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IN-VITRO SCREENING PROTOCOL FOR RESISTANCE TO COMMON POTATO SCAB

By

Jarred E. Driscoll

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ABSTRACT

IN-VITRO SCREENING PROTOCOL FOR RESISTANCE TO COMMON POTATO SCAB, *STREPTOMYCES SCABIES*

By

Jarred E. Driscoll

Common potato scab (*Streptomyces scabies*) produces cork-like lesions on the outer surface of the potato (*Solanum tuberosum* L.) tuber, decreasing the marketability of the tubers. A protocol that is both efficient and effective in discerning resistant from susceptible varieties would be valuable. Four methods were used to assess *S. scabies* disease reaction on a set of potato clones (Liberator, Russet Burbank, Jacqueline Lee, and MSG227-2). The methods included, two spore suspensions with one applied to etiolated tuber sprouts and the other applied to potato tuber discs (PTDs), a thaxtomin A (TA) tuber assay, and a greenhouse assay. There was no host tissue response to either spore suspension assay and no correlation for resistance could be drawn with the TA tuber assay. Two years of field data were also collected for scab reaction on a segregating tetraploid population (MSL603, 162 individuals). Fifteen individuals from this population were selected and tested using the greenhouse assay. This assay showed a favorable response to the disease and was chosen as the method for comparing disease reaction with MSL603 population field results. Inoculated soil was used for the greenhouse study at a concentration of 3 X 10^8 CFU/cc. The greenhouse study did not correlate to the 2004 field season but did moderately correlate to 2005 ($r = 0.3823$, $p = 0.0102$) and with the averaged ratings from the 2005 and 2004 field trials ($r = 0.2503$, $p = 0.0413$). The greenhouse assay may provide data that would compliment the field data in identifying resistant clones.

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LITERATURE REVIEW

The Potato

Though not as old or as well documented as other crops such as wheat and barley, the history of potato dates back thousands of years. Originating in Bolivia and the Peruvian highlands, humans began cultivating the potato about 8,000 years ago (Ross, 1986). Domestication came first in South America. The first Europeans to see the potato were the Spanish around the 1520s to 1530s during their conquest. The Spanish brought the potato back with them, where it spread throughout Europe. Now, more than 400 years later, the potato is grown worldwide, with more than 3,200 varieties commercially available (Hamerester and Hils, 2003).

Today, the cultivated potato (*Solanum tuberosum* L.) ranks fourth in worldwide importance after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aesitvum* L.) (FAOSTAT data, 2004). Worldwide, the potato is grown on over 18.6 million hectares and production has reached over 323 million metric tons with China leading potato production for all countries with 73 million MT. (FAOSTAT data, 2005). The U.S. produces over 19 million MT on more than 438,000 ha. The National Agricultural Statistics Service (2005) ranked the state of Michigan 9th in potato production in the U.S. with 589,680 MT. In 2005, Michigan planted 17,806 ha. worth over $106 million.

The potato can be found under an extremely wide range of habitats; from tropical and subtropical to desert, temperate, and mountainous regions. It is used for animal feed, extraction of specific derivatives such as starch, and most importantly as a human food source.

Nutritionally, potato is rich in vitamin C, potassium, and B complex vitamins. The vitamin C content provides a significant source for an adult's daily requirement. By itself, potato is a good source of energy and is comparatively lower than other cooked foods such as cassava, plantain, and beans (Woolfe, 1987). The protein content, however, of a cooked potato is comparable to that of rice (Paul and Southgate, 1978).

The fresh weight content of potato is approximately 80% water and 20% dry matter. Dry matter content varies among potato varieties. The largest constituent of dry matter content in potato is starch at roughly 70% (Burton, 1966). Potato starch is used in manufacturing for adhesives, the textile and food industries, and producing derived goods such as alcohol and glucose (Woolfe, 1987). The ash content of a potato is composed of inorganic residues and contains minerals (Mg, Ca, and Na) and trace elements (Cu, Fe, and Zn), comprising roughly 4-6% of dry matter (True et al. 1978).

According to Hawkes (1990), there are 224 wild and 7 cultivated species within *Solanum* section *Petota* (Solanaceae). The vast majority of species are tuber-bearing (Spooner and Hijmans, 2001). Spooner et al. (2004) describe two major epicenters of potato germplasm diversity. The first stretches from the U.S. southwest to the Mexican central highlands, and the second region is the Andes of South America. The latter region claims the majority of diversity found in potato.

It is generally accepted that the basic chromosome number for the genus *Solanum* is 12. There is a wide range of ploidy levels in wild tuberiferous bearing species. Ploidy levels range from diploid (2n=2x=24) to hexaploid (2n=6x=72). The cultivated potato is tetraploid (2n=4x=48) and the wild progenitors are diploid (2n=2x=24).

Many wild potato species have yet to be characterized, and as a consequence, their value in breeding is limited. There has been, however, a fair amount of resistance breeding through the use of wild germplasm. Traditionally, 4x-2x crosses have been made. The significance of endosperm balance number (EBN) becomes apparent when trying to incorporate agronomically valuable traits, such as disease resistance, into a cultivated background. For transmission to occur, both parents must have a congruent EBN, meaning the cultivated parent should have normal gamete reduction (2n), while the diploid parent must maintain unreduced gametes (2n).

Potato is infected by numerous diseases including late blight (*Phytophthora infestans*) *There* are numerous diseases that infect potato. It is estimated that total loss of potato yield worldwide due to pests and diseases is at 22% (Ross, 1986). Total losses of potato as a consequence of disease and pest issues will undoubtedly vary. Certain pests and diseases are more common in occurrence or have the capacity to cause greater loss and are therefore deserving of more attention.

There are several well documented and important bacterial diseases of potato. Since potato tubers are formed below ground, they are subjected to a wide range of soil-borne pathogens. *Erwinia carotovora* spp. is responsible for such diseases as black leg, soft rot, and aerial stem rot (Banks, 2004). Many of the soft-rot bacteria produce pectolytic enzymes that begin the process of cell maceration (Agrios, 1997). Bacterial ring rot (*Clavibacter michiganensis* subs. *sepedonicus*) overwinters primarily in infected seed pieces and is not killed by freezing (Banks, 2004). Symptomless carriers of this disease are possible as not all plants exhibit the interveinal chlorosis, curling and browning on leaves. *Streptomyces scabies*, the casual agent of common potato scab, is also a soil-born bacteria that infects tubers. In certain years, susceptible tubers to common potato scab may be rendered unmarketable.

Common Potato Scab and its Biology

There are over 400 identified species of *Streptomyces* and only a fraction of these are plant pathogenic (Loria et al., 1997). Those that are pathogenic infect a wide range of hosts. The host range of *S. scabies* is confined to below ground root and tuber cops such as radish (*Raphanus sativus*), parsnip (*Pastinaca sativa*), beet (*Beta vulgaris*), carrot (*Daucus carota*), and potato (*Solanum tuberosum*) (Goyer and Beaulieu, 1997). The pathogen has even incited pod wart disease on the hulls of peanut, leaving a raised, black lesion on the outer hull (Loria et al., 1997). The genus *Streptomyces* are most well known for their ability to produce medicinally important antibiotics. More than two-thirds of naturally derived antibiotics are produced as secondary metabolites by *Streptomyces* species (Loria et al., 2003).

Of the bacterial diseases, *Streptomyces scabies* is one of the major pathogens that infect potato. The pathogen produces necrotic, corky-textured lesions on the outer surface of the potato. The lesions can vary in their appearance as being raised, surface or pitted. Chip processors consider pitted lesions a chip defect because the pit will be apparent in the chip. Surface and raised lesions present less of a problem as potatoes are peeled before they are chipped, thereby removing the lesions. Since the marketplace for potatoes is quality driven, the presence of scab lesions, especially those which are pitted, on the outer surface of the potato for both table and chipping varieties significantly lessen their marketability.

Roland Thaxter (1890) first isolated the pathogen from infected potatoes in Connecticut, and classified it as the fungus, *Oospora scabies*. Some 50 years later it was reclassified as *Streptomyces scabies,* a bacterium (Waksman and Henrici, 1943).

Streptomyces scabies is a gram positive, filamentous bacterium. It is a saprophyte which may over-winter either in soil or on the surface of tubers and can serve as next year's inoculum source. Inoculum may also be spread by water and wind-blown spores. Once present in the soil, *S. scabies* may survive indefinitely (Agrios, 1997). The pathogen has long been known to persist in drier soils with pH between 5.2 and 7.0 (Waksman, 1921). *Streptomyces scabies* has branched mycelium that have few if any cross walls. Its sporogenous hyphae develop into corkscrew-like spiral chains. When spores are mature, they develop melanin pigmentation.

Sporogenous hyphae release their spores and germinate when they land on a suitable host. Germ tubes from these spores primarily invade young tubers through lenticels, but will also take advantage of stomata and wounds. As illustrated by Loria et al. (2003), the invasion of natural openings within the root structure may not always be necessary. They observed short, branch-like secondary hyphae that were perpendicular to the primary hyphae. Invasion of the tuber surface was in a uniform pattern, a short distance from the branch point. This would suggest that the branch-like hyphae are the penetration structures. This is in contrast to other root pathogens as they are not usually able to invade intact tissue, but rather through natural openings. Once penetration occurs through several layers of cells, the pathogen is able to derive its nutrient needs from the dead cell material it has infected. It breaks down insoluble material such as lignocellulose and chitin.

The infection process is facilitated by the phytotoxin thaxtomin. Named by King et al. (1989) after Roland Thaxter, the pathogen's discoverer, thaxtomins are a class of 4-nitroindol-3-yl-containing 2,5-dioxopiperazines that have been positively correlated with scab infection.

Bukhalid and Loria (1997) cloned the gene *nec1* from a pathogenic strain of *S. scabies* and expressed it in the non-pathogenic *S. lividans*. The transformed *S. lividans* was able to produce necrotic lesions and colonized tuber tissue. The *nec1* gene produces an extracellular water-soluble compound that induces lesions. They proposed that *nec1* is physically linked to designated ORF*tnp*, which produces an amino acid sequence similar to that of the *Staphylococcus aureus* family of transposonases. This family is noted for horizontal transfers of antibiotic resistance genes, suggesting that *nec1* was horizontally incorporated into what are now plant pathogenic species of *Streptomyces*.

Loria et al. (1998) presented more evidence supporting the *nec1* gene in pathogenicity. Using Southern analysis, they analyzed 43 strains from three major plant pathogenic species of *Streptomyces* (*S. scabies*, *S. acidiscabies*, and *S. turgidiscabies*) and probed with *nec1*. They found that all 43 strains hybridized to a single fragment, suggesting that the *nec1* gene may be conserved among plant pathogen species of *S. scabies*. All of these strains with the exception of one produced thaxtomin. This result would also suggest that *nec1* is involved as a pathogenicity factor, and while it may not be directly involved with thaxtomin synthesis, it leads to the possibility of being linked to a putative thaxtomin gene.

Loria et al. (1997) described 11 different chemical structures of thaxtomin from plant pathogenic species of *Streptomyces*. In particular, thaxtomin A (TA) has warranted the most attention because this is the toxin that is produced in most abundance by *S. scabies* spp.

The mode of action for thaxtomin still remains unknown. Currently there are two hypotheses. Both deal with a cell wall target; aiding in the break-down of cellular barriers either directly or indirectly. Deposition or composition of the cell wall is disrupted by abnormal cell plate formation during cytokinesis as observed in onion root tip cells (Fry and Loria, 2002). They suggested that thaxtomin targets cell walls either directly or indirectly for both monocotyledonous and dicotyledonous crops. Loria et al. (2003) observed severe hypocotyl stunting in radish. In onion the same was observed with binucleate cells, and in tobacco severe hypertrophy was apparent. They hypothesized that if thaxtomin most likely has a cell wall target, then the phytotoxin may aid hyphae in cell penetration by compromising cell wall integrity. Recent work on the resistance mechanism to common scab is the detoxification process of thaxtomin. This involves, at least in part, the glucosylation of the phytotoxin thaxtomin (King and Lawrence, 2000; Acuna et al., 1998). Acuna et al. (2001) examined the fate of ^{14}C TA in tubers. 'Nooksack,' a scab-resistant cultivar, as well as 'Atlantic', a scab-tolerant cultivar, showed detectable quantities of glucosilated TA or A-ß di-O-glucoside (TAG). The susceptible cultivar 'Ranger Russet' did not show detectable amounts of TAG and therefore was not able to utilize glucosyltransferase in replacing –OH groups with glucose, therefore making the cultivar susceptible.

While most of the work has concentrated on the role thaxtomins have relatively little has been done with degradative enzymes. McQueen and Schottel (1987) showed that a heat stable esterase produced by pathogenic *S. scabies* was able to break down potato suberin. Although seemingly rare, it has also been shown that TA is not required in order for *S. scabies* to be pathogenic on potato (El-Sayed, 2000). Beausejour and Beaulieu (2004) performed N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis on *S. scabies.* They characterized melanin deficient mutants from wild strain EF – 35 and noted how this deficiency related to TA production. They found that while some mutants had reduced virulence and became nonpathogenic, they were still able to produce TA in oat bran broth. This work suggests that other factors besides TA, such as nec1, contribute to *S. scabies* pathogenicity.

Although *S. scabies* is probably the best documented plant pathogenic species of the *Streptomyces* genus, there are several other plant pathogenic species of *Streptomyces*. *Streptomyces acidiscabies* causes symptoms identical to those of *S. scabies* at a pH as low as 4.5. *Streptomyces ipomoeae,* infects sweet potato (*Batatas ipomoeae*), a disease known as soil rot. This disease, facilitated by thaxtomin C, can cause a reduction in vine growth as well as yield. *Streptomyces turgidiscabies* was first documented in Japan on the island of Hokkaido (Miyajima et al., 1998). This species produces comparable lesions to *S. scabies* but is dissimilar in DNA-DNA homology and in ribosomal DNA sequence (Miyajima et al., 1998; Takeuchi et al., 1996).

