This is to certify that the
thesis entitled

VIRULENCE AND FUNGICIDE SENSIVITY OF
PHYTOPHTHORA CACTORUM ISOLATED FROM
AMERICAN GINSENG

presented by

Shaunta Nichelle Hill

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Plant Pathology

Mary K. Hausbeck
Major Professor's Signature

4/27/05
Date

Virulence and fungicide sensitivity of *Phytophthora cactorum* isolated from American ginseng

By

Shaunta Nichelle Hill

A THESIS

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

MASTER OF SCIENCE

Department of Plant Pathology

2005

Abstract

Virulence and fungicide sensitivity of *Phytophthora cactorum* isolated from American ginseng

By

Shaunta Nichelle Hill

Foliar blight and root rot of American ginseng (*Panax quinquefolium*) caused by *Phytophthora cactorum* has been managed with the fungicide mefenoxam. During 2003 and 2004, commercial ginseng gardens were monitored in Wisconsin (WI) and Michigan (MI) as well as research gardens at Michigan State University (MSU). Following periods of cool, wet weather, reddening of leaflets, root rot, plant wilting and death were observed. Diseased plants were sampled and *P. cactorum* was isolated. A minimum of 104 *P. cactorum* isolates were recovered each year. Isolates were screened for sensitivity to mefenoxam by measuring radial mycelial growth on 100-ppm mefenoxam-amended V8 agar. For 2003, 79, 48 and 85% of isolates collected from MSU, MI and WI plants were resistant to mefenoxam, respectively. In 2004, 83 and 91% of isolates recovered from MSU and WI plants and seedlings were resistant to mefenoxam, respectively. Isolates were tested for virulence using an apple fruit bioassay. All were virulent. Alternative products were tested against *P. cactorum*. Mefenoxam was the least effective while dimethomorph, mancozeb with zoxamide and copper hydroxide were the most effective against *P. cactorum*. *Phytophthora cactorum* is an important pathogen in MI and WI gardens. The development of isolates resistant to mefenoxam is a new finding. Currently un-registered fungicides may be useful in managing *P. cactorum* diseases.

Dedication

To my circle of support: Jacqueline, Anita, Shirley, Sebren and Deja. Words can't express my gratitude for your presence, support and love. To the teachers and mentors from my past, who helped to guide my path and Ms. Lenora Ashford, thanks for the science talk!

Acknowledgements

I would like to thank the people who contributed to the completion of this research. To my major professor, Dr. Mary Hausbeck, thank you for your guidance and the opportunity to study plant pathology. To Dr. Annemiek Schilder and Dr. Joe Vargas, my committee members; thank you for your support towards this degree and knowledge that you have shared. I wish to also thank Dr. Marcia Ewers for insisting I apply to Grad School and Blair R. Harlan, Sheila Linderman and Jeffrey A. Woodworth who offered technical assistance.

I would like to acknowledge the USDA Cooperative State Research, Education, and Extension Service; Pest Management Alternatives Program and the IR-4: Biopesticide Research Grant as funding sources for the duration of this research. Also, I would like to acknowledge the Ginseng Growers of Michigan, Ginseng Board of Wisconsin and Wisconsin Ginseng Growers Association for their commitment and patience with this project.

A special acknowledgement to my family and friends. Thank you for your love and support thought my graduate program and in everyday aspects of my life.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

Literature Review

History of Ginseng

Ginseng has long been used as a "cure-all" medicine by the Chinese. The first reference to ginseng appeared in Chinese literature in the year 1 B.C. (Putnam, 1984). In 1709, the first Westerner, French missionary Father Jartoux, became aware of both the ginseng plant and the great esteem in which it was held (Putnam, 1984).

Father Jartoux recorded his memories of China and descriptions of ginseng in the Memoir of the Royal Academy of Paris in 1714 which was translated into English and published in the Philosophical Transactions of the Royal Society of London (Hardacre, 1968; Putnam, 1984). In 1716, Father Joseph Francis Lafitau observed American ginseng in his hometown of present day Montreal and confirmed his identification based on descriptions by Father Jartoux (Beyfuss, 1998). After ginseng was found in Canada, the search for it began in the United States. In 1751, ginseng was found in New York, Massachusetts and Vermont (Nash, 1898; Beyfuss, 1998) and soon after the harvesting of ginseng for trade began. In 1773, the ship "Hingham" sailed from Boston to China with 55 tons of wild ginseng on board (Keller, 1998). The first shipment of wild ginseng to China after the American Revolution was reported to have been made by John Jacob Astor from New York in 1782 (Keller, 1998).

With its rising popularity and the amount exported increasing yearly, the wild-ginseng population began to dramatically decline and cultivation became the focus. In 1870, Abraham Whisman of Virginia tried unsuccessfully to cultivate ginseng. It wasn't until 1885, that George Stanton was successful. George Stanton is credited as being the "father of ginseng cultivation", not only because he was able to keep his gardens healthy,

but because he was able to understand the shade requirements and stratification needs of the plant (Harding, 1936). With the cultivation of ginseng the trade market remained viable and flourished. In the 19th century, nearly 21,000 tons of American ginseng were exported between 1821 and 1983 (Baranov, 1966; Keller, 1998). From 1992 to 2001, the export value was over 1.8 billion dollars and in 2002, exports rose to 33.6 million, up 34% from 2001 (USDA Tropical products, world markets and trade, 2003).

With exports of ginseng increasing in the U.S. since commercialization, the number of ginseng farms also increased. The first census report to include ginseng was taken in 1954. At that time the U.S. Census of agriculture reported only 5 ginseng growers with a total of 21 acres (Carlson 1986). The next census report that included ginseng was published in 1992 and included 824 ginseng farms (U.S. 1997 Census of Agriculture – State Data.). In 1997, the number of farms harvesting ginseng increased to 1,081. In 2002, the number of farms harvesting ginseng decreased to 588 (U.S. 2002 Census of Agriculture – State Data).

To protect wild populations of American ginseng, the U.S. government regulates its harvest. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was initiated by the United States in 1977. It regulates the commercial trade of endangered species and monitors the trade of species that are at risk of becoming endangered (Beyfuss, 1998). Under the terms of CITES, the U.S. Fish and Wildlife Service monitors wild ginseng populations and issues permits for wild and cultivated ginseng importing and exporting (Pritts, 1995).

Since the start of CITES, wild ginseng has been deemed endangered in Maine and Rhode Island, threatened in Michigan and New Hampshire, of "special concern" in

Connecticut, Tennessee, Massachusetts and North Carolina and "vulnerable" in New York and Pennsylvania (USDA, NRCS, 2002).

