INHIBITION OF *OPHIOGNOMONIA CLAVIGIGNENTI-JUGLANDACEARUM* BY *JUGLANS* SPECIES BARK EXTRACTS

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Abstract.—A rapid and reliable screening technique is needed for selecting trees with resistance to butternut canker. In a laboratory assay, reagent grade naphthoquinones and crude bark extracts of *Juglans* species variously inhibited spore germination and growth of *Ophiognomonia clavigignenti-juglandacearum*, the causal fungus of butternut canker. The in vitro disc assay revealed that the level of inhibition varied by naphthoquinones and by extracts from different species of *Juglans* and selections of butternut. Ranking the trees by the level of inhibition approximated their level of resistance observed in past assays based on challenging the trees with the fungus through wounds and their response to natural infection in the field. Butternut is known to produce naphthoquinone compounds with antimicrobial activity. These compounds, if produced at different concentrations, may account for the observed range of inhibition levels in the assay and variation in canker resistance among selections of butternut in the field. This assay may have potential use for selecting butternut with disease resistance for conservation and restoration purposes.

Concern over the rapid loss of butternut (*Juglans cinerea*) to butternut canker caused by *Ophiognomonia clavigignenti-juglandacearum* (OCJ) (=*Sirococcus clavigignenti-juglandacearum*) (Broders and Boland 2011) has increased since the disease was first reported in 1967. Investigators in the United States and Canada are examining the potential conservation of individual trees that may have resistance to the disease (Schlarbaum et al. 2004). Occasionally, one to several healthy butternut trees are found growing among groups of similarly aged diseased and dying butternut, and it has been speculated that these trees may have resistance to the disease and value for breeding and restoration of the species (Ostry and Woeste 2004).

Differences in susceptibility to OCJ among *Juglans* species and selected butternuts have been demonstrated using artificial wound inoculation tests (Orchard et al. 1982; Ostry and Moore 2007, 2008). Among the species tested, heartnut (*Juglans ailantifolia* var. *cordiformis*) and black walnut (*J. nigra*) were among the least susceptible and Persian walnut (*Juglans regia*) was the most susceptible. Inoculations of putative resistant butternuts found significant differences between accession, month of inoculation, and fungal isolate (Ostry and Moore 2008). Resistance mechanisms among different *Juglans* species have been only minimally explored. It has widely been observed that butternut x heartnut hybrids, often referred to as "buarts," are more resistant to canker than pure butternuts (Hoban et al. 2009). One hypothesis is that the thicker periderm of heartnut provides resistance against the fungus, and the high phenolic production of black walnut confers disease resistance to that species (Nair 1999).

The capability of plants to produce chemical substances involved in resistance to pathogens has been extensively studied. Phenolics such as salicylic acid are well known as signal molecules for both the hypersensitive response and systemic acquired

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resistance (Klessig and Malamy 1994). Disease resistance may be correlated with an increase in these and other substances, and chemical assays for detecting disease resistance have been developed. Baiocchi et al. (1994) found varying levels of phenolics among poplars displaying different levels of resistance to *Discosporium populeum*. Bucciarelli et al. (1999) found that aspen phenotypes resistant to *Entoleuca mammata* (=*Hypoxylon mammatum*) produced wound callus rich in phenolics that was absent in the susceptible phenotypes. Reservatrol production has been investigated as a possible indicator of resistance in grapevines to *Plasmopara viticola* and *Botrytis cinerea* (Barlass et al. 1987, Jeandet et al. 1992). Gao and Shain (1995) found that different levels of a polygalacturonase inhibitor in American and Chinese chestnut explained the difference in levels of resistance of these species to *Cryphonectria parasitica*, the cause of chestnut blight.

Evidence indicates that there are substances in butternut bark that have substantial fungicidal and antimicrobial properties. Butternut bark extracts were the most antagonistic and had the broadest spectrum of activity of the tree species tested against several human pathogenic bacteria (Omar et al. 2000) and fungi (Ficker et al. 2003).

It is generally established that *Juglans* species contain a number of structurally related, double-ring compounds called naphthoquinones. Many naphthoquinones have been found to inhibit the growth of plant pathogens. Several naphthoquinones known to be present in walnut husks including 1,4-naphthoquinone, juglone, menadione, and plumbagin were found effective against *Aspergillis flavius* (Mahoney et al. 2000). Naphthoquinones also inhibited the growth of several human pathogenic bacteria (Park et al. 2005, 2006).

The most predominant and most thoroughly studied naphthoquinone is juglone. It has long been observed that walnut trees are detrimental to the growth of certain plants such as alfalfa, apples, and tomatoes grown in close proximity. Root exudates were implicated and the substance was found to be juglone (Davis 1928, Massey 1925). It is responsible for the allelopathic effect of black walnut and butternut, and is present in the roots, leaves, fruit hulls, and bark of both species (Heimann and Stevenson 1997). Pure juglone and crude extract from green walnut hulls have been found inhibitory against a wide range of microorganisms including bacteria, filamentous bacteria, algae, and dermaphytes (Krajci and Lynch 1978). Juglone was an effective inhibitor of *Botrytis cinerea*, *Cladosporium herbarum*, and *Fusarium avenaceum* (Hadacek and Greger 2000). Inhibition of the growth of the wood-rotting fungus *Pleurotis sajor-caju* (Curreli et al. 2001) and the pecan scab fungus, *Fusicladium effusum* (Windham and Graves 1981) has also been demonstrated. It has been suggested that, compared to pecan, black walnut's high levels of juglone may be responsible for its greater resistance to scab (Hedin et al. 1979).

In a study comparing both leaf pathogens and non-pathogens of black walnut, juglone was more effective against the non-pathogens (*Gnomonia quercina*, *G. platani*, and *Sclerotinia sclerotiorum*) and one pathogen (*Cristulariella moricola*) than against the other two pathogens, *Cylindrosporium juglandis* and *Gnomonia leptostyla* (Cline and Neely 1984). This finding may indicate tolerance to juglone among some *Juglans* pathogens and higher concentrations of juglone are required to inhibit their growth. In that study the juglone concentration in leaves was dependent on leaf age; young leaves had a higher juglone concentration and were more resistant to anthracnose fungi than older leaves.