Management of Common Potato Scab

Irrigation

Irrigation has long been known to limit scab infection. Sanford (1923) found that soil moisture was either directly or indirectly responsible for the control of scab. He also noted that there were infection differences for tubers grown under dry conditions versus those under moist conditions. On dry soil, tubers were heavily infected with scab, while in moist soil, potatoes were only slightly diseased. He also suggested that there was a critical time period when the disease was hindered by moisture. Lewis (1970) later defined this time period as five weeks for the soil to remain moist after tuber initiation. If the soil was kept moist with a water potential greater than $-10\ J\ kg^{-1}$ for this amount of time, there was a "striking reduction in infection." Lapwood et al. (1966, 1970a, 1970b) not only corroborated these findings by stating that irrigation has a significant role in disease suppression, but also determined when a potato is physiologically most susceptible. They looked at internode elongation during tuber maturation, and found that infection of internodes four and five were of the greatest interest because infection at these internodes caused the greatest blemishing. Although irrigation may provide an effective means for managing scab on a small scale, it may not always be the most practical, especially for soils with low water holding capacity.

Soil Amendments

Soil amendments including manure, lime, and cover crops have shown to produce inconsistent results with the control of scab. Conn and Lazarovits (1998) spread cattle, liquid swine and chicken manures on commercial potato fields. They found that incidence of scab after the first year of application declined, but a general increase in incidence thereafter ensued for the remaining two years of the study. Their conclusion was that aside from the type of manure being used, soil type is an integral part of disease presence.

The use of ammonium lignosulfonate (ALS) as a soil amendment was shown to have some promise in reducing incidence of scab. Soltani et al. (2001) recorded a reduction in scab severity by 50-80% on all four commercial growing sites in Ontario, Canada. They found a decline in soil pH, and an increase in soil microbe density. Lignosulfonate is a byproduct of the sulfite method for manufacturing paper. This is a complex macromolecule with negatively charge molecular groups (sulfonate, hydroxyl, phenolic, and carboxyl). As a by-product of the paper making process, it has a high nitrogen and carbon content, and has been observed to increase availability of macro and micronutrients to plants (Alkanani and MacKenzie, 1996; Meier et al. 1993).

Fertilizer treatments have been used in treating common potato scab. Sturz et al. (2004) reported that the application of fertilizer amendments can alter rhizobacterial communities that correlate with a varying degree of antibiosis against *S. scabies.* Ammonium sulfate $(NH4)_2SO4)$ was applied at a rate of 379 kg/ha. for two years. While field plots had a 10% decrease in scab incidence, they suggest that the decrease in soil pH by the fertilizer created a competitive advantage for those species that are antagonistic to *S. scabies.*

Chemical Control

Chemical and other antimicrobial compounds have been used and vary in their degree of success. Chemical treatments such as 3,5-D or benzoic and picolinic acids tend to cause plant injury (McIntosh et al., 1988). The chemical pentachloronitrobenzene (PCNB) or Blocker has been used, although it can be phytotoxic at higher concentrations. Curwen and Groskopp (1978) applied PCNB at a high rate (20 lbs/A) during a two year study on Russet Burbank. They noted that while the incidence of scab was significantly reduced, a smaller tuber size was apparent. In another study, antimicrobial compounds were tested for their efficacy on common potato scab (Hollister, 2005). Scab isolates DP, 88-21, and Onaway were grown on potato dextrose agar (PDA) with four antimicrobial compounds, vancomycin, gentamicin, PCNB, and copper sulfate. Of the four antimicrobial compounds only two, gentamicin (10 ppm) and vancomycin (1.0 ppm), were effective at low doses in controlling *S. scabies* while PCNB (1,000 ppm) and copper sulfate (100 ppm) were effective at higher doses. At higher concentrations PCNB and copper sulfate decreased yield as the compounds were phytotoxic. Neither vancomycin or gentamicin are registered for use on potatoes in the U.S.

Antagonistic Species

Sanford (1926) first suggested that certain microbes having "antibiotic qualities" could inhibit the development of scab. Antagonistic species of *Streptomyces* have been used as biological control agents against *S. scabies* (Liu et al., 1995). Liu et al. (1995)

found that two suppressive strains, *S. diastatochromogenes* strain PonSSII and *S. scabies* strain PonR, significantly reduced scab incidence on potato cv. Norchip compared to the control. Subsequently, Liu et al. (1996) isolated 22 disease suppressive isolates from both conducive and suppressive soils. These isolates exhibited more antibiotic activity against pathogenic scabies than compared to the standard suppressive strains PonSSII and PonR.

Some work has been done in the area of spontaneous mutants resistant to the antibiosis effect. Neeno-Eckwall and Schottel (1999) looked at the frequency with which these mutants form. They used the mutant strain *S. scabies* RB4, which they noted was able to grow in cultures of the suppressive strain PonSSII. From these cultures they selected eleven mutant strains. Two of the eleven mutants lost the ability to produce thaxtomin and compared to RB4 overall thaxtomin production decreased by 75%. They describe an inoculated soil assay whereby disease severity decreased compared to that of parent strain RB4. There is evidence that these antagonistic species of *S. scabies* are abundant. Lui et al. (1996) assayed 93 randomly chosen isolates from Red Pontiac, Cobbler and other breeding lines. Forty-six strains came from suppressive soils in Grand Rapids, MN; 9 from conducive soils at Grand Rapids, MN; 27 from conducive soil at Becker MN; 8 from a production field at ND; and 3 from production field at Big Lake MN. Of the 93 strains, 22 produced more antibiotic activity than PonR and PonSSII, and they were nonpathogenic.

Breeding Resistance and Genetics

Breeding for resistance to common potato scab is probably the most effective way to combat the disease (McKee, 1958). Genetic improvement for a variety of agronomically important traits including yield, chipping quality and disease and insect resistance has been successful in diploid species (Spooner and Bamberg, 1994). Reddick (1953) stated that there are plants resistant to scab and the resistance is heritable.

Certain wild species have resistance to common scab. Working with diploids as compared to tetraploids, presents a sensible solution for resistance management for several reasons as pointed out by Dionne and Lawrence (1961). First, since the number of chromosomes is reduced by half, resistance is easier to identify in the homozygous state. Second, incomplete dominance or dosage effect may also be exhibited. This would help to characterize moderate resistance. Third, the number of individuals within a population should theoretically be less to identify the favorable recombinations necessary for resistance. Lastly, wild diploid potato germplasm holds the key to disease resistance for cultivated varieties. Wild potato germplasm display a greater range of resistance to disease and pests than do their cultivated counterparts (Hawkes, 1992).

A number of studies have been conducted in characterizing scab resistance. Reddick (1939) pointed out that *S. commersonii, S. chacoense, S. caldasii* var. *glabrescens, and S. jamesii* all have resistance to common scab.

Once resistance has been characterized it may be crossed back to the tetraploid level. Studies have examined the transmission of resistance by using diploid interspecific hybrids (Tai et al., 1996; Murphy et al., 1995). Material was selected based on their resistance to scab as well as their ability to produce 2n gametes, allowing the resistance to be transferred to the tetraploid level. Jansky and Rouse (2003) reported similar results with transmission of scab resistance while broadening the resistance to two other diseases, early die (*Verticillium dahliae*) and early blight (*Alternaria solani*). By combining genetically diverse backgrounds of wild material (*S. berthaultii*, *S. tarijense* and *S. chacoense)* and crossing back to the tetraploid level, disease resistant progeny were produced. Dionne and Lawrence (1961) looked at incorporating scab resistance into the susceptible diploid *S. phureja*. They took a scab resistant clone of *S. chacoense* and crossed it with *S. phureja*. The most resistant F_1 individuals were crossed to produce the F_2 generation. The F_2 was back-crossed (BC) to *S. phureja,* the recurrent parent. The resistant BC_1 individuals were observed for their resistance to scab. They concluded that resistance to the disease is not inherited in a simple manner, but that many factors are involved. They did report that all scab resistant selections had a high level of solanine, making them bitter.

The genetic inheritance for resistance to common potato scab is not clearly understood. Resistance is believed to be quantitative (Cipar and Lawrence, 1972). Alam (1972) hypothesized that two loci are responsible for scab resistance. At one locus (Sc_1), one or more dominant alleles confer resistance while at the other locus (sc_1) a homozygous recessive condition confers resistance. Relatively little has been studied with regards to a marker based system for the resistance to scab. This is understandable since there is considerable environmental influence in the outcome of scab infection, and that resistance scores for hybrids may vary from one year to year.

Testing for resistance

An effective and efficient screening technique for scab resistance in a breeding program is needed to identify resistant breeding lines and cultivars. Traditionally, field trials have been used to distinguish resistant from susceptible clones; however, results can be strongly influenced by environmental conditions.

There have been many attempts to reduce environmental variabilities and/or improve the accuracy of the screening process. One immediate solution to controlling environmental variability is to quantify inoculum levels. Keinath and Loria (1991) observed a positive relationship between inoculum density and severity of scab produced on potato. Jellis (1974) controlled in-field soil moisture by covering field rows with plastic tunnels. He reported a greater difference between resistant and susceptible varieties, while maintaining less variability between replicates compared to the traditional approach. Hooker (1950) developed a method for observing an in-vitro scab infection. By planting a seed piece in a pot with a hole in the side covered up by moss, he then inverted the pot and placed it on a saucer. Scab infections could then be viewed on a daily basis.

Early studies that tried to incite scab while testing for resistance commonly used naturally infected field soil. Wiersema (1970) reported having developed a reliable greenhouse test using field soil in 3L plastic pots. Watering was done by subirrigation instead of overhead watering thereby leaving the top layer of soil in the pot drier and inciting a greater degree of disease. According to Bjor (1974), field soil produced variable results, and by simply inoculating a sand mixture, results became more consistent. He grew potato seed pieces in pots with an inoculated sand mixture and controlled the water supplied. Results from this experiment were satisfactory in that scab severity scores corresponded to what had been observed in the field and from prior experience. Later, Bjor and Roer (1980) produced a method for testing resistance to potato scab by growing seed pieces in the greenhouse. Using 3 L pots, they planted two susceptible varieties (Kerrs Pink and Bintje) and two resistant lines (FxAq-1 and T-64-12-28) with field or inoculated sand. They found that by using inoculated sand, infection severity was consistent compared to field soil. They also found that resistance could be determined based on two to three pots per line.

The aim of this research is to gather information on a series of different methods to screen for resistance to common potato scab. Specifically, the thesis objectives are to:

1. Gather data on the four different methods that will be employed to discern resistance to *S. scabies*: thaxtomin A assay, tuber disc inoculation, potato sprout inoculation, and a greenhouse assay with infested soil.

2. Use the most efficient protocol on a population that is segregating for scab resistance (MSL603 population) and compare those results with the results obtained from the field evaluations for this segregating population.

MATERIALS AND METHODS

Plant Material

Four potato clones were chosen to assess each screening method. The breeding line MSG227-2 is a round white chip processing line and is resistant to scab. Jacqueline Lee is a commercial release from the Michigan State potato breeding program (Douches et al., 2001a). This variety has an oval type with cream colored flesh, resistant to late blight and is susceptible to common potato scab. Liberator is also from the Michigan State potato breeding program (Douches et al., 2001b). This chip processing variety is a scab resistant round white. Russet Burbank was added as a scab resistant check variety. Table 1 demonstrates how these clones were rated for both the field trial and greenhouse assay. Table 2 shows scab ratings from the Michigan State University Potato Breeding Program for the test lines Jacqueline Lee, MSG227-2, Russet Burbank, and Liberator for the years 1998 through 2005. An average rating was calculated for those years and an overall standing of resistance obtained. This data was used to establish each line's level of resistance so that the work from this research could be cross-checked with each line's known reaction to scab.

Table 1. Rating scale used to classify the severity of scab infection on potato tubers. Field tubers and greenhouse assayed tubers were rated in accordance with table. Ratings ranged from 0 – 5 and were based on percent lesion coverage and the amount of pitting present.

Scab Severity Rating Scale

Rating	Description
0	No scab
1	Superficial or infrequent surface scab with 1-10 % coverage
2	Moderate surface scab 11-25 % coverage with no pitting
3	Small infrequent pits, with or without surface scab 26-50 % surface coverage
4	Serious pitted scab, with 51-75 % coverage
5	Severe pitted scab that may be accompanied by surface or raised lesions >75 %

Table 2. Overall Scab Ratings[1] from 1998-2005 of clones used in thaxtomin A, spore suspension and greenhouse assays.

	Year								
Potato Line	1998	1999	2000	2001	2002	2003	2004	2005	Ave. Rating
Jacqueline Lee	3.3	3.5	2.5	2.3	2.7	2.5	2.8	2.3	2.7
MSG227-2	1.0	1.1	1.8	0.3	0.5	0.5	0.8	1.0	0.9
Liberator	1.5	1.0	0.5	0.3	0.0	0.0	0.3	0.8	0.6
Russet Burbank	1.0	1.0	1.0	0.6	1.0	0.5	0.8	N/A	0.8

[1] Ratings are based on a 0 - 5 scale. A rating of 0 has no scab (resistant), a rating of 3 has <10% pitting and is susceptible and a rating of 5 is very susceptible with severe pitting and >75% lesion coverage.