Botanical Description

Ginseng is a member of the Araliaceae family and found in the genera *Panax* and *Eleutherococcus*. Siberian ginseng (*Eleutherococcus senticosus*) is not true ginseng (Pritts, 1995). *Panax* is derived from the Greek word *panakos* (a panacea) meaning "cure all". The common name ginseng literally means "root of man" because the root of this plant often resembles the shape of a human body (Mindell, 1992). Of the several species of ginseng included in the *Panax* genus, only Oriental ginseng (*Panax ginseng*) and North American ginseng (*Panax quinquefolium*) are important to the ginseng market. Oriental ginseng refers to plants native to North Korea and China while American ginseng is native to Canada and the eastern and midwestern United States.

Ginseng is a unisexual erect perennial plant with a canopy that can reach 30-40 cm in height and requires only 30% of full sunlight (Proctor, 1996). The total number of leaves on a ginseng plant corresponds to the plant's age (Proctor, 1996). The canopy of a mature ginseng plant consists of three or four compound leaves arranged in a whorl. Each leaf contains five ovate, saw-toothed leaflets. The upper three leaflets are larger than the lower two with the leaves referred to as prongs. There are five leaflets on each leaf. An immature plant has one or two prongs and adds an additional leaf every few years until maturity. A first year seedling has one leaf with three leaflets. In the Midwest, the cultivation season for ginseng is late April through late October (Polczinski, 1982; Pritts, 1995; Hausbeck, 2004).

Ginseng has a single umbel inflorescence that forms at the terminus of the stem (Putnam, 1984). The umbel has 5-50 small greenish-white perfect flowers (Putnam, 1984) that are self-fertile and each capable of producing a small green kidney-shaped berry after fertilization. The berries ripen and turn crimson red in late summer or early fall (Harding, 1936; Pritts, 1995). Each berry contains two seeds approximately 0.5 cm in diameter and is slightly longer than it is wide. Stratification is needed for ginseng seed and is generally eighteen to twenty-two months in duration (Proctor and Bailey, 1987). During stratification, the endocarp of the berry remains tightly closed, opening slightly along its suture a month or so before germination (Putnam, 1984). The series of warm and cold temperatures over the 18-22 month stratification period is necessary for the embryo to grow and mature (Putnam, 1984; Proctor and Louttit, 1995; Harrison et al., 2000).

Ginseng has a fibrous taproot, which can be 7-13 cm long (Anonymous, 2000). The outer skin of the root is generally light to golden brown and the interior is white to light yellow in color. The root is also forked, fleshy, ringed, necked and wrinkled, thus its nickname of "old man's roots". The neck of ginseng refers to its rhizome at the crown of the root. It is on this rhizome that the bud for the next year's shoot develops during the spring; with the bud expanding so that the stem, rather than the leaves, is seen first (Putnam, 1984). This bud remains dormant during the winter months (Harrison et al., 2000). Root age can also be determined by counting the rings (scars from winter dormancy and annual abscission of the foliage) on the neck or crown of the root.

Cultivation

Ginseng can be grown as wild, wild-simulated, woods-cultivated, and cultivated. Wild ginseng is found in its native habitat, usually a hardwood forest. Wild-simulated ginseng is seeded and grown under approximately 70% natural shade. Woods-cultivated ginseng is similar to wild simulated ginseng; however, the area is first cleared by removing plants and leaf litter that would naturally compete with or hinder ginseng growth. Cultivated ginseng is grown under shade cloth in a cleared field. Both cultivated and woods-grown ginseng require intensive maintenance including mulch such as straw or hay, weeding, and fungicide use.

Ginseng grading and selection for market is based on its cultivation method and its appearance. Wild or woods-grown ginseng is preferred over cultivated ginseng because of its rarity. Therefore, the bulk of the market consists of ginseng cultivated under shade-cloth. Roots that appear old, large, and thick are preferred. The root should also be coarse in texture and have many rings, a white interior and golden brown exterior (Pritts, 1995).

Ginseng roots are generally harvested after four years under shade cloth, six to eight years when woods-cultivated, and after eight to fifteen years of growing in a wild-simulated system (Pritts, 1995). It is illegal to harvest wild ginseng without special permits. Ginseng can be harvested by hand or by use of mechanical diggers and once roots are retrieved, they are washed, dried and prepared for market.

Environmental and nutrient requirements

Ginseng grows best in loamy, light textured, well-drained soil with a pH of 5.5 to 6.5 (Pritts, 1995). The crop also requires 20 to 60 inches of precipitation annually and a minimum cold period (<49° F) of sixty days to break dormancy each spring (Pritts, 1995).

Mulch is typically added to the plant beds after seeding (Putnam, 1984) and is used for frost protection. Mulch also helps to maintain the soil moisture and prevent soil temperature from rising too rapidly in the spring (Polczinski, 1982). Mulch suppresses weed growth, acts as an organic fertilizer by helping retain the soil humus level, and plays a role in preventing soil erosion (Polczinski, 1982).

Nitrogen requirements for ginseng gardens increase with the age of the garden at approximately 40, 60, 80 and 100 kg N/ha for 1, 2, 3, and 4-year gardens, respectively (British Columbia Ministry of Agriculture, Food and Fisheries, 2003). Nitrogen is responsible for foliar growth of the plant and nitrate is the preferred and most readily available form. Symptoms of nitrogen deficiency include slow growth and pale green to yellow leaflets. (British Columbia Ministry of Agriculture, Food and Fisheries, 2003; Curran, 1985).

The recommended rate of phosphorus for ginseng is based on the recommended amount used for root vegetables (15 lb phosphate) (P_2O_5) /acre (Harrison et al., 2000). Symptoms of phosphorus deficiency include purpling or reddening of the leaflets, and reduced growth (British Columbia Ministry of Agriculture, Food and Fisheries, 2003). The recommended rate of potassium is also based on the amount used for root vegetables (60 lb potash) (K_2O) /acre, (Harrison et al., 2000). Potassium deficiency symptoms include slow growth, mottling, bronzing and drying of leaf tips and margins (British Columbia Ministry of Agriculture, Food and Fisheries, 2003).

Chemistry and Medicinal Use

The traditional western attitude has been that ginseng varieties offer similar benefits (Pritts, 1995). However, the Chinese believe that the two types (American and Oriental) represent yin and yang. American ginseng is believed to be yin, which in Chinese tradition is negative, cold, and dark (Duke, 1989). Oriental ginseng is considered to be yang, which is positive, warm, and light (Duke, 1989). Traditionally, an overly yang person was encouraged to eat more yin food and an overly yin person should have more yang food (Duke, 1989). This practice was believed to establish a natural balance, considered to be important for the body, whereas all diseases were considered to result from imbalances (Chen, 2002).

Ginseng contains at least eight or ten different saponins or ginsenosides, which are the main active ingredients. The range of saponins and ginsenosides vary and distinguishes the varieties. Other constituents of ginseng include panaxic acid, essential oils, vitamins, saccharides and various kinds of amino acids. American ginseng contains slightly more saponins but less proteinous and oily substances than Oriental ginseng (Hou, 1978).