The objective of this study was to test reagent grade naphthoquinones and crude bark extracts of *Juglans* species and a *Juglans* hybrid for their effects against OCJ using a disc diffusion assay. Bark variables examined included month of collection and tissue age. We tested the hypothesis that *Juglans* species and selections contain various concentrations of juglone and/or related naphthoquinones, and that these

compounds influence resistance to OCJ. The purpose of this work was to examine the potential use of this assay to select butternut with resistance to butternut canker.

MATERIALS AND METHODS

Disc Diffusion Assay with Reagent Grade Naphthoquinones

A preliminary disc assay was conducted using several related naphthoquinones including juglone, 1,4-naphthoquinone, plumbagin, menadione, and lawsone obtained from Sigma-Aldrich (St. Louis, MO). The naphthoquinones were dissolved in 95 percent ethanol and applied to sterile 6.5-mm-diameter cellulose discs at a rate of 0, 5, 10, 20, 50, and 100 µg per disc. The assay procedure used was similar to the standard antibiotic sensitivity test (Barry 1964). To each petri plate containing malt agar, a spore suspension equivalent to 4 x 10^5 OCJ spores per plate was added and spread over the surface evenly using a sterile, bent plastic rod. Eight discs of naphthoquinone were tested for each treatment level. Plates were placed in the dark and incubated at 20 °C. After incubation for 72 hours, the fungal growth was clearly visible on the plates as a solid lawn, except for a clear inhibition zone around the discs. The diameter of each of these inhibition zones (including the disc) was measured, and samples with no inhibition were recorded as 6.5 mm, the diameter of the disc. The experiment was repeated once.

Plant Material

All bark samples were collected from a plantation near Rosemount, MN, consisting of 10- to 12-year-old trees. Species included *J. cinerea*, *J. nigra*, *J. ailantifolia* var. *cordiformis*, and the hybrid *J. cinerea* x *J. ailantifolia*. These trees included both non-selected, seed-propagated butternut of unknown origin and grafted trees selected for possible disease resistance (Table 1). Bark samples were collected monthly from April through October. A minimum of three 30-cm lengths (0.5 to 2.5 cm diameter) of 4- to 6-year-old branches per tree were collected each month.

Bark Extraction

In 2006, branches were divided by bark age: current year, 1- to 2-year-old, and 3- to 4-year-old bark. Outer (green layer) bark was discarded and only the inner, fibrous bark was used. Current year bark was collected starting in June. The following extraction procedure according to Omar et al. (2000) was used. Bark was air dried and ground in a Wiley mill to a fineness of a 20 mesh screen (0.8 mm). For the extraction, the bark powder was soaked in 95 percent ethanol at a rate of 3 g per 15 ml for 48 hours, and the resultant extractives were filtered and air dried. A total of 190 extract samples from 10 trees (Table 1) were prepared and stored at -20 °C. Extracts were prepared once and two experiments were performed on samples of the same extract.

In 2010, the inner bark of 1- to 6-year-old branches was used. The extraction procedure was modified somewhat to reduce heating and oxidative processes during grinding and to increase yield of extractives. Bark was ground with dry ice and stored at -70 °C. The extraction process was begun by mixing 1 g of bark powder in 10 ml of cold (-20 °C) 95 percent ethanol and soaking the mixture overnight. Mixtures

Table 1.—Source trees for bark extracts, Rosemount, MN.

Accession	Species/Selection	Year tested 2006	2010
NB03	Non-selected *J. cinerea*[a]	X	X
NB04	Non-selected *J. cinerea*	X	X
NB10	Non-selected *J. cinerea*	X	X
NB11	Non-selected *J. cinerea*	X	X
NB16	Non-selected *J. cinerea*	X	X
SB01	Selected *J. cinerea*[b]		X
SB20	Selected *J. cinerea*		X
SB22	Selected *J. cinerea*	X	X
SB54	Selected *J. cinerea*		X
SB60	Selected *J. cinerea*		X
SB67	Selected *J. cinerea*		X
SB148	Selected *J. cinerea*	X	X
XX128	*J cinerea* x *J. ailantifolia*[b]	X	X
HN133	*J. ailantifolia* var *cordiformis*[b]	X	X
WA01	*J. nigra*[a]	X	X

[a]Open-pollinated seed origin.

[a]Grafted orchard trees, source tree selected for potential resistance to *Ophiognomonia clavigignenti-juglandacearum*.

were then agitated at room temperature for 24 hours, centrifuged, and the supernatant removed. Two successive extractions of 10 ml of 95 percent ethanol each were performed on the same bark powder and added to the original aliquot for a total of 30 ml of combined extract. Extracts were dried under vacuum until near dryness, and then air-dried to a tarry consistency. A total of 105 samples from 15 trees (Table 1) were prepared once and stored at -70 °C. Three experiments were performed with each extract sample.

Fungal Cultures

Cultures of OCJ isolated from butternut cankers collected in Wisconsin and Minnesota (Table 2) were grown on malt agar petri plates at 20 °C in the dark until sporulation occurred (30 days). Sporulating cultures were flooded with sterile water and rubbed lightly with a sterile, bent plastic rod to dislodge spores. Spore concentration was adjusted using a hemacytometer. Spores from two separate isolates were used in 2006 and mixed spores from four isolates were used in the 2010 experiments.

Bark Extract Disc Diffusion Assay

Bark extracts were resuspended in 95 percent ethanol and applied to sterile 6.5 mm cellulose discs at a rate of 2 mg per disc, then air dried. Ethanol controls were also prepared. To each malt agar petri plate, a spore suspension equivalent to 4 x 10^5 OCJ spores per plate was added and spread over the surface evenly using a sterile, bent plastic rod. Four discs of each extract were placed equidistant on each malt agar plate, two plates for each combination of month, tree accession and isolate. Plates were incubated at 20 °C in the dark. After 72 hours, the diameter of inhibition zones was measured. The experiment was repeated once in 2006 and twice in 2010.