Growth of *Streptomyces scabies* cultures

The production and extraction of thaxtomin was followed as described by Goyer et al. (1999), with some modifications. Step cultures were made starting with 25 ml flasks of tryptic soy broth (TSB, soybean-casein digest) (Sigma-Aldrich Co.), made as directed by the manufacturer, and shaken overnight at 30 °C. Cultures were centrifuged and pellets from each tube were diluted with five volumes fresh TSB and mixed well. An aliquot of 100 µl from this mixture was used to inoculate every 25 ml of oat bran broth (OBB). Oat bran broth was made by boiling 40 g of oat bran in 1 L of distilled water for 15 minutes. Inoculated OBB cultures were placed on a rotary shaker for 8 d at 28 °C. Cultures were centrifuged and supernatant collected for thaxtomin extraction.

Isolation and Purification of Thaxtomin

A 1:1 volume of OBB culture and ethyl acetate were mixed and the upper phase removed. The ethyl acetate portion containing thaxtomin was dried and extracted with 100 µl of ethyl acetate. Crude thaxtomin was fractionated twice using silica gel thin layer chromatography (TLC). Both were fractionated with chloroform and methanol. The first used a 9:1 and then 7:3, respectively. The purified thaxtomin was scraped off and resuspended in methanol.

Potato Tuber Disc Assays:

Spore Assays

Spore suspensions were made using two week old cultures of *S. scabies*. Five milliliters of double distilled water was added to each cultured Petri dish. Spores were then quantified using a hemacytometer and the concentration was adjusted to 1 x 10^6 spores/ml. A spray dispenser was used to apply the suspension onto PTDs. Potato tuber discs were made the same way as with the thaxtomin assay. Digital photographs were recorded at 12 h intervals for 72 h. Potato tuber discs were kept at 28 °C.

Surface sterilized tubers were placed in the dark at 10 °C until etiolated sprouts were approximately 7.5 cm in length. A spray dispenser was used to apply the spore suspension onto the sprouts. Tubers were then placed in a culture vessel with sterilized vermiculite and kept in the dark at 28 °C. Digital photographs were recorded three times: days 1, 8, and 25. Etiolated sprouts were kept at 28 °C. Both spore suspension assays were conducted as a completely randomized design with three replications.

Thaxtomin Assay

Six concentrations of thaxtomin A were used (0 μg, 5 x 10^{-1} μg, 5 x 10^{-2} μg, 5 x 10^{-3} μg, 5 x 10^{-4} μg, and 5 x 10^{-5} μg) on potato tuber disks (PTDs) that were placed in Petri dishes. Potato tuber discs were made using a 2.5 cm cork borer. Thin slices were cut to 0.5 cm thick. Two layers of moistened filter paper were placed below the PTDs to keep the tuber discs from drying. Purified thaxtomin was applied to each PTD in 10 μl aliquots by using filter paper (6 mm diameter). The filter paper was air dried before being placed on PTDs and approximately 14 ul of sterile distilled water was added to each treatment to ensure good contact between the filter paper and PTD. Petri dishes were wrapped in Parafilm and digital photographs were taken at 12 h intervals. Potato tuber disc scores ranged from 0-5 and were based on the intensity of necrosis that was visually observed. The percent of theoretical coverage based on the size of the filter paper disc was also taken into account. Ratings were scored as follows; a score of 0 had no observable level of necrosis, a rating of 1 had < 10 % of very slight necrosis, a rating of 2 had 11 – 25 % necrosis with darker flecking, a rating of 3 had more pronounced necrosis in a determined area with 26 – 50 % coverage, a rating of 4 had 51 – 75 % necrosis covering the surface of the PTD and a 5 rating had the darkest necrosis observed with >75 % surface coverage (Fig. 1). The assay was a completely randomized design with four replications.

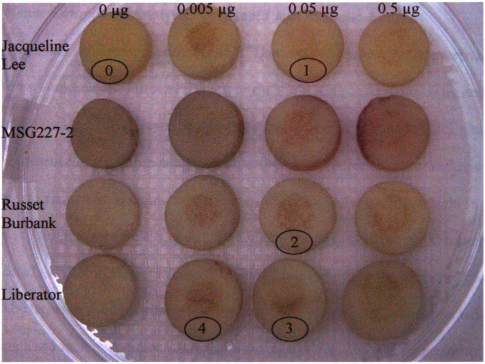

Fig. 1. Direct application of thaxtomin A (TA). Three concentrations of TA were placed on potato tuber discs (PTDs); 0.005 µg, 0.05 µg, and 0.5 µg. Discs were evaluated for percent coverage of necrotic lesions. Examples of ratings are displayed 0 - 4 on the PTDs. Ratings were recorded after 48 h.

Plant Defense Compound Analysis: Terpenoid and Phenolic Analysis

A separate thaxtomin experiment of PTDs was used to observe the presence of plant defense compounds. Fifteen PTDs from each line were tested at the 0 μg and 0.005 μg concentration level. Thin layer chromatography was employed using silica plates to detect the presence of terpenoid and phenolic compounds. After 48 h the surface of each tuber disc was removed. Weights were taken of each line and shaken overnight in methanol (10 ml per gram fresh weight). Samples were vacuum dried and the methanol was collected. Chloroform was added (0.25 ml/g fresh weight) to the methanol and mixed. The terpenes (bottom phase) and the phenols (upper phase) were extracted and dried using a nitrogen evaporator. Samples were reconstituted by adding methanol at 1 ml/10 g fresh weight. Scopoletin, chlorogenic acid and caffeic acid were used as control compounds for both terpenoid and phenolic phases. Test and control compounds were applied to TLC plates with 50 μl and 100 μl blots for terpenoid and phenolic analysis respectively. The terpenoid analysis ran in ethyl acetate: cyclohexanes (1:1). Plates were sprayed with vanillin and sulfuric acid mixture and heated at 120 °C for 10 minutes. Ethyl acetate, formic acid, acetic acid, and water (100:11:11:27, v/v) comprised the solvents for the phenolic analysis. Plates were sprayed with a mixture of 2 ml p-nitroaniline in 2N HCl, 0.5 ml sodium nitrate, and 15 ml sodium acetate. After the phenol plate was dried a 10 % sodium carbonate solution was sprayed. Upon drying, the plates were viewed under UV light using the Spectroline® Transilluminator, model TR-302 at 302 nm. Digital photographic images were then recorded.

Field Nursery Study of MSL603 Mapping Population

A segregating population (MSL603) for scab resistance was generated from the cross Jacqueline Lee x MSG227-2. The MSL603 population consisted of 162 individuals in 2004 and 160 individuals in 2005. Field trials were planted in 2004 and 2005 at Michigan State University's Soils Farm in East Lansing, MI. In 2004 tuber seed pieces were planted on May, 7 and rated on the week of September 6th. In 2005 plots were planted on May, 9 and rated on the week of September 19. The trial was planted as a randomized complete block design with five hill plots and four replications. Plots were mechanically harvested and the tubers were laid on the surface in the field. Tubers were visually scored by a team of three. Ratings were based on a 0-5 scale with 0 - 1 classified as resistant, a score of 3 is susceptible and a rating of 5 is highly susceptible (Figs. 2 - 4). Ratings were based on lesion coverage and lesion type, with higher scores given to tubers with pitted lesions (Table 2).

Fig. 2. An example of a resistant plot with a rating of 1.0. Plots were rated on a scale from 0 – 5 with a rating of 0 having no scab, a rating of 3 is susceptible and a rating of 5 is very susceptible.

Fig. 3. An example of a plot with less than 10 % pitting and scab rating of 3. Plots were rated on a scale from 0 – 5 with a rating of 0 having no scab, a rating of 3 is susceptible and a rating of 5 is very susceptible.

Fig. 4. An example of a susceptible scab plot. This plot was given a rating of 5 due to deep and severe pitting and coverage. Plots were rated on a scale from 0 – 5 with a rating of 0 having no scab, a rating of 3 is susceptible and a rating of 5 is very susceptible.

Greenhouse Assay

Fifteen individuals were selected from the MSL603 mapping population for a greenhouse assay, including the parents. These individuals were chosen for the study based on the two years of replicated field trial data. Five individuals from three classes; resistant, moderately resistant, and susceptible were included for the greenhouse assay, including the parents.

A greenhouse protocol developed by Dr. Leslie Wanner, USDA/ARS, Beltsville, MD. This protocol was followed closely with some modifications (L. Wanner, pers. comm.). Cultures of *S. scabies* were grown on yeast media extract (YME) plates for two weeks. Spore concentration was quantified using a hemocytometer and was adjusted to 1 X 10^6 spores/ml. One milliliter of spore suspension was used to step culture into 50 ml of liquid yeast media extract (YME), and was incubated in a shaker for three days at 28 °C. Cultures were centrifuged (5000 g for 5 minutes) in 10 ml batches. Each culture was resuspended in distilled water and added to its own culture vessel with sterilized vermiculite and 50 ml of Say's solution. Vermiculite was sterilized by autoclaving three times for one hour every other day at 121 °C. The weight of the vermiculite was recorded for later inoculum quantification. Culture vessels were used to incubate the vermiculite for approximately 14 d at 28 °C. Each vessel was gently shaken every other day to mix inoculum. After the 14 d incubation, samples from all culture vessels were plated on YME by mixing 1 g of inoculated vermiculite in 9 ml of sterile distilled water. This sample was incubated for two days at 28 °C to assess concentration and ensure that no contamination was present. Concentrations were determined by plating four 10 µl dilutions (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) onto YME. Colony forming units (CFU) were then counted.

The preliminary greenhouse screening of the four test lines, Jacqueline Lee, Russet Burbank, Liberator, and MSG227-2 was conducted at two concentrations; 5×10^8 spores/pot and 5×10^9 spores/pot. The concentration for testing the progeny of the MSL603 population was 3×10^8 CFU/cc. To standardize concentration, approximately 900 ml of equal parts of sterilized soil and sand were added to bleached 2 L pots. Soil and sand were sterilized by autoclaving three times for three hours every other day at 121 °C. Inoculated vermiculite was mixed into the second 900 ml mixture of sterilized soil and sand. Controls were given uninoculated, sterilized vermiculite. Concentrations were maximized per pot by dividing the total amount of cultured inoculum by the number of pots. Potatoes were surface sterilized with 5 % bleach solution, and planted between the first and second layers of soil and sand mixture. Upon emergence, potatoes were given a supplemental 16 h photoperiod. Plant watering was reduced and the soil was allowed to dry out between each watering application. After approximately 14 weeks, tubers were harvested and visually graded in the same manner as field tubers. This is based on scab severity which accounts for two criteria; percent of lesion coverage and lesion type. An overall average for lesion coverage was given for each pot and each pot was given an overall rating of 0-5 (Table 2). A score of 0 - 1 is considered resistant, > 1 - 2.5 moderately resistant, and > 2.5 - 5 susceptible. Tubers with pitting incurred a higher score than those without pitting. This was a completely randomized design with six replications.

Statistical Analysis

Potato tuber disc assays using the spore suspensions failed to yield any scorable results, so no statistics were employed. All analysis of variance were performed using the SAS general linear model procedure at $\alpha = 0.05$ (SAS 2002). Necrosis ratings from PTD assays using direct thaxtomin application were analyzed by two-way factorial analysis of variance for potato variety x thaxtomin concentration. Field scab ratings and greenhouse scab ratings were each tested using one-way analysis of variance and Fisher's Protected Least Significant Difference for means separation ($\alpha = 0.05$).

Pearson's correlation coefficients were tested using the SAS correlation procedure at $\alpha = 0.05$ (SAS 2002). Data were compared for two years (2004 and 2005) of field ratings, the 2004-2005 average field rating and the greenhouse rating for the 15 select MSL603 individuals.

RESULTS

Spore Assays:

Potato Tuber Disc

The spore suspension assay showed no visual signs of infection on the PTDs. A general overall darkening of each PTD could be observed with no color distinction between lines (Fig. 5).

Etiolated Tuber Sprouts

The inoculation of etiolated tuber sprouts with a spore suspension did not produce visual symptoms of scab infection. No differences were observed between controls and treatments.

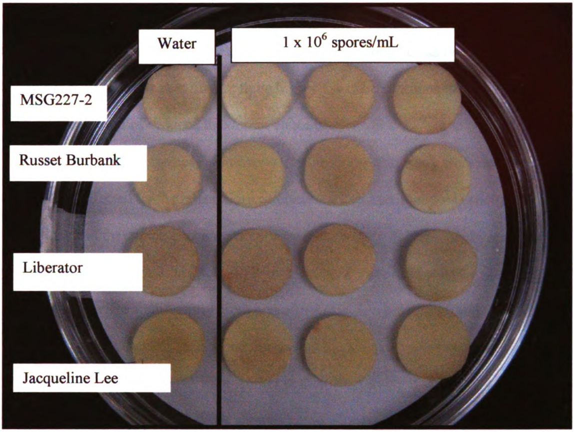

Fig. 5. Potato tuber discs (PTDs) inoculated with a scab spore suspension (1 X 10^6 spores/ml) after 48 h. Untreated PTDs are shown in the far left hand column under the water heading. Treated PTDs are shown in the three far right-hand columns.

Thaxtomin Assay

The potato tuber disc assay using direct thaxtomin application on the four potato clones with differing levels of field scab resistance was conducted as two separate runs. The first run assayed the four lines at three higher TA concentrations (0.5 to 5 x 10^{-3} μg), and the second run had reduced thaxtomin concentrations (5 x 10^{-3} μg to 5 x 10^{-5} μg).

Severity ratings for necrosis observations from the first assay were not significantly affected by the interactions of concentration and line ($P = 0.4649$). Both potato lines and thaxtomin concentrations were significantly different for overall main effects means (Table 3). Liberator and Russet Burbank were similar in the observed level of necrosis and were significantly more necrotized than Jacqueline Lee and MSG227-2 (Fig. 6). Russet Burbank typically had the darkest necrotic lesions of all lines (Fig. 7). Jacqueline Lee and MSG227-2 were similar in their level of necrosis across all concentrations. The untreated PTDs showed no necrosis.

The results from the second TA assay were difficult to interpret as significant interactions between concentration and line were observed ($P = 0.0001$, Table 3). Liberator and Russet Burbank had a significantly higher level of necrosis for all concentrations except at the 5 X 10^{-5} μg and 0 μg levels compared to Jacqueline Lee and MSG227-2 (Fig. 8). No necrosis was observed for all lines at the lowest concentration (Fig. 9).

Table 3. Analysis of Variance P-values for necrosis ratings of concentration and variety using Potato Tuber Disc assays with direct thaxtomin A (TA) application. Two test runs were used to examine reaction to TA; one using higher concentrations and a second using lower concentrations.

Run	Variable	p-value
1-High TA	Concentration	< 0.0001
1-High TA	Variety	0.0029
1-High TA	Conc. X Variety	0.4649
2-Low TA	Concentration	< 0.0001
2-Low TA	Variety	< 0.0001
2-Low TA	Conc. X Variety	<0.0001

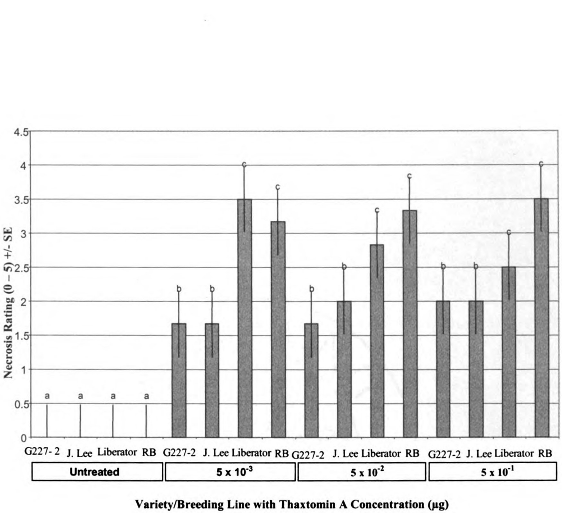

Fig. 6. Graphical representation of direct application of thaxtomin A (TA) on potato tuber discs (PTDs). This is the first and higher of two sets of concentrations used in determining TA reaction on PTDs. Four test clones were used; MSG227-2, Jacqueline Lee, Liberator and Russet Burbank. The rating scale ranged from 0 – 5, with 0 having no necrosis and a PTD with a rating of 5 having >75% necrotic coverage. Four concentrations were employed; from left to right 0 µg, 5 x 10^{-3} µg, 5 x 10^{-2} µg, and 5 x 10^{-1} µg.

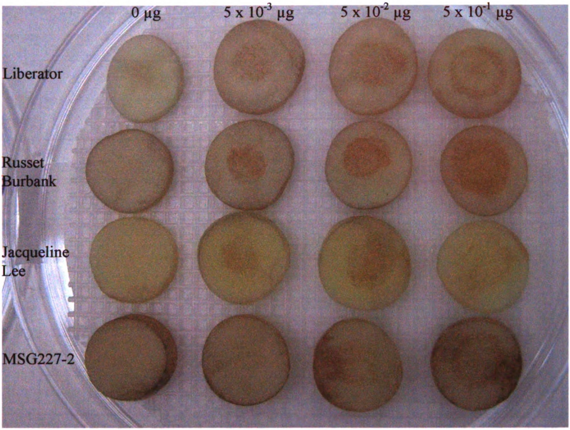

Fig. 7. Potato tuber disc (PTD) assay using direct thaxtomin A (TA) application. This is the first and higher of two sets of concentrations used in determining TA reaction on PTDs. Assay demonstrates the four potato test clones Liberator, Russet Burbank, Jacqueline Lee, and MSG227-2 at four thaxtomin A concentrations (0 µg, 5×10^{-3} µg, 5×10^{-2} µg, and 5×10^{-1} µg) after 48 h.

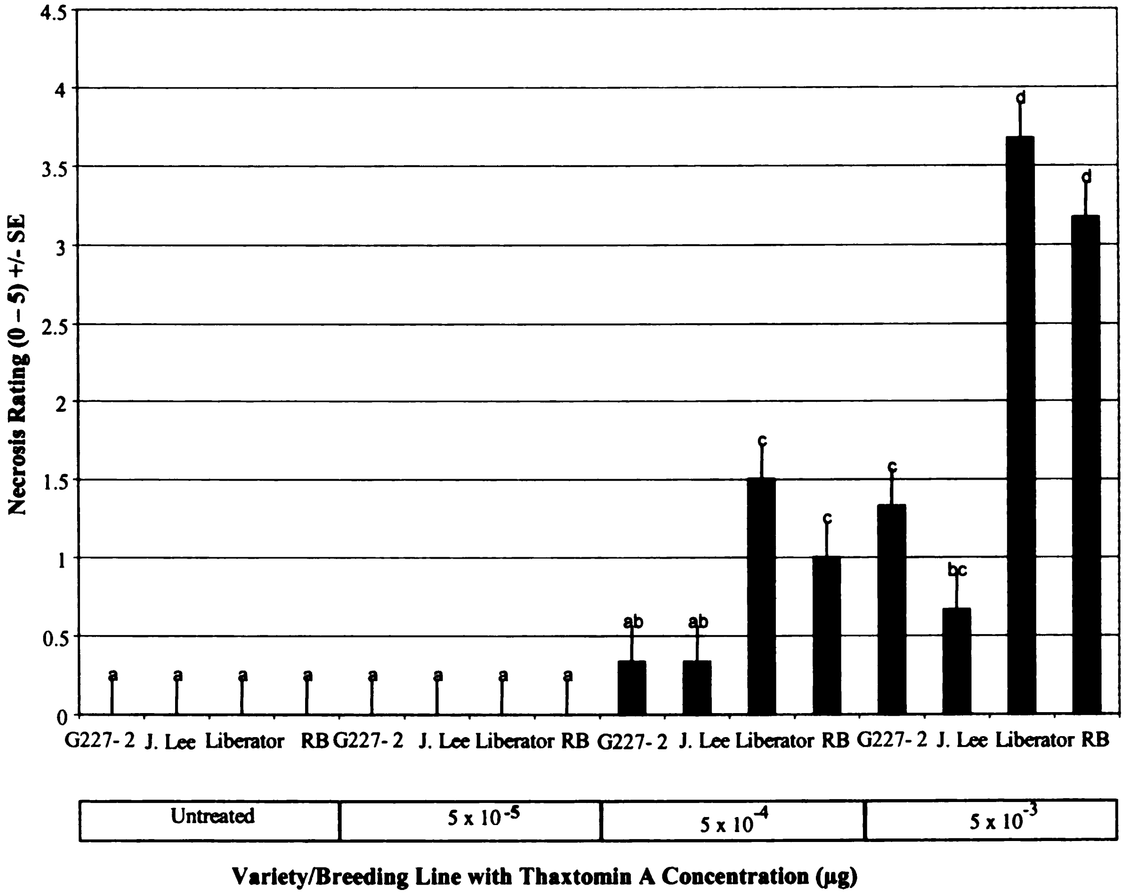

Fig. 8. Graphical representation of direct application of thaxtomin A (TA) on potato tuber discs (PTDs). This is the second and lower of two sets of concentrations used in determining TA reaction on PTDs. Four test clones were used; MSG227-2, Jacqueline Lee, Liberator and Russet Burbank. The rating scale ranged from 0 – 5, with 0 having no necrosis and a PTD with a rating of 5 having >75% necrotic coverage. Four concentrations were employed; from left to right 0 μg, 5×10^{-5} μg, 5×10^{-4} μg, and 5×10^{-3} μg.

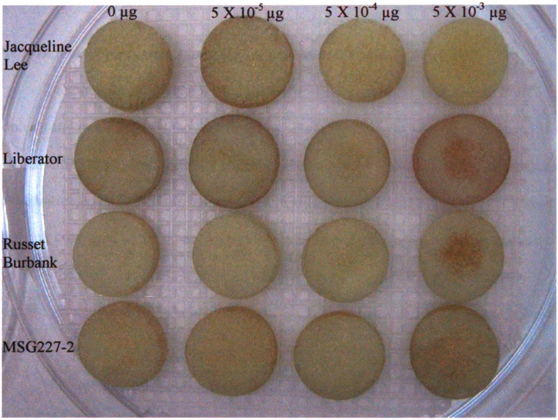

Fig. 9. Potato tuber disc (PTD) assay using direct thaxtomin A (TA) application. This is the second and lower of two sets of concentrations used in determining TA reaction on PTDs. Assay demonstrates the four potato test clones Liberator, Russet Burbank, Jacqueline Lee, and MSG227-2 at four TA concentrations; from left to right 0 µg, 5 x 10^{-5} µg, 5 x 10^{-4} µg, and 5 x 10^{-3} µg) after 48 h.

Plant Defense Compound Analysis: Terpenoid and Phenolic Analysis

The phenolic analysis revealed that chlorogenic acid was produced for both controls and treatments for the four breeding lines (Figs. 10 and 11). Though bands were not quantified the overall intensity appears to be stronger for the treated PTDs compared to the untreated PTDs. Some banding difference occurred with MSG227-2 and Russet Burbank compared to the other two test lines. Both had banding in the upper portion of the phenolic analysis plates, suggesting that there are differences in plant defense compounds among lines. These differences were more prominent on the treated phenolic plate.

No terpenoid compounds were observed for the controls. Line MSG227-2 had the only band present at the upper most portion of the plate (Fig. 12).

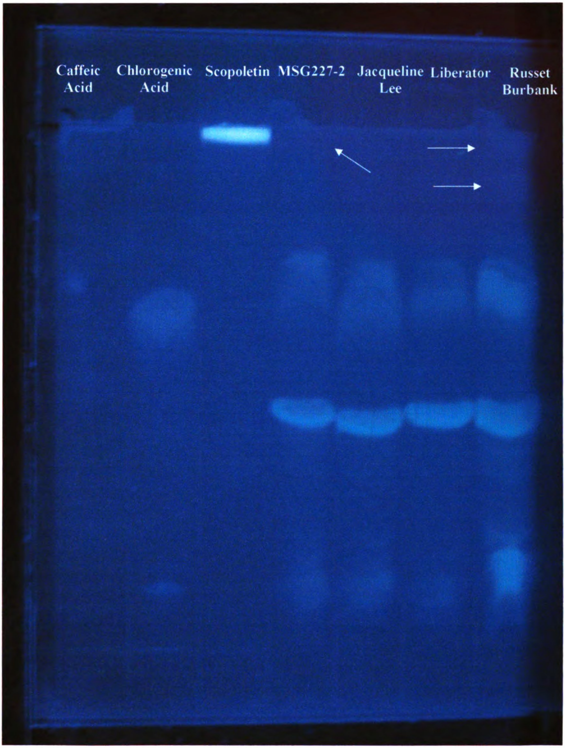

Fig. 10. Phenolic analysis on silica gel thin layer chromatography (TLC) of control potato tuber discs (PTDs). Potato tuber discs were assayed using a direct application of thaxtomin A (TA) at 5 x 10^{-3} µg after 48 h for the presence of plant defense compounds. Plates were viewed here under ultraviolet light (302 nm). Untreated test lines produced chlorogenic acid. Arrows depict unique banding for the clones MSG227-2 and Russet Burbank.

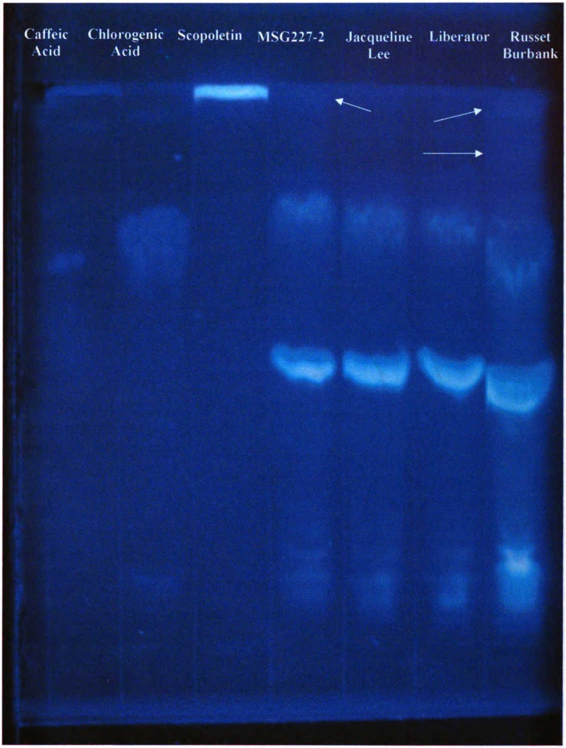

Fig. 11. Phenolic analysis on silica gel thin layer chromatography (TLC) of treated potato tuber discs (PTDs). Potato tuber discs were assayed using a direct application of thaxtomin A (TA) at 5 x 10^{-3} µg after 48 h for the presence of plant defense compounds. Plates were viewed here under ultraviolet light (302 nm). Treated test lines produced chlorogenic acid. Arrows depict unique banding for the clones MSG227-2 and Russet Burbank.

