	0.58	$0.40<P<0.50$	I03.1550	61:36	5:3	0.01	$0.90<P<1.00$
		1:1	2.98	$0.08<P<0.10$			1:1	6.44	$P<0.02$
aaccaa302	41:51	5:3	12.63	$P<0.0005$	aggcaa87	47:50	5:3	8.17	$P<0.005$
		1:1	1.09	$0.30<P<0.40$			1:1	0.09	$0.80<P<0.90$
acccac500	53:45	5:3	2.97	$0.08<P<0.10$	aacctt145	68:30	5:3	1.98	$0.15<P<0.20$
		1:1	0.65	$0.40<P<0.50$			1:1	14.73	$P<0.02$
aaccaa195	58:39	5:3	0.30	$0.60<P<0.70$					
		1:1	3.72	$0.05<P<0.08$					

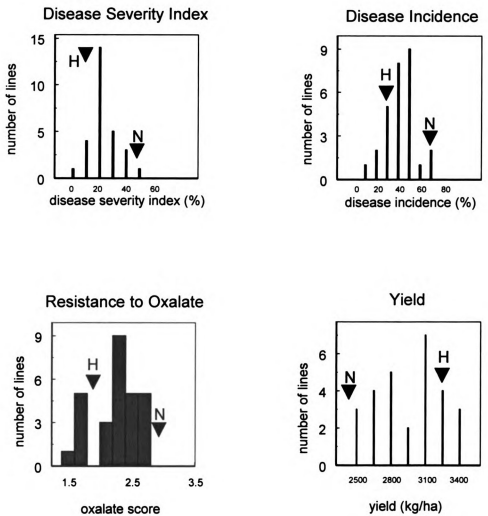

Figure B1. Phenotypic distribution across four environments for 28 recombinant inbred lines of the Huron/Newport population evaluated for marker association to disease severity index, disease incidence, resistance to oxalate, and yield (see Chapter 2 and 3). Location of mean values for Huron (H) and Newport (N) are located at arrows.

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