Table 2.—*Ophiognomonia clavigignenti-juglandacearum* isolates used in assays.

Isolate	Origin	Year used 2006[a]	Year used 2010[b]
1344	Forest Co., WI	X	
1347	Kanabec Co., MN	X	
1384	Goodhue Co., MN		X
1385	Ramsey Co., MN		X
1387	Langlade Co., WI		X
1388	Forest Co., WI		X

[a]Isolates were used separately.
[b]Isolates were mixed.

Statistical Analyses

The 3-day inhibition zone measurements were subjected to analysis by means and standard errors, one-way ANOVA and via PROC MIXED (Enterprise Guide 4.2, SAS Institute, Cary, NC). Tree accession was included as the fixed effect and month of bark harvest, experiment, and isolate (for the 2006 data) were included as random effects; least square means separation tests were conducted using a Tukey-Kramer procedure with a significance level of 0.05.

RESULTS

Disc Diffusion Assay with Reagent Grade Naphthoquinones

The assay revealed that OCJ spore germination and growth was inhibited to varying degrees by the reagent grade naphthoquinones (Fig. 1). Menadione, 1, 4-naphthoquinone, and plumbagin were highly effective against OCJ. Juglone was also inhibitory, but to a lesser extent. Lawsone was minimally inhibitory and the ethanol controls exhibited no inhibition of spore germination or fungal growth.

Bark Extract Disc Diffusion Assay

In 2006 bark extracts from the current year branch growth had a significantly ($p < 0.0001$) weaker inhibitory effect than older bark, with the inhibitory effect of extracts from 1- to 2-year-old bark nearly identical to extracts of the 3- to 4-year-old bark (Fig. 2). The level of inhibition in the first experiment was greater than in the second experiment ($p < 0.0001$), presumably because of degradation of the compounds in storage. However, the difference had no effect on the ranking of the accessions in the experiments. The level of inhibition varied by OCJ isolate with isolate 1347 being inhibited less than isolate 1344 ($p < 0.0001$) (Fig. 2). Isolate, however, had no effect on the ranking of the accessions (data not shown).

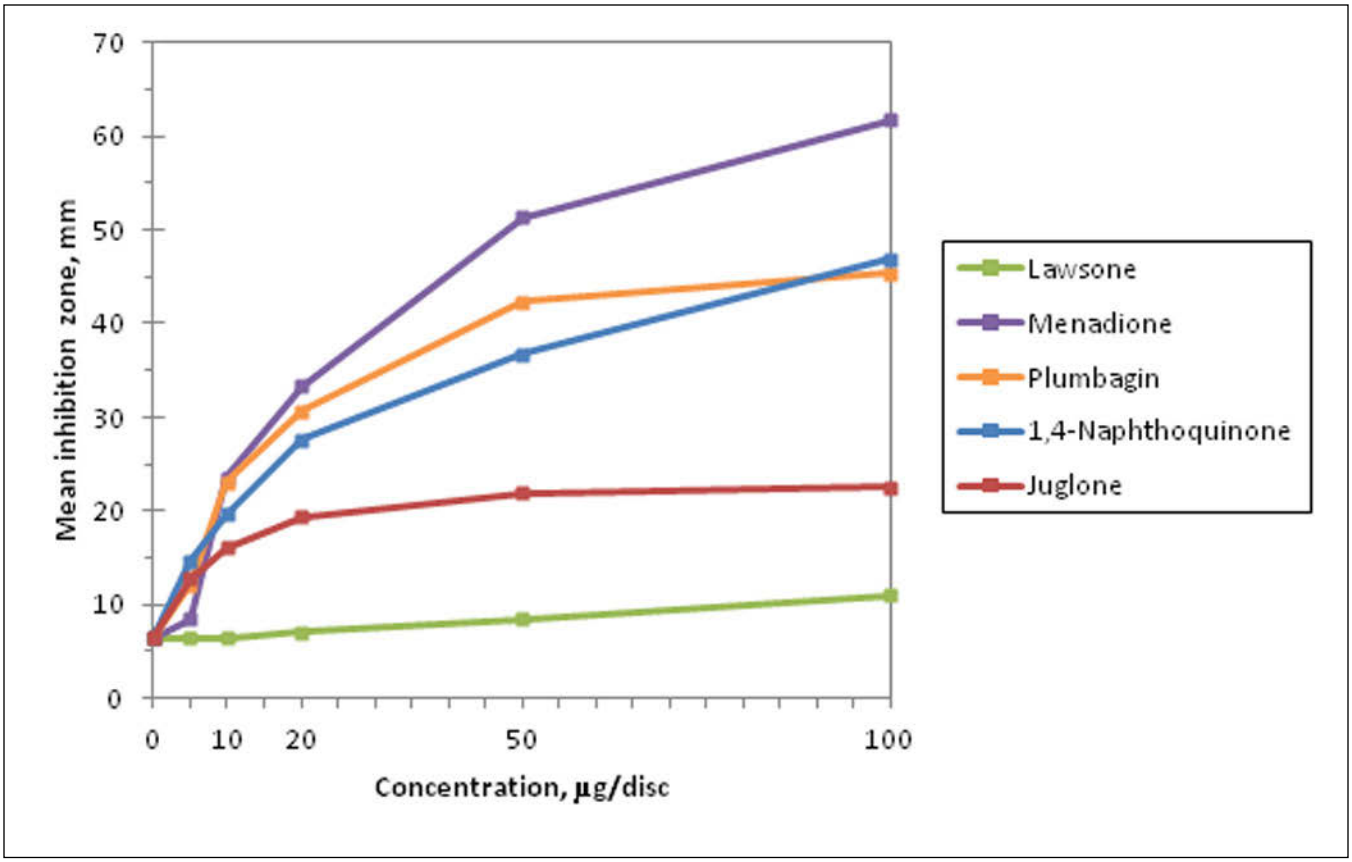

Figure 1.—Inhibition of *Ophiognomonia clavigignenti-juglandacearum* by reagent grade naphthoquinones. Data points are means of two experiments, n=16, SE=0.725.