Ginseng in the United States was not considered to have medicinal value until about 1905 (Pritts, 1995). Ginseng has been studied as a treatment option for heart disease, cancer, obesity and more recently diabetes. Research conducted at St. Michael's Hospital and the University of Toronto concludes that American ginseng reduces blood sugar (Vuksan et al., 2000; Ireland, 2000; Devitt, 2000 and 2003). Also Sotaniemi et al. (1995) reported that ginseng might be a useful therapeutic adjunct in the management of non-insulin-dependent diabetes. Sotaniemi et al. (1995) also stated that ginseng therapy

elevated mood, improved psychophysical performance, reduced fasting blood glucose, and body weight.

Diseases of ginseng

Ginseng is susceptible to a number of pathogens that can cause foliar blights, stem blights and root rots. Some of the most devastating pathogens include *Alternaria panax*, *Botrytis cinerea*, *Fusarium spp.*, *Cylindrocarpon destructans*, *Rhizoctonia solani* and *Phytophthora cactorum*.

Alternaria panax can cause leaf blight and stem rot. A typical symptom of Alternaria blight is spotting and yellowing of the leaves (Davis and Shoemaker, 1999). The spots, in which clusters of Alternaria conidia form, appear on the leaves in rings of yellowed tissue. Infection may also occur at the base of the stem resulting in premature stem dieback or defoliation and browning or reddening of the stem. Elongated, yellow to light brown spots on the stems may also be present. Disease is favored by humid and wet conditions. The pathogen is capable of overwintering in plant debris.

Another important foliar blight of ginseng is caused by *Botrytis cinerea*. Botrytis produces sclerotia that are capable of over wintering. Symptoms of Botrytis blight include an obvious gray fuzzy mat of conidia on the leaves, lesions, and water soaking (Hausbeck, 2004). Disease is favored by continuous leaf wetness and a dense canopy (Brun, 1999). Under wet conditions, lesions can expand rapidly, completely cover leaflets and blight healthy leaflets. Conidia are readily produced on diseased tissue and may be spread by wind, splashing rain, or human and machinery movement within the garden.

Cylindrocarpon destructans can cause damping off, crown and root rot of ginseng (Hausbeck, 2004). Infection normally starts near the root tip and progress upward often

destroying the entire root. A classic symptom of *C. destructans* root rot is "disappearing root", whereby the root tissue disintegrates due to rot. Another symptom of *C. destructans* rot is a reddish-brown to dark-brown rusty colored canker on the root. These cankers are often dry and cracked (Hudelson, 2002).

Rhizoctonia solani infection of ginseng results in symptoms similar to Cylindrocarpon and includes a rusty discoloration of the root and crown with reddish - brown cankers on the roots. Infection by *Fusarium* spp. can cause stem and crown infections, although root rots are most common resulting in red streaking of the tissue. When Fusarium root rot is advanced, the foliage wilts and becomes reddened.

Phytophthora cactorum

Phytophthora cactorum (Lebert & Cohn) J. Schröt, is considered to be the most devastating fungal disease on ginseng because it can destroy entire ginseng gardens in a few weeks (Darmono et al., 1991).

Phytophthora cactorum is a homothallic oomycete with a wide host range. This species infects more than 200 plant species in 150 genera representing 60 plant families (Erwin and Ribeiro, 1996). *Phytophthora cactorum* was first reported from rotting cacti (*Cereus giganteus* and *Melocactus nigrotomentosus*) in Czechoslovakia by Lebert and Cohn in 1870 (Erwin and Ribeiro, 1996). On ginseng, *P. cactorum* can cause leaf blight and/or root rot. Van Hook first documented root rot of ginseng by *P. cactorum* in the United States in 1906, (Putnam, 1984; Erwin and Ribeiro, 1996). In 1915, Rosenbaum published an in-depth treatise on Phytophthora disease of ginseng (Putnam, 1984). In 1915, Phytophthora root rot was one of the most serious diseases confronting ginseng growers (Duke, 1989). During 1984 in Wisconsin, up to 50% of plants in some gardens

were lost to *P. cactorum* (Duke, 1989). *Phytophthora cactorum* is also known for causing leather rot in strawberry and collar rot in apples.

Phytophthora cactorum has aseptate mycelium. The optimum growing temperature is 25° C. *Phytophthora cactorum* produces oospores, sporangia, zoospores, and chlamydospores, all of which require free water for germination. Oospores are sexual resting spores and are generally 15-36 μm in size (Erwin and Ribeiro, 1996). When conditions are favorable for germination, oospores produce mycelium, which can then form sporangia. Germination in the field begins after a dormant period during the winter at soil temperatures of about 7.5° C and then increases rapidly within 22 days as the soil temperature increases to 13° C (Erwin and Ribeiro, 1996).

Sporangia are asexual structures, and can germinate directly. The sporangia are ovoid, papillate and borne terminally (Erwin and Ribeiro, 1996). They can also germinate indirectly by division of the contents within the spore into an indefinite number of zoospores (Ribeiro, 1978). Zoospores are released from the sporangium through a pore at the tip (Ribeiro, 1978). Producing zoospores gives *P. cactorum* an increased potential for epidemics. They may also be dispersed passively in flowing water over long distances, contaminating growing sites and irrigation water (Erwin and Ribeiro, 1996). Zoospores are asexual, kidney-shaped spores that move by using one tinsel and one whiplash flagellum.

Zoospores exhibit strong attraction to certain amino acid exudates produced by roots (Ribeiro, 1978). Wounds, succulent tissues, and shoot tips are the most susceptible to infection. When zoospores come into contact with an appropriate surface, they become circular, lose their flagellum, secrete a cell wall (encyst) and form a germ tube to

infect the plant and initiate mycelial growth if conducive conditions exist (Ribeiro, 1978). Zoospores typically penetrate a host directly through roots or through stomata and they can form spherical haustoria (Ribeiro, 1978).

Chlamydospores are round to ovoid vegetative asexual resting spores, which germinate by germ tubes (Tucker, 1967) and can form within or at the tips of hyphae. Historically, chlamydospores of *P. cactorum* have been observed infrequently. Darmono and Parke (1990) have suggested that this may be the result of in vitro conditions. The authors reported that the optimal temperature for chlamydospores production by *P. cactorum* was at 4° C and although chlamydospores were produced at 24 and 28° C, they were less abundant. Darmono and Parke (1990) suggested that chlamydospores may be able to form during the winter months under natural conditions and contribute to overwintering survival of *P. cactorum* thereby serving as an inoculum source for the following growing season.

An early symptom of *Phytophthora* infection on ginseng is a drooping leaflet (Erwin and Ribeiro, 1996). Dark green water-soaked lesions may appear on leaflets (Duke, 1989; Proctor, 1996). Purpling or reddening of the leaflets may be observed if infection occurs late in the season. Phytophthora leaf blight can also resemble symptoms resulting from heat and drought injury, Alternaria leaf blight, or frost damage.