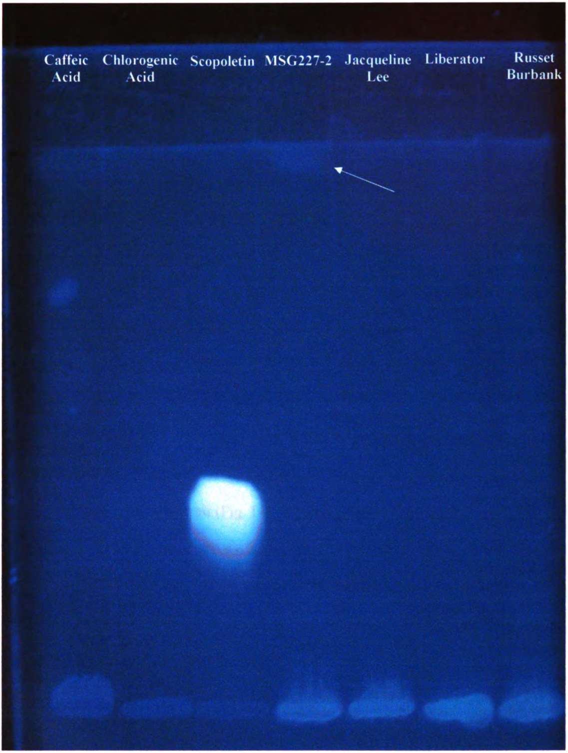

Fig. 12. Terpenoid analysis on silica gel thin layer chromatography (TLC) of treated potato tuber discs (PTDs). Potato tuber discss were assayed using a direct application of thaxtomin A (TA) at 5 x 10^{-3} µg after 48 h for the presence of plant defense compounds. Plates were viewed here under ultraviolet light (302 nm). Arrow depicts unique banding for MSG227-2.

2004 and 2005 Field Data

Field data for the 2004 season revealed that scab pressure was generally higher compared to 2005 (Fig. 13). Data was gathered on the MSL603 population (162 progeny) for years 2004 and 2005 (Table 4). Least significant differences (LSD) for scab rating in 2004 were 1.4 and 1.1 for 2005. Michigan State breeding line G227-2 ranked 3rd in 2004 and ranked 6th in 2005 while Jacqueline Lee ranked 58th and 146th, respectively. In 2004 MSL603-272 was the most resistant line in the population and in 2005 it ranked 3rd. Shaded lines were chosen for advancement to the greenhouse screen by comparing both years of data. Resistance classes (resistant, moderately resistant and susceptible) were developed based on LSDs. Lines that fell within the same LSD for both years were selected.

The distribution of scab ratings for all 162 progeny in the MSL603 population ranged from 1.2 to 4.5 in 2004. The distribution of the progeny follows a typical bell-shaped curve of a quatitative trait (Fig. 14). A similar bell-shaped distribution was observed in 2005 with a range of 0.9 – 3.6. Transgressive segregants were observed at the susceptible end of the distribution, however, no progeny more resistant than MSG227-2 were observed at the resistant end of the distribution. The scab ratings for all progeny in 2005 show a distribution skewd towards less infection (Fig. 15).

Weather data was recorded by Michigan Automated Weather Network (MAWN, 2006). Weather data for June, 2004 indicated that the monthly daytime high temperature was nearly 5 °C cooler compared to 2005. In addition 20 mm of less rainfall than 2005 was recorded for the same time period (Table 5). Similar trends were observed for the month of July with lower average monly high temperatures (25.8 °C) for 2004 compared to 2005 (28.4 °C). Morevover, 14 mm less precipitation fell in 2004 than 2005 for July.

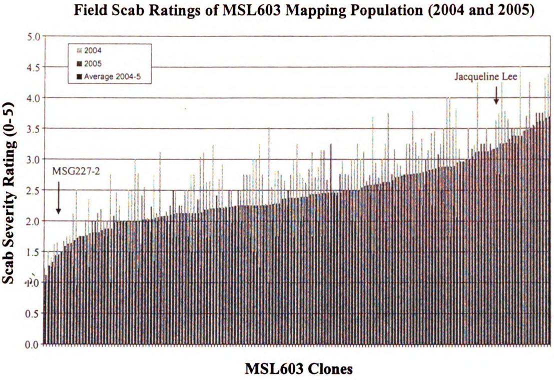

Fig. 13. 2004 and 2005 field results of the MSL603 mapping population, demonstrating higher disease pressure for the 2004 season compared to 2005. The sigmoidal-like curve represents the average score for both years. The parents are located at opposite ends of the graph. Field ratings ranged from 0 – 5 with 0 having no scab and 5 being susceptible.

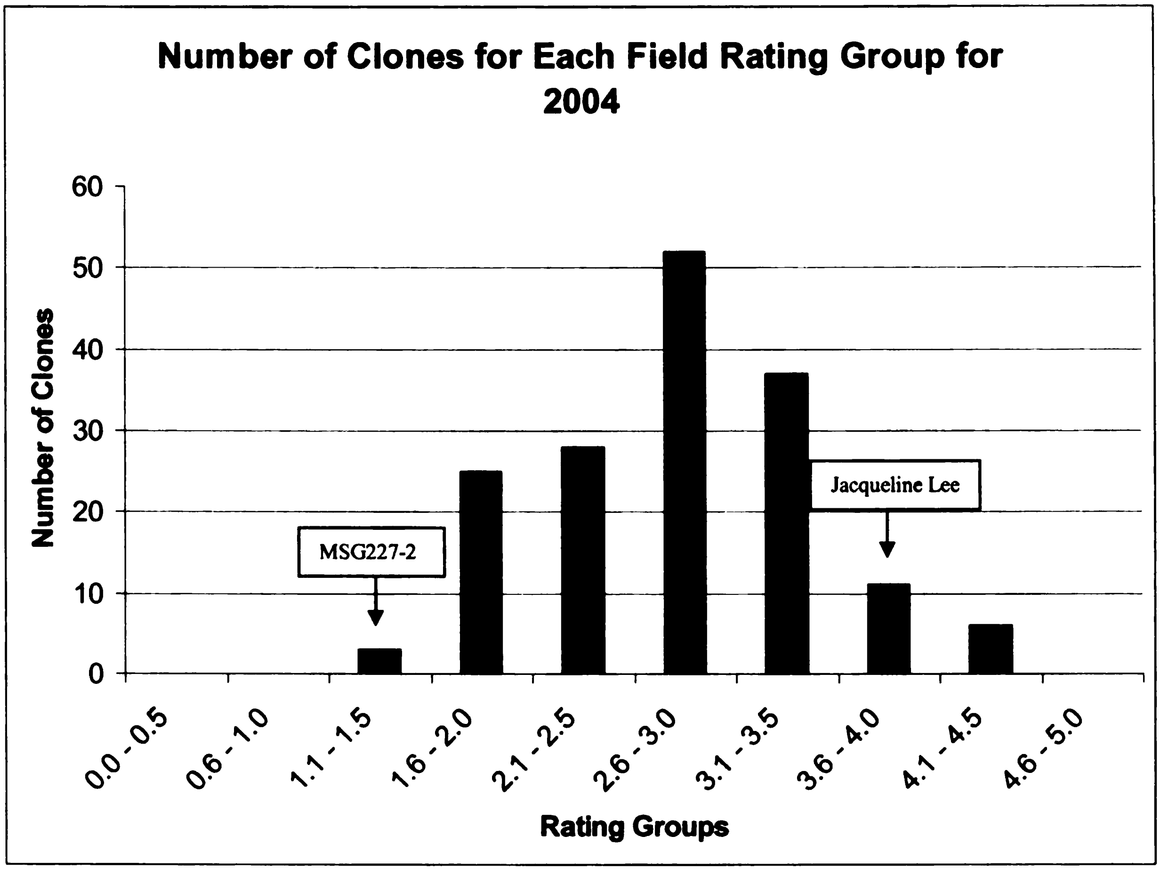

Fig. 14. Frequency distribution of 2004 field scab ratings for all 162 progeny of MSL603. Clones are grouped based on 0.5 ratings on a scale from 0 – 5. The parents MSG227-2 (resistant) and Jacqueline Lee (susceptible) are also shown.

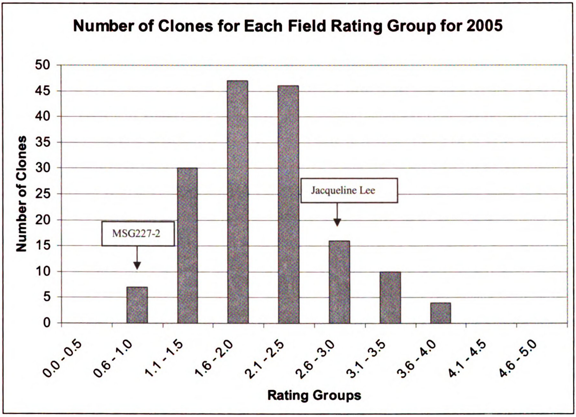

Fig. 15. Frequency distribution of 2005 field scab ratings for all 162 progeny MSL603. Clones are grouped based on 0.5 ratings on a scale from 0 – 5. The parents MSG227-2 (resistant) and Jacqueline Lee (susceptible) are also shown.

Table 4. Field results for MSL603 population for 2004 and 2005. Sorted by line, the LSD* for scab rating in 2004 was 1.4. The LSD for scab rating in 2005 was 1.1. Highlighted lines were chosen for the greenhouse assay. Lines were chosen based on their level of resistance between both years.

	2004	2005
LINE	RATING	RATING
J. Lee	2.6	3
G227-2	1.5	1
150	3.5	3.3
151	2.9	2.5
152	2.6	2.8
153	4.3	2.8
154	3.7	3
156	2.8	1.5
157	3.2	1.2
158	2.5	2
159	2.9	3.2
160	2.9	1.5
161	3	1.2
162	2.6	2.2
163	3	1.8
164	1.8	1.5
165	3.6	2.8
166	2.2	2
167	1.6	2
169	2.8	1.8
170	2.7	2.2
171	3.2	1.8
172	2.5	1.8
174	2.3	2.5
175	2	2.5
176	3.8	1.5
178	3.5	2.5
N/A	3	N/A
180	2.7	2.1
181	2.5	1.5
182	4.3	3
183	2.8	2
184	2.5	2.4
185	2.2	1.5
186	4	1.8
187	2.9	2.3
189	2	2.5
190	3.5	2.3
191	3	1.5
192	2.8	1.8
193	2.7	3.1
194	3.8	2.8

	2004	2005
LINE	RATING	RATING
197	3.2	2.5
198	3.3	1.8
199	2.6	1.8
200	3	2.3
201	2.8	2.8
202	2.8	1.8
203	2.2	2.3
204	2.3	1.7
205	3.8	2.8
207	2.4	1.3
208	3.2	2.7
209	2	2.3
211	3.1	2
212	3.3	2.3
213	3	3.3
214	3.2	3
215	2.8	2.4
217	2.9	2
218	1.8	1.8
219	1.4	1.3
220	1.8	1.8
221	2.5	1.8
222	2.8	1.8
223	3	2
224	1.8	1.5
225	3.3	2.3
226	3.3	1.5
227	2.6	1.7
229	3.5	2.3
231	3.2	2.3
233	3.8	2.1
234	4.5	2.3
235	2.7	2.5
236	2	1.8
238	2.8	2.3
258	2.3	2
260	2.1	2
261	2.7	2.1
262	3.3	2.3
263	2.8	2.2
264	2.5	1.5
265	3.5	1

Table 4. Continued.

	2004	2005
LINE	RATING	RATING
266	2.5	0.9
267	1.9	2.1
268	3.7	1.5
271	1.7	3.3
272	1.2	1
273	N/A	3
274	1.6	1.3
275	1.7	2.5
276	2	2.3
277	3	3.3
278	3.8	1.7
279	3.3	3.5
281	1.7	1.5
282	1.8	2.2
283	2.9	2.6
285	3.1	1.3
286	3	1
287	2.8	1.8
288	2.8	2
291	4.4	3
293	3.3	1.3
294	1.6	2.4
295	2.8	1.5
296	3	2.1
297	2.1	2
298	4.3	2.3
299	2	1.8
N/A	2.1	N/A
303	3.4	2.3
305	2.2	2.2
308	3	2
312	2.8	1.4
314	3.4	3.8
315	2.5	2.4
316	2	2
317	N/A	1.5
318	3.2	1.8
319	3.3	2.5
320	2.5	2
321	3.5	2.8

	2004	2005
LINE	RATING	RATING
322	3.5	2.2
323	3.7	3.3
324	3.3	2.3
325	2.1	1.3
326	3.1	1.8
N/A	3.5	N/A
330	2.5	1.5
331	4.2	2
332	2	1.5
N/A	3.2	N/A
335	2.7	2.5
336	2.5	2
337	2.5	1.7
338	2.8	1.8
339	2.3	2.2
340	3.5	3.8
341	2.8	2
342	1.9	2.4
344	3.3	2
345	3.5	3.3
346	2.8	1
347	3	2
351	3	3.3
354	2.8	1.2
355	1.7	1.3
356B	1.6	2
357	2.7	2.3
359	2.9	2
362	2	2
363	2.2	2.8
365	2.1	1.5
366	3.1	2.5
368	2.7	1.8
371	3.1	1
372	4	1.8
376	2.5	1.5
377	3.5	3.8
378	3	1.5
380	2	2.4
381	3.5	3.6

* Least Significant Differences (LSD) were calculated at alpha = 0.05.

Table 5. Monthly Temperature and Precipitation Data for East Lansing, MI for 2004 and 2005 Field Seasons

	2004			2005		
Month	Max T °C	Min T °C	Prec mm	Max T °C	Min T °C	Prec mm
April	15.8	3.1	14	16.4	2.7	20
May	20.8	8.8	205	18.3	5.6	33
June	23.8	12.2	89	28.2	16.0	109
July	25.8	14.8	102	28.4	15.7	116
August	23.9	12.8	87	27.9	15.9	16
September	25.0	10.8	27	25.5	11.6	77
October	15.5	4.7	49	16.5	5.4	17
Mean	21.5	9.6	573	23.0	10.4	388

Greenhouse Screen

Both MSG227-2 and Jacqueline Lee were rated as being more susceptible in the greenhouse assay compared to their overall field average (Table 6). The breeding line MSG227-2 was rated 0.5 points (on a scale of 0 – 5) higher in the greenhouse and Jacqueline Lee was rated 0.2 points higher compared to field ratings, whereas all MSL603 selected progeny had a lower greenhouse rating compared to their field scores.

Two progeny from the MSL603 population, MSL603-274 and MSL603-272, were among the more resistant clones from this group (Fig. 16). Jacqueline Lee was the most susceptible and MSG227-2 ranked 13^{th} in order of resistance.

Field data from the MSL603 lines in 2005 were moderately correlated ($r = 0.3823$) to greenhouse assayed tubers ($P = 0.0102$, Fig. 17). Greenhouse ratings were not correlated with the 2004 field season ($P = 0.1169$). When field ratings for the MSL603 individuals were averaged over both years a significant correlation ($r = 0.2503$) was observed with the greenhouse assay ($P = 0.0413$, Fig. 18).

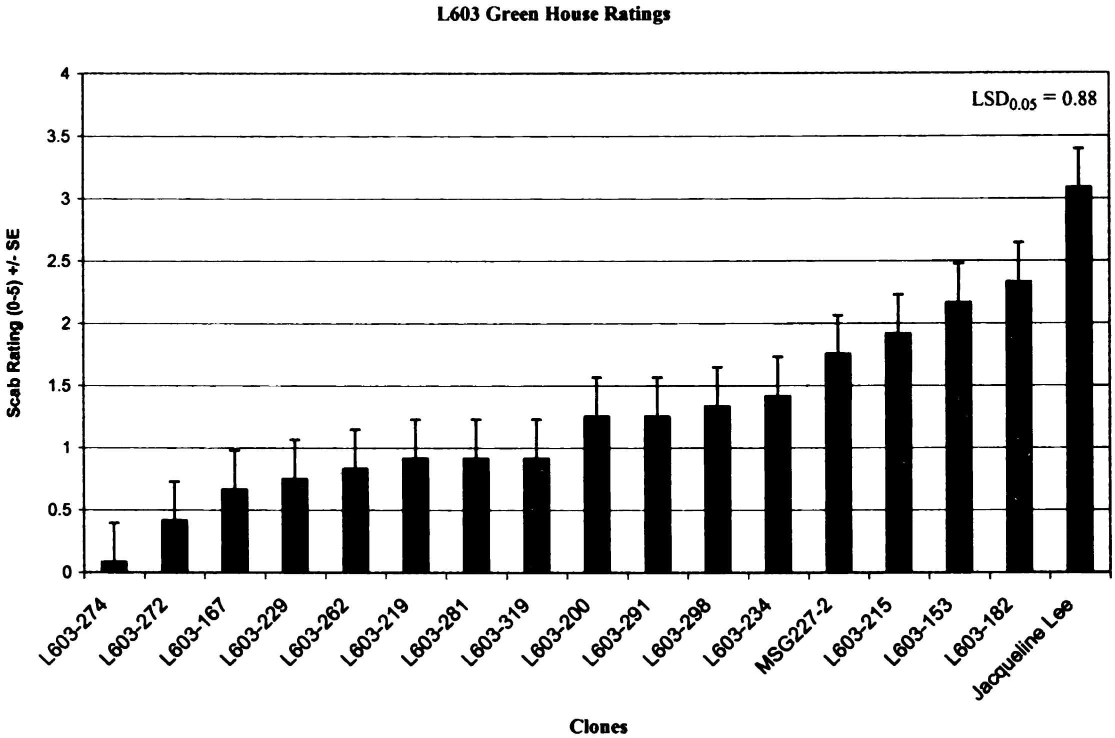

Fig. 16. Ratings for the 15 select MSL603 progeny and parents tested in the greenhouse. All lines were tested in artificially infested soil (3 x 10^8 CFU/cc) and were rated based on percent lesion coverage and the amount of pitting. Ratings were based on a scale of 0 -5 with 0 having no scab and 5 having sever scab. The parents are represented by the darker shaded bars.

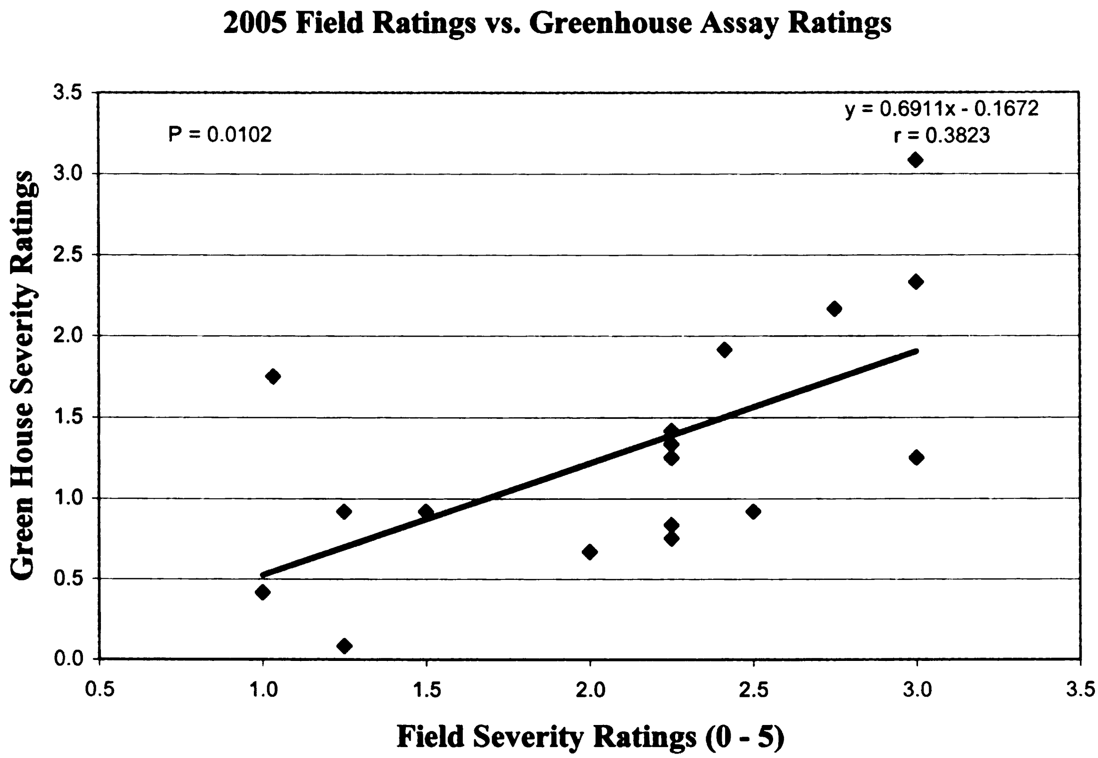

Fig. 17. A moderate correlation between the 2005 field season and greenhouse assayed tubers from the MSL603 mapping population on infested soil (3 x 10^8 CFU/cc). Graph displays the 15 select progeny from the MSL603 population with parents.

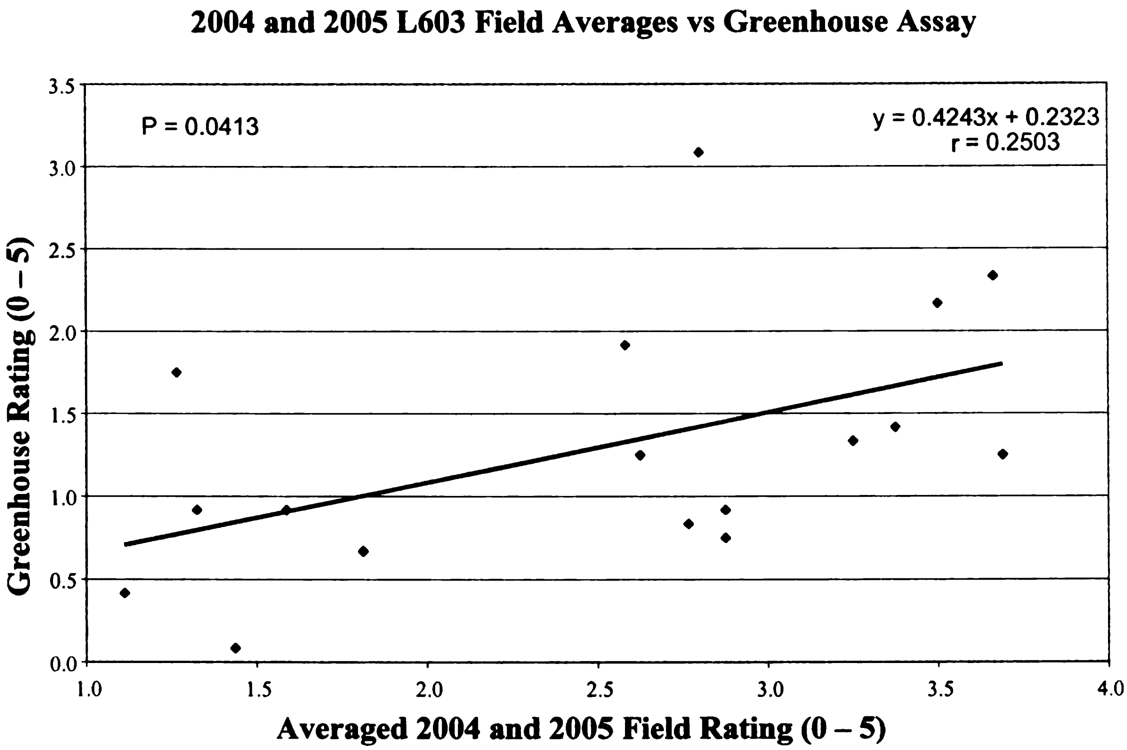

Fig. 18. Graph demonstrating a moderate correlation between averaged scab field ratings for 2004 - 2005 and greenhouse ratings for the 15 select progeny from the MSL603 population and their parents. The greenhouse screen used infested soil with a concentration of 3 x 10^8 CFU/cc.

Table 6. Three resistance classes of the MSL603 progeny for the field seasons 2004 and 2005. Fifteen individuals were selected from the population and divided into three resistance classes. Greenhouse ratings[1] are compared here with 2004, 2005, and their combined field rating average. Parents Jacqueline Lee and MSG227-2 are also included.

	Line	2004	2005	Field Average	Greenhouse
Resistant	167	1.6	2.0	1.8	0.7
	219	1.4	1.3	1.4	0.9
	272	1.2	1.0	1.1	0.4
	274	1.6	1.0	1.3	0.1
	281	1.7	1.5	1.6	0.9
Susceptible	200	3.0	2.3	2.7	1.3
	215	2.4	2.8	2.6	1.9
	229	2.3	3.5	2.9	0.8
	262	2.3	3.3	2.8	0.8
	319	2.5	3.3	2.9	0.9
Susceptible	234	4.5	2.3	3.4	1.4
	291	4.4	3.0	3.7	1.3
	182	4.3	3.0	3.7	2.3
	298	4.3	2.3	3.3	1.3
	153	4.3	2.8	3.6	2.2
Parents	Jacqueline Lee	2.6	3.1	2.9	3.1
	MSG227-2	1.5	1.0	1.3	1.8

[1] Ratings were based on a 0 to 5 scale with 0 = no disease and 5 = most severe symptoms. A rating of 3 or higher signifies susceptibility.

DISCUSSION

Spore Suspension Assays:

Etiolated Tuber Sprouts

Since no scab induced lesions resulted from the etiolated tuber assay, we believe that a closer look should be taken at the set-up of the study. While temperature (28 °C) was optimum for the germination of spores sprayed onto the sprouts, relative humidity was not observed as a factor. This may account for the absence of scab induced lesions, as spores would have dried and not germinated. Further investigations into the etiolated sprout test should take into account relative humidity by covering the container holding the sprouts, while still allowing for passive air flow.

Potato Tuber Discs

Potato tuber discs did not produce scab-like lesions or noticeable signs or symptoms of infection. Relative humidity may have also been a factor in the outcome of this experiment. While PTDs were in Petri dishes they still dried out well before 48 h. It may be possible that residual suberin and/or starch remained on the PTDs even after their wash in sterile distilled water. This would account for a layer of protection against germinating spores from the suspension.

Thaxtomin A

The use of TA as a means for assessing pathogenicity of *S. scabies* with a susceptible clone (Chippewa) has previously been documented (Loria et al., 1995). The authors used a direct application of TA on PTDs and produced lesions quite similar to what has been documented here. One standard TA test compound was used at 1 μg, clearly showing signs of necrosis and crude oatmeal broth extracts containing TA (0.17 μg to 4.25 μg/ ml) also produced necrotic lesions. This is in contrast with what was observed in this study since it was the resistant clones that produced more necrotic activity than the susceptible. Also, MSG227-2 (resistant) behaved statistically similar to Jacqueline Lee (susceptible).

Goyer et al. (1999) used a serial dilution of thaxtomin A at 0 μg, 1 μg, 10 μg, and 100 μg on the cv. Russet Burbank. They found that the severity of necrosis reflected the concentration that was applied to the disc. Observational data was taken on the four test lines with TA concentrations; 100 μg, 10 μg, 1 μg and 0.1 μg (appendix, Fig. 6). This largely corroborates the findings of this TA study especially with the dilutions placed on Russet Burbank, which consistently produced necrotic lesions. It is interesting to note that the *S. scabies* strain (DPZ) used in this study was isolated from a deep pitted lesion on Russet Burbank. The ability of *S. scabies* to produce differential virulence among pathogenic strains has been documented (Loria et al. 1995). Since all lines displayed necrosis at these higher concentrations it was determined that this was not an efficient use of thaxtomin and therefore much lower concentrations should be used.

This study was expanded to evaluate the four test lines (Jacqueline Lee, Liberator, MSG227-2, and Russet Burbank) with known levels of resistance to common scab. Results from the TA PTD assay indicate that there was no correlation between known field resistance and the degree of necrosis observed for either resistant or susceptible lines. Thaxtomin A does not consistently necrotize cultivars with known levels of resistance. Liberator and Russet Burbank are the two most resistant cultivars of the four test lines. Both expressed a more intense necrotic reaction to TA than Jacqueline Lee or MSG227-2 at all concentrations except at the 5×10^{-5} µg concentration. Moreover, a line such as MSG227-2, which has a similar scab rating to Russet Burbank over an eight year period (Table 1), did not develop the level of necrosis that is consistent with the resistant cultivars (Liberator and Russet Burbank). Instead, MSG227-2 is comparable to Jacqueline Lee, the susceptible of the four test lines, in its reaction to TA. From previous field data (Table 1) we would expect to see consistent differences. If TA were able to distinguish resistance from susceptibility, then we would expect the resistant lines to either develop or not develop necrotic lesions depending on level of resistance. We conclude that TA is not useful in discriminating resistant from susceptible potato clones.

It has been documented that thaxtomin A plays a major role in the pathogenicity of *S. scabies* since a positive correlation exists between pathogenicity and ability to produce TA (King et al. 1991). Other factors, aside from TA, are most likely involved in pathogenicity. Relatively little work, as compared to the work that has been accomplished with thaxtomins, have concentrated on degradative enzymes. McQueen and Schottel (1987) showed that a heat stable esterase produced by pathogenic *S. scabies* was able to break down potato suberin.

Although rare, it has also been shown that TA is not required for *S. scabies* to be pathogenic on potato (El-Sayed, 2000). Beausejour and Beaulieu (2004) performed N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis on *S. scabies*. They characterized melanin deficient mutants from wild strain EF – 35 and determined how this deficiency related to TA production. They found that while some mutants had reduced virulence and became nonpathogenic, they were still able to produce TA in oat bran broth. This work suggests that other factors in addition to TA contribute to *S. scabies* pathogenicity.

The work done by Loria et al, (1998) suggests that the *nec1* gene, which produces an extracellular water-soluble compound that induces scab lesions, is likely to be a stonger pathogenicity factor than TA. This gene has been cloned (Bukhalid and Loria 1997) and has been used in transforming a non-pathogenic species (*S. lividans*) to pathogenic. If this gene can be purified and expressed it may be evaluated as a screening tool for resistant clones of potato.

Field assessment of scab: 2004 and 2005

Scab pressure was more severe in 2004 compared to 2005 (Fig. 13). This is evidenced by more progeny with less infection in 2005 and a higher number of progeny with more infection in 2004. In 2004 the scab rating ranged from 1.2 to 4.5, while in 2005, the range was from 0.9 to 3.8. The resistant parent, MSG227-2 and the susceptible parent Jacqueline Lee, had ratings of 1.5 and 2.6 in 2004 and 1.0 and 3.1 in 2005, respectively. The skewd frequency of those individuals in 2005 with a more resistant rating compared to 2004 further corroborates variability in disease severity for the field evaluations between both years (Fig. 15). The correlation between the scab ratings for 2004 and 2005 was very low ($r = 0.09$, $p = <0.0001$) (data not shown). This demonstrates the strong environmental variability associated with these ratings, despite employing an experienced team for visual scab ratings.

Progeny from the MSL603 population were chosen based on their rating data for both field seasons. Using LSDs, three resistance classes were identified; resistant, moderately resistant, and susceptible. Individuals that were within the same resistance class for both years were advanced to the greenhouse assay. Some discretion was given to 2004 when selecting susceptible clones since the most susceptible clone in 2005 was rated a 3.8.

Weather data was observed for two critical months (June and July) during the time of tuberization. Ambient air temperature was cooler in 2004 than in 2005 for June and July. This is less conducive to the incidence of scab. Less precipitation fell for the months of June and July for 2004, which is more conducive to the formation of the disease. Differences in disease pressure between years could be linked to precipitation.

Greenhouse Assay

A greenhouse screen of the four test lines demonstrated that soil inoculum concentrations were too low to attain statistical differences between lines. Higher concentrations were then used on the fifteen select MSL603 progeny. Despite using higher concentrations of inoculum, scab incidence was not great enough to stongly discriminate between resistant and intermediate classes of progeny. Future greenhouse tests should employ higher inoculum concentrations than 3 x 10^8 CFU/cc and to monitor inoculum concentration levels during the onset of tuberization.

The greenhouse test of the 15 select MSL603 progeny produced lesions that were comparable in appearance to those found on field grown tubers. The scab ratings from the progeny of the MSL603 population when tested in the greenhouse, moderately correlated ($r = 0.3823$, $p = 0.0102$) with the 2005 field scab ratings. The greenhouse ratings did not correlate with the 2004 field scab ratings. The averaged field ratings for the 2004 and 2005 field seasons held a weaker correlation ($r = 0.2503$, $p = 0.0413$). The significant correlation between 2005 scab ratings and the greenhouse test may be due to the lower scab inoculum levels in the greenhouse pot test. Keinath and Loria (1991) found that scab severity is positively correlated to inoculum concentrations ranging from 5.5 x 10^2 to 6.8 x 10^5 CFU/g soil-sand under greenhouse conditions. In another study, Loria and Kempter (1986) obtained results with a much lower concentration (3 x 10^6 CFU/ml) of inoculum. They used stem cuttings and seed pieces to assess disease reaction on cultivars Chippewa, Katahdin, and Superior under greenhouse conditions. Although lesions were similar to those found on field grown tubers, they were not able to generate moderate or deeply pitted lesions. Either way, it is clear that the amount of inoculum present for potential infect on potato is confounded by, environmental factors and/or cultivar resistance. Bjor and Roer (1980) found that their inoculated coarse sand greenhouse assay performed more reliably that their field test. They also found that the most resistant varieties retained their resistance across thirty-six different *S. scabies* isolates.

The most resistant progeny (MSL603-274 and MSL603-272) from the MSL603 population behaved similarly when comparing field and greenhouse ratings (Table 5). Transgressive segregants were observed in both field and greenhouse ratings. Of the two years of field data, five MSL603 progeny were more susceptible to common potato scab than Jacqueline Lee, the susceptible parent. When comparing average progeny ratings from the field with Jacqueline Lee data from Table 2 (over an eight year period), 9 clones are more susceptible than the check. When comparing field data for resistant progeny with the resistant parent (MSG227-2), only one clone was more resistant (MSL603-272). It may be worth while to look at other populations segregating for scab resistance to see if resistant transgressive segregants can be indentified.

It is important to identify scab resistant clones in a breeding program. The greenhouse assay is time and labor intensive, therefore, it may be used to test only a limited number of clones. The field evaluation is less resource intensive, but does have environmentally influenced variability, limiting our ability to quickly identify resistant clones. Less resources are needed to run a field test compared to greenhouse scab assay. To take advantage of both screens the breeder could first evaluate a large number of clones in the field and then select only the clones with less infection for further evaluation in the greenhouse. If we look at the MSL603 population (Fig. 15) 38 of the 162 progeny had a scab rating of 1.5 or less. This removed over 75 % of the progeny from further evaluation. The greenhouse test may identify clones that were misclassified as resistant from the lower scab infection group.

CONCLUSIONS

The focus of this study was to find a simple and reliable method for screening potato lines for resistance to common potato scab. Four methods were tested in this study; a greenhouse assay, TA assay, and two spore suspensions: one applied to etiolated tuber sprouts and a second applied to PTDs. Both spore suspension protocols did not produce observable results. No correlation could be found between the necrosis level of PTDs and field resistance for the TA assay. Moreover, the high level of soft rot infection present in both PTD assays (thaxtomin A and spore suspension) indicated that neither method would be effective in identifying resistant individuals. The greenhouse study produced lesions comparable to field grown tubers. A moderate correlation was found between field data and the greenhouse assay using selected progeny from the MSL603 population. The results from this study did not fully eliminate the amount of variability found in traditional field scab tests. The greenhouse results indicate that the average field ratings from both years were always higher than the greenhouse ratings for all progeny, except for the parents.

Further investigation is needed to find a suitable resistance screen to common potato scab. In addition, limiting the amount of variation in disease ratings from an in-vitro experiment compared to scab nursery field trials should be of primary importance, especially since field trials have been the traditional means for rating scab resistance. This will likely be the most challenging part during the evaluation of the resistance screen since water, temperature and clone resistance will remain as key variables during disease progression.

APPENDIX

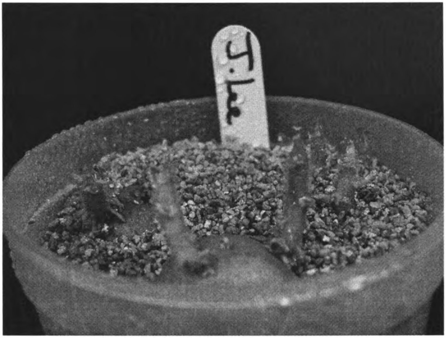

Fig. 19. Day 1 of the spore suspension treatment of tuber sprouts with Jacqueline Lee.

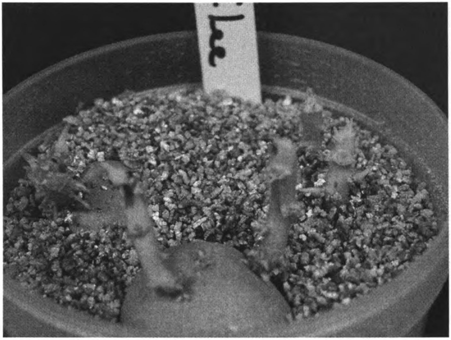

Fig. 20. Day 25 of the treated sprouts of Jacqueline Lee.

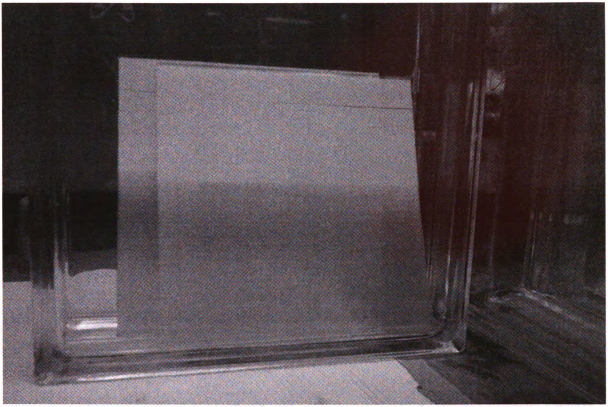

Fig. 21. Purifying thaxtomin on silica gel thin layer chromatography plates as they run in the migration solvents chloroform and methanol.

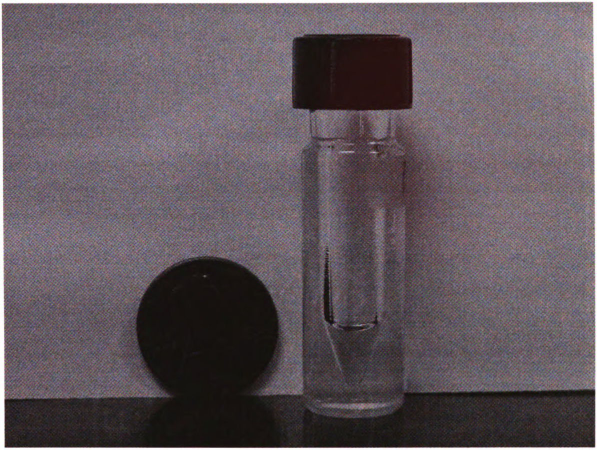

Fig. 22. Purified thaxtomin.

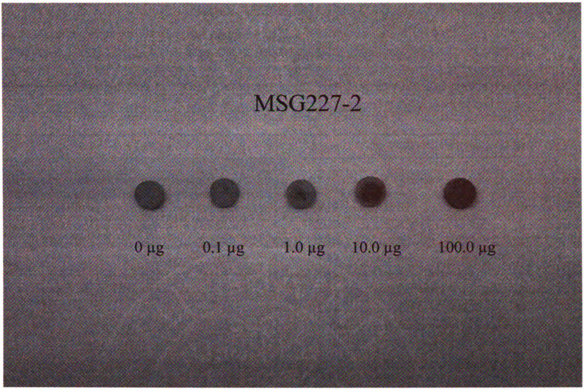

Fig. 23. Observational data taken on MSG227-2 using a direct application of TA on PTDs. Five concentrations were used; 0 µg, 0.1 µg, 1.0 µg, 10.0 µg, and 100.0 µg.

Table 7. Field data from the 2004 field season. Rating, percent coverage, and number of replications (N) were recorded. Data is sorted by parent first and then by rating. The LSDs for scab rating and percent scab coverage is 1.4 and 21.0 respectively. (LSD alpha = 0.05).

LINE	RATING	% Cov.	N	LINE	RATING	% Cov.	N
J. Lee	2.6	16.5	4	204	2.3	11.2	4
MSG227-2	1.5	10.2	4	258	2.3	21.4	4
272	1.2	6.5	4	339	2.3	15.1	4
219	1.4	11.9	4	207	2.4	17.7	5
167	1.6	8.2	4	330	2.5	19.4	1
274	1.6	8.9	4	172	2.5	20.7	4
294	1.6	10.1	4	181	2.5	22.2	4
356B	1.6	12.1	4	184	2.5	17.9	4
355	1.7	11	4	221	2.5	15.9	4
271	1.7	3.9	3	266	2.5	15	4
281	1.7	13.6	4	315	2.5	27.9	4
275	1.7	18.2	4	320	2.5	28.8	4
164	1.8	9.1	4	336	2.5	15.6	4
218	1.8	10.8	4	337	2.5	16.1	4
220	1.8	7.1	4	376	2.5	9.8	4
224	1.8	9.2	4	264	2.5	19.5	5
282	1.8	21.4	4	158	2.5	18.4	3
267	1.9	13.8	1	152	2.6	17.3	4
342	1.9	15.4	4	162	2.6	17.9	3
175	2	10.7	4	227	2.6	18.8	4
276	2	8.7	4	199	2.6	18.5	3
189	2	9.1	4	261	2.7	19.9	4
209	2	8.1	4	335	2.7	19.3	4
236	2	10.7	4	357	2.7	19.1	2
299	2	11.4	4	193	2.7	19.6	3
316	2	9.7	4	368	2.7	17.6	4
362	2	13	4	180	2.7	22.4	4
380	2	6.9	4	235	2.7	37.6	4
332	2	13.5	4	170	2.7	26.6	3
260	2.1	14.8	4	156	2.8	9.6	4
301	2.1	13.7	3	169	2.8	14.7	4
325	2.1	8.7	3	183	2.8	15.9	4
297	2.1	21.8	4	201	2.8	19.1	4
365	2.1	13.1	4	215	2.8	17.3	4
166	2.2	18.2	2	222	2.8	20.2	4
363	2.2	20.1	2	238	2.8	15.1	4
185	2.2	13.3	2	287	2.8	19.8	4
305	2.2	15.1	4	295	2.8	20.7	4
203	2.2	18.1	2	312	2.8	14.5	4
174	2.3	18.5	4	338	2.8	13.3	4

Table 7. Continued.

LINE	RATING	% Cov.	N
341	2.8	13.7	4
346	2.8	23.6	4
263	2.8	25	3
288	2.8	16	4
354	2.8	24.3	4
202	2.8	33.8	2
192	2.8	25	3
217	2.9	17.6	4
359	2.9	22.7	5
283	2.9	28.4	4
151	2.9	25.9	4
159	2.9	26.3	4
160	2.9	17	4
187	2.9	15.8	2
223	3	34.7	4
163	3	20.8	4
179	3	25.8	4
191	3	18.4	4
200	3	21.6	4
277	3	16.1	4
286	3	17.6	4
296	3	20.4	4
308	3	18	4
347	3	32.5	4
351	3	26.2	4
378	3	23.1	4
213	3	21.5	4
161	3	34.4	3
366	3.1	19.6	4
285	3.1	12.5	3
326	3.1	22.5	3
371	3.1	22.3	3
211	3.1	32.9	4
171	3.2	27.1	4
197	3.2	37.4	4
318	3.2	24.6	4
231	3.2	48	2
333	3.2	35.2	2
208	3.2	21.2	5
214	3.2	21.8	4
157	3.2	20.2	2
198	3.3	18.3	4

LINE	RATING	% Cov.	N
212	3.3	28.8	4
226	3.3	27	4
279	3.3	24.4	4
293	3.3	31.7	4
319	3.3	24.6	4
324	3.3	28.8	4
344	3.3	31.4	4
262	3.3	30.3	5
225	3.3	19.9	3
303	3.4	55.5	4
314	3.4	31.9	3
322	3.5	23.9	1
328	3.5	23.7	1
340	3.5	30.1	4
377	3.5	29.8	4
150	3.5	26.1	4
178	3.5	21.8	4
229	3.5	36.5	4
321	3.5	25.6	4
345	3.5	18.6	4
381	3.5	16	4
190	3.5	34.2	4
265	3.5	22.6	4
165	3.6	31.7	4
154	3.7	37.3	4
268	3.7	42.4	4
323	3.7	29.6	4
176	3.8	35.2	4
205	3.8	29.1	4
194	3.8	31.9	3
278	3.8	32.3	3
233	3.8	32.5	4
186	4	38.3	4
372	4	40.1	4
331	4.2	42.9	3
153	4.3	33.6	4
298	4.3	42.6	4
182	4.3	54.5	3
291	4.4	49.1	3
234	4.5	55.6	4

Table 8. Field data from the 2005 field season. Rating, percent coverage, and number of replications (N) were recorded. The LSDs for scab rating and percent scab coverage is 1.1 and 17.5 respectively. (LSD alpha = 0.05).

LINE	RATING	% Cov.	N	LINE	RATING	% Cov.	N
J. Lee	3	17.9	8	337	1.7	7.5	3
MSG227-2	1	8.9	7	163	1.8	13.3	4
266	0.9	2.5	3	169	1.8	11.8	4
265	1	4	4	171	1.8	15.3	4
272	1	4.8	4	172	1.8	11	4
286	1	1.8	4	186	1.8	12.3	4
346	1	5.5	4	192	1.8	19	4
371	1	5	2	198	1.8	24	4
157	1.2	4.6	1	202	1.8	10.3	4
161	1.2	6.5	3	221	1.8	11.3	4
354	1.2	4.8	3	222	1.8	17.3	4
207	1.3	7.3	4	236	1.8	13.5	4
219	1.3	5.3	4	287	1.8	11.5	4
274	1.3	7	4	299	1.8	10.8	4
285	1.3	4.8	4	318	1.8	18.3	4
293	1.3	7.5	4	326	1.8	13.3	4
325	1.3	5.3	4	368	1.8	11	4
355	1.3	5	4	372	1.8	10.3	4
312	1.4	9.1	5	199	1.8	13.7	5
268	1.5	10.5	2	218	1.8	10.1	5
156	1.5	11.5	4	220	1.8	12.9	5
160	1.5	14.5	4	338	1.8	9.5	5
164	1.5	15.8	4	336	2	7	2
176	1.5	6.8	4	341	2	23.5	2
181	1.5	20.3	4	158	2	8.5	4
185	1.5	9.3	4	166	2	12.3	4
191	1.5	8.5	4	167	2	14.8	4
224	1.5	7.8	4	183	2	15.3	4
226	1.5	14.5	4	211	2	15.8	4
264	1.5	11.8	4	217	2	19.5	4
281	1.5	8.8	4	223	2	10	4
295	1.5	9	4	258	2	18	4
317	1.5	10.3	4	260	2	29.3	4
330	1.5	8.8	4	288	2	22	4
332	1.5	15.5	4	297	2	27	4
365	1.5	14.5	4	308	2	12.8	4
376	1.5	6	4	316	2	13.8	4
378	1.5	13.5	4	320	2	23.5	4
204	1.7	18.9	3	331	2	18.3	4
227	1.7	18.8	3	344	2	21	4
278	1.7	14.2	3	347	2	22	4

Table 8. Continued.

LINE	RATING	% Cov.	N
356B	2	13.8	4
359	2	16.5	4
362	2	19.8	4
233	2.1	19.9	5
180	2.1	16.5	3
261	2.1	11.2	3
267	2.1	13.5	3
296	2.1	17.8	3
170	2.2	18.1	5
162	2.2	15.6	1
282	2.2	27.5	3
305	2.2	14.8	3
322	2.2	24.2	3
263	2.2	27.5	2
339	2.2	13.5	2
187	2.3	8.8	4
190	2.3	20.8	4
200	2.3	20	4
203	2.3	18	4
209	2.3	25	4
212	2.3	14	4
225	2.3	23	4
229	2.3	19.5	4
231	2.3	15.5	4
234	2.3	17.5	4
238	2.3	14.8	4
262	2.3	16.3	4
276	2.3	25.3	4
298	2.3	14	4
303	2.3	12.5	4
324	2.3	16	4
357	2.3	14.8	4
342	2.4	15.1	5
294	2.4	20.9	3
380	2.4	20.5	3
184	2.4	13.5	3
215	2.4	15.8	3
315	2.4	16.5	3
151	2.5	30.5	4

LINE	RATING	% Cov.	N
174	2.5	12.8	4
175	2.5	13.8	4
178	2.5	19.8	4
189	2.5	21	4
197	2.5	13.5	4
235	2.5	32.5	4
275	2.5	27.3	4
319	2.5	17	4
335	2.5	28.5	4
366	2.5	17.5	4
283	2.6	26.2	3
208	2.7	16.2	3
153	2.8	18.3	4
165	2.8	15.3	4
194	2.8	19.3	4
201	2.8	26.8	4
205	2.8	22.3	4
321	2.8	26	4
155	2.8	23.5	1
363	2.8	14.5	1
273	3	23.5	2
154	3	27.5	4
182	3	20.8	4
214	3	29.5	4
291	3	42.8	4
193	3.1	26.5	3
159	3.2	20.6	1
150	3.3	33	4
213	3.3	22.3	4
271	3.3	30	4
277	3.3	28.3	4
323	3.3	44.8	4
345	3.3	27.3	4
351	3.3	29.5	4
279	3.5	22	4
381	3.6	30.5	5
314	3.8	29.5	4
340	3.8	30	4
377	3.8	44.8	4

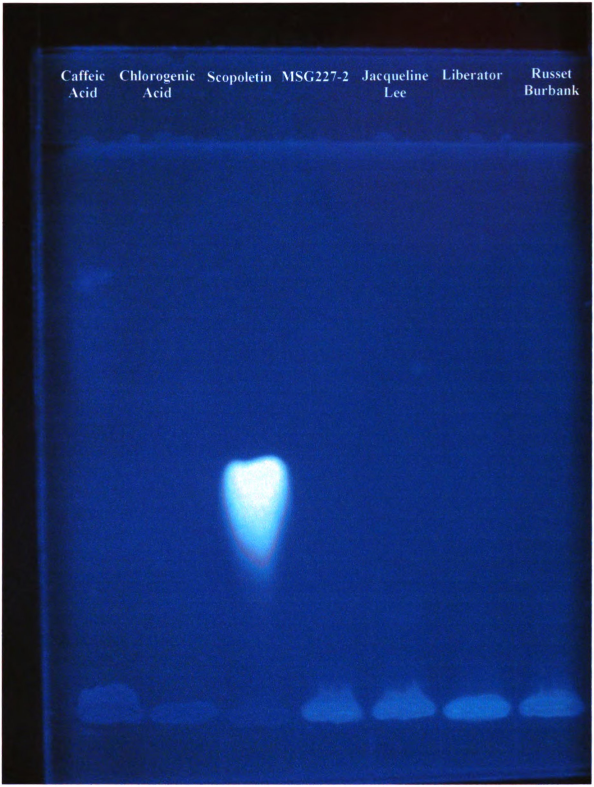

Fig. 24. Terpenoid analysis on thin layer chromatography. Figure demonstrates the four control PTD test lines assayed after 48 h under ultraviolet light (302 nm).

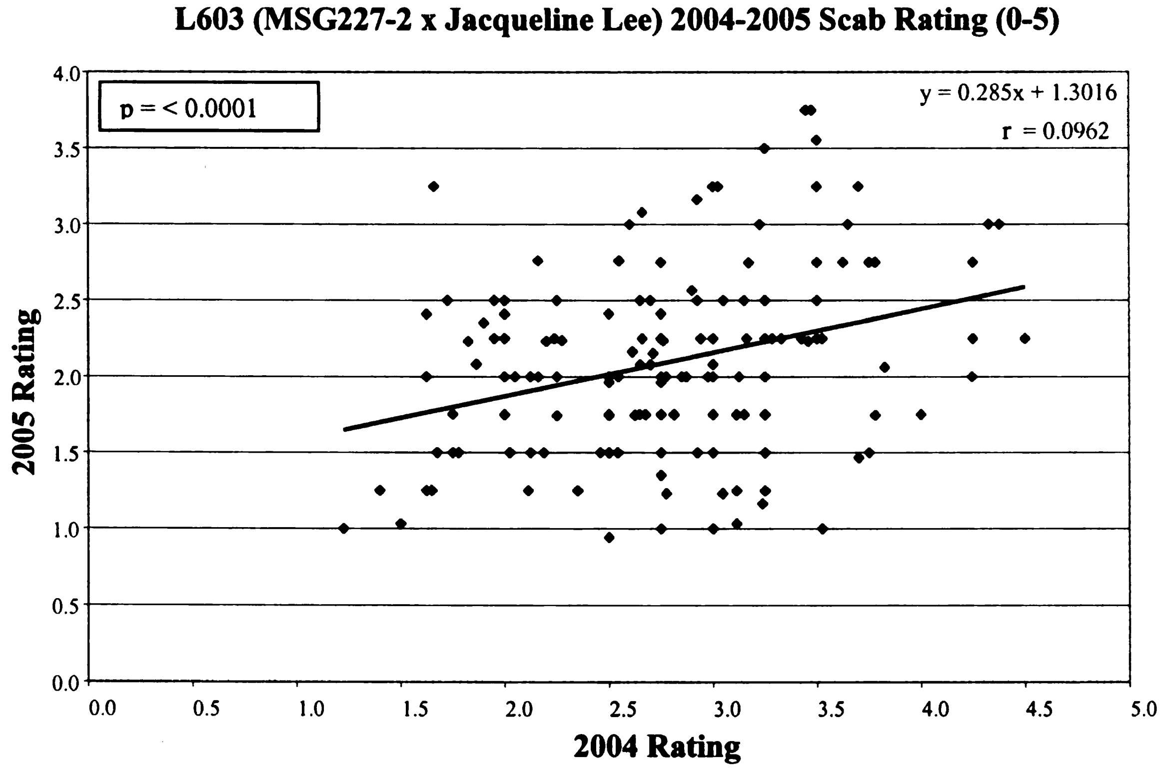

Fig. 25. Weak correlation for ratings between field seasons 2004 and 2005.

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