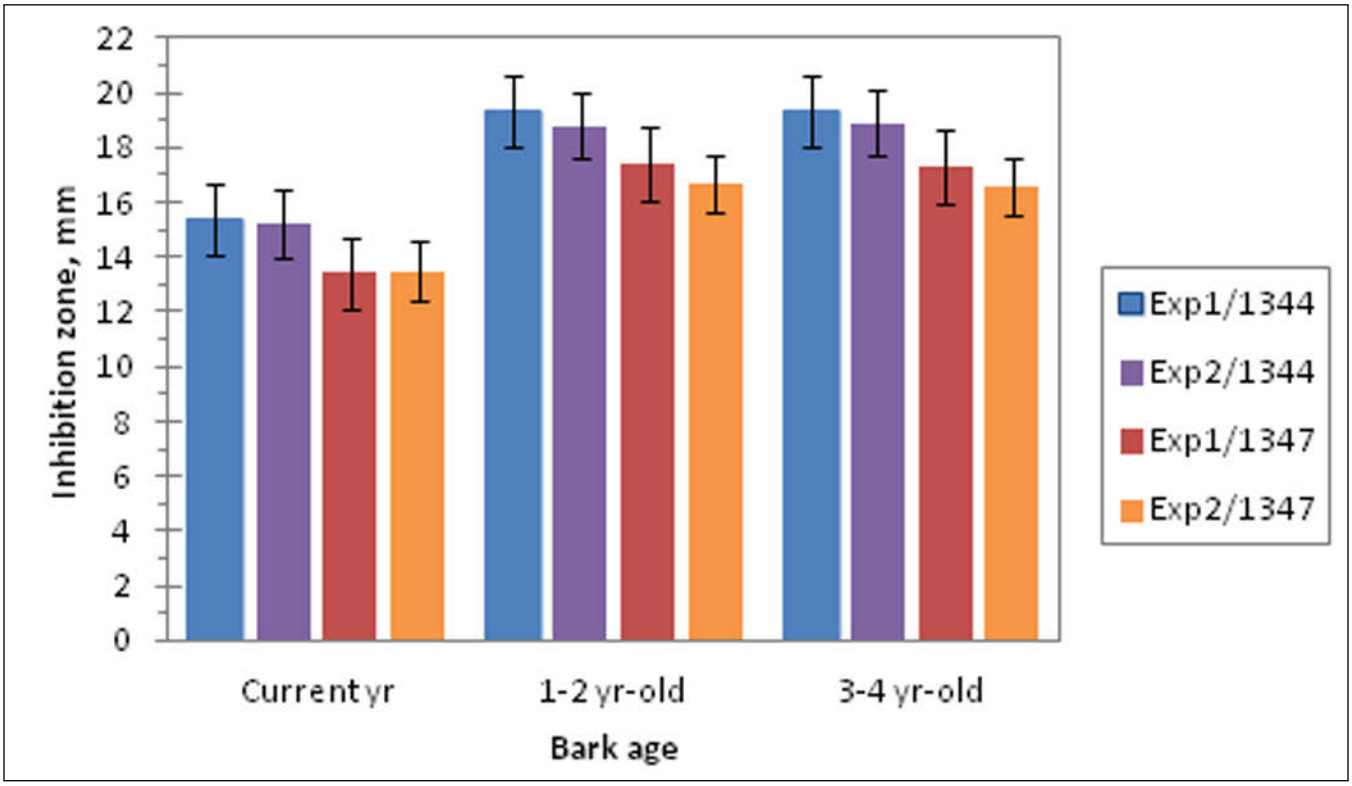

Figure 2.—Bark extract inhibition of *Ophiognomonia clavigignenti-juglandacearum* comparing bark age and isolate, April-October, 2006. Data from all collection months and accessions combined.

There was a difference ($p < 0.0001$) in inhibition by month of bark collection (Fig. 3). In 2006 inhibition by extracts peaked in May. Inhibition reached another peak in August and September. The level of inhibition using extracts from bark collections in August and September were not significantly different from each other. In 2010 there was no peak in inhibition in May, however the later trend was similar to 2006, with the inhibitory activity peaking with late summer and early fall bark collections (Fig. 3). Although there was a difference ($p < 0.0001$) between the three experiments in 2010, the ranking of the accessions in terms of the size of the inhibition zone in each experiment was similar (Fig. 4).

The inhibitory effect of bark extracts varied by *Juglans* species and accession (Table 3, Fig. 5). Most of the nonselected butternut lines (NB) ranked lower than the selected, putative resistant lines (SB). For example, inhibition by extracts of NB16 and NB03 consistently ranked among the lowest in both years, while inhibition of bark extract from SB22 was greater than all other accessions in 2006 and continued to rank among the highest in 2010. Extracts from the hybrid consistently ranked high in inhibition, and the walnut and the heartnut were somewhat variable, ranking moderate to low.

In 2010 the differences in rankings of the accessions, species and the hybrid was most clearly evident using the extracts from the August bark collection (Fig. 5). While bark extracts from all accessions yielded peak inhibition in late summer or early fall, the inhibition of extracts from the selected butternuts peaked earlier than the non-selected butternuts.

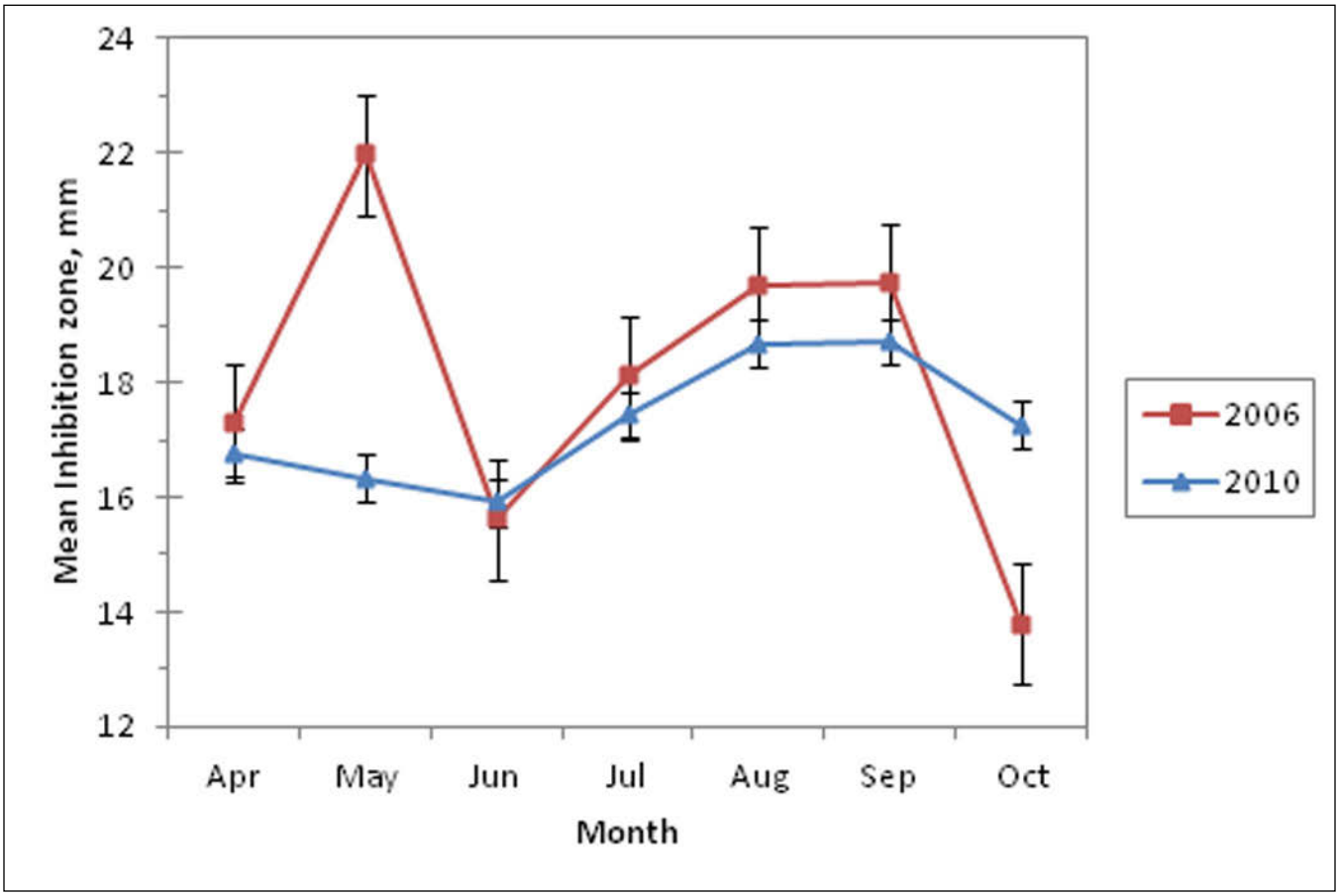

Figure 3.—Inhibition of *Ophiognomonia clavigignenti-juglandacearum* comparing experiment year and month of bark collection. Data from all accessions combined. The experiment was repeated once in 2006 and twice in 2010.

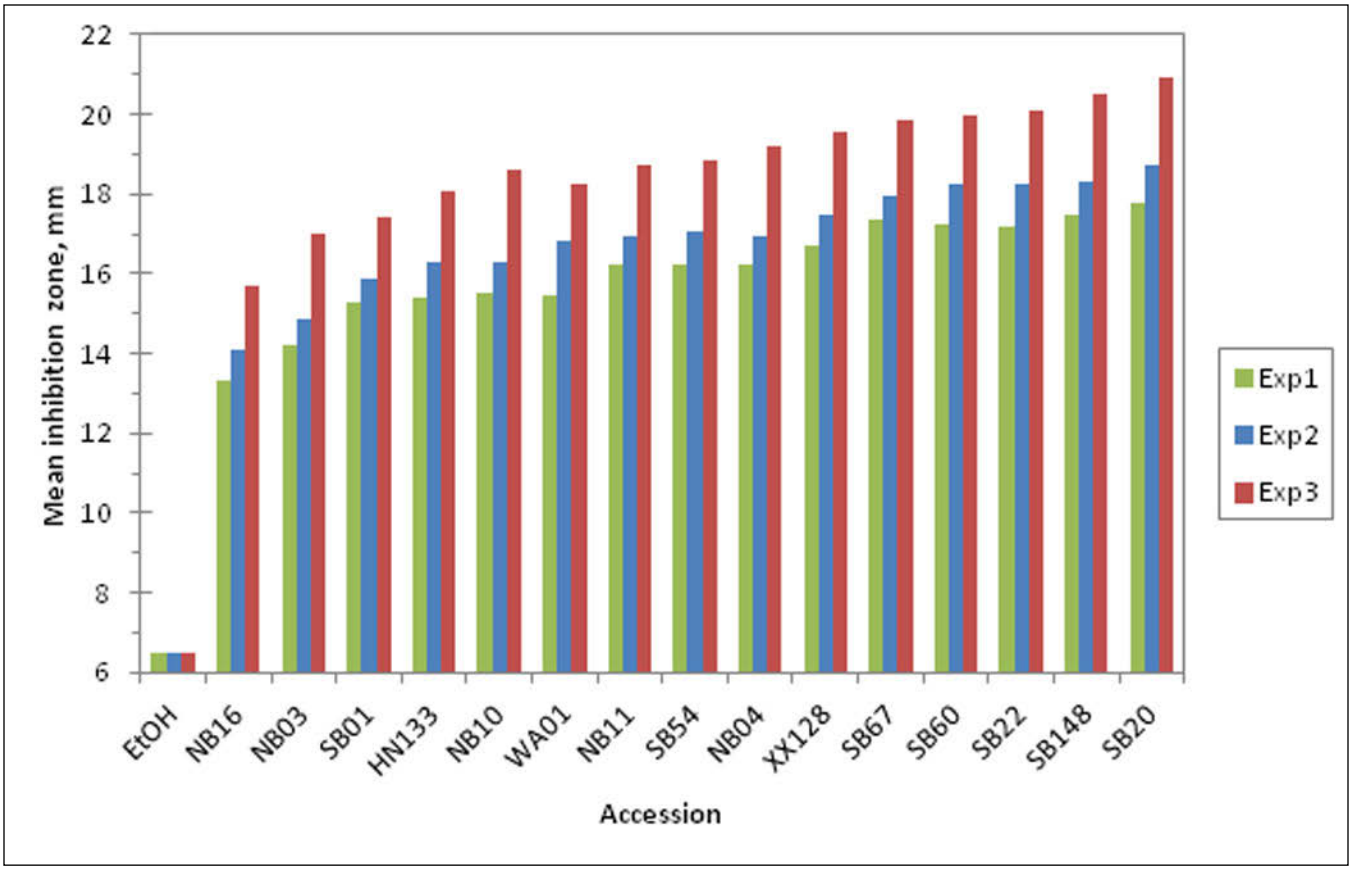

Figure 4.—Inhibition of *Ophiognomonia clavigignenti-juglandacearum* comparing tree accession and experiments in 2010. NB=Nonselected butternut, HN=heartnut, WA=walnut, XX=hybrid, SB=selected butternut, EtOH=ethanol control.

Table 3.—Bark extract inhibition of *Ophiognomonia clavigignenti-juglandacearum*.

2006[a]				2010[b]			
	Inhibition zone, mm				Inhibition zone, mm		
Accession	Mean[c]	Range	SE	Accession	Mean[c]	Range	SE
NB16	14 a	10.5-18	0.208	NB16	16 a	13-19	0.223
WA01	18 b	13.5-22	0.209	NB03	16 a	14-20	0.221
NB03	18 b	13.5-23	0.217	NB10	17 ab	13-20	0.250
NB04	20 c	15-26	0.233	HN133	18 bc	15-21	0.199
HN133	20 c	16-26	0.201	NB04	18 c	13-22	0.389
SB148	21 c	16-25	0.215	SB01	18 c	14-23	0.311
NB10	21 cd	15-28	0.287	NB11	18 c	14.5-21	0.288
NB11	21 de	16.5-28	0.237	WA01	19 cd	16-22	0.239
XX128	21 e	17-29	0.252	SB54	19 cd	16-22	0.212
SB22	23 f	18-29	0.247	SB148	20 de	17-23	0.234
				SB22	20 ef	17-23	0.202
				XX128	20 ef	17-23	0.211
				SB60	20 ef	16.5-25	0.364
				SB67	21 ef	17-26	0.334
				SB20	21 f	17-24	0.241

[a]Data for August/September collection, 1- to 4-year-old bark, 2 isolates and 2 experiments combined, n (number of discs) = 128/accession.
[b]Data from August/September collection, 3 experiments combined, n (number of discs) = 48/accession.
[c]Values with the same letter do not differ significantly according to Tukey-Kramer's least squares means test ($p<0.05$).

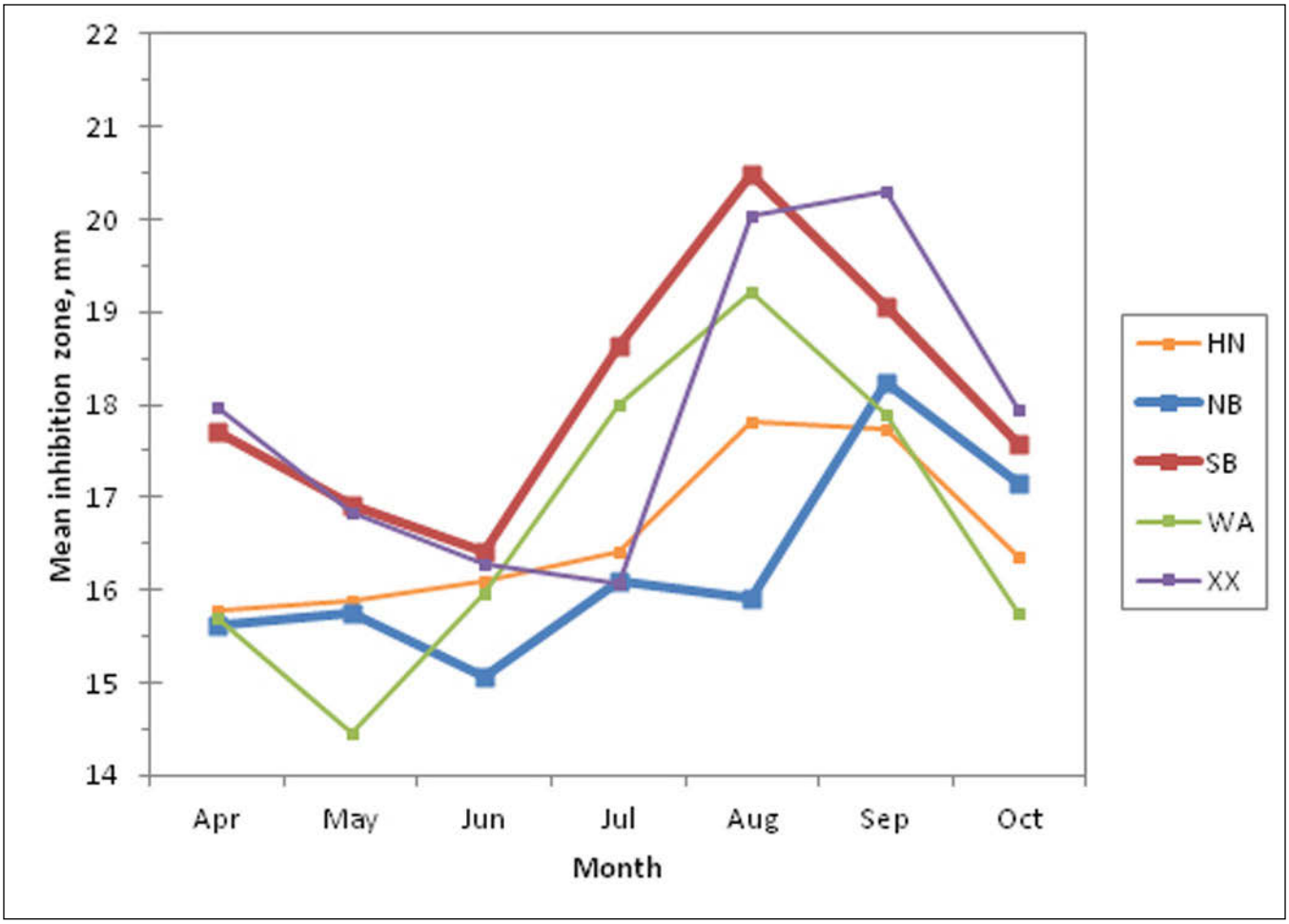

Figure 5.—Mean inhibition of *Ophiognomonia clavigignenti-juglandacearum* comparing month of bark collection and species, 2010. HN=heartnut, NB=Nonselected butternut, SB=selected butternut, WA=walnut, XX=hybrid.

DISCUSSION

Restoration of butternut will require a reliable procedure to select trees that have resistance to butternut canker. Some success has been reported by investigators challenging trees directly with the pathogen in common garden orchards. However, propagating candidate trees, establishing orchards and testing trees in this manner is time and cost prohibitive in most cases. A rapid, repeatable test that distinguishes highly disease resistant trees from susceptible trees is needed.

The preliminary work reported in this paper offers an encouraging approach to screen butternut for canker resistance using crude bark extracts against the pathogen employing a rapid in vitro assay. We demonstrated that naphthoquinone compounds known to be produced in *Juglans* species are inhibitory to spore germination and growth of OCJ.

A range in the size of the inhibitory zones resulting from bark extracts of different *Juglans* species and butternut selections suggests that different inhibitory compounds or quantities of these compounds produced by individual trees may be responsible for the level of fungal growth inhibition. It remains to be determined whether these compounds play a role in resistance to the fungus in nature that could explain the observed variation in disease severity among butternut.

The highest level of inhibition was obtained using extracts from bark collected in late summer and fall. Fall was also the period of greatest separation of susceptible and resistant butternut selections based on artificial inoculations of trees with the fungus (Ostry and Moore 2008). This suggests that this may be a key period for butternut trees to produce active defense compounds. We are investigating the temporal changes in the level of inhibition of bark extracts among the known resistant and susceptible butternut selections to determine if a late summer-fall assay of bark extracts could be used to differentiate levels of compounds among trees in order to select trees that may be resistant to the canker disease.

Results of the disc assay produced results similar to screening 7- to 11-year-old trees in the field by introducing the fungus into wounds (Ostry and Moore 2008). We obtained a range of reactions among unknown butternut selections and those selected for putative resistance to OCJ. Butternut selections known to be disease-free or nearly so in the field (SB accessions) generally ranked higher than nonselected (NB) accessions based on the size of the inhibition zones in the laboratory assay.

We are currently working with Dr. Adrian Hegeman in the Department of Horticultural Science at the University of Minnesota using metabolomics (analytical measurement of secondary metabolites) to identify the active compounds in the crude extracts used in the assay reported in this paper. Juglone and plumbagin have been positively identified in the extracts. Bark extracts from additional trees in our butternut archive collection will be used in future assays and results compared to previous canker resistance screening tests of these trees and to the original source tree health in the field to validate the utility of this assay.

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LITERATURE CITED

Baiocchi, C.; Marengo, E.; Roggero, M.A.; Giacosa, D.; Vietto, L.; Toccori, S. 1994. **A chromatographic and chemometric study of the bark phenolic compounds of two poplar clones with different resistance to *Discosporium populeum*.** Chromatographia. 39: 482-489.

Barlass, M.; Miller, R.M.; Douglas, T.J. 1987. **Development of methods for screening grapevines for resistance to infection by downy mildew. II. Reservatrol production.** American Journal of Enology and Viticulture. 38: 65-68.

Barry, A.L. 1964. **The routine antibiotic disc-plate sensitivity tests. I. Variations in the size of inoculum.** American Journal of Medical Technology. May-June: 153-161.

Broders, K.D.; Boland, G.J. 2011. **Reclassification of the butternut canker fungus, *Sirococcus clavigignenti-juglandacearum*, into the genus *Ophiognomonia*.** Fungal Biology. 115: 70-79.

Bucciarelli, B.; Ostry, M.E.; Fulcher, R.G.; Anderson, N.A.; Vance, C.P. 1999. **Histochemical and microspectrophotometric analyses of early wound responses of resistant and susceptible *Populus tremuloides* inoculated with *Entoleuca mammata* (*Hypoxylon mammatum*).** Canadian Journal of Botany. 77: 548-555.

Cline, S.; Neely, D. 1984. **Relationship between juvenile-leaf resistance to anthracnose and the presence of juglone and hydrojuglone glucoside in black walnut.** Phytopathology. 74: 185-188.

Curreli, N.; Sollai, F.; Massa, L.; Comandini, O.; Rufo, A.; Sanjust, E.; Rinaldi, A.; Rinaldi, A.C. 2001. **Effect of plant-derived naphthoquinones on the growth of *Pleurotus sajor-caju* and degradation of the compounds by fungal cultures.** Journal of Basic Microbiology. 41: 253-259.

Davis, E.F. 1928. **The toxic principle of *Juglans nigra* as identified with synthetic juglone, and its toxic effects on tomato and alfalfa plants.** American Journal of Botany. 15: 620.

Ficker, C.E.; Arnason, J.T.; Vindas, P.S.; Alvarez, L.P.; Akpagena, K.; Gbeassor, M.; De Souza, C.; Smith, M.L. 2003. **Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts.** Mycoses. 46: 29-37.

Gao, S.; Shain, L. 1995. **Activity of polygalacturonase produced by *Chryphonectria parasitica* in chestnut bark and its inhibition by extracts from American and Chinese chestnut.** Physiological and Molecular Plant Pathology. 46: 199-213.

Hadacek, F.; Greger, H. 2000. **Testing of antifungal natural products: methodologies, comparability of results and assay choice.** Phytochemical Analysis. 11: 137-147.

Hedin, P.A.; Langhans, V.E.; Graves, C.H., Jr. 1979. **Identification of juglone in pecan as a possible factor of resistance to *Fusicladium effusum*.** Journal of Agricultural Food Chemistry. 27: 92-94.

Heimann, M.F.; Stevenson, W.R. 1997. **Walnut and butternut toxicity.** Urban Phytonarian Series Bull. A3182. Madison, WI: University of Wisconsin Extension. 2 p.

Hoban, S.M.; McCleary, T.S.; Schlarbaum, S.E.; Romero-Severson, J. 2009. **Geographically extensive hybridization between the forest trees American butternut and Japanese walnut.** Biology Letters. 5: 324-327.

Jeandet, P.; Sbaghi, M.; Bessis, R. 1992. **The production of reservatrol (3,5,4'-trihydroxystilbene) by grapevine in vitro cultures, and its application to screening for grey mould resistance.** Journal of Wine Research. 3: 47-57.

Klessig, D.F.; Malamy, J. 1994. **The salycylic acid signal in plants.** Plant Molecular Biology. 26: 1439-1458.

Krajci, W.M.; Lynch, D.L. 1978. **The inhibition of various micro-organisms by crude walnut hull extracts and juglone.** Microbios Letters. 4: 175-181.

Mahoney, N.; Molyneux, R.J.; Campbell, B.C. 2000. **Regulation of aflatoxin production by naphthoquinones of walnut (*Juglans regia*).** Journal of Agricultural Food Chemistry. 48: 4418-4421.

Massey, A.B. 1925. **Antagonism of the walnuts (*Juglans nigra* L. and *J. cinerea* L.) in certain plant associations.** Phytopathology. 15: 773-784.

Michler, C.H.; Pijut, P.M.; Jacobs, D.F.; Meilan, R.; Woeste, K.; Ostry, M.E. 2006. **Improving disease resistance of butternut (*Juglans cinerea*), a threatened fine hardwood: a case for single-tree selection through genetic improvement and deployment.** Tree Physiology. 26: 121-128.

Nair, V.M.G. 1999. **Butternut canker - an international concern.** In: Raychaudhuri, S.P.; Maramorosch, K., eds. Biotechnology and plant protection in forestry science, New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.: 239-252.

Omar, S.; Lemonier, B.; Jones, N.; Ficker, C.; Smith, M.L.; Neema, C.; Towers, G.H.N.; Goel, K.; Arnason, J.T. 2000. **Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional medicine.** Journal of Ethnopharmacology. 73: 161-170.

Orchard, L.P.; Kuntz, J.E.; Kessler, K.J., Jr. 1982. **Reaction of *Juglans* species to butternut canker and implications for disease resistance.** In: Black walnut for the future: Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 27-31.

Ostry, M.E.; Moore, M. 2007. **Natural and experimental host range of *Sirococcus clavigignenti-juglandacearum*.** Plant Disease. 91: 581-584.

Ostry, M.E.; Moore, M. 2008. **Response of butternut selections to inoculation with *Sirococcus clavigignenti-juglandacearum*.** Plant Disease. 92: 1336-1338.

Ostry, M.E.; Woeste, K. 2004. **Spread of butternut canker in North America, host range, evidence of resistance within butternut populations and conservation genetics.** In: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.; et al., eds. Black walnut for a new century, Proceedings, Sixth Walnut Council research symposium. Gen. Tech. Rep. NC-243. U.S. Department of Agriculture, Forest Service, North Central Research Station: 114-120.

Park, B.-S.; Kim, J.-R.; Lee, S.-E.; Kim, K.-S.; Takeoka, G.R.; Ahn, Y.-J.; Kim, J.-H. 2005. **Selective growth-inhibiting effects of compounds identified in *Tabebuia impetiginosa* inner bark on human intestinal bacteria.** Journal of Agricultural Food Chemistry. 53: 1152-1157.

Park, B.-S.; Lee, H.-K.; Lee, S.-E.; Piao, X.-L.; Takeoka, G.R.; Wong, R.; Ahn, Y.-J.; Kim, J.-H. 2006. **Antibacterial activity of *Tabebuia imetiginosa* Martius ex DC (Taheebo) against Helicobacter pylori.** Journal of Ethnopharmacology. 105: 255-262.

Schlarbaum, S.E.; Anderson, R.L.; Ostry, M.E.; Brosi, S.L.; Thompson, L.M.; Clark, S.L.; van Manen, F.T.; Spaine, P.C.; Young, C.; Anagnostakis, S.A.; Brantley, E.A. 2004. **An integrated approach for restoring butternut to eastern North American forests.** In: Li, Bailian; McKeand, S., eds. Forest genetics and tree breeding in the age of genomics: progress and future, IUFRO Joint Conference of Division 2; 2004 November 1-5; Charleston, SC: 156-158.

Windham, G.; Graves, C.H., Jr. 1981. **Reaction of isolates of *Fusicladium effusum* to juglone.** Phytopathology. 71: 913.