Infection of ginseng foliage is promoted by wet conditions (Pritts, 1995). Spores can be splashed along with soil particles to adjacent plants, and contact with tools can also move spores (Pritts, 1995). Infections initiated on leaves can move down the stem, causing collapse and separation from the root crown.

Excess water and poorly drained soils can predispose the roots to rot. High moisture and cool temperatures favor infection of stems by *P. cactorum*, which moves

rapidly down to the root (Erwin and Ribeiro, 1996). Initial indications of root rot caused by *P. cactorum* include root discoloration and a spongy texture. Secondary decay often occurs due to invasion of soft rot bacteria. Instead of having a white and firm interior, the root turns brown with a soft or liquid texture, a sign of the vascular tissue breakdown. Secondary roots may separate or be discolored. Late season infections in mature gardens may go unnoticed until the roots are harvested and dried (Pritts, 1995).

Disease Management

Primary control of *P. cactorum* begins with sanitation. By using clean seed, destroying diseased plants and using good cultural practices, *P. cactorum* infections can be reduced. Any equipment used in diseased beds should be thoroughly washed and disinfected with a soap or bleach solution and allowed to dry before moving it to healthy beds (Pritts, 1995). Diseased plants should be removed from a garden and should include healthy plants within eighteen inches of the infected plants (Pritts, 1995). When disease pressure is high, fungicide applications may be necessary.

Fungicide options for *P. cactorum* have been available for use on ginseng for approximately the past 20 years. Metalaxyl (trade name: Ridomil ®; Syngenta Crop Protection Inc., Greensboro, NC), a systemic fungicide specific against *Pythium* and *Phytophthora* spp. was introduced in 1977 (Duke, 1989). Metalaxyl is in the acylamide class and affects RNA synthesis.

Preliminary research by Putnam and Mitchell (1984) showed that metalaxyl could reduce disease incidence by 55-66% on three-year-old plants. Laboratory studies verified that metalaxyl inhibited both growth and reproduction of *P. cactorum* when grown on an artificial medium (Putnam and Mitchell, 1984; Duke, 1989). Metalaxyl also increased

yield by 50% with a single spray in early June at an application rate of 0.25 kg a.i./ha (Putnam and Mitchell, 1984; Erwin and Ribeiro, 1996).

Mefenoxam, (trade name: Ridomil Gold ®; Syngenta Crop Protection Inc.) introduced in 1997, is the R-enantiomer of metalaxyl. Like metalaxyl, mefenoxam is a systemic fungicide specific against *Pythium* and *Phytophthora* spp. Mefenoxam also inhibits RNA synthesis, which results in the prevention of spore production and inhibition of mycelial growth (Syngenta Crop Protection Inc., 2004).

Two formulations of Ridomil Gold® are available for use on ginseng. Ridomil Gold® EC (emulsifable concentration) and Ridomil Gold GR (granular). The labeled rate for Ridomil Gold® EC on ginseng is 12 ounces per acre as a drench in 100-400 gal. of water (Syngenta Crop Protection Inc., 2004). For the Ridomil Gold® GR on ginseng the rate is 15 lbs. per acre applied uniformly to the soil surface in the spring before the plants begin growing (Syngenta Crop Protection Inc., 2004).

Fungicide Resistance

Dekker (1987) defines fungicide resistance as a condition where strains of a sensitive species, become (usually by mutation) significantly less sensitive to a fungicide. Application of the fungicide selects for the resistant strains, and thus favors their multiplication. Problems with fungicide resistance have increased since selective fungicides were introduced (Dekker, 1987). Resistance to mefenoxam among some *Phytophthora* spp. has been documented on crops such as cucumbers, potato, and soybean (Lamour and Hausbeck, 2000; Taylor et al., 2002; Malvick, 2003).

Development of fungicide resistance by a fungus may increase based on the chemical and its use over time and depends on the reproductive mode of the fungus.

Reproduction in oomycetes occurs with two gametangia per mating strain: an oogonium, which can hold one or several eggs, and an antheridium, which fertilizes the oogonium (Heffer, 2002). Homothallic fungi such as *P. cactorum* have one mating type for reproduction. A strain sensitive to a fungicide will produce sensitive progeny and a resistant strain will produce resistant progeny.

Methods for determining fungicide resistance can be accomplished via laboratory, greenhouse and field tests. For all tests, potentially fungicide-resistant strains must be compared with the sensitive wild-type fungus (Dekker, 1987). A common laboratory technique for resistance screening is to use a radial growth of mycelium on fungicide-amended agar medium. This technique uses small agar blocks with mycelium taken from the periphery of an actively growing fungal colony that are placed on either agar media amended with the fungicide (test plate) or on agar media with no fungicide (control) (Dekker, 1987). The toxicity of the fungicide is evaluated by measuring and comparing the diameter of the colony growth on the test and control plates (Dekker, 1987).

Utkhede and Gupta (1988) studied resistance to metalaxyl by in vitro selection of *P. cactorum* strains exhibiting resistance. Single spore isolates were maintained on corn meal agar (CMA) with fungicide and incubated at 18° C for 4 weeks after inoculation with a 5mm *P. cactorum* agar plug. Fungicide concentrations of the test plates were 100, 150, 200 and 250 $\mu g \cdot ml^{-1}$ of metalaxyl. All plates were measured after 4 weeks at 18° C. Utkhede and Gupta (1988) determined that exposure to a gradual increase in metalaxyl concentration resulted in the development of isolates that exhibited in vitro resistance to metalaxyl. Further, a linear relationship exists between the concentrations of metalaxyl and the inhibition of *P. cactorum* growth. Inhibition (%) of *P. cactorum* on CMA plates

amended with 50, 100,150, 200 and 250 $\mu g \cdot ml^{-1}$ of metalaxyl was 18± 7, 32 ± 10, 35± 8, 42± 8, and 33± 9, respectively.

Jeffers and Schnabel (2004) reported that *P. cactorum* isolated from strawberry roots and crowns was resistant to mefenoxam. Mefenoxam sensitivities were determined by monitoring the radial mycelial growth of the isolates on clarified V8 juice agar amended with 100-ppm mefenoxam. All isolates were resistant to mefenoxam with growth on fungicide-amended plates obtaining 73- 89% of the unamended control plates. Prior to the research presented in this thesis, research has not been conducted to determine if resistance is a problem with *P. cactorum* isolated from ginseng.

Introduction

American ginseng (*Panax quinquefolium*) is an unisexual, erect, perennial plant (Proctor, 1996). Ginseng has been used extensively in oriental countries as a traditional medicine (Anonymous, 2000). Today it is more common to the find the root ground into a powder or used as an extract for use in cosmetic and food products including toothpaste, lipstick, soft drinks, energy drinks, tea, coffee, baked goods and vitamins (Pritts, 1995).

Ginseng is propagated by seed. A stratification period of 18 – 22 months is required for growth and maturation of the embryo (Putman, 1984; Proctor and Louttit, 1995; Harrison et al., 2000). Ginseng requires shade and is commonly cultivated using a natural or artificial canopy. More than 95% of the commercial ginseng grown in the U.S. is cultivated in Wisconsin (Adam, 2004). Currently, Wisconsin's 400 ginseng growers cultivate 2,100 acres of ginseng producing 500 to 1,700 lb/acre, which represents 10% of the world's supply of ginseng (Hausbeck, 2004). At approximately $20 to $40/lb, ginseng is a high value crop. Michigan has a relatively new ginseng industry with significant acreage established in 1995 (Hausbeck, 2004). Most of the production is located in the Upper Peninsula and is grown under a natural forest canopy. Based on current prices, woods-grown cultivated ginseng represents a Michigan inventory of over $50 million (Hausbeck, 2004).

Phytophthora cactorum (Lebert & Cohn) J. Schröt, causes foliar blight and root rot, which may destroy an entire ginseng garden in a few weeks (Darmono et al., 1991) (Figure 1). *Phytophthora cactorum* was first documented in association with ginseng in

the U.S. in 1906 (Van Hook, 1906; Erwin and Ribeiro, 1996) and has become an increasing concern among Wisconsin and Michigan ginseng growers (Hausbeck, 2004).

Phytophthora cactorum is a homothallic oomycete and produces oospores, chlamydospores, sporangia, and zoospores, all of which require free water for germination. Oospores (15-36 μm) and chlamydospores (39.7 μm) are potential overwintering structures (Darmono and Parke, 1990; Erwin and Ribeiro, 1996). Germination of oospores in the field generally occurs during temperatures of 7.5° C to 13° C (Erwin and Ribeiro, 1996). Ovoid sporangia can undergo a division of contents into swimming zoospores. Zoospore production increases the potential for disease epidemics. They may be disseminated passively in flowing water over long distances, contaminating growing sites and irrigation water (Erwin and Ribeiro, 1996).

Cool temperatures and high moisture favor foliar infections of ginseng (Darmono et al., 1991; Pritts, 1995). During periods of warm, sunny weather when the diseased tissues dry rapidly, the disease is temporarily arrested (Rosenbaum, 1915; Erwin and Ribeiro, 1996). Foliar infection results in dark green water-soaked lesions and wilting and reddening of leaflets. When the stem becomes infected, disease can progress rapidly down the stalk (Erwin and Ribeiro, 1996). Root rot symptoms include root discoloration and a spongy texture. Secondary roots may separate and also be discolored.

Phytophthora cactorum infects more than 200 plant species in 150 genera representing 60 plant families, and isolates are generally not host specific (Erwin and Ribeiro, 1996). Darmono et al. (1991) determined that isolates of *P. cactorum* from ginseng gardens and forest soils in Wisconsin were pathogenic to ginseng. Darmono et al. (1991) also investigated the potential of using apple cotyledons and ginseng leaflets as

baits for *P. cactorum.* All of the selected garden and forest isolates colonized both the apple cotyledons and the ginseng leaflets *in vitro;* however, the recovery of *P. cactorum* on either bait was not a good predictor in assessing pathogenicity or the degree of virulence to ginseng seedlings. Tucker (1967) used wounded apple fruit to conduct pathogenicity studies with isolates of *P. cactorum* recovered from beech (*Fagus* sp.), yellow Turk Cap lily (*Lilium candidum*), apple (*Pyrus malus*), rhubarb (*Rheum rhaponticum*), peony (*Paeonia sp.*) and pine (*Pinus* sp.). Apple fruit may also be used for *P. cactorum* detection and isolation (Erwin and Ribeiro, 1996).

Historically, *P. cactorum* diseases (foliar blight and root rot) have been managed with frequent use of the registered fungicides, metalaxyl and/or mefenoxam (Putnam and Mitchell, 1984). Recently, Jeffers and Schnabel (2004) indicated that *P. cactorum* isolated from strawberry roots and crowns was resistant to mefenoxam. Resistance to mefenoxam among other *Phytophthora* spp. has been documented on cucumbers, potato, and soybean (Lamour and Hausbeck, 2000; Taylor et al, 2002; Malvick, 2003). Other products registered for Phytophthora foliar blight and root rot of ginseng include fosetyl-al (Aliette) and salts of phosphorous acid (Phostrol), which offer a moderate level (60–74%) of control against *P. cactorum* (Hausbeck, 2004).

The objectives of this research were to: 1) determine the prevalence of *P. cactorum* in Wisconsin and Michigan ginseng gardens, 2) screen *P. cactorum* isolates for sensitivity to mefenoxam, 3) determine the pathogencity and virulence of the isolates, and 4) screen alternative chemical products for efficacy against *P. cactorum.*

Material and Methods

Isolation and culture of *P. cactorum*:

From April to August 2003, American ginseng plants with *P. cactorum* symptoms (reddened leaflets and spongy roots) were collected from 34 cultivated and woods-grown commercial ginseng gardens in Wisconsin and Michigan, and from ginseng research plots at the Plant Pathology and Horticulture farms located at Michigan State University, East Lansing, MI. From May to August 2004, six cultivated commercial ginseng gardens were sampled in Wisconsin and one research ginseng garden was sampled at the MSU Plant Pathology research farm. In addition, greenhouse seedlings growing in a research greenhouse on the campus of Michigan State University exhibiting wilting, blackened stems and damping off were sampled.

Samples (seedling and 2,3-year old plants) were washed with distilled water and then sprayed with ethanol (70%). Tissue samples (approximately 2x2 mm) of leaves, stems and roots were embedded in 2% water agar (Sigma-Aldrich Corp., St. Louis, MO) amended with ampicillin (2 ml/L) (Sigma-Aldrich Corp.) in 100x15 mm petri dishes (VWR International, West Chester, PA). Cultures were incubated at room temperature (23-25° C) and evaluated for characteristic *P. cactorum* hyphal growth and sporangia (Erwin and Ribeiro, 1996) after 24 hours. Hyphal tips were transferred to 4% V8 (Campbell Soup Company, Camden, NJ) agar until axenic cultures were obtained. Cultures were incubated at room temperature under lab lighting for 4-5 days to stimulate sporangia production for zoospores. Single-zoospore isolates were obtained by flooding agar plates with 20 ml of 10° C sterilized distilled water, incubating the plates at room temperature for 25 minutes, and plating 5 drops of the zoospore suspension onto water agar plates (100x15 mm). The zoospore suspension was spread onto water agar plate

with a sterile hockey stick. Single germinating zoospores were transferred to 20% V8 agar for long-term storage.

All cultures were stored at 13° C in 1.5 ml sterile micro-centrifuge tubes (Dot Scientific Inc., Burton, MI) containing 1 ml of sterilized distilled water and one hemp seed (Carolina Biological Supply Co., Burlington, NC). Cultures were also placed into long-term storage at 13° C on V8 (20%) agar media amended with benomyl (0.050 g) (Du Pont, Wilmington, DE), rifampicillin (2 ml/L) (Sigma-Aldrich Corp.) and ampicillin (2 ml/L) in petri plates (35x10 mm) (VWR International).

Laboratory screening for mefenoxam sensitivity:

Isolates were removed from long-term storage, transferred to V8 (20%) agar petri plates (100x15 mm), and allowed to grow at room temperature (23-25° C) until cultures were a minimum of 40 mm in diameter. Four agar plugs (7 mm) were removed from each culture plate. One agar plug was placed in the center of each of two V8 (20%) agar plates and each of two V8 (20%) agar plates amended with 100-ppm mefenoxam (Syngenta Crop Protection Inc, Greensboro, NC). The plates were incubated at room temperature for 3 days and the radial mycelial growth (cm) recorded. Thirty-five isolates recovered in 2003 and 13 recovered in 2004 were also screened on 1000-ppm mefenoxam at 3 and 5 days with the radial mycelial growth diameter (cm) measured.

During the mefenoxam screening, ten isolates produced mycelial growth between 23 and 50% on the mefenoxam-amended agar relative to the unamended control plate. Because the sensitivity of the isolates was determined by the presence or absence of growth on 100-ppm mefenoxam, an additional assay using apple fruit was designed to determine the mefenoxam sensitivity. Standards including a known mefenoxam resistant

(growth 100% of the control) and mefenoxam sensitive (0% of the control) isolates were used.

Isolates were grown on V8 (20%) agar at room temperature until cultures reached 40 mm in diameter and then agar plugs (7 mm) were removed. Five commercial 'McIntosh' apple fruit were used to test each isolate. Prior to inoculation, apples (approximately 110 g) were washed in warm water with Joy dish soap (Procter & Gamble, Cincinnati, OH) to remove residue. The apples were wounded to the depth of a 10 mm approximately 45 mm down from the stem by a pushpin dipped in ethanol (70%). Three of the five apples were treated with mefenoxam (0.75 pt/100gal) until runoff with a hand-pump compressed air sprayer. The remaining two apples served as controls. After the treated apples dried, one plug of the appropriate *P. cactorum* isolate was placed over the wound site. Agar plugs (and wound sites for control apples) were then covered with a 1.5 ml micro-centrifuge tube and secured to the apple with petroleum jelly.

Inoculated apples were placed in clean aluminum foil pans with paper towels saturated with water. The pans were covered with plastic wrap and the apples were incubated for one week at room temperature under fluorescent lights. The average temperature inside the trays was 30° C and was monitored using a data logger (Watch Dog 100-Temp 2K, Spectrum technologies, Inc, Plainfield, IL). After one week, the diameter (in cm) of lesion coverage for each apple was recorded and averaged. *Phytophthora cactorum* infection was confirmed through isolation from the apple tissue following the termination of the experiment.

Screening for virulence:

Isolates of *P. cactorum* were tested for virulence on apple fruit (Tucker, 1967) and compared to other *Phytophthora* spp. including *P. citricola*, *P. gonapodyides* and an uninoculated control. *Phytophthora citricola* and *P. gonapodyides* were chosen because they cause root and fruit rot to apple, respectively (Erwin and Ribeiro, 1996). Each *Phytophthora* isolate was screened for virulence on four commercial 'McIntosh' apple fruit (Erwin and Ribeiro, 1996) arranged in a completely randomized design. Apples were prepared and wounded as previously described. Agar plugs (7 mm) were made for each *P. cactorum* isolate and *Phytophthora* spp. standard. One agar plug was then placed over each wound site and covered with a 1.5 ml micro-centrifuge tube. The micro-centrifuge tubes were secured to the apple with petroleum jelly.

The apples were incubated in the same manner as described for the mefenoxam-sensitivity apple fruit assay. After one week, the area of the fruit covered with a necrotic lesion (%) was estimated as 0, 25, 50, 75 or 100%. The virulence ratings of the apples were averaged for each isolate and the trial was conducted twice. After rating for disease, the apples were surface disinfested by wiping with ethanol (70%). Tissue samples (approximately 2x2 mm) were removed from the apple and embedded into water agar to confirm *P. cactorum* as the causal agent of the observed disease.

Efficacy of fungicides using apple fruit and ginseng seedlings:

McIntosh apple fruit. Commercial 'McIntosh' apples were used to assess the ability of various chemical treatments (Table 1) including reduced risk products and biopesticides to protect against disease caused by a mefenoxam-resistant isolate of *P. cactorum*. Apples were prepared and wounded as previously described. Immediately following

wounding, the apples (six per treatment) were treated with fungicide until runoff with a hand-pump compressed air sprayer. Treated apples were allowed to dry before they were inoculated and placed in aluminum foil pans for incubation. Inoculation and incubation were accomplished as previously described. Apples were arranged in a completely randomized design.

Table 1. Products tested for efficacy on apple fruit against *P. cactorum* recovered from American ginseng in experiment 1

Product	Active Ingredient	Manufacturer	Formulation Used (per 100 gallons)	Classification[z]
Acrobat 50 WP	dimethomorph	BASF Corp. Research Triangle, NC	6.4 oz (189.2 ml)	E chemical
Aliette WDG	fosetyl-al [w]	Bayer Crop Science, Research Triangle, NC	5 lb (2,268 g)	-
Gavel 75DF	mancozeb + zoxamide[y]	Dow Agroscience Indianapolis, IN	2 lb (907.2 g)	B2 carcinogen
Kocide 2000 DF	copper hydroxide[x]	Griffin LLC Valdosta, GA	3 lb (1,361 g)	No rating
Phostrol 4.32EC	phosphorus salts[w]	NuFarm Specialty Prod. Lobeco, SC	4.5 pt (2,041 g)	Biopesticide
Ranman 400SC	cyazofamid	ISK Bioscience Corp. Concord, OH	1.5 fl oz (44.4 ml)	Reduced risk
Reason 500SC	fenamidone	Bayer Crop Science Research Triangle, NC	4 fl oz (118.3 ml)	Reduced risk
Ridomil Gold EC	mefenoxam[w]	Syngenta Crop Proc. Greensboro, NC	0.75 pt (354. 9 ml)	Reduced risk

[w]Labeled for ginseng and *P. cactorum*.
[x]Labeled for ginseng.
[y]Section 18. Exemption of federal and state agencies for emergency use.
[z]Human risk assessment: B2 carcinogen= Likely human carcinogen; C carcinogen= Possible human carcinogen for which there is limited animal evidence; D carcinogen= There is inadequate evidence to determine carcinogenicity in humans; E chemical= Evidence of non-carcinogenicity in humans.

Table 1 con't. Products tested for efficacy on apple fruit against *P. cactorum* recovered from American ginseng in experiment 2

Product	Active Ingredient	Manufacturer	Formulation Used (per 100 gallons)	Classification[z]
Acrobat 50 WP	dimethomorph	BASF Corp. Research Triangle, NC	6.4 oz (189.2 ml)	E chemical
Bravo Weather Stick 6F	chlorothalonil	Syngenta Crop Proc. Greensboro, NC	3 pt (1,419.5 ml)	B2 carcinogen
Cabrio EG	pyraclostrobin	BASF Corp., Research Triangle, NC	4 oz (118.3 ml)	Reduced risk
Curzate 60DF	cymoxanil	DuPont Wilmington, DE	8 fl oz (236.6 ml)	E chemical
Dithane 75DF	mancozeb	Dow Agroscience Indianapolis, IN	1.5 lb (680.4 g)	B2 carcinogen
Endorse 2.2WP	polyoxin D zinc salt	Cleary Chemical Corp. Dayton, OH	2.2 lb (998.0 g)	Biopesticide
Omega 500F	fluazinam,	Syngenta Crop Proc. Greensboro, NC	4 oz (118.3 ml)	Reduced risk
Quadris	azoxystrobin[x]	Syngenta Crop Proc. Greensboro, NC	12.3 fl oz (363.8 ml)	Reduced risk
Pristine 38WG (BAS 516)	pyraclostrobin + boscalid	BASF Corp, Research Triangle, NC	4 oz (118.3 ml)	Reduced risk
Previcur Flex 6F	propamacarb	Bayer Crop Science Research Triangle, NC	19.2 fl oz (567.8 ml)	D carcinogen
Ridomil Gold EC	mefenoxam[w]	Syngenta Crop Proc. Greensboro, NC	0.75 pt (354. 9 ml)	Reduced risk
Tanos 50DF	famoxadone + cymoxanil	DuPont Wilmington, DE	10 oz (295.7 ml)	E chemical
Zoxium	zoxamide	Dow Agroscience Indianapolis, IN	5.0 oz (147.9 ml)	Reduced risk

[w]Labeled for ginseng and *P. cactorum*.
[x]Labeled for ginseng.
[y]Section 18. Exemption of federal and state agencies for emergency use.
[z]Human risk assessment: B2 carcinogen= Likely human carcinogen; C carcinogen= Possible human carcinogen for which there is limited animal evidence; D carcinogen= There is inadequate evidence to determine carcinogenicity in humans; E chemical= Evidence of non-carcinogenicity in humans.

After one week, the diameter (in cm) of lesion coverage for each apple was recorded and then averaged for the six apples per treatment. The number of apples infected for each treatment was also recorded. After rating, apples were wiped with 70% ethanol solution and samples were taken to confirm infection by *P. cactorum*. Each experiment was conducted three times. Data were subjected to analysis of variance (ANOVA) and when significant treatment effects were indicated, treatment means were compared by Fisher's Least Significant Difference (LSD) test at $P = 0.05$ (SAS Institute Inc. Cary, N.C.).

Ginseng seedlings. Stratified ginseng seed (*Panax quinquefolium*) were mixed in silica sand and stored at 3° C until germination. Seeds that initiated germination were hand planted into 72-cell flats containing silica sand in greenhouse seedlings growing in a research greenhouse on the campus of Michigan State University. Following emergence, seedlings were carefully removed from the sand and observed for disease symptoms. Any seedling showing root discoloration was discarded. Healthy seedlings were hand planted into 89 x 64 mm pots (Hummert International, Earth City, MO) with autoclaved medium particle vermiculite (Therm-O-Rock Inc., Chandler, AZ) and medium grain sand (at a 25% to 75% ratio respectively) in the research greenhouses at Michigan State University. Plants were maintained in the greenhouse under 63% shade cloth (Hummert International) with temperatures between 17.8 and 25.6° C and irrigated as needed.

A mefenoxam-insensitive isolate of *P. cactorum* was grown under lab lighting for 7 days on dilute V8 agar. Six plates with sporulating *P. cactorum* were flooded with 20 ml of 10° C sterilized distilled water and then incubated at room temperature for 25 minutes to release zoospores. Fungicide treatments were applied as a drench in sufficient

volume to displace approximately 10% of the liquid in the pots. Plants were arranged in a blocked design with 8 plants per treatment. Two experiments were conducted. A 2 ml zoospore suspension was injected into the soil at the base of each seedling immediately after the fungicide treatments were applied. For the first experiment, the plants were treated and inoculated on 26 April and disease ratings were taken on 5, 8, and 10 May. For experiment 2, the plants were treated and inoculated on 3 May and disease ratings were taken on 12, 18 and 27 May. Disease ratings were based on a 1 to 5 scale, where 1=healthy, 3= collapse of the stem, and 5=dead. Data were subjected to analysis of variance (ANOVA) and when significant treatment effects were indicated, treatment means were compared by Student-Newman-Keuls test at P =0.05 (SAS Institute Inc. Cary, N.C.).

Detection of *P. cactorum* on ginseng seed:

Detection from seedlings. Stratified ginseng seeds were planted in the research greenhouses at Michigan State University in new 80 mm deep (104 oz) aluminum foil pans (Handi-foil Corporation, Wheeling, IL) with autoclaved medium particle vermiculite and medium grain sand (at a 25% to 75% ratio respectively). After emergence, 200 tissue samples were taken from seedlings exhibiting wilting and blackened stems. Samples (approximately 2x2 mm) of stems and roots were embedded in 2% water agar amended with ampicillin (2 ml/L) in 100x15 mm petri dishes. Cultures were incubated at room temperature (23-25° C) and evaluated for characteristic *P. cactorum* hyphal growth and sporangia (Erwin and Ribeiro, 1996) after 24 hours. All isolates were prepared and maintained as previously described.

Koch's postulates were conducted for *P. cactorum* isolates recovered from ginseng seedlings. Stratified ginseng seed was germinated in the greenhouse in silica

sand. After two weeks of growth (height of 25 mm), seedlings with healthy leaves and roots were selected. Seedlings were aseptically transferred to capped 25 x 70 mm (30 ml) glass vials containing 5 ml of water agar amended with ampicillin (2 ml/L) (Reid, 2000). Seedlings were observed for 2 days under fluorescent lights for disease. Several *P. cactorum* isolates recovered from ginseng (three collected from mature plants in 2003 and seven collected from seedlings in 2004) were grown on V8 agar until 40 mm in diameter and then agar plugs (7 mm) were removed. One agar plug of each isolate was placed at the base of the appropriate seedling stem at the agar line. Each isolate was tested twice. Seedlings were incubated in the laboratory under florescent light. After one week, tissue samples were removed from the seedlings and embedded in water agar with ampicillin (2 ml/L). *Phytophthora cactorum* exhibiting characteristic growth and structures on the isolation media (gnarled mycelium, ovoid and papillate sporangia and oospores with paragynous antheridium) was recovered.

Koch's postulates were also conducted with seedlings planted into 89 x 133 mm pots with silica sand. Seedlings were inoculated at the base of the stem as previously described. Seedlings were placed inside plastic bags with paper towel saturated with water and incubated in the laboratory. After one week, tissue samples were removed from the seedlings and embedded in water agar with ampicillin (2 ml/L). *Phytophthora cactorum* exhibiting characteristic growth and structures on the isolation media was recovered.

Detection from seed. Stratified ginseng seeds were obtained from five commercial ginseng gardens. Twenty-five seeds from each of the sampled seed lots were rinsed in distilled water to remove sand particles and embedded in 2% water agar amended with

ampicillin (2 ml/L) in 100x15 mm petri dishes. After 2 days, plates were examined and sub-cultured for fungal identification. The study was conducted twice.

Results

Isolation and culture of *P. cactorum*:

Over the course of the study, *P. cactorum* was a significant problem in Michigan and Wisconsin and 162 isolates were collected (Table 2). In 2003, 26 ginseng gardens with symptomatic plants (wilted, reddened leaves and discolored root) were surveyed in Wisconsin. Pathogens recovered included *Fusarium* spp., *Pythium* spp, *Cylindrocarpon destructans* and *P. cactorum*. In 2004, six gardens with high *P. cactorum* disease pressure were surveyed. Only one of the six had previously been sampled in 2003. In Wisconsin, 47% (15) of 32 ginseng gardens had incidence of *P. cactorum* from 2003 and 2004. *Phytophthora cactorum* was recovered from 83% of the sampled ginseng gardens in Michigan during 2003. Additional *P. cactorum* isolates (56) were recovered from Michigan State University research greenhouse seedlings in 2004 (Table 2).

Laboratory screening for mefenoxam sensitivity:

Insensitivity to mefenoxam was detected in the majority (76 and 88% for 2003 and 2004, respectively) of samples collected. Nearly all isolates (93%) recovered from the greenhouse seedlings were insensitive to mefenoxam (Table 3). Two isolates recovered in 2003 from Wisconsin ginseng plants produced mycelial growth at 25 and 49% of their control on the mefenoxam-amended V8 agar. When these two isolates were screened during the mefenoxam-sensitivity apple assay, they produced lesions at 8 and 26% of their controls respectively. Because growth in vitro and in vivo was less than 50% of the control, both isolates were classified as sensitive to mefenoxam.

Eight isolates recovered in 2004 from Wisconsin seedlings produced mycelial growth less than 50% of their controls during the in vitro assay. To determine

mefenoxam sensitivity of the eight isolates, they were screened on mefenoxam-treated apple fruit. Four isolates produced mycelial growth of 36, 42, 47 and 44% respectively, on the mefenoxam-amended V8 agar (relative to the control) produced lesions greater than 50% of their controls on the apple fruit and were classified as insensitive to mefenoxam. The remaining 4 isolates with 23, 23, 35 and 38% of mycelial growth on the mefenoxam-amended V8 agar did not produce lesions of 50% or greater than the controls on the apple. Lesion coverage was measured at 0% for 3 of the 4 isolates and 24% for the isolate having 38% growth on amended agar. These isolates were classified as sensitive to mefenoxam.

Screening for virulence:

Of 111 isolates collected in 2003 that were screened for virulence to 'McIntosh' apple fruit, 84% produced lesions spanning 25% of the surface fruit area (Table 4). In 2004, 103 isolates were screened and nearly all produced lesions spanning 25% of the apple fruit (Table 4).

Efficacy of fungicides using apple fruit and ginseng seedlings:
'McIntosh' apple fruit. In experiment 1, significant differences (P=0.05) were observed among the treatment means (Table 5). Dimethomorph (Acrobat), copper hydroxide (Kocide), mancozeb + zoxamide (Gavel) and fenamidone (Reason) consistently yielded disease rating less than the untreated control in each replication. In 2 of the 3 replications, phosphorus salt (Phostrol) and cyazofamid (Ranman) provided significantly better control than the untreated. No significant differences were detected among the untreated and the remaining products.

Significant differences (P=0.05) were also observed in the second experiment among treatments (Table 6). Dimethomorph (Acrobat) provided complete protection

against *P. cactorum* in each replication and was significantly better at managing disease than the untreated control. In 2 of the 3 replications, mancozeb (Dithane) and zoxamide (Zoxium) were significantly better than the untreated control.

Ginseng seedlings. Disease pressure was severe in both experiments, with 75 and 87.5% of the untreated inoculated plants dead at the final observation. Copper hydroxide (Kocide) completely prevented plant death in each experiment. Dimethomorph (Acrobat) limited plant death to 25% (Table 7).

Plant death was limited to 25% of total plants for phosphorus salts (Phostrol) and fosetyl - al (Aliette) treated plants in the first experiment. However, in the second experiment, plant death reached unacceptable levels with plant death at 75% or greater. Mancozeb (Dithane) and mancozeb + zoxamide (Gavel) limited plant death to 37.5% at the final rating for both experiments. Fenamidone (Reason) limited plant death to 25% in the first experiment, but was at 50% for the second. Mefenoxam (Ridomil Gold), the industry standard, performed poorly in both experiments due to the mefenoxam-insensitivity of the isolate. All other treatments had $\geq$50% plant death at the final evaluation and offered little disease control (Table 7).

Detection of *P. cactorum* on ginseng seed:

Detection from seedlings. Greenhouse-grown ginseng seedlings were sampled for disease. Tissue excisions were taken from approximately 195 seedlings, and placed onto amended water agar. Fifty-six *P. cactorum* isolates were identified based on morphological characteristics. Ten isolates (7 of which were recovered from the seedlings) were selected and used to inoculate healthy ginseng seedlings. After 1 week of initiating Koch's Postulates, symptoms (wilting and blacken stems) matching those

exhibited on greenhouse seedlings and seedling recovered from the field were observed. Disease symptoms on seedlings began at the base of the stem at the inoculation site. Wilting and twisting of stems were observed 3 days after inoculation. Four days after inoculation, root discoloration was observed. Stems turned brown, and later blackened at 6 days post inoculation. Disease tissue was excised to confirm *P. cactorum* based on morphological characteristics (gnarled mycelium, ovoid and papillate sporangia and oospores with paragynous antheridium).

Detection from seed. In addition to *P. cactorum*, four fungi were recovered from direct plating of 125 (5 seed lots) stratified ginseng seeds: *Pythium* spp, *Fusarium* spp. *Septonema* sp. and *Cylindrocarpon destructans*. These four fungi are known ginseng pathogens and were identified by morphological characteristics. In the first experiment, all seeds yielded growth of *Fusarium* spp. and *Cylindrocarpon destructans*. Eighty percent of seeds were infected with *Septonema* sp. and 60% with *Pythium* spp. Phytophthora cactorum was recovered from only 20% of the seeds. In the second experiment, only 4 pathogens were identified: *Pythium* spp, (on 40% of seed), *Fusarium* spp. (<1%), *Septonema* