This is to certify that the
dissertation entitled

A SEARCH FOR TOLERANCE TO BLACK ROOT ROT IN
STRAWBERRY

presented by

CHRISLYN ANN PARTICKA

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Plant Breeding & Genetics/Horticulture

James F Hancock
Major Professor's Signature

12/8/05

Date

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.
MAY BE RECALLED with earlier due date if requested.

DATE DUE	DATE DUE	DATE DUE

A SEARCH FOR TOLERANCE TO BLACK ROOT ROT IN STRAWBERRY

By

Chrislyn Ann Particka

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment for the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture/Plant Breeding and Genetics

2005

ABSTRACT

A SEARCH FOR TOLERANCE TO BLACK ROOT ROT IN STRAWBERRY

By

Chrislyn Ann Particka

Black root rot (BRR) is a widespread disease of strawberry (*Fragaria ×ananassa* Duchnesne) that causes the death of feeder roots and the degradation of structural roots. The major causal organisms of black root rot include *Rhizoctonia fragariae* Husain and W.E. McKeen, *Pythium* Pringsh. spp. and *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven. The current method of control for black root rot is methyl-bromide fumigation; however, methyl bromide is scheduled to be phased out in 2005, and its effects are short-lived in matted-row systems. The objectives of the first study were to measure levels of tolerance to black root rot in 20 strawberry genotypes and to determine which pathogens were present in the soil. The genotypes were planted in four blocks each of methyl-bromide fumigated and nonfumigated soil, and were evaluated for crown number, number of flowers per crown, yield, and average berry weight over two years. The results showed that all three pathogens were present in the field, and that there was a significant genotype × fumigation interaction for yield and crown number in both years. The cultivars Bounty, Cabot, and Cavendish, all released from the breeding program in Nova Scotia, displayed tolerance to the pathogens that cause BRR. The objective of the second study was to determine the heritability of BRR tolerance. Nine genotypes were chosen from the previous study to use as parents: three that

displayed high tolerance to BRR ('Bounty', 'Cabot', and 'Cavendish'), three that displayed intermediate tolerance ('Guardian', 'Midway', and 'Winona'), and three that displayed little or no tolerance ('Jewel', LH50-4, and 'Mesabi'). The progeny from a diallel cross were grown on fumigated and nonfumigated soil and evaluated for crown number, flower number, and yield. Results showed no interaction between treatment and family, indicating that breeding for increased tolerance to BRR will be difficult.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. James Hancock for the opportunity to earn my PhD under his direction. His support, guidance, humor, and wealth of knowledge that he has so willingly shared has made this a wonderful learning experience. I also thank the other members of my committee, Dr. Amy Iezzoni, Dr. James Kelly, and Dr. Annemiek Schilder for their helpful input and advice.

Pete Callow also deserves an enormous thank-you, for all of his help in every aspect of my research, and for answering the multitude of questions I had. Somehow, Pete (and Jim) managed to make even the most mundane tasks fun. I would also like to thank all of the other members of the Hancock lab, especially Sedat Serçe and Cholani Weebadde, for your friendship and camaraderie. Sedat deserves a special thank-you for all of his help in analyzing my data.

I would also like to thank all of my friends and colleagues, including Mercy and Jim Olmstead, Audrey and Chris Sebolt, Roberto Lopez, Liz Driscoll, Nat Hauck, and Angie Durhman. Special thanks also goes to the support staff, including Lorri Busick, Bill Chase, Joyce Lockwook, Kristi Lowrie, Sherry Mulvaney, Sharon Roback, and Gary Winchell.

Finally, I thank my family, especially my parents, Steve and Julie Drake, for your love and support, and my husband, Michael Particka, for making my life complete.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

°C	Degrees Celsius
ANOVA	Analysis of Variance
BRR	Black root rot
cm	Centimeter
d	Day
ha	Hectare
g	Gram
kg	Kilogram
L	Liter
μg	Microgram
m	Meter
ml	Millileter
PDA	Potato Dextrose Agar

CHAPTER ONE

LITERATURE REVIEW

Introduction

Black root rot (BRR) is a serious disease of strawberry (*Fragaria ×ananassa* Duchnesne) that causes the death of feeder roots, the degradation and blackening of structural roots, and an overall decrease in plant vigor and productivity (Maas, 1998). BRR has been reported in strawberry in the United States, Canada, Europe, and Australia (Raski, 1956). One of the earliest reports of BRR in Michigan was by Coons (1924), who noted that infected roots blackened and the cortex peeled off. Many believe that BRR has now replaced red stele as the most serious disease of strawberry in the Northeast (Pritts and Wilcox, 1990).

As BRR does not affect the crown of the plant, it can be distinguished from diseases that do, such as *Phytophthora cactorum* (Lebert & Cohn) Schröt. and *Colletotrichum fragariae* A. N. Brooks (Wing et al., 1994). BRR can be separated from Verticillium wilt (*Verticillium albo-atrum* Reinke & Berthier) as Verticillium wilt is most severe in the first year of growth and the outer leaves of the infected plants wilt and die while the inner leaves remain healthy (Maas, 1998). BRR-infected plants, by contrast, rarely show symptoms during the first year of growth, and leaves of all ages wilt (Maas, 1984).

Biotic factors associated with BRR

Rhizoctonia

Many biotic factors have been associated with BRR. *Rhizoctonia* DC. spp. are some of the major fungi associated with BRR, and were first associated with the disease by Zeller (1932). Zeller isolated *Rhizoctonia solani* Kühn as well as other fungi from root lesions, but *R. solani* was the only one that demonstrated pathogenicity. Many other early researchers identified or suspected *R. solani* or other *Rhizoctonia* species as the cause of BRR (Coons, 1924; Hildebrand and Koch, 1936; Katznelson and Richardson, 1948; Miller, 1948; Rich and Miller, 1963).

A new species of *Rhizoctonia*, *R. fragariae* Husain and W.E. McKeen, was described in the mid-1960s (Husain and McKeen, 1963a). They believed *R. fragariae* had been overlooked in the past because of its close resemblance to *R. solani*, which was common and well-known at that time. The new species was differentiated from *R. solani* as well as other *Rhizoctonia* species as it did not produce any sclerotia. Pea (*Pisum sativum* L.), bean (*Phaesolus vulgaris* L.), tomato (*Lycopersicon esculentum* Mill.), carrot (*Dacus carota* L.), and sunflower (*Helianthus annuus* L.) were found to be hosts of *R. fragariae*. Husain and McKeen (1963b) showed that strawberry roots exude a substance that contains many amino acids (glycine, threonine, alanine, serine, and tyrosine) which is stimulatory to *R. fragariae*.

Since Husain and McKeen's report describing *R. fragariae*, it has been associated with BRR in strawberries in many areas of the United States and other countries (Abad et al., 1999; D'Ercole et al., 1989; LaMondia and Martin, 1988; Szczygiel and Profic-Alwasiak, 1989.). In a survey of strawberry fields in Massachusetts, Drozdowski (1987) found that binucleate *Rhizoctonia*-like fungi (such as *R. fragariae*) were the predominant fungal pathogens in the root systems. Maas (1998) stated that *R. fragariae* may be the most widespread pathogen of BRR. In Michigan field surveys, *R. fragariae* was the only fungus consistently isolated from plants showing symptoms of BRR and is currently believed to be the main fungal pathogen that causes BRR (C. Osborn, personal communication).

Although *R. fragariae* is found in most strawberry plantings, infected plants do not always show symptoms of BRR. Ribeiro and Black (1971) sampled plants from fields in West Virginia, Maryland, Pennsylvania, and Arkansas that showed symptoms of BRR as well as adjacent plants showing no symptoms of the disease. Although *R. fragariae* was isolated from all symptomatic plants, it was also isolated from 80% of the healthy-looking plants. The authors concluded that *R. fragariae* exists with strawberry plants as an endophytic mycorrhizal fungus, and only when certain environmental or nutritional conditions occur does the fungus become pathogenic.

Pythium

Many *Pythium* species have also been associated with BRR, with *Pythium ultimum* Trow being the most common (Wilhelm, 1998). One of the first reports of *Pythium* in strawberries was in 1930, when Plakidas isolated nine different strains of the fungus from strawberry roots in Louisiana and found that one strain was more pathogenic than the rest. However, none of the strains were identified. In Ontario, Hildebrand and Koch (1936) and in England, Berkley and Lauder-Thomson (1934), isolated *Pythium* (in addition to other pathogens) from field-grown strawberries as well. Nemec and Sanders (1970) surveyed strawberry fields in Illinois in order to identify the different *Pythium* species associated with strawberries there. Eight different species were found, with *P. irregulare* Buisman, *P. perniciosum* Serbinow, and *P. sylvaticum* W.A. Campbell and J.W. Hendrix being isolated most frequently. A study in Japan found *P. sylvaticum, P. ultimum, P. spinosum* Sawada, and *P. oedochilum* Drechs. to be the most common *Pythium* species in strawberry fields, although *Rhizoctonia* species were more prevalent (Watanabe et al., 1977). In a Massachusetts survey (Drozdowski, 1987), *Pythium* was only occasionally found in the wettest areas.

Other fungi

Over the years that BRR has been studied, many other fungi besides *Rhizoctonia* and *Pythium* have been identified as possible causes of the disease. Strong and Strong (1931) indicated through isolation and inoculation studies that *Coniothyrium fuckelli* Sacc. and *Hainesia lythri* (Desmaz.) Höhn were common causal organisms of BRR in Michigan. Isolation studies by Hildebrand (1934) showed that *Fusarium* spp., *Ramularia* spp., and *Pythium* spp. were most often found in diseased strawberry roots in plantings in the Niagara Peninsula. In Britain, Berkley and Lauder-Thomson (1934) found five fungi capable of damaging strawberry roots, each of which caused very similar damage. They were: *C. fuckelli, H. lythri, Cylindrocarpon radicicola* Wollenweb., *Fusarium* *orthoceras* Appel. and Wollenweb., and *Pachybasium candidum*. Miller (1948) discovered that *Fusarium* Wr. and *Ramularia* Unger non Roussel were two of the most prevalent and pathogenic fungi of strawberry roots in Oregon. In Italy, *Verticillium dahliae* Kleb., *Idriella lunata* P.E. Nelson and K. Wilh., and *Cylindrocarpon destructans* (Zinssmeister) Scholten were shown to be components of BRR in addition to *Rhizoctonia* and *Pythium* (D'Ercole et al., 1989).

Root lesion nematode

Nematodes, specifically the root lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven], have been associated with strawberries since Steiner first reported finding them in strawberry fields in Florida in 1931. Berkley and Lauder-Thomson (1934) and Hildebrand and Koch (1936) were some of the earliest researchers to link nematodes to BRR, although the species of nematode was not mentioned. In the mid-1950s through the early 1960s, researchers worldwide found *P. penetrans* as well as other species of *Pratylenchus* in strawberry fields having problems with BRR including Klinkenberg in the Netherlands (1955), Goheen and Bailey in Massachusetts (1955), Chapman in Kentucky (1956), Riggs et al. in Arkansas (1956), and Townshend in Ontario (1962). *P. penetrans* has also been found in the roots of wild plants of the alpine strawberry, *F. vesca alpina* (Klinkenberg, 1955), the wood strawberry, *F. vesca* L. (Townshend, 1958), and the Virginiana strawberry, *F. virginiana* Duch. (Goheen and Braun, 1956).

In the late 1950s and early 1960s, researchers established the pathogenicity of *P. penetrans* but were not able to clearly link it with the symptoms of BRR. Townshend (1962) found that while *P. penetrans* did considerable damage to strawberry roots, it was not the same sort of damage attributed to BRR. He suggested that the symptoms of BRR were caused by fungi and bacteria, and that the nematode was a precursor of the root rot. A later study by Townshend (1963) proved that *P. penetrans* was pathogenic to strawberry by following Koch's postulates. A soil fumigation study found that high populations of *P. penetrans* were associated with low plant vigor, while low populations were associated with high plant vigor, but BRR symptoms were not described (Braun and Keplinger, 1960). Goheen and Smith (1956) found that high numbers of root lesion nematodes can produce injury and that it is a primary parasite of strawberry. Raski (1956) found that even very high levels of nematodes did little damage to roots, and concluded that *P. penetrans* was not an important factor in BRR.

Other nematodes

A number of nematodes other than *P. penetrans* have been associated with BRR. Chapman's (1956) survey of strawberry fields in Kentucky found many nematode species (*Meloidogyne hapla* Chitwood, *Tylenchorhynchus claytoni* Steiner, *T. dubius* Cobb, *Xiphinema americanum* Cobb, *Paratylenchus* spp., and *Helicotylenchus nannus* Steiner), although *Pratylenchus* spp. were found most often. However, only *M. hapla* was a known pathogen of strawberry other than *Pratylenchus* spp., and no pathogenicity tests were conducted for the other nematodes. In Massachusetts, the root-knot nematode, *Meloidogyne* spp., was found to live in strawberry roots, although it did not thrive (Bailey, 1956). A later study by Edwards et al. (1985) found that *M. hapla* parasitized and reproduced in the 12 cultivars tested, with a wide range of effects on root growth. Some of the cultivars ('Apollo', 'Catskill', 'Delite', 'Earliglow' and 'Prelude') were unaffected by the nematode.

Rhizoctonia and P. penetrans complex

Although nematodes and fungi have been separately implicated as the cause of BRR, most researchers now believe that BRR is caused by a disease complex between the fungi and the nematodes. Chen and Rich (1962) were the first to conduct studies to determine if such a relationship exists. They found that fungi infected areas of the root damaged by *P. penetrans* more readily than healthy tissue and that *P. penetrans* moved away from roots as fungi invaded the tissue. LaMondia and Martin (1989) conducted a study to determine the effect of *P. penetrans*, *R. fragariae*, and temperature on the severity of BRR, and found that root infection by *P. penetrans* consistently raised the severity of BRR caused by *R. fragariae*. The fungus alone caused 25-36% root rot at 10 °C and 30-38% at 20 °C. Feeding by nematodes, however, increased root rot to 36-52% at 10 °C and 70-82% at 20 °C.

The root lesion nematode has also been shown to interact with Verticillium wilt. A study by Abu-Gharbieh et al. (1962) showed that 'Dixieland', which is highly susceptible to *Verticillium,* developed disease symptoms more quickly and more severely when inoculated with both *Verticillium* and *P. penetrans* than when inoculated with *Verticillium* alone. However, an interaction was not apparent when cultivars were used that were moderately or highly resistant to *Verticillium*.

Interactions between nematodes and fungi have been shown in many other crops besides strawberry. In cotton, studies have shown a link between Fusarium wilt and the sting nematode (*Belonolaimus gracilis* Steiner) (Holdeman and Graham, 1954), the reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) (Neal, 1954) and *Meloidogyne incognita* var. *acrita* (Kofoit and White) Chitwood (Martin et al., 1955). In a study where tobacco plants inoculated either alone with fungi that were either considered non-pathogens of tobacco or were not important on plants beyond juvenile stage or in combination with *M. incognita* showed that none of the pathogens [*Pythium ultimum, Curvularia trifolii* (Kauffm.) Boedijn, *Botrytis cinerea* Pers.:Fr., *Aspergillus ochraceus* K. Wilh., *Penicillium martensii* Biourge, and *Trichoderma harzianum* Rifai] caused disease unless the nematode was present (Powell et al., 1971). Some plants inoculated with *T. harzianum* and nematodes were so damaged that they were near death at the end of the study.

Abiotic factors associated with BRR

Many different abiotic factors have been associated with BRR in addition to the biotic factors. Fletcher (1917) attributed most BRR to winter injury, but also indicated that poor culture, lack of fertility, plant crowding, insufficient mulch, and wet soils were partial causes of the disease. Smith and Horne (1922) believed BRR was caused by waterlogged soil or sudden drying of the soil.

Miller (1948) thought BRR was due to desiccation of the roots during transplanting.

While the abiotic factors have since been found to not cause BRR alone, they may affect disease development. The establishment and growth of pathogens that cause the disease can be influenced by certain abiotic factors. For example, Zeller (1932) found that *Rhizoctonia* spp. were more common in light soils, but disease symptoms were more severe in clay loam soils. *Pythium* spp. are also favored by fine-textured soils (Hendrix and Campbell, 1973). In contrast, Klinkenburg (1955) found that the root lesion nematode was more common in sandy soils. Soil moisture also affects fungi and nematodes. *Pythium* spp. are most often associated with very wet soils (Watanabe et al., 1977). Wing et al. (1995a) found that soil compaction, fine-textured soils, age of the planting, successive years of strawberry monoculture, flat beds, use of the herbicide terbacil (3-tert-butyl-5-chloro-6-methyluracil), and non-use of the fungicide metalaxyl (methyl-N-(2,6-dimethylphenyl)-N-(2-methoxyacetyl)-DL-alaninate) all increased incidence of BRR. A recent study by Mervosh and LaMondia (2004), however, found that the use of terbacil at up to four times the maximum amount allowed per year did not increase incidence of *P. penetrans* or *R. fragariae*, nor did it reduce the health of perennial, structural, or feeder roots.

Control of BRR

Researchers have been struggling to find an effective way to control BRR in matted row systems since it was first described in the early 1900s. Early researchers recommended a variety of cultural control measures. Coons (1924) suggested rotation with grain crops, as they seemed to reduce *Rhizoctonia* infestation in soils. He also suggested selecting healthy planting stock, protecting plants from winter damage, incorporating mulch, and ensuring adequate drainage, but seemed to imply that such measures were somewhat futile. Strong and Strong (1930) also suggested selecting vigorous plants with white roots as planting stock and careful handling of plants during setting to keep roots from drying out. In another article in 1931, they included crop rotation and breeding for resistance as additional control measures.

Chemical control

Even today, about 80 years after BRR was first reported, no long-lasting, effective control measures have been developed for matted row culture. Fumigation with methyl bromide and chloropicrin have been reported to give good control in annual systems, especially where *Rhizoctonia* predominates (Maas, 1998). However, the effects of fumigation wear off in perennial matted row systems as pathogen populations reestablish over time. Some have even speculated that fumigation could ultimately result in an increase in pathogen pressure in perennial matted row systems, as fumigation kills beneficial soil microbes, leaving nothing to compete with pathogens that either survive in low numbers or are reintroduced to the field. Methyl bromide is scheduled to be phased out (USDA, 2000), and while cost-effective chemical alternatives to methyl bromide have been identified, none provide the full spectrum of control that methyl bromide does (Fennimore et al., 2003; Shaw and Larson, 1999).

Non-chemical control

Many researchers have looked to crop rotation and cover crops to help mitigate the effects of BRR. Morgan and Collins (1964) studied the effect of different cover crops and organic soil amendments on *P. penetrans* populations. They found that while actively growing timothy sod resulted in the highest nematode populations, composted timothy hay resulted in the lowest. Peat moss also significantly lowered nematode populations, and manure and coniferous sawdust had a slight effect as well. LaMondia et al. (2002) found that rotation to 'Saia' oats (*Avena strigosa* Schreb.) and Triple S sorghum-sudangrass [*Sorghum bicolor* Durra × *S. sudanense* (Piper) Stapf.] suppressed both *R. fragariae* and *P. penetrans*, while 'Garry' oats (*Avena sativa* L.) suppressed *R. fragariae* but increased *P. penetrans.* In a related study, Elmer and LaMondia (1999) combined 'Saia' oats, 'Garry' oat, or 'Triple S' sorgho-sudangrass with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$. The combination of 'Saia' oats and $(NH_4)_2SO_4$ resulted in less root damage, larger plants, and earlier harvest than if 'Saia' oats were combined with $Ca(NO_3)_2$ or if another crop was combined with $(NH_4)_2SO_4$. Application of sorgho-sudangrass in combination with $(NH_4)_2SO_4$ didn't affect disease severity or yield, but reduced nematode numbers. Use of 'Garry' oat reduced disease severity and *R. fragariae* infection, but did not affect yield or plant growth. Recent studies conducted at Cornell University found that fumigation with methyl bromide resulted in the highest yield, but a rotation of kale (*Brassica oleracea* L.)/sweet corn [*Zea mays* L. var. *saccharata* (Sturt.) Bailey]/rye (*Secale cereale* L.) also proved to be effective (Seigies, 2004).

Overall, rotations involving multiple species fared much better than rotations involving single species.

Elmer and LaMondia (1995) also studied the effect of mineral nutrition alone on BRR. They compared $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ supplemented with KCl, $CaCl_2$, K_2SO_4, or $CaSO_4$ combined with and without a slow-release micronutrient product. Overall, plants fertilized with $(NH_4)_2SO_4$ had less disease and higher yields, but no differences were seen in nematode densities. The use of K or Cl salts and the use/non-use of micronutrients had no effect on disease severity or yield.

Researchers in Israel studied the use of biological control agents in commercial strawberry fields and nurseries (Elad, et al., 1981). In the study, they investigated the effectiveness of the mycoparasitic fungus *Trichoderma harzianum* in controlling *R. solani*. In nursery plots, *T. harzianum* reduced the disease severity of *R. solani* by 18-46% and reduced the infestation of the soil by up to 92%. In commercial fields, plants treated with *T. harzianum* before planting resulted in a 21-37% increase in early yield, and when treatments were combined in the nursery and fruiting fields, a 20% increase in yield was observed.

Current research at Michigan State University has shown promising results from use of biocontrol agents in both greenhouse and field studies (A. Schilder, personal communication). A controlled greenhouse study tested a number of commercial biocontrol agents as well as isolates of *Paenibacillus macerans* Schardinger Ash from cranberry fruit and *Trichoderma* spp., and found that one of the *Trichoderma* spp. isolates, T-10, was the most effective as it had a positive significant impact on all parameters measured. Field studies tested a number of different products, including Quadris (a strobilurin-type fungicide), in two different sites, and showed mixed results. In the first site, there were no significant differences between the untreated control and any of the products used. At the second site, five products, Quadris, Polyversum (a beneficial *Pythium oligandrum* Drechs.), T-10, Primastop (a commercial formulation of *Gliocladium catenulatum* Gilman & Abbott), and DiTera [a nematicidal fungus called *Myrothecium verrucaria* (Albertini & Schweinitz:Fr.) Ditmar] produced significantly higher yields than the untreated control.

Genetic control

The most effective way to control BRR would be to identify or to develop tolerant strawberry cultivars. A few studies have been undertaken to identify genotypes with tolerance to the pathogens causing BRR, but most new eastern cultivar releases have not been screened.

Potter and Dale (1994) conducted studies to determine if resistance to *P. penetrans* alone could be found in strawberry, and showed that 'Guardian' had the highest resistance overall. Further studies showed considerable variation among 19 cultivars, with the four most resistant being 'Pajaro', 'Chandler', Annapolis', and 'Glooscap' (Dale and Potter, 1998). Interestingly, all these have the California cultivar Lassen in their pedigree.

Recent studies by Pinkerton and Finn (2005) evaluated the resistance and tolerance of a number of strawberry cultivars and wild genotypes (*F. chiloensis* and *F. virginiana* subspecies) to both *P. penetrans* and *M. hapla* (northern root knot nematode). The results showed that both cultivated and wild genotypes had considerable resistance to *M. hapla*, and that resistance to *P. penetrans* was less common. However, they did note that using wild genotypes as sources of resistance would be of little value as resistance to both nematodes can be found in *Fragaria ×ananassa.*

Wing et al. (1995b) found that 'Tristar' 'Earliglow' and 'Midway' had the healthiest roots in a field infected primarily with *Pythium* spp. However, disease incidence in this field was relatively low as no above-ground symptoms were observed, and *Rhizoctonia* spp. and the root lesion nematode were absent.

Studies in California on fumigated and nonfumigated soils that measured leaf number, plant diameter, yield, fruit weight, and fruit appearance did not identify any genotypes with strong tolerance to sublethal levels of soil pathogens (Larson and Shaw, 1995; Shaw and Larson, 1996). In the first study, the interaction between genotype and fumigation was not significant, but this interaction was significant in the second study. The authors pointed out, however, that the interaction explained only 2% to 5% of the variance, and concluded that the lack of fumigation affected all cultivars similarly. In the first study, only California genotypes were tested, and in the second study, nine genotypes from outside California (six of which were from the USDA program in Maryland) and nine California genotypes were used, which represented only a small fraction of the germplasm grown.

Studies in Michigan identified only modest differences in tolerance based on yield, fruit weight, crown and runner number, and root and crown appearance in fields infested with *Pythium* spp., *Rhizoctonia* spp., *Idriella lunata* P.E. Nelson & K. Wilh., *Meloidognye hapla*, and *P. penetrans* (Hancock et al., 2001). This study included four California cultivars, 11 eastern US cultivars, and 12 *F. virginiana* Duchnesne F_1 hybrids. Although no significant genotype × fumigation interactions were found, there was a significant species source × fumigation interaction for fruit weight at $P \leq 0.01$, and for yield and runner number at $P \leq 0.10$. Also, the *F. virginiana* hybrids performed better overall than the eastern or California cultivars, and the authors concluded that some tolerance might exist in *F. virginiana*, which could be used to improve current cultivars.

Further studies using *F. virginiana* and *F. chiloensis* wild selections as well as *F. ×ananassa* cultivars showed positive results (C. Osborn, personal communication). Field studies in methyl bromide fumigated and nonfumigated soils did not find any genotypes with high levels of tolerance to BRR. However, the wild genotypes performed better overall for a number of yield parameters than did the cultivars. In controlled greenhouse studies using the same genotypes, plants were inoculated with *Rhizoctonia* spp. and *P. penetrans* alone and in combination. The results showed that that Frederick 9 and NC 95-1-1 (both *F. virginiana* genotypes) were resistant to *P. penetrans* and that NC 96-48-1 (another *F. virginiana* genotype) was resistant to the mixed infection.

Objectives

***1) Measure the levels of tolerance to BRR in 20 diverse strawberry genotypes [Chapter 2, Published in the Journal of the American Society for Horticultural Science, 130(5):688-693.*]

Nineteen old and new cultivars from six different breeding programs across the United States and Canada along with one *F. virginiana* selection from Montana were screened for tolerance to BRR. The genotypes were evaluated for crown number, number of flowers per crown, yield, and average berry weight over two years. Genotypes were considered tolerant if the percent reduction between fumigated and nonfumigated soil was low for most parameters, and if they performed well overall on nonfumigated soil.

2) Determine the heritability of tolerance to BRR (Chapter 3).

Nine genotypes were chosen that represented the range of tolerance to BRR—three that were identified as being highly tolerant, three that were highly intolerant, and three that were intermediate. The genotypes were mated in a diallel crossing scheme and each of the resulting progeny were evaluated on fumigated and nonfumigated soil for crown number, number of inflorescences per crown, number of flowers per inflorescence, yield, and average berry weight over one year.

CHAPTER TWO

FIELD EVALUATION OF STRAWBERRY GENOTYPES FOR TOLERANCE TO BLACK ROOT ROT ON FUMIGATED AND NONFUMIGATED SOIL

Introduction

Black root rot (BRR) is a widespread disease of strawberry (*Fragaria ×ananassa*) that causes an overall decrease in productivity due to the death of feeder roots and the degradation of structural roots (Maas, 1998). One of the earliest reports of BRR was by Coons (1924), who noted that the roots of infected plants were blackened and the cortex peeled off. By the 1950s, BRR had been reported in the United States, Canada, Europe, and Australia (Raski, 1956). Many strawberry researchers now believe that BRR has replaced red stele (*Phytophthora fragariae* Hickman) as the most serious root disease of strawberry in the Northeastern USA (Pritts and Wilcox, 1990).

Many biotic factors have been associated with BRR. *Rhizoctonia fragariae* Husain and W.E. McKeen was first described as a causative organism of BRR in 1963, and since then, it has been associated with BRR in many areas of the United States and other countries (Abad et al., 1999; D'Ercole et al., 1989; Husain and McKeen, 1963a). Maas (1998) concluded that *R. fragariae* is the most widespread pathogen that causes BRR, although many *Pythium* species have also been associated with the disease (Nemec and Sanders, 1970). *Pythium ultimum* Trow is considered to be the most common one (Wilhelm, 1998). The root lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven] was first associated with strawberry in 1931, and was linked to BRR by many researchers in the mid-1950s to early 1960s (Klinkenberg, 1955; Steiner, 1931; Townshend, 1962).

While *R. fragariae, Pythium* spp., and *P. penetrans* have been separately implicated as the cause of BRR, the disease is often caused by a complex of all three. Chen and Rich (1962) found that fungi infected areas of the root damaged by *P. penetrans* more readily than healthy tissue, and that the nematodes moved away from the roots as fungi invaded. LaMondia and Martin (1989) determined that infection by *P. penetrans* consistently raised the severity of BRR caused by *R. fragariae*.

Several abiotic factors have also been associated with BRR. Fletcher (1917) attributed BRR to winter injury, poor culture, lack of fertility, plant crowding, insufficient mulch, and wet soils. Wing et al. (1995a) found that soil compaction, fine-textured soils, age of the planting, successive years of strawberry monoculture, use of the herbicide terbacil, and non-use of raised beds and of the fungicide metalaxyl all increased incidence of BRR.

Currently, there are no effective, long-lasting control measures for BRR in perennial matted row culture. Fumigation with methyl bromide and chloropicrin has been reported to give good control in annual systems (Maas, 1998); however, its effects gradually wear off in perennial matted row systems as pathogen populations reestablish over time. Some have even suggested that fumigation could ultimately result in an increase in BRR in perennial systems, as fumigation kills beneficial soil microbes, leaving nothing to compete with the pathogens that either survive in low numbers or are reintroduced to the field

(Pritts and Wilcox, 1990). Methyl bromide is scheduled to be phased out (USDA, 2000), and while cost-effective chemical alternatives to methyl bromide have been identified, none provide the full spectrum of control that methyl bromide does (Fennimore et al., 2003; Shaw and Larson, 1999).

Many researchers have looked to crop rotation and cover crops to help mitigate the effects of BRR. Morgan and Collins (1964) studied the effect of different cover crops and organic soil amendments on *P. penetrans* populations and found that composted timothy hay (*Phleum pratense* L.) was most effective in reducing nematode numbers. LaMondia et al. (2002) found that rotation to 'Saia' oats (*Avena strigosa* Schreb.) and Triple S sorghum-sudangrass [*Sorghum bicolor* Durra × *S. sudanense* (Piper) Stapf.] suppressed both *R. fragariae* and *P. penetrans*, while 'Garry' oats (*Avena sativa* L.) suppressed *R. fragariae* but increased *P. penetrans.* Recent studies conducted at Cornell University found that fumigation with methyl bromide resulted in the highest yield, but a rotation of kale (*Brassica oleracea* L.)/sweet corn [*Zea mays* L. var. *saccharata* (Sturt.) Bailey]/rye (*Secale cereale* L.) also proved to be effective (Seigies, 2004). Overall, rotations involving multiple species fared much better than rotations involving single species.

The most effective way to control BRR would be to identify or to develop tolerant strawberry cultivars. A few studies have been undertaken to identify genotypes with tolerance to the pathogens causing BRR, but most new eastern cultivar releases have not been screened.

Potter and Dale (1994) conducted studies to determine if tolerance to *P. penetrans* alone could be found in strawberry, and showed that 'Guardian' had the highest resistance overall. Further studies showed considerable variation among 19 cultivars, with the four most resistant being 'Pajaro', 'Chandler', Annapolis', and 'Glooscap' (Dale and Potter, 1998). Interestingly, all these have the California cultivar Lassen in their pedigree.

Wing et al. (1995b) found that 'Tristar' 'Earliglow' and 'Midway' had the healthiest roots in a field infected primarily with *Pythium* spp. However, disease incidence in this field was relatively low as no above ground symptoms were observed, and *Rhizoctonia* DC. spp. and the root lesion nematode were absent.

Studies in California on fumigated and nonfumigated soils that measured leaf number, plant diameter, yield, fruit weight, and fruit appearance did not identify any genotypes with strong tolerance to sublethal levels of soil pathogens (Larson and Shaw, 1995; Shaw and Larson, 1996). In the first study, the interaction between genotype and fumigation was not significant, but this interaction was significant in the second study. The authors pointed out, however, that the interaction explained only 2% to 5% of the variance, and concluded that the lack of fumigation affected all cultivars similarly. In the first study, only California genotypes were tested, and in the second study, nine genotypes from outside California (six of which were from the USDA program in Maryland) and nine California genotypes were used, which represented only a small fraction of the germplasm grown.

Studies in Michigan identified only modest differences in tolerance based on yield, fruit weight, crown and runner number, and root and crown appearance in fields infested with *Pythium* spp., *Rhizoctonia* spp., *Idriella lunata* P.E. Nelson & K. Wilh., *Meloidognye hapla* Chitwood, and *P. penetrans* (Hancock et al., 2001). This study included four California cultivars, 11 eastern US cultivars, and 12 *F. virginiana* Duchnesne F_1 hybrids. Although no significant genotype × fumigation interactions were found, there was a significant source × fumigation interaction for fruit weight at $P \leq 0.01$, and for yield and runner number at $P \leq 0.10$. Also, the *F. virginiana* hybrids performed better overall than the eastern or California cultivars, and the authors concluded that some tolerance might exist in *F. virginiana*, which could be used to improve current cultivars.

Herein we describe a field screen for BRR tolerance that included both old and new cultivars from six breeding programs, as well as one wild genotype. We focused on yield and vigor of the above-ground portion of the plants evaluated with and without fumigation, and a number of cultivars developed in Nova Scotia were shown to have considerable tolerance.

Materials and Methods

We evaluated 19 cultivars and one *F. virginiana* genotype (Table 1). The planting was established in June of 2002 at the Horticulture Teaching and Research Center in Holt, Mich., on soil that had been in strawberries for over five years. Plants were obtained from commercial nurseries, and were planted in a split-plot design with four plots each of nonfumigated soil and soil that had been treated with a mixture of 2 methyl bromide : 1 chloropicrin (weight : weight) injected at a rate of 392 $kg \cdot ha^{-1}$. Three plants of each genotype were planted in each plot, with 46 cm within-row spacing and 61 cm between-row spacing. The plots received supplemental irrigation as needed, and weeds were controlled through use of pre-emergence herbicides and mechanical methods. At the end of the first fruiting season, we selected 10 genotypes that appeared to represent the range of variation for tolerance to BRR (Table 1), and the rest of the genotypes were removed to simplify plot maintenance.

The plants were allowed to runner freely, with sufficient training to keep plants of different genotypes separated. In the fall of year one (2002-2003 season) and year two (2003-2004 season), the total number of crowns was recorded in each plot (8 Nov. and 24 Oct., respectively). Individual plants were not counted, but most were represented by one or two crowns. In the spring of year one and year two, the number of flowers per crown was counted (27 May and 21 May, respectively). During the fruiting season each year (11 June-9 July 2003 and 3 June-29 June 2004), fruit that were at least 60% ripe were harvested and weighed weekly. A random 25-berry sample was taken from each genotype in each plot to determine average fruit weight. Crown and yield data were divided by the number of surviving mother plants for analysis, as in a few instances, not all of the original plants survived.

In both years, root samples were taken from randomly-selected plants to determine presence of plant parasitic nematodes (14-21 July 2003 and 12-17 May 2004) and fungal pathogens (14 July 2003 and 28 Sept. 2004). For nematode isolation, two plants were randomly selected from the 10 genotypes included in both years of the study from each of the plots (for a total of 160 plants). Plants were dug using a hand spade and were placed in plastic bags on ice and then stored at 1.5 °C until they were processed the following day. Nematodes were extracted from root tissue following the flask-shaker method (Bird, 1971). To make up a 1-g sample, approximately 0.5 g of roots were selected from the two plants from each plot, for a total of 80 composite samples. For fungal isolation, approximately 100 plants were randomly selected from the same 10 genotypes in both fumigated and nonfumigated plots, but samples were not kept separate for genotype or treatment. Plants were dug using a hand spade and were placed in plastic bags on ice and stored at 1.5 °C until they were processed later in the day or the following day. Fungi were isolated from roots by washing the root systems in running water, and selecting root segments that had visible lesions. The root segments were cut into 1-cm long sections, surface-sterilized in 1% NaOCl for three minutes, rinsed three times in sterile distilled water, and were blotted dry on sterile filter paper. At least 80 segments were used both times fungi were isolated. The root pieces were placed on selective medium [1/4 strength acidified PDA supplemented with ampicillin (50µg/ml) and streptomycin (20µg/ml)] and were transferred to fresh media as needed for identification. Fungi were identified based on the morphology of hyphae and of spores (Barnett & Hunter, 1998; Maas, 1998).

Model variance components due to fumigation, genotype, and fumigation × genotype treatment interaction and error were estimated as in Larson and Shaw (1995). The analysis of variance was conducted using the GLM function of

SAS (SAS Institute, Cary, N.C.), with years being analyzed separately, as half the genotypes were removed after the first year.

Results and Discussion

Pathogens present

Over 90% of the nematodes isolated were the root lesion nematode, *Pratylenchus* Filipjev spp. Fumigation with methyl bromide was effective at killing the root lesion nematode, and they did not move back into fumigated areas during the study. In samples from fumigated soil, 5% contained the root lesion nematode in year one, and only 8% did in year two; numbers ranged from two to 54 per gram of root tissue across years, with an average of two. One hundred percent of the samples from nonfumigated soil contained the root lesion nematode in year one, while 85% of the samples contained nematodes in year two. Root lesion nematode numbers ranged from two to 152 nematodes per gram of root from nonfumigated soil, with an average of 28. No significant differences in nematode number were observed across genotypes in either year (P = 0.200, df=9 in year 1; P = 0.249, df=9 in year 2).

Rhizoctonia spp. and *Pythium* spp. were the most common pathogenic fungi present in the soil. In year one *Pythium* spp. were found in many more samples than *Rhizoctonia* spp., but unfortunately, the relative percentages were not recorded. In year two, *Rhizoctonia* spp. was isolated from 43% of the root segments, while *Pythium* spp. was isolated from only one sample. *Cylindrocarpon destructans* (Zinnsmeister) Scholten, which has also been associated with BRR (Wilhelm, 1998), was also isolated from three root segments in year two. Differences in the most common type of pathogen found between year 1 (samples were collected on 14 July) and year 2 (samples were collected on 28 Sept.) is most likely due to the difference in sampling date. *R. fragariae* is not parasitic on strawberry roots under warm temperatures, and is therefore replaced by other pathogens during the spring and summer (Husain and McKeen, 1963a).

Overall cultivar performance

In year one, mean yields across treatments were highest for 'Mesabi', 'Cabot', 'Bounty', 'Brunswick', 'Annapolis', and 'Cavendish', all of which produced over 900 g per plant (Table 2). Each of these genotypes produced their high yields in different ways. 'Mesabi' ranked in the top third of all genotypes for crown number, number of flowers/crown, and fruit weight. 'Cabot' ranked only in the upper half of all genotypes for crown number and number of flowers/crown, but had the largest fruit. 'Bounty' ranked in the bottom third for fruit weight, but was in the top third for crowns number and number of flowers/crown. 'Brunswick' was intermediate for number of flowers/crown and fruit weight, but ranked in the top third for crown number. 'Annapolis' was only intermediate for number of flowers/crown, but ranked in the top third for crown number and fruit weight. 'Cavendish' was intermediate for crown number, but ranked in the top third for number of flowers/crown and fruit weight.

In year two, 'Brunswick' and 'Annapolis' were dropped from the study, but 'Mesabi', 'Cabot', Bounty', and 'Cavendish' remained among the highest producers, joined by 'Jewel' and 'Winona'. Again, their high yields were achieved in different ways. 'Bounty' remained near the bottom for fruit weight, but was one of the highest for crown number and number of flowers/crown. 'Cabot' had the largest fruit and was in the top third for crown number, but had only intermediate number of flowers/crown. 'Cavendish' ranked high for number of flowers/crown and fruit weight, but was low for crown number. 'Mesabi' was intermediate for all three yield components. 'Jewel' and 'Winona' had intermediate crown numbers and numbers of flowers/crown, but were in the top third for fruit weight.

Effects of fumigation on yield components of genotypes

Fumigation resulted in increased yield in both years of the study; yield on fumigated soil was 46% higher ($P < 0.001$) in year one and 33% higher ($P < 0.001$) in year two (Table 2). Crown number was also significantly higher on fumigated plots. In year one, fumigation resulted in 47% more crowns ($P < 0.001$), and in year two, crowns were increased by 41% ($P < 0.001$). Individual fruit weight was significantly higher on nonfumigated plots in year two ($P = 0.019$), but by just 10%. The number of flowers per crown was not significantly different between fumigated and nonfumigated plots in either year.

The genotype × fumigation interaction for yield was significant in both years, indicating that some genotypes are more tolerant to BRR than others (Table 2). The interaction explained 46% of the variance in year one, and 26% in year two. In year one, the highest overall producers, 'Bounty', 'Brunswick', 'Cabot', and 'Cavendish', had high yields (866-790 g) on nonfumigated soil, and their yields were reduced by only 25-35% without fumigation (Figure 1). In contrast, 'Annapolis', and 'Mesabi' had only moderate yields on nonfumigated soil (572 and 483g, respectively), and their yields were reduced by 60 to 70%. In year two, 'Cabot', 'Bounty', and 'Cavendish' remained the highest producers on nonfumigated soil (over 800g) and their yields were reduced by less than 20% without fumigation (Figure 4). Yields of 'Mesabi', 'Jewel', and 'Winona' were again less than 600g on nonfumigated soil and were reduced by over 50% without fumigation. This indicates that while 'Mesabi', 'Jewel', and 'Winona' are vigorous and have high yield potentials, they have little tolerance to the pathogens that cause black root rot. 'Bounty', 'Brunswick', 'Cabot', and 'Cavendish' all have high yield potentials and are tolerant to black root rot.

The genotype × fumigation interaction was significant for crown number in both years, mirroring the yield data (Table 2). The interaction explained 61% of the variance in year one, and 26% in year two. In year one, 'Cavendish' had among the highest crown numbers (11) on nonfumigated soil, and had only 7% fewer crowns than fumigated soil (Figure 2). 'Bounty', 'Brunswick', 'Cabot', and 'Annapolis' also had very high crown numbers (9-13), but their numbers were reduced by 40-50% without fumigation. 'Mesabi' had a rather low number of crowns on nonfumigated soil (6), and the crown numbers were reduced by 68%.

The number of flowers per crown was not significant for the genotype × fumigation interaction in either year (Table 2). This suggests that the number of flowers per crown is not involved in the yield reductions seen on nonfumigated soil.

The fumigation × genotype interaction was significant for fruit weight in year one (Table 2); however, the interaction explained only 6% of the variance, and fumigation did not appear to have a consistent effect across genotypes. Average berry weight was higher on nonfumigated soil for seven genotypes and lower for 13 genotypes (Figure 3). Many authors have reported a significant decrease in berry size on nonfumigated soil that was strongly associated with yield reductions (Hancock et al., 2001; Larson and Shaw, 1995; Shaw and Larson, 1996;). Our results indicate that while berry weight can be reduced on nonfumigated soil, the effect is modest (10% or less) and variable across genotypes, making reductions in crown number much more important in effecting yields on nonfumigated fields of matted row cultivars.

Potential for breeding new cultivars resistant to BRR

Based on our studies, we feel there is sufficient variability to breed for increased tolerance to BRR. Especially impressive are the cultivars from the breeding program of Agriculture and Agri-Food Canada in Nova Scotia—all eight of those cultivars included in the study were in the top half for yield on nonfumigated soil in year one (Figure 1), and in the second year of the study, the four that were included were ranked first, second, third, and fifth (Figure 4). These cultivars also were the most tolerant to BRR, being among the lowest in percent reduction on nonfumigated soil. This means there is opportunity to combine vigor and tolerance to increase overall performance in soils infested with the pathogens that cause BRR.

In previous studies conducted in California and Michigan, little genetic diversity was found that could be utilized in increasing tolerance to soil pathogens (Hancock et al., 2001; Larson and Shaw, 1995; Shaw and Larson, 1996), but the top-performing cultivars from Nova Scotia ('Bounty,' 'Brunswick,' 'Cabot,' and 'Cavendish') were not used in these studies. In examining the pedigrees of the cultivars from Nova Scotia, it was not evident that there was a single source of tolerance; no one genotype was present in these cultivars that was not also present in cultivars from other programs. However, methyl bromide has not been used in the Nova Scotia breeding plots (A. Jamieson, personal communication), as it has in most other breeding programs, suggesting that tolerant genotypes can be selected in the presence of pathogen pressure.

Table 1. Strawberry genotypes grown on fumigated and nonfumigated soil at Holt, Mich. from 2002-2004 to evaluate tolerance to black root rot.

Genotype	Year released	Parentage	Origin
Allstar	1981	US 4419 × MDUS 3184	USDA-Beltsville, Maryland
Annapolis	1984	K74-5 × Earliglow	AAFC[y], Kentville, N.S.
Bounty[z]	1972	Jerseybelle × Senga Sengana	AAFC, Kentville, N.S.
Brunswick	1999	Cavendish × Honeoye	AAFC, Kentville, N.S.
Cabot[z]	1998	K78-5 × K86-19	AAFC, Kentville, N.S.
Cavendish[z]	1990	Glooscap × Annapolis	AAFC, Kentville, N.S.
Chandler	1983	Douglas × Cal 72.361-105	University of California, Davis
Earliglow	1975	MDUS 2359 × MDUS 2713	USDA-Beltsville, Maryland
Evangeline	1999	Honeoye × Veestar	AAFC, Kentville, N.S.
Gov. Simcoe	1985	Holiday × Guardian	HRIO[x], Simcoe, Ontario
Guardian[z]	1969	NC 1768 × Surecrop	USDA-Beltsville, Maryland
Honeoye	1979	Vibrant × Holiday	NYSAES[w], Geneva, New York
Jewel[z]	1985	NY 1221 × Holiday	NYSAES, Geneva, New York
Kent[z]	1981	K 68-5 × Raritan	AAFC, Kentville, N.S.
LH50-4[z]	-	-	Native *F. virginiana* from Montana
Mesabi[z]	2000	Glooscap × MNUS 99	University of Minnesota, St. Paul
Midway[z]	1960	Dixieland × Temple	USDA-Beltsville, Maryland
Mira	1995	Scott × Raritan	AAFC, Kentville, N.S.
Surecrop	1956	Fairland × MDUS 1972	USDA-Beltsville, Maryland
Winona[z]	1995	Earliglow × MNUS 52	University of Minnesota, St. Paul & USDA-Beltsville, Maryland

[z] Included in both years

[y] Agriculture and Agri-Food Canada

[x] Horticulture Research Institute of Ontario

[w] New York State Agricultural Experiment Station

Table 2. ANOVA and variance components for yield, crown number, flower number, and fruit weight of strawberry genotypes in Holt, Mich. on fumigated and nonfumigated soil. Plants were set in 2002 and evaluated in 2002-2003 (Year 1) and 2003-2004 (Year 2).

Source	Yield (g/plant)[z]		Crowns (no./plant)[z]		Flowers (no./crown)		Avg. fruit weight (g)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Fumigation								
Fumigated	902.9	825.5	15.8	39.3	8.7	14.5	10.7	8.2
Non-fumigated	484.8	556.7	8.4	23.2	9.7	13.0	10.3	7.4
Genotype								
Allstar	595.4	-	8.8	-	8.8	-	11.4	-
Annapolis	995.8	-	13.6	-	8.5	-	12.9	-
Bounty	1062.7	875.8	18.1	35.0	9.3	18.5	8.4	6.0
Brunswick	1051.5	-	15.5	-	8.5	-	11.2	-
Cabot	1081.1	973.0	12.3	29.3	8.3	12.1	16.6	12.7
Cavendish	900.0	886.3	10.9	20.4	9.1	15.6	12.9	10.2
Chandler	280.5	-	8.7	-	5.1	-	10.3	-
Earliglow	737.0	-	10.4	-	9.4	-	10.1	-
Evangeline	696.2	-	14.8	-	6.8	-	10.3	-
Gov. Simoce	605.2	-	11.6	-	7.9	-	8.9	-
Guardian	378.6	491.8	15.1	30.2	6.5	14.5	7.3	6.9
Honeoye	681.4	-	10.2	-	8.0	-	10.4	-
Jewel	796.2	966.4	11.1	24.6	8.9	15.5	13.8	9.4
Kent	719.2	591.0	7.5	13.8	10.5	19.2	11.7	8.0
LH50-4	61.7	76.4	19.5	91.0	28.4	9.9	1.4	1.0
Mesabi	1104.5	952.1	13.2	24.5	10.4	13.9	11.7	7.5
Midway	269.2	264.7	7.3	15.7	6.8	8.4	7.9	5.8

Table 2 (cont'd)

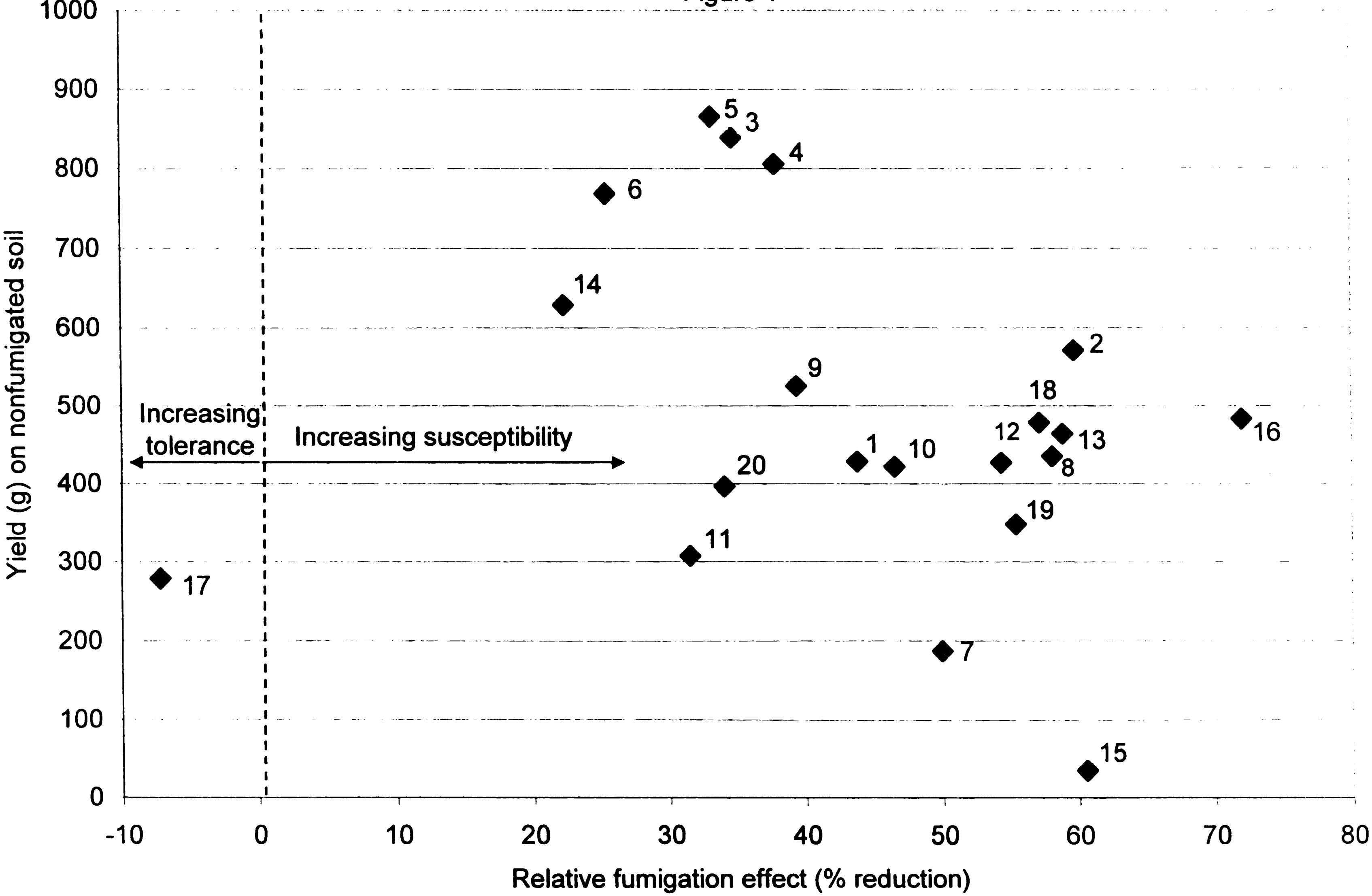
Mira	798.3	-	12.6	-	8.2	-	12.1	-
Surecrop	564.3	-	10.7	-	7.6	-	8.5	-
Winona	498.7	833.5	10.0	27.7	7.9	10.1	12.0.	10.4
Mean	693.9	691.1	12.1	31.3	9.2	13.8	10.5	7.8
ANOVA								
Significance *(P)*								
Genotype (G)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.027	< 0.001	< 0.001
Fumigation (F)	< 0.001	0.001	< 0.001	< 0.001	0.045	0.313	0.262	0.019
G × F	0.004	0.023	0.008	0.005	0.052	0.501	0.050	0.365
σ^2	75,446 (33)[y]	109,546 (41)	17.8(37)	153.0 (19)	7.5 (25)	50.1 (69)	4.6 (34)	2.1 (17)
σ^2_g	34,717 (15)	55,086 (21)	0.0 (0)	360.4 (44)	20.1 (67)	9.5 (13)	8.1 (59)	9.9 (80)
σ^2_{gxf}	103,633 (46)	68,058 (26)	29.5 (61)	209.7 (26)	1.4 (5)	0.0 (0)	0.8 (6)	0.3 (3)
σ^2_p	227,558	264,132	48.1	811.4	30.0	72.2	13.7	12.4

[z] Yield and crown number are expressed as the amount per surviving mother plant in a plot.

[y] Values in parentheses are percentages of the total phenotypic variance due to random effects, σ^2p; variance components labeled 0 where negative.

Figure1. Plot showing yield and relative fumigation effect (percent reduction on nonfumigated soil compared to fumigated soil) in year 1 for 20 genotypes grown at Holt, Mich. Yield is expressed as the amount/surviving mother plant in a plot. Positive values for percent reduction indicate the value was lower on nonfumigated soil, while negative values indicate the value was higher on nonfumigated soil. The genotypes were 1) 'Allstar', 2) 'Annapolis', 3) 'Bounty', 4) 'Brunswick', 5) 'Cabot', 6) 'Cavendish', 7) 'Chandler', 8) 'Earliglow', 9) 'Evangeline', 10) 'Governor Simcoe', 11) 'Guardian', 12) 'Honeoye', 13) 'Jewel', 14) 'Kent', 15) LH50-4, 16) 'Mesabi', 17) 'Midway', 18) 'Mira', 19) 'Surecrop', 20) 'Winona'.

Figure 1



1000
900
800
700
600
500
400
300
200
100
0
-10
0
10
20
30
40
50
60
70
80
Yield (g) on nonfumigated soil
Relative fumigation effect (% reduction)
Increasing tolerance
Increasing susceptibility
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure 2. Plot showing crown number and relative fumigation effect (percent reduction on nonfumigated soil compared to fumigated soil) in year 1 for 20 genotypes grown at Holt, Mich. Crown number is expressed as the amount/surviving mother plant in a plot. Positive values for percent reduction indicate the value was lower on nonfumigated soil, while negative values indicate the value was higher on nonfumigated soil. The genotypes were 1) 'Allstar', 2) 'Annapolis', 3) 'Bounty', 4) 'Brunswick', 5) 'Cabot', 6) 'Cavendish', 7) 'Chandler', 8) 'Earliglow', 9) 'Evangeline', 10) 'Governor Simcoe', 11) 'Guardian', 12) 'Honeoye', 13) 'Jewel', 14) 'Kent', 15) LH50-4, 16) 'Mesabi', 17) 'Midway', 18) 'Mira', 19) 'Surecrop', 20) 'Winona'.

Figure 2

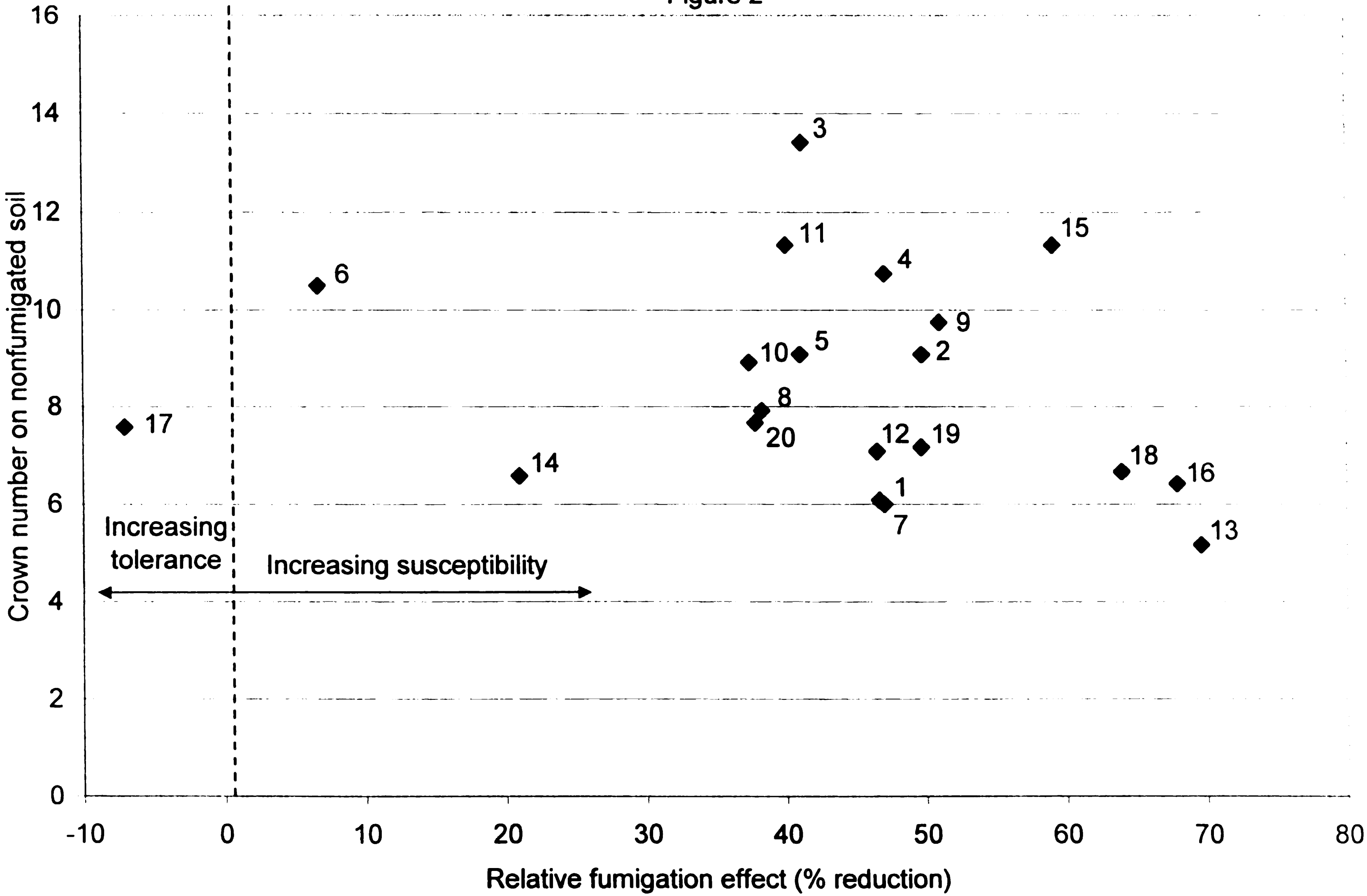
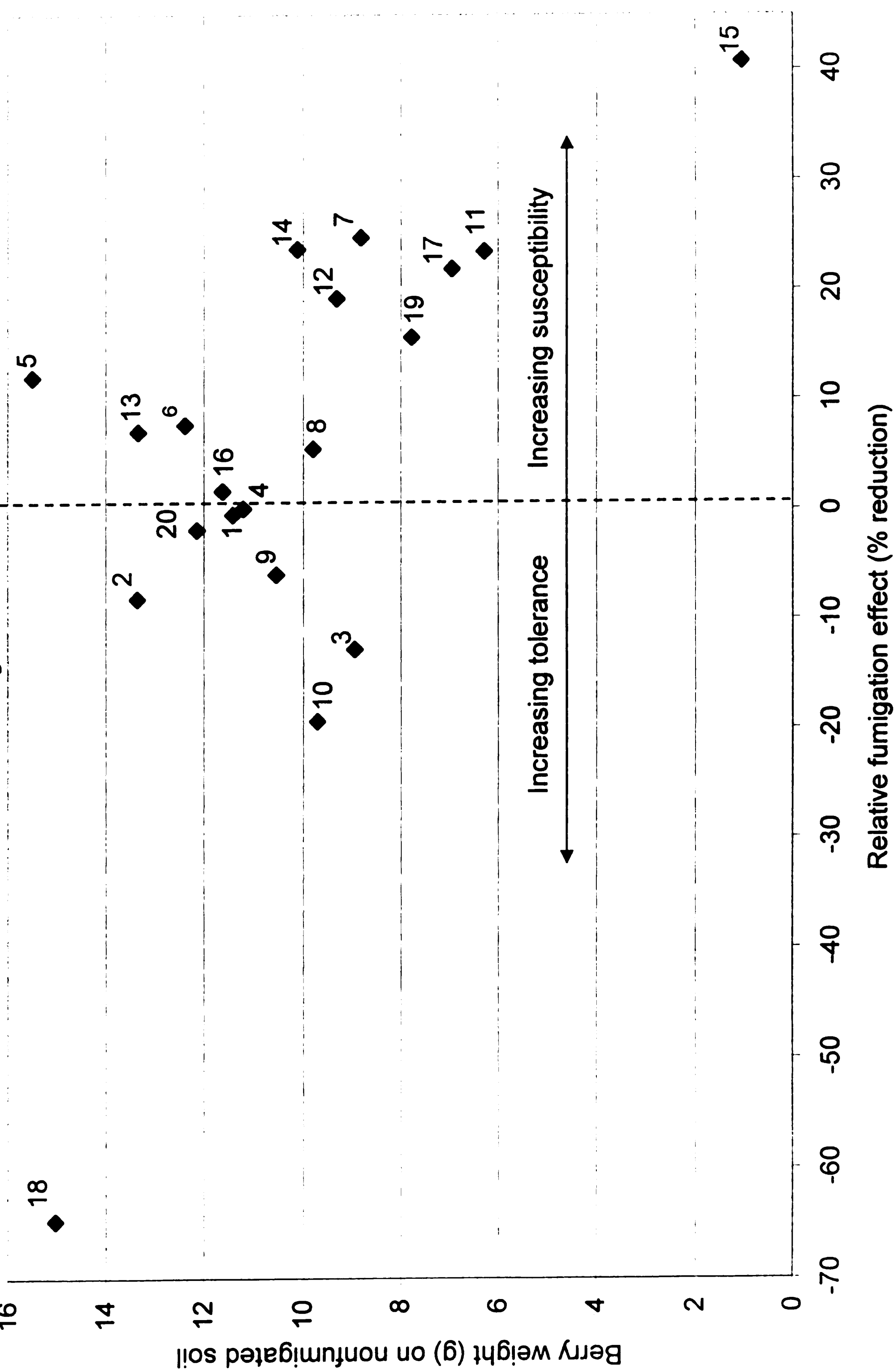

16
14
12
10
8
6
4
2
0
-10
0
10
20
30
40
50
60
70
80
Crown number on nonfumigated soil
Relative fumigation effect (% reduction)
Increasing tolerance
Increasing susceptibility
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure 3. Plot showing average berry weight and relative fumigation effect (percent reduction on nonfumigated soil compared to fumigated soil) in year 1 for 20 genotypes grown at Holt, Mich. Positive values for percent reduction indicate the value was lower on nonfumigated soil, while negative values indicate the value was higher on nonfumigated soil. The genotypes were 1) 'Allstar', 2) 'Annapolis', 3) 'Bounty', 4) 'Brunswick', 5) 'Cabot', 6) 'Cavendish', 7) 'Chandler', 8) 'Earliglow', 9) 'Evangeline', 10) 'Governor Simcoe', 11) 'Guardian', 12) 'Honeoye', 13) 'Jewel', 14) 'Kent', 15) LH50-4, 16) 'Mesabi', 17) 'Midway', 18) 'Mira', 19) 'Surecrop', 20) 'Winona'.

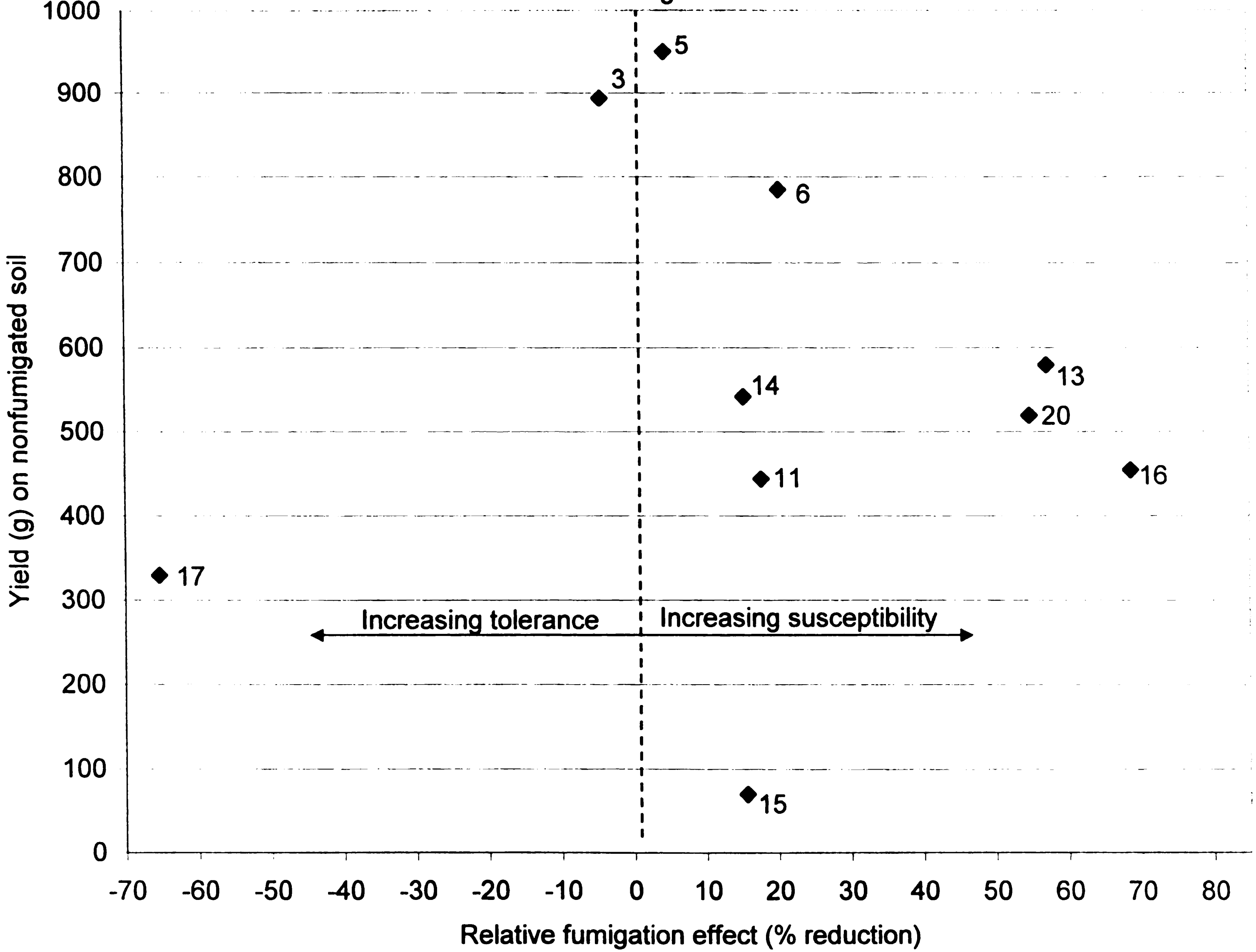
Figure 3



Berry weight (g) on nonfumigated soil
0
2
4
6
8
10
12
14
16
Relative fumigation effect (% reduction)
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
Increasing tolerance
Increasing susceptibility
18
5
13
6
2
20
1
16
4
9
8
3
10
14
12
7
19
17
11
15

Figure 4. Plot showing yield and relative fumigation effect (percent reduction on nonfumigated soil compared to fumigated soil) in year 2 for 10 genotypes grown at Holt, Mich. Yield is expressed as the amount/surviving mother plant in a plot. Positive values for percent reduction indicate the value was lower on nonfumigated soil, while negative values indicate the value was higher on nonfumigated soil. The genotypes were 3) 'Bounty', 5) 'Cabot', 6) 'Cavendish', 11) 'Guardian', 13) 'Jewel', 14) 'Kent', 15) LH50-4, 16) 'Mesabi', 17) 'Midway', 20) 'Winona'.

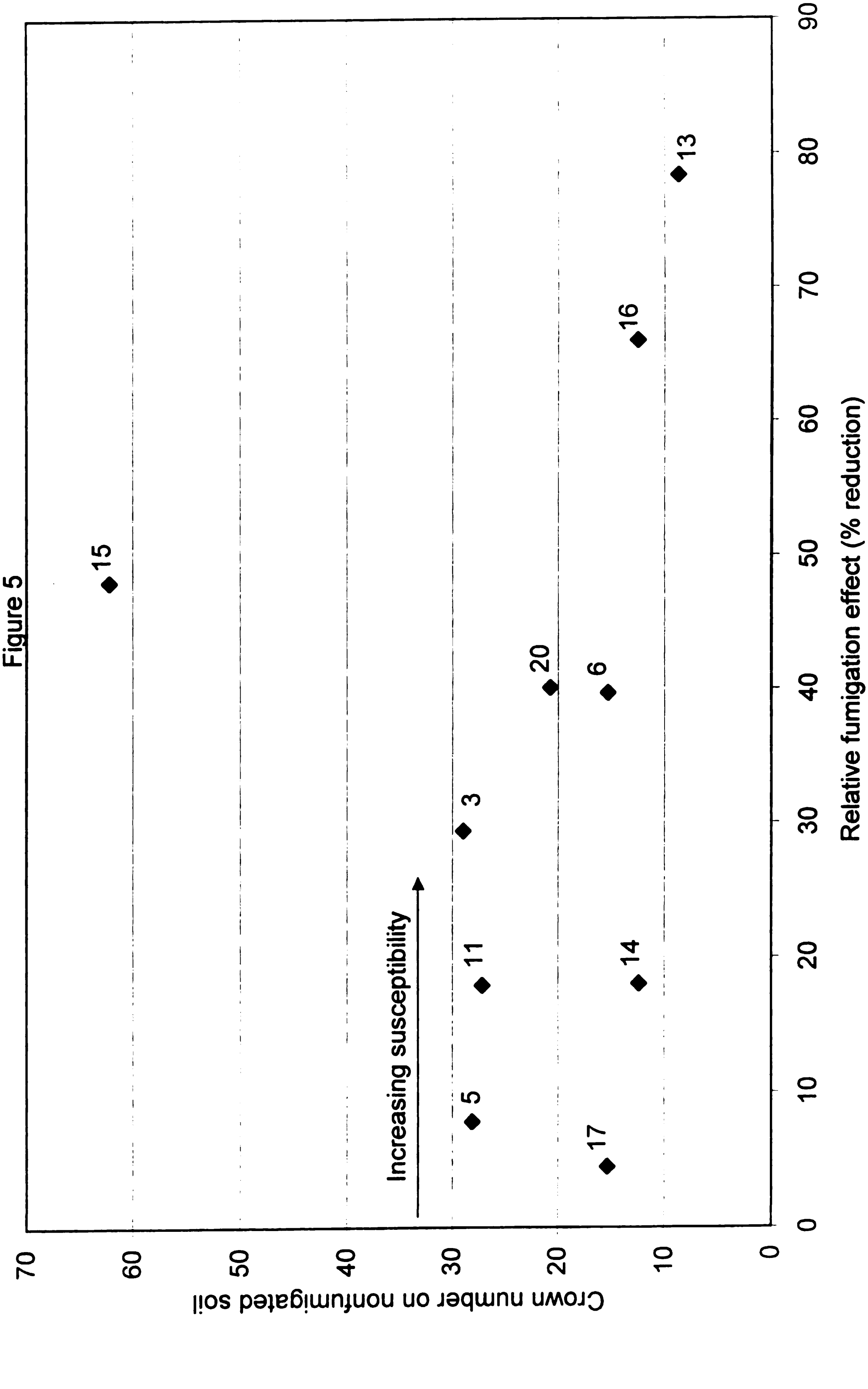
Figure 4



Yield (g) on nonfumigated soil
Relative fumigation effect (% reduction)
Increasing tolerance
Increasing susceptibility
1000
900
800
700
600
500
400
300
200
100
0
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
3
5
6
14
11
13
20
16
17
15

Figure 5. Plots showing crown number and relative fumigation effect (percent reduction on nonfumigated soil compared to fumigated soil) in year 2 for 10 genotypes grown at Holt, Mich. Crown number is expressed as the amount/surviving mother plant in a plot. Positive values for percent reduction indicate the value was lower on nonfumigated soil, while negative values indicate the value was higher on nonfumigated soil. The genotypes were 3) 'Bounty', 5) 'Cabot', 6) 'Cavendish', 11) 'Guardian', 13) 'Jewel', 14) 'Kent', 15) LH50-4, 16) 'Mesabi', 17) 'Midway', 20) 'Winona'.

Figure 5



Crown number on nonfumigated soil
0
10
20
30
40
50
60
70
Relative fumigation effect (% reduction)
0
10
20
30
40
50
60
70
80
90
Increasing susceptibility
15
20
6
3
11
14
5
17
16
13

CHAPTER THREE

BREEDING FOR INCREASED TOLERANCE TO BLACK ROOT ROT IN STRAWBERRY

Introduction

Black root rot (BRR) is a widespread disease of strawberry (*Fragaria ×ananassa*) that causes the death of feeder roots and the degradation of structural roots, resulting in an overall decrease in productivity (Maas, 1998). By the 1950s, BRR had been reported in many areas of the world (Raski, 1956), and most strawberry researchers now believe that BRR has replaced red stele (*Phytophthora fragariae* Hickman) as the most serious root disease of strawberry in the Northeastern USA (Pritts and Wilcox, 1990).

Many biotic factors have been associated with BRR. *Rhizoctonia* DC. spp. was first linked to BRR in the early 1930s (Zeller, 1932), and *Rhizoctonia fragariae* Husain and W.E. McKeen was first described as a causative organism of BRR in 1963 (Husain and McKeen, 1963a). Since then, *R. fragariae* has been associated with BRR in many areas of the United States and other countries (Abad et al., 1999; D'Ercole et al., 1989; Szczygiel and Profic-Alwasiak, 1989). Although *R. fragariae* is the most widespread pathogen that causes BRR (Maas, 1998), various *Pythium* species have also been identified as causal organisms (Nemec and Sanders, 1970). *Pythium ultimum* Trow is considered to be the most common one (Wilhelm, 1998). The root lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven] was first associated with strawberry in the early 1930s (Steiner, 1931), and Townshend (1963) proved it was pathogenic to strawberry by following Koch's postulates.

Although the three pathogens have been separately implicated as the cause of BRR, it is now believed to be a disease complex between the fungi and nematodes. Fungi are able to infect areas of the root damaged by *P. penetrans* more easily than healthy tissue, and nematodes move away from roots after fungi invade (Chen and Rich, 1962). LaMondia and Martin (1989) found that the severity of *R. fragariae* infection is raised due to infestation by *P. penetrans.*

In addition to biotic factors, several abiotic factors have been associated with BRR. Wing et al. (1995a) found that soil compaction, fine-textured soils, age of the planting, successive years of strawberry monoculture, use of the herbicide terbacil, and non-use of raised beds and of the fungicide metalaxyl all increased incidence of BRR. A recent study by Mervosh and LaMondia (2004), however, found that use of Terbacil at up to four times the maximum recommended rate each year did not increase incidence of *P. penetrans* or *R. fragariae*, nor did it reduce the health of perennial, structural, or feeder roots.

No completely effective chemical control measures have been identified for BRR in matted row culture. In annual systems, fumigation with methyl bromide and chloropicrin is highly effective, especially where *Rhizoctonia* predominates (Maas, 1998). However, pathogens ultimately reestablish in perennial systems, and some believe that fumigation can result in an increase in BRR as fumigation also kills beneficial soil microbes, leaving nothing to compete with invading pathogens (Pritts and Wilcox, 1990). Methyl bromide is being phased out by the end of this year (USDA, 2000), and no cost-effective chemicals have been identified that provide the same level of control as methyl bromide (Fennimore et al., 2003; Shaw and Larson, 1999).

Developing strawberry cultivars with tolerance to BRR would be an effective control measure. Potter and Dale (1994) showed that 'Guardian' had a high level of resistance to *P. penetrans,* and further studies showed considerable variation among 19 cultivars, with the four most resistant being 'Pajaro', 'Chandler', Annapolis', and 'Glooscap' (Dale and Potter, 1998). Interestingly, those four cultivars have 'Lassen', a California cultivar, in their background.

In a field primarily infected with *Pythium* spp., 'Tristar', 'Earliglow', and 'Midway' had the healthiest roots (Wing et al., 1995b). However, *Rhizoctonia* spp. and *P. penetrans* were not in the field and no disease symptoms were observed on above-ground portions of the plants, indicating that disease pressure was low.

Two studies in California that measured leaf number, plant diameter, yield, fruit weight, and fruit appearance on fumigated and nonfumigated soils did not identify any genotypes with strong tolerance to sublethal levels of soil pathogens (Larson and Shaw, 1995; Shaw and Larson, 1996), although a significant interaction between genotype and fumigation was observed in the second study. The authors concluded that lack of fumigation affected all cultivars similarly as the interaction only explained 2% to 5% of the variance. However, only a small fraction of the strawberry germplasm grown in the USA was represented in these studies. Only California genotypes were tested in Larson and Shaw (1995), and in Shaw and Larson (1996), nine genotypes from outside California (six of which were from the USDA program in Maryland) and nine California genotypes were used.

Another study in Michigan using a broader array of germplasm (four California cultivars, 11 eastern US cultivars, and 12 *F. virginiana* Duch. F_1 hybrids) also identified only modest differences in tolerance based on yield, fruit weight, crown and runner number, and root and crown appearance. Fields used in the study were infested with *Pythium* spp., *Rhizoctonia* spp., *Idriella lunata* P.E. Nelson & K. Wilh., *Meloidognye hapla* Chitwood, and *P. penetrans* (Hancock et al., 2001). Although no significant genotype × fumigation interactions were found, there was a significant source × fumigation interaction for fruit weight at $P \leq 0.01$, and for yield and runner number at $P \leq 0.10$. The authors concluded that some tolerance might exist in *F. virginiana* that could be used to improve cultivars, as *F. virginiana* hybrids performed better overall than the eastern or California cultivars.

In the most recent study conducted in Michigan, 20 strawberry genotypes were screened for field tolerance to BRR, including old and new cultivars from six North American breeding programs as well as one wild genotype (Chapter 2). The genotypes were evaluated for crown number, number of flowers per crown, yield, and average berry weight in both fumigated and nonfumigated soil for two years. A significant genotype × fumigation interaction was observed for crown number and yield in both years of the study, explaining 46% and 26% of the variance for yield in years 1 and 2, respectively, and 61% and 26% of the variance for crown number in years 1 and 2, respectively. Interestingly, 'Bounty', 'Cabot', and 'Cavendish', all from the breeding program in Nova Scotia, displayed the highest level of tolerance to BRR. These cultivars had not been previously tested for their tolerance to BRR.

In the experiments outlined here, progeny populations were created to determine the amount of genetic variability for BRR tolerance, where the Nova Scotia cultivars were used as parents along with six other genotypes. Duplicate daughter plants of the progeny population were grown on fumigated and nonfumigated soil and were evaluated for crown number, flower number, and yield.

Materials and Methods

We chose nine genotypes from the previous study (Chapter 2) to use as parents: three that displayed high tolerance to BRR ('Bounty', 'Cabot', and 'Cavendish'), three that displayed intermediate tolerance ('Guardian', 'Midway', and 'Winona') and three that displayed low tolerance ('Jewel', LH 50-4, and 'Mesabi') (Table 3). The genotypes were crossed in diallel mating scheme with no selfs, and reciprocal crosses were grouped into the same family. Thirty-two out of 36 possible families were generated, as four crosses were not successful and did not produce any progeny (Table 4).

Crosses were made on greenhouse-grown plants in the spring of 2003 by removing stamens with a pair of sharp tweezers then transferring pollen to the stigma with a camel hair paint brush or fingernail. Pollinated flowers were covered with cheesecloth for 48 hours to reduce the chance of outside pollination. In most instances, fresh pollen was collected from open flowers for crosses, but occasionally pollen was used that had been previously collected and stored in 1.5 mL Eppendorf tubes at –16 °C. Fruit were allowed to ripen on the plant, then were harvested for seed collection. Seeds were sown on moist, sterilized potting soil and placed in a growth chamber at 4 °C with continuous inflorescent light until they began to germinate. The seedlings were then transferred into growth chambers at ~ 20 °C until they reached the 4 - 6 leaf stage, when they were potted in 10 × 10 × 12-cm pots. These were held in a greenhouse in Holt, Mich. under natural daylengths and temperatures (5.5 - 38 °C) until they were planted into a field at the Horticulture Teaching and Research Center in Holt.

On 2 June 2004, the field was fumigated with Vapam (metam sodium) in 1.2-m strips, alternating between fumigated and nonfumigated bands, at a rate of 89 $L \cdot ha^{-1}$. The field was planted on 21 June 2004. Plants in each family were evenly divided into five replications, and the plants (hereafter referred to as mother plants) were set on the border between the fumigated and nonfumigated strips, with 1.07 m between plants. Beginning on 24 June 2004, two runners from each of the mother plants were trained into the middle of the fumigated and nonfumigated strips, and daughter plants were established. The runners connecting the mother and daughter plants were cut beginning on 1 Aug. 2004, and the mother plants were dug up and removed from the field on 31 Aug. and 1 Sept. 2004. The field received supplemental irrigation as needed, weeds were controlled through use of preemergence herbicide and mechanical methods, and fertilizer was applied on 10 Sept. at a rate of 1.8 $kg \cdot ha^{-1}$ N.

Plants were allowed to runner freely, with sufficient training to keep the runners of each daughter plant separate from the runners of other daughter plants in the field. The number of runner plants produced by each daughter plant was counted on 21-28 Oct. 2004, after the first killing frost of the year. Crowns were also counted on each daughter plant and all of the runner plants (crown number), but crown counts and plants counts were essentially as over 90% of the plants had only one crown due to the young age of the planting. The number of inflorescences per daughter plant and all of the runner plants was counted on 23-31 May 2005 before flowers were fully open. This number was divided by the crown number to obtain mean number of inflorescences/crown. The number of flowers per inflorescence were counted on five randomly-selected runner plants from each mother plant on 9 and 10 June after all flowers were open and fruit development had begun and an average flower number/inflorescence value was calculated. Ten fruit were harvested from each daughter plant and the runner plants on 20-27 June 2005 and weighed to calculate mean fruit weight. Total yield was calculated by multiplying crown number, number of inflorescences/ crown, number of flowers/inflorescence, and mean fruit weight.

Root samples were taken from randomly-selected plants to determine presence of fungal pathogens on 15 Aug. 2005 and plant parasitic nematodes on 29 Aug. 2005. For fungal isolation, ≈ 10 plants were randomly selected from both fumigated and nonfumigated soil from each of the five blocks and kept separate for treatment and block. Plants were dug using a hand spade and were placed in plastic bags on ice then stored at 1.5 °C until they were processed the following day. Fungi were isolated from roots by washing the root systems in running water and selecting root segments that had visible lesions. The root segments were cut into 1-cm long sections, surface-sterilized in 1% NaOCl for three minutes, rinsed three times in sterile distilled water, and were blotted dry on sterile filter paper. The root pieces were placed on selective medium [1/4 strength acidified PDA supplemented with ampicillin (50µg/ml) and streptomycin (20µg/ml)] and were transferred to fresh media as needed for identification. Fungi were identified based on the morphology of hyphae and of spores (Barnett & Hunter, 1998; Maas, 1998). For nematode isolation, four plants were randomly selected from the front, middle, and back area of each of the five blocks, and were kept separate for treatment, block, and area of the field, for a total of 120 plants. Soil was also collected from each of these areas for a total of 30 samples. Plants and soil were dug using a hand spade and were placed in plastic bags on ice and stored at 1.5 °C until they were processed the following day. Nematodes were extracted from root tissue following the flask-shaker method, except that a 0.05% NaOCl solution was used in place of the ethyl-mecuric-chloride dihydrostreptomycin sulfate solution (Bird, 1971). To make up a 1-g sample needed, ~0.25g of roots were selected from each of the four-plant samples, for a total of 30 composite samples. Nematodes were isolated from roots using a modified centrifugal flotation procedure with nested sieves (Jenkins, 1964).

Model variance components were estimated due to fumigation, family, and family × genotype treatment interaction and error as in Larson and Shaw (1995). The analysis of variance was conducted using the GLM function of SAS (SAS Institute, Cary, N.C). DIALLEL-SAS05 (Zhang et al., 2005), a program based on Griffing's and Gardner-Ebhart Analyses, was used to calculate GCA and SCA effects. Three parents, 'Guardian', LH 50-4, and 'Mesabi' were not included in this analysis, as they did not produce sufficient progeny for a full diallel analysis.

Results and Discussion

Analysis of all the families

In the ANOVA of all the families, significant differences were observed for both treatment and family, but not for the interaction between treatment and family. Overall means for most of the parameters measured were higher on fumigated soil than nonfumigated soil (Table 5). Crown number was 20% higher ($P < 0.001$), mean individual fruit weight was 9% higher ($P = 0.012$), and total yield was 26% higher ($P < 0.001$). Only the number of inflorescences/crown and flowers/inflorescence were not significantly different between fumigated and nonfumigated soil ($P = 0.992$ and $P = 0.876$, respectively). In our previous study in Michigan, we found that mean yield reduction due to lack of fumigation was 46% in year one, and 33% in year 2. Average berry weight was smaller only in the second year by just 10%, and crown number was reduced by 47% in the first year and 41% in the second year. (Chapter 2). The lower reduction in berry weight than crown number in both studies suggests that losses in runner production (and therefore daughter plants and crowns) plays a more important role in yield reduction associated with the lack of fumigation than loss of berry size.

Significant differences ($P < 0.001$) were also observed between families for all the parameters measured (Table 5). Mean crown numbers varied from 9.1 crowns for 'Cabot' × LH 50-4 to just 1.6 crowns for 'Bounty' × 'Guardian'. The rest of the eight families that had LH 50-4 as a parent had higher crown numbers than all other families. This is not surprising, as LH 50-4 is a wild *F. virginiana* selection and produces a large number of new daughter plants from runners (Hancock et al., 2001).

Mean individual fruit weight varied widely from the largest fruit of 16.1 g for 'Cabot' × 'Cavendish' to a low of 2.7 g for LH 50-4 × 'Midway'. The eight families with the lowest fruit weight each had LH 50-4 as a parent, which is expected because of the small fruit size of LH 50-4.

The number of inflorescences/crown varied significantly across families, but showed less variability than crown numbers or fruit weight. The number of flowers/inflorescence ranged from a high of 9.3 for 'Bounty' × LH 50-4 to a low of 6.4 for 'Guardian' × 'Winona'.

Total yield varied across families from a 1419.9 g average for the progeny of 'Bounty' × 'Cabot' to just 241.4 g for 'Mesabi' × 'Midway'. Other high-yielding families were 'Cavendish' × 'Jewel', 'Bounty' × 'Cavendish', 'Bounty' × 'Winona', and 'Cabot' × 'Cavendish'. Fruit weight appeared to be the factor that contributed most to high yield, as three of the high-yielding families ranked high for fruit weight ('Bounty' × 'Cabot', 'Cavendish' × 'Jewel', and 'Cabot' × 'Cavendish') and the other two were in the middle ('Bounty' × 'Cavendish' and 'Bounty' × 'Winona'). Crown number contributed more modestly to high yield, with 'Bounty' × 'Cabot', 'Bounty' × 'Cavendish', and 'Bounty' × 'Winona' all having mid-range crown numbers. The high number of flowers/inflorescence found in 'Bounty' × 'Cavendish' and 'Bounty' × 'Winona' also contributed to high yield.

Analysis of the complete diallel

In the ANOVA used to calculate general combining ability (GCA) and specific combining ability (SCA) among the smaller set of families, treatment effects were significant for crown number ($P < 0.001$) and total yield ($P < 0.001$), but were not for inflorescences/crown ($P = 0.757$), flowers/inflorescence ($P = 0.737$) or mean individual fruit weight ($P = 0.085$) (Table 6). Similar to the analysis with all of the families, crown number was 26% higher, and yield was 28% higher on fumigated soil. There were also significant differences ($P < 0.01$) among families for crown number, berry weight, and total yield which mirrored the full family analysis.

GCA was significant ($P \leq 0.01$) for all parameters, and SCA was significant for crown number ($P = 0.014$), fruit weight ($P < 0.001$), and total yield ($P = 0.007$), but not for the number of inflorescences/crown (P = 0.987) or the number of flowers/inflorescence ($P = 0.399$) (Table 6). These results are as expected, as numerous studies on strawberries have uncovered a great deal of genetic variability for most yield components (Hancock, 1999).

While there was considerable genetic variability observed among families for most of the yield components, there appeared to be little genetic variability for resistance to BRR. Treatment × family interactions were not significant in the diallel analysis, as was the case in the analysis with the complete set of families. Similarly, all of the treatment × GCA and treatment × SCA interactions were not significant, except for a treatment × GCA interaction for berry weight ($P = 0.049$). This result was surprising as we previously observed significant differences in tolerance among the cultivars used as parents in this comparison (Chapter 2).

It may be that the difference in disease pressure between fumigated and nonfumigated plots was not as great in this study as in the previous one, and as a result made it more difficult to recognize tolerant types. Average yield was 46% higher due to fumigation in the first year after fumigation in the previous study, as opposed to 26% in this study. The fumigated strips in this study were only 1.2-m wide and bordered by nonfumigated strips on each side, so pathogens did not have to move very far in order to reestablish in fumigated soil. In the previous study, 11.5 × 4.5-m split plots (of 11.5 × 9-m plots) were fumigated, so there was a larger barrier to pathogens moving into fumigated soil from nonfumigated soil. We can also not rule out the possibility that Vapam, which was used in this study, is a less-effective fumigant than methyl-bromide, which was used in the previous study (Shaw and Larson, 1999).

Overall, the number of nematodes found in fumigated and nonfumigated areas was not significantly different ($P = 0.2848$), nor were the number found in soil alone ($P = 0.4786$) or roots alone ($P = 0.2295$) (Table 7). The only significant difference between fumigated and nonfumigated areas was for the number of root lesion nematodes found in root samples ($P = 0.0127$). The majority of nematodes from soil samples were ring nematodes (*Criconemella xenoplax* De Grisse & Loof), while the majority of nematodes found in root samples were root lesion nematodes. When the root and soil samples were combined, 93% from fumigated areas contained nematodes, while 100% from nonfumigated areas contained nematodes. In the previous field studies using methyl bromide and chloropicrin, very few samples from fumigated soil contained nematodes (5% and 8% the first and second year after fumigation, respectively), and they were found in very low numbers (Chapter 2).

Rhizoctonia sp. was the most common pathogenic fungi found in root samples, and was found equally in both fumigated and nonfumigated soil (Table 8). *Fusarium* sp. were also commonly found, both from fumigated and nonfumigated samples. *Fusarium* sp. is generally considered a weak pathogen in strawberries (A. Schilder, personal communication), and has been shown to interact with *P. penetrans* in the BRR complex (Maas, 1998). Overall, 48% of the root samples containing fungi were from fumigated areas, and 54% were from nonfumigated areas. In the previous study, both *Rhizoctonia* sp. and *Pythium* sp. were found, but *Rhizoctonia* sp. was more common (Chapter 2).

In conclusion, it appears that pathogen pressure had equalized in the fumigated and nonfumigated plots by the end of the first fruiting season, even though positive fumigation effects were observed on most yield components. Apparently, the plants in this study were under less pathogen pressure than those in our previous one. The data obtained in this study suggest that genetic improvement in tolerance to BRR will be difficult to achieve, expect perhaps under heavy pathogen pressure. Part of the difficulty in identifying tolerant types is likely due to the fact that BRR is a disease complex involving three different pathogens and the relative pressure from these pathogens can vary from year to year and site to site (Wing et al., 1994). Several other studies have also reported limited genetic variation in levels of tolerance to BRR (Hancock et al., 2001; Larson and Shaw, 1995; Shaw and Larson, 1996). The key to finding cultivars that will perform well in BRR-infested soils will be to select in soils with a long history of no fumigation.

Table 3. Strawberry genotypes with high, medium, and intermediate tolerance to black root rot used as parents in a diallel mating scheme.

Genotype	Year released	Parentage	Origin
High tolerance			
Bounty	1972	Jerseybelle × Senga Sengana	AAFC[z], Kentville, N.S.
Cabot	1998	K78-5 × K86-19	AAFC, Kentville, N.S.
Cavendish	1990	Glooscap × Annapolis	AAFC, Kentville, N.S.
Intermediate tolerance			
Guardian	1969	NC 1768 × Surecrop	USDA-Beltsville, Md.
Midway	1960	Dixieland × Temple	USDA-Beltsville, Md.
Winona	1995	Earliglow × MNUS 52	Univ. of Minnesota, St. Paul and USDA-Beltsville, Md.
Low tolerance			
Jewel	1985	NY 1221 × Holiday	NYSAES[y], Geneva, NY
Mesabi	2000	Glooscap × MNUS 99	Univ. of Minnesota, St. Paul
LH 50-4	---	---	Native *F. virginiana* from Montana

[z] Agriculture and Agri-Food Canada.

[y] New York State Agricultural Experiment Station

Table 4. Diallel mating scheme showing the number of progeny generated from each cross. Note that there were no selfs, and that reciprocal crosses were grouped into the same family.

Genotype	Cabot	Cavendish	Guardian	Jewel	LH 50-4	Mesabi	Midway	Winona
Bounty	50	10	19	50	50	8	28	8
Cabot		50	50	8	50	0	32	41
Cavendish			0	50	50	0	50	50
Guardian				0	14	24	10	5
Jewel					50	23	39	50
LH 50-4						50	50	50
Mesabi							8	11
Midway								50

Table 5. Analysis of variance and variance components with all families for crown number, inflorescence number, flowers per inflorescence, fruit weight, and total yield of strawberry families grown in Holt, Mich., on fumigated and nonfumigated soil. Plants were set in 2004 and evaluated over one year.

Source	Crown number	Inflorescence per crown	Flowers per inflorescence	Fruit weight	Total yield
Fumigation					
Fumigated	5.0	2.4	7.8	8.6	711.0
Nonfumigated	4.0	2.4	7.7	7.8	526.3
Family					
Bounty × Cabot	4.3	2.9	7.8	14.9	1419.9
Bounty × Cavendish	4.1	2.7	8.3	9.2	870.3
Bounty × Guardian	1.6	3.1	8.0	7.4	341.0
Bounty × Jewel	2.4	3.0	8.5	9.0	541.9
Bounty × LH50-4	7.0	2.5	9.3	3.4	580.8
Bounty × Mesabi	2.3	3.5	7.8	7.9	443.8
Bounty × Midway	2.6	3.1	8.2	7.1	510.8
Bounty × Winona	5.0	2.3	8.5	9.4	840.7
Cabot × Cavendish	2.8	2.4	7.0	16.1	804.6
Cabot × Guardian	5.0	1.6	6.6	11.1	575.8
Cabot × Jewel	2.2	2.5	7.3	12.9	445.9
Cabot × LH50-4	9.1	2.1	8.3	4.6	761.4
Cabot × Midway	1.8	2.9	7.0	8.6	365.9
Cabot × Winona	4.2	2.0	6.4	13.8	794.4
Cavendish × Jewel	3.3	2.8	7.8	13.2	979.2
Cavendish × LH50-4	6.6	2.7	8.3	4.1	578.3
Cavendish × Midway	1.9	2.9	6.7	9.2	398.8
Cavendish × Winona	3.7	2.4	7.0	11.2	727.4
Guardian × LH50-4	8.5	1.9	7.6	2.8	334.6
Guardian × Mesabi	2.7	2.5	7.5	8.6	490.3
Guardian × Midway	1.6	4.3	7.3	6.3	315.8
Guardian × Winona	2.6	3.0	6.4	9.4	412.4
Jewel × LH50-4	8.4	2.3	9.1	3.1	579.8
Jewel × Mesabi	3.5	2.3	7.9	11.3	795.6
Jewel × Midway	2.4	3.0	7.1	7.1	449.5
Jewel × Winona	2.5	2.6	7.9	9.9	544.4

LH50-4 × Mesabi	6.3	2.9	8.3	3.6	564.0
LH50-4 × Midway	5.5	3.0	8.3	2.7	376.5
LH50-4 × Winona	6.2	2.5	7.6	3.2	363.8
Mesabi × Midway	1.8	3.4	6.8	6.4	241.4
Mesabi × Winona	3.3	2.6	6.8	11.1	665.6
Midway × Winona	3.4	2.6	7.2	8.3	482.7
Mean	4.0	2.7	7.6	8.3	581.2
ANOVA					
Significance (P)					
Treatment (T)	0.001	0.992	0.876	0.012	< 0.001
Block/F	0.009	0.002	< 0.001	< 0.001	0.081
Family (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
T x F	0.928	0.966	0.576	0.362	0.527

[z] Crown number and total yield are expressed per daughter plant and all of her runner plants.

Table 6. Analysis of variance and variance components with the smaller set of families for crown number, inflorescence number, flowers per inflorescence, fruit weight, and total yield of strawberry families grown in Holt, Mich., on fumigated and nonfumigated soil. Plants were set in 2004 and evaluated over one year.

Source	df	Crown number	Inflorescence per crown	Flowers per inflorescence	Fruit weight	Total yield
Treament (T)	1	26.1**	0.05	0.1	17.0	1346699.6**
Block/T	8	1.8	0.74	1.0	16.0**	167445.5
Family (F)	14	9.4**	0.95	4.5**	79.6**	811099.7**
GCA	5	19.3**	2.34**	11.0**	193.7**	1389329.1**
SCA	9	4.1*	0.16	1.0	15.0**	500359.4**
T x F	14	1.4	0.44	1.0	7.5	73205.1
T x GCA	5	2.5	0.46	1.2	12.8*	122867.9
T x SCA	9	1.1	0.42	1.0	5.8	49319.0
Error	110	1.7	0.56	0.9	5.5	105503.5

[z] Crown number and total yield are expressed per daughter plant and all of her runner plants.

Table 7. Number of and significance for plant pathogenic nematodes in root and soil samples from strawberries grown in Holt, Mich., on fumigated and nonfumigated soil. Plants were set in 2004 and evaluated over one year.

Treatment	Soil						Root			Overall Total
	Root lesion	Ring	Dagger	Stubby root	Root knot (juvenile)	Total	Root lesion	Root knot (juvenile)	Total	
Fumigated	7	141	13	35	82	278	32	115	147	425
Nonfumigated	10	203	144	50	3	410	228	46	274	684
Significance (*P*)	0.8546	0.9155	0.6765	0.2867	0.2261	0.4786	0.0127	0.5979	0.2295	0.2848

Table 8. Percentage of root samples containing plant parasitic fungi from strawberries grown on fumigated and nonfumigated soil in Holt, Mich. Plants were set in 2004 and evaluated over one year.

	Treatment	
Pathogen	**Fumigated**	**Nonfumigated**
Rhizoctonia	9.8	11.8
Epicoccum	9.8	7.8
Fusarium	11.8	13.7
Trichoderma	2.0	-
Alternaria	2.0	-
Unknown	11.8	19.6

APPENDIX

EVALUATION OF STRAWBERRY PEDIGREES TO DETERMINE IF BLACK ROOT ROT TOLERANCE CAN BE TRACED TO A COMMON ANCESTRY

Introduction

The most effective way to control BRR would be to identify or to develop tolerant strawberry cultivars. Previous studies identified little genetic variability in tolerance to BRR in field screens (Hancock et al., 2001; Larson and Shaw, 1995; Shaw and Larson, 1996), but a very limited amount of the strawberry germplasm was evaluated. Therefore, we screened 20 different strawberry genotypes from six breeding programs for field tolerance to BRR (Chapter 2). The genotypes were evaluated for crown number, number of flowers per crown, yield, and average berry weight in both fumigated and nonfumigated soil for two years. The results showed that there was a significant genotype × fumigation interaction for crown number and yield in both years of the study. Interestingly, 'Bounty', 'Cabot', and 'Cavendish', all released from the breeding program in Nova Scotia, displayed the highest level of tolerance to BRR.

A study by Sjulin and Dale (1987) evaluated the genetic diversity of strawberry cultivars released from North American breeding programs from 1960 to 1985. They traced the pedigrees of the cultivars back to the founding clones, calculated the genetic contribution each founding clone made to the cultivar, and clustered them using Ward's method (Ward, 1963). The cultivars clustered into nine groups that were highly related to geographic origin, most strongly between cultivars developed within California (two groups) and those developed outside of California (seven groups).

In order to determine if tolerance (or lack thereof) to BRR can be attributed to a specific origin, we traced the pedigrees of the 19 cultivars included in the field screen (Chapter 2) and conducted analyses similar to Sjulin and Dale's (1987).

Materials and Methods

The 19 cultivars included in the study were all released from breeding programs in North America from 1960-2000 (Table 9). The pedigrees of the cultivars were traced using Brooks and Olmo (1997), Darrow (1937, 1966), Etter (1920), Hedrick (1925), internet sources and published cultivar descriptions. Personal communication from strawberry researchers was used as well. Pedigrees were traced to a point where the parentage of a genotype was either not known or differed between sources, and those genotypes were referred to as founding clones (Table 10).

The genetic contribution was calculated as in Sjulin and Dale (1987). However, unlike Sjulin and Dale, we did not assume that genotypes selected from open-pollinated populations were self-pollinated, and instead designated the unknown parent as the pollen parent of that particular genotype (Table 10). We also did not sum together and enter as a single variable those founding clones whose genetic contributions were always equal, and Excel® (Microsoft Corporation, Redmond, Wash.) spreadsheets were used to calculate the genetic contribution. The "multivariate" and "cluster observation" functions of MINITAB were used for the cluster analysis.

Results and Discussion

The three BRR tolerant cultivars, 'Bounty', 'Cabot', and 'Cavendish' (Chapter 2), did not cluster together and in fact were quite distant in the cluster analysis (Figure 6). Therefore, we were not able to identify a source of BRR tolerance. The genes for tolerance likely come from a source in the background of most cultivars.

Cluster analysis also did not indicate that cultivars grouped according to geographic origin (Figure 6). For example, the cultivars from the Nova Scotia breeding program did not group closely together, nor did the cultivars from the USDA program in Maryland. The lack of distinct cluster groups can partially be explained because 18 of the 57 founding clones appeared in at least 18 of the 19 cultivars included in the study (Table 11). These 18 founding clones accounted for approximately 50 – 90% of the genetic contribution of each cultivar. Another possible reason for the lack of cluster groups could be because of the small number of cultivars included in the study. In Sjulin and Dale's (1987) study, the pedigrees of 134 cultivars were traced.

Some cultivars were found on distinct branches, separated from most other cultivars (Figure 6, Table 11). 'Bounty' did not loosely group with any other cultivars because 'Markee' accounts for 25% its genetic composition. 'Chandler' fell onto a distinct branch because 'Aberdeen', which accounts for 11 – 25% of the genetic contribution of all other cultivars, is absent from it. Also, 'Chandler' has a large amount of 'Middlefield' and the pollen parent of 'Nich Ohmer' in its background. MN 2374 accounts for 13% of the genetic contribution of 'Mesabi', causing it to be on a distinct branch. MN 2374 is a selection from the University of Minnesota breeding program from 1960s when Wayne Wilcox was selfing cultivars such as 'Dunlap' and 'Trumpeter' for up to six generations (J. Luby, personal communication). Unfortunately, the exact background of MN 2374 is unknown. 'Kent' fell onto a distinct branch due to the unique contributions from Seedling TD8 and 'Bubach'. 'Jewel' fell into its own branch due to a 13% contribution from 'Markee', as well as small contributions from other founding clones such as 'Early Jersey Giant' and 'Inèpuisable'.

Table 9. Strawberry cultivars included in the pedigree study to determine if tolerance to black root can be traced to a specific origin. These same cultivars were evaluated in a field study to determine their level of tolerance to black root rot (Chapter 2).

Genotype	Year released	Parentage	Origin
Allstar	1981	US 4419 × MDUS 3184	USDA-Beltsville, Maryland
Annapolis	1984	K74-5 × Earliglow	AAFC[z], Kentville, N.S.
Bounty	1972	Jerseybelle × Senga Sengana	AAFC, Kentville, N.S.
Brunswick	1999	Cavendish × Honeoye	AAFC, Kentville, N.S.
Cabot	1998	K78-5 × K86-19	AAFC, Kentville, N.S.
Cavendish	1990	Glooscap × Annapolis	AAFC, Kentville, N.S.
Chandler	1983	Douglas × Cal 72.361-105	University of California, Davis
Earliglow	1975	MDUS 2359 × MDUS 2713	USDA-Beltsville, Maryland
Evangeline	1999	Honeoye × Veestar	AAFC, Kentville, N.S.
Gov. Simcoe	1985	Holiday × Guardian	HRIO[w], Simcoe, Ontario
Guardian	1969	NC 1768 × Surecrop	USDA-Beltsville, Maryland
Honeoye	1979	Vibrant × Holiday	NYSAES[x], Geneva, New York
Jewel	1985	NY 1221 × Holiday	NYSAES, Geneva, New York
Kent	1981	K 68-5 × Raritan	AAFC, Kentville, N.S.
Mesabi	2000	Glooscap × MNUS 99	University of Minnesota, St. Paul
Midway	1960	Dixieland × Temple	USDA-Beltsville, Maryland
Mira	1995	Scott × Raritan	AAFC, Kentville, N.S.
Surecrop	1956	Fairland × MDUS 1972	USDA-Beltsville, Maryland
Winona	1995	Earliglow × MNUS 52	University of Minnesota, St. Paul & USDA-Beltsville, Maryland

[z] Agriculture and Agri-Food Canada

[y] Horticulture Research Institute of Ontario

[x] New York State Agricultural Experiment Station

Table 10. Origin and mean genetic contribution (GC) of the founding clones to the 19 North American strawberry cultivars included in the pedigree study to determine if tolerance to black root rot can be traced to a specific origin.

Clone no.	Founding clones	Origin	Mean GC (%)
1	Belmont	Massachusetts, 1880	4
2	British Queen	England, 1840	<1
3	*F. virginiana* N.J. Scarlet	New Jersey, 1868	9
4	Jucunda	England, 1854	1
5	Methven Scarlet	England, 1815	5
6	Sharpless	Pennsylvania, 1872	6
7	White Carolina	England, before 1806	2
8	Wilson	New York, 1851	3
9	Pollen parent of Keen's Seedling	--	4
10	Pollen parent of Keen's Imperial	--	2
11	Pollen parent of Hoffman	--	1
12	Pollen parent of Goliath	--	<1
13	Pollen parent of Neunan	--	<1
14	Pollen parent of Clyde	--	3
15	Pollen parent of Vicomtesse	--	2
16	Aberdeen	New Jersey, before 1917	18
17	Missionary	Virginia, 1900	13
18	Pollen parent of Black Prince	--	2
19	William Belt	Ohio, 1888	1
20	Pollen parent of Portia	--	1
21	Chesapeake	Maryland, 1903	2
22	Middlefield	Connecticut, 1890	2
23	Pollen parent of unnamed seedling in pedigree of Rose Ettersburg	--	<1
24	*F. chiloensis* (unnamed)	Unknown, before 1905	<1
25	*F. chiloensis* (unnamed)	Peru, before 1905	<1
26	Frith	Scotland, 1918	2
27	Glendale	Ohio, 1971	<1
28	Jersey Queen	New Jersey, 1878	<1
29	Parry	New Jersey, 1880	<0.1
30	Chair's Favourite	England,	<0.1
31	Banner	Massachusetts, 1890	<1
32	Ettersburg121	California, 1905	1
33	Pollen parent of Nich Ohmer	--	2
34	Marshall	Massachusetts, 1890	<1
35	Teutonia	Germany (?)	<1
36	Unser Fritz	Germany, 1872	<1
37	Pollen parent of König Albert von Sachsen	--	<1
38	Cassandra	Ontario, 1906	<1
39	Markee	Germany, before 1942	3
40	Pocomoke	Maryland, 1902	1
41	Pollen parent of Aroma	--	<1
42	Pearl	New Jersey, 1889	<1

43	SeedlingTD8	Scotland, before 1945	<1
44	Bubach	Illinios, 1882	<0.1
45	Cal. 1021	California, before 1930	<0.1
46	Cal. BH14	California, before 1930	<0.1
47	Early Jersey Giant	New Jersey, 1907	<0.1
48	*F. chiloensis* from Cape Mendocino	California, before 1905	<0.1
49	*F. virginiana* glauca	Unknown, before 1905	<0.1
50	Inèpuisable	France, 1870	<0.1
51	Longworth	Ohio, 19848	<0.1
52	Lucie Boisselot	France, before 1930	<0.1
53	MN 2374	Minnesota, before 1970	<1
54	Streamliner	Oregon, 1938	<1
55	Pollen parent of US 235 op	--	<0.1
56	Pollen parent of St. Joseph	--	<0.1
57	Pollen parent of Louis Gauthier	--	<0.1

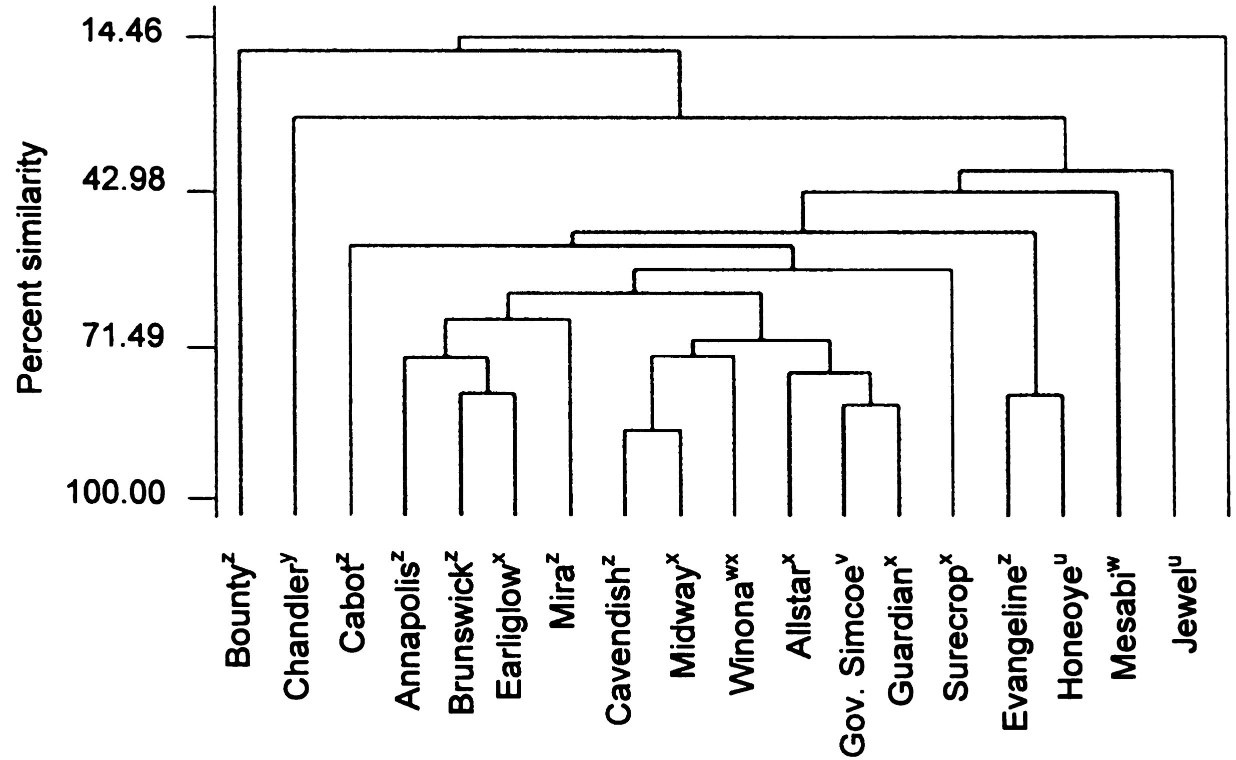
Table 11. Cultivars and the genetic contribution of the founding clones[z] included in the pedigree study to determine if tolerance to black root rot can be traced to a specific origin.

Founding clone	Cultivar																		
	Bou	Cha	Cab	Ann	Bru	Ear	Mir	Cav	Mid	Win	All	Gov	Grd	Sur	Eva	Hon	Mes	Knt	Jwl
1	2	3	4	4	4	4	4	3	4	4	4	4	4	4	4	4	2	3	3
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	6	9	9	9	9	10	9	7	10	10	10	10	10	10	11	10	6	8	7
4	1	1	4	1	1	1	1	1	1	4	1	1	1	1	2	2	2	1	1
5	3	5	5	5	5	5	5	4	5	5	5	5	5	5	6	6	3	4	4
6	11	6	6	6	6	6	6	7	5	5	6	5	5	7	6	6	6	6	8
7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	6	3	2	2	4	2	3	3	2	2	2	2	2	3	5	4	3	2	4
9	3	4	4	4	4	4	4	4	4	4	5	4	4	5	5	4	3	4	4
10	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2
11	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	2
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	2	3	4	4	4	4	4	3	4	4	4	4	4	4	4	4	2	3	3
15	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	1	2	2
16	13	.	17	22	20	25	15	17	25	24	16	20	19	25	24	23	11	20	16
17	.	6	15	12	12	13	18	7	19	9	13	26	19	13	8	16	8	11	21
18	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
19	.	2	1	2	1	3	1	1	3	2	2	1	2	.	.	.	1	1	.
20	.	2	1	2	1	3	1	1	3	2	2	1	2	.	.	.	1	1	.
21	.	1	2	2	1	3	.	1	3	4	14	4	8	.	.	.	1	.	.
22	1	13	1	2	2	.	4	3	.	.	.	1	.	.	1	1	3	4	1
23	.	1	1	1	1	1	.	1	1	1	1	1	1	.	.	.	1	.	.
24	.	1	1	1	1	2	.	1	2	1	1	1	1	.	.	.	1	.	.
25	.	1	1	1	1	1	.	1	1	1	1	1	1	.	.	.	1	.	.
26	.	.	2	2	1	3	3	1	.	4	6	3	6	13	.	.	2	.	.
27	2	.	1	1	1	.	1	1	.	.	.	1	.	.	1	1	1	1	1
28	2	.	1	1	1	.	1	1	.	.	.	1	.	.	1	1	1	1	1
29	.	1	1	1	1	1	.	1	1	1	1	1	1	.	.	.	1	.	.
30	1	.	1	1	1	.	1	1	.	.	.	1	.	.	1	1	1	1	1
31	.	6	1	1	1	.	2	2	.	.	.	.	.	.	.	.	2	3	.
32	.	3	1	4	3	.	2	6	.	.	.	.	.	.	.	.	4	1	.
33	.	13	1	2	2	.	4	3	.	.	.	.	.	.	.	.	3	4	.
34	.	8	.	1	1	.	1	7	.	.	.	.	.	.	.	.	1	.	.
35	6	.	1	.	1	.	.	2	.	.	.	.	.	.	.	.	2	.	3
36	3	.	1	.	1	.	.	1	.	.	.	.	.	.	.	.	1	.	2
37	3	.	1	.	1	.	.	1	.	.	.	.	.	.	.	.	1	.	2
38	.	.	1	.	2	.	.	5	.	.	3	.	.	.	.	.	3	.	.
39	25	.	2	.	3	.	.	.	.	.	.	.	.	.	.	.	6	.	13

40	.	.	.	.	3	.	3	.	.	.	.	.	.	.	9	6	.	.	.
41	.	.	.	.	1	.	1	.	.	.	.	.	.	.	2	2	.	.	.
42	.	.	1	.	.	.	.	.	.	2	.	.	.	.	.	.	.	5	.
43	.	.	1	.	.	.	.	.	.	3	.	.	.	.	.	.	.	6	.
44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
45	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
48	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
49	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	13	.	.
54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
55	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
57	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1

[z] See Table 10 for the names of the founding clones.

Figure 6. Cluster dendrogram based on the genetic contribution of the founding clones to the 19 strawberry cultivars included in the pedigree study to determine if tolerance to black root rot can be traced to a specific origin.



[z] Agriculture and Agri-Food Canada, Nova Scotia

[y] University of California, Davis

[x] USDA-Beltsville, Maryland

[w] University of Minnesota, St. Paul

[v] Horticulture Research Institute of Ontario, Simcoe

[u] New York State Agricultural Experiment Station, Geneva

Literature Cited

Abad, Z.G., F.J. Louws, and G.E. Fernandez. 1999. *Rhizoctonia* and *Pythium* species associated with black root rot of strawberries in North Carolina. Phytopathol. 89(6):S1 (Abstr.)

Abu-Gharbieh, W., E.H. Varney, and W.R. Jenkins. 1962. Relationship of meadow nematode to Verticillium wilt of strawberries. Phytopathol. 52: 921. (Abstr.)

Bailey, J.S. 1956. Does the root-knot nematode (*Meloidogyne* sp.) thrive in the roots of strawberry plants in Massachusetts? Plant Dis. Rptr. 40(1):44. (Abstr.)

Barnett, H.L. and B.B. Hunter. 1998. Illustrated genera of imperfect fungi. 4th Edition. APS Press, St. Paul MN.

Berkeley, G.H. and I. Lauder-Thomson. 1934. Root rots of strawberry in Britain: the "black lesion" type of strawberry root rot. Jour. Pom. and Hort. Sci. 12:222-246.

Bird, G.W. 1971. Influence of incubation solution of the rate of recovery of *Pratylenchus penetrans* from cotton roots. J.Nematol. 3:378-385.

Braun, A.J. and J.A. Keplinger. 1960. The pathogenicity of meadow nematodes as determined by the growth of strawberry plants in a commercial planting. Phytopathol. 50:239. (Abstr.)

Brooks, R.M. and H.P. Olmo. 1997. The Brooks and Olmo Register of fruit and nut varieties. ASHS Press, Alexandria, Va.

Chapman, R.A. 1956. Plant parasitic nematodes associated with strawberries in Kentucky. Plant Dis. Rptr. 40:179-183.

Chen, T.A. and Rich, A.E. 1962. The role of *Pratylenchus penetrans* in the development of strawberry black root rot. Plant Dis. Rptr. 46(12):839-843.

Coons, G.H. 1924. Black root rot of strawberry. Mich. Agr. Expt. Sta. Quarterly Bul. 7(1): 25-26.

Dale, A. and J.W. Potter. 1998. Strawberry cultivars vary in their resistance to northern lesion nematode. J. Nematol. 30:577-580.

Darrow, G.M. 1937. Strawberry improvement, p. 445-495. In: U.S. Department of Agriculture (ed.). Yearbook of Agriculture. United States Government Printing Office, Washington, D.C.

Darrow, G.M. 1966. The strawberry. Holt, Rinehart, and Winston, New York.

D'Ercole, N., P. Nipoti, and D. Manzali. 1989. Research on the root rot complex of strawberry. ActaHort 265:497-506.

Drozdowski, J. 1987. A survey of fungi associated with strawberry black root rot in commercial fields. Adv. in Strawberry Production 6:47-49.

Edwards, W.H., R.K. Jones, and D.P. Schmitt. 1985. Host suitability and parasitism of selected strawberry cultivars by *Meloidogyne hapla* and *M. incognita*. Plant Dis. 69:40-42.

Elad, Y., I. Chet, and Y. Henis. 1981. Biological control of *Rhizoctonia solani* on strawberry fields by *Trichoderma harzianum*. Plant and Soil 60:245-254.

Elmer, W.H. and J.A. LaMondia. 1995. The influence of mineral nutrition on strawberry black root rot. Adv. in Strawberry Res. 14:42-48.

Elmer, W.H. and J.A. LaMondia. 1999. Influence of ammonium sulfate and rotation crops on strawberry black root rot. Plant Dis. 83(2):119-123.

Etter, A.F. 1920. Ettersburg strawberries, describing varieties and breeding methods. A.F. Etter, Ettersburg, Calif.

Fennimore, S.A., M.J. Harr, and H.A. Ajwa. 2003. Weed control in strawberry provided by shank- and drip-applied methyl bromide alternative fumigants. HortScience 38(1): 55-61.

Fletcher, S. 1917. Strawberry Growing. MacMillan, New York.

Goheen, A.C. and J.S. Bailey. 1955. Meadow nematodes in strawberry plantings in Massachusetts. Plant Dis. Rptr. 39(11):879-880.

Goheen, A.C. and A.J. Braun. 1956. Some parasitic nematodes associated with wild strawberry plants in woodlands in Maryland. Plant Dis. Rptr. 40(1):43.

Goheen, A.C. and J.B. Smith. 1956. Effects of inoculation of strawberry roots with meadow nematodes, *Pratylenchus penetrans*. Plant Dis. Rptr. 40(2):146-149.

Hancock, J.F., P.W. Callow, S. Serçe, and A.C. Schilder. 2001. Relative performance of strawberry cultivars and native hybrids on fumigated and nonfumigated soil in Michigan. HortScience 36(1):136-138.

Hedrick, U.P. (ed.). 1925. The small fruits of New York. N.Y. State Agr. Expt. Sta. Rpt. 1925, Pt. II.

Hendrix, FF., Jr. and W.A. Campbell 1973. Pythiums as plant pathogens. Annu. Rev. Phytopathol.11:77-98.

Hildebrand, A.A. 1934. Recent observations on strawberry root rot in the Niagra Peninsula. Can. J. Res. 11:18-31.

Hildebrand, A.A. and L.W. Koch. 1936. A microscopical study of infection of the roots of strawberry and tobacco seedlings by micro-organisms of the soil. Can. J. Res. 14:11-26.

Holdeman, Q.L. and T.W. Graham. 1954. Effect of the sting nematode on expression of Fusarium wilt in cotton. Phytopathol. 44:683-685.

Husain, S.S. and W.E. McKeen. 1963a. *Rhizoctonia fragariae* sp. nov. in relation to strawberry degeneration in Southwestern Ontario. Phytopathol. 53:532-540.

Husain, S.S. and W.E. McKeen. 1963b. Interactions between strawberry roots and *Rhizoctonia fragariae*. Phytopathol. 53:541-545.

Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Rptr. 48:692.

Katznelson, H. and L.T. Richardson. 1948. Rhizosphere studies and associated microbiological phenomena in relation to strawberry root. Scientific Agr. 28:293-309.

Klinkenberg, C.H. 1955. Nematode diseases of strawberries in the Netherlands. Plant Dis. Rptr. 39(8):603-606.

LaMondia, J.A. and S.B. Martin. 1988. Plant parasitic nematodes and fungi are an unhealthy alliance against strawberries. Frontiers of Plant Science, Conn. Agr. Exp. Sta. Rpt. 40(2):3-5.

LaMondia, J.A. and S.B. Martin. 1989. The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. Plant Dis. 73:107-110.

LaMondia, J.A., W.H. Elmer, T.L. Mervosh, and R.S. Cowles. 2002. Integrated management of strawberry pests by rotation and intercropping. Crop Protection 21:837-846.

Larson, K.D. and D.V. Shaw. 1995. Relative performance of strawberry genotypes on fumigated and nonfumigated soils. J. Amer. Soc. Hort. Sci. 120(2):274-277.

Maas, J.L. 1984. Compendium of strawberry diseases. 1st edition. APS Press, St. Paul, Minn.

Maas, J.L. 1998. Compendium of strawberry diseases. 2nd edition. APS Press, St. Paul, Minn.

Martin, W.J., L.D. Newsom, and J.E. Jones. 1955. Relationship of nematodes of the genera *Meloidogyne*, *Tylenchorhynchus*, *Helicotylenchus*, and *Trichodorus* to the development of Fusarium wilt in cotton. Phytopathol .45:349. (Abstr.)

Mervosh, T.L. and J.A. LaMondia. 2004. Strawberry black root rot and berry yield are not affected by terbacil herbicide. HortScience 39:1339-1342.

Miller, P.W. 1948. Relation of desiccation to the development of black root rot of strawberries. Plant Dis. Rptr. 32(7):315-316.

Morgan, G.T. and W.B. Collins. 1964. The effect of organic treatments and crop rotation on soil populations of *Pratylenchus penetrans* in strawberry culture. Can. J. Plant Sci. 44:272-275.

Neal, D.C. 1954. The reniform nematode and its relationship to the incidence of Fusarium wilt of cotton at Baton Rouge, La. Phytopathol.44: 447-450.

Nemec, S. and H. Sanders. 1970. Pythium species associated with strawberry root necrosis in southern Illinois. Plant Dis. Rptr. 54(1):49-51.

Particka, C.A. and J.F. Hancock. 2005. Field evaluation of strawberry genotypes for tolerance to black root rot on fumigated and nonfumigated soil. J. Amer. Hort. Sci. 130(5):688-693.

Pinkerton, J. and C.E. Finn. 2005. Response of strawberry species and cultivars to the root-lesion and northern root-knot nematodes. HortScience 40(1):33-38.

Potter, J.W. and A. Dale. 1994. Wild and cultivated strawberries can tolerate or resist root-lesion nematode. HortScience 29(9):1074-1077.

Powell, N.T., P.L. Meléndez, and C.K. Batten. 1971. Disease complexes in tobacco involving *Meloidogyne incognita* and certain soil-borne fungi. Phytopathol. 61:1332-1337.

Pritts, M. and W.F. Wilcox. 1990. Black root rot disease of strawberry. Cornell Small Fruits Nswl. 5(4): 1-2.

Raski, D.J. 1956. *Pratylenchus penetrans* tested on strawberries grown on black-root-rot soil. Plant Dis. Rptr. 40(8): 690-693.

Riberio, O.K. and L.L. Black. 1971. *Rhizoctonia fragariae*: A mycorrhizal and pathogenic fungus of strawberry plants. Plant Dis. Rptr. 55:599-603.

Rich, S. and P.M. Miller. 1963. Efficiency of infective propagules of *Rhizoctonia solani* in reducing vigour of strawberry plants. Nature 197: 719-720.

Riggs, R.D., D.A. Slack, and J.P. Fulton. 1956. Meadow nematode and its relation to decline of strawberry plants in Arkansas. Phytopathol. 46:24. (Abstr.)

Seigies, A.C. 2004. Overcoming strawberry replant disorder through diversified rotations.. MS Thesis, Dept. of Horticulture, Cornell Univ., Ithaca.

Shaw, D.V. and K.D. Larson. 1996. Relative performance of strawberry cultivars from California and other North American sources in fumigated and nonfumigated soils. J. Amer. Soc. Hort. Sci. 121(5):764-767.

Shaw, D.V. and K.D. Larson. 1999. A meta-analysis of strawberry yield response to preplant soil fumigation with combinations of methyl bromide-chloropicrin and four alternative systems. HortScience 34(5):839-845.

Sjulin, T.M. and A. Dale. 1987. Genetic diversity of North American strawberry cultivars. J. Amer. Hort. Sci. 112(2):375-385.

Smith, E.H. and W.T. Horne. 1922. Strawberry root rot. Annu. Rpt. Cal. Agr. Expt. Sta. 1921:90.

Steiner, G. 1931. *Tylenchus pratensis* de Man on tobacco, tomato, and strawberry. Plant Dis.Rptr. 15:106-107.

Strong, F.C. and M.C. Strong. 1930. Black root rot of strawberry ruins plantations. Mich. Agr. Expt. Sta. Quarterly Bul. 12(3):75-79.

Strong, F.C. and M.C. Strong. 1931. Investigations on the black root rot of strawberries. Phytopathol. 21:1041-1060.

Szczygiel, A. and H. Profic-Alwasiak. 1989. Studies on the interaction between nematodes and fungi in infecting strawberry plants. ActaHort 256:561-568.

Townshend, J.L. 1958. The effect of *Pratylenchus penetrans* on a clone of *Fragaria vesca*. Can. J. Bot. 36:683-685.

Townshend, J.L. 1962. The root lesion nematode *Pratylenchus penetrans* in strawberry in the Niagra Peninsula and Norfolk County in Ontario. Can. J. Plant Sci. 42:75-78.

Townshend, J.L. 1963. The pathogenicity of *Pratylenchus penetrans* to strawberry. Can. J. Plant Sci. 43:75-78.

U.S. Department of Agriculture. 2000. Economic implications of the methyl bromide phaseout. Agr. Info. Bul. No. 756, Washington, D.C.

Ward, J.H. 1963. Hierarchical grouping to optimize and objective function. J. Amer. Stat. Assn. 58:236-244.

Watanabe, T., K. Hashimoto, and M. Sato. 1977. Pythium species associated with strawberry roots in Japan, and their role in strawberry stunt disease. Phytopathol. 67:1324-1332.

Wilhelm, S. 1998. Black root rot, p. 53. In: J. L. Maas (ed.) Compendium of strawberry diseases. 2nd edition. The American Phytopathological Society, St. Paul, Minn.

Wing, K.B., M.P. Pritts and W.F. Wilcox. 1994. Strawberry black root rot: a review. Adv. in Strawberry Res. 13:13-19.

Wing, K.B, M.P. Pritts, and W.F. Wilcox. 1995a. Biotic, edaphic, and cultural factors associated with strawberry black root rot in New York. HortScience 30(1):86-90.

Wing, K.B., M.P. Pritts, and W.F. Wilcox. 1995b. Field resistance of 20 strawberry cultivars to black root rot. Fruit Var. J. 49(2):94-98.

Zeller, S.M. 1932. A strawberry disease caused by *Rhizoctonia*. Ore. Agr. Expt. Sta. Bul. 295:1-22.

Zhang, Y., M.S. Kang, and K.R. Lamkey. 2005. DIALLEL-SAS05: A comprehensive program for Griffing's and Gardner-Eberhart analyses. Agronomy J. 97(4):1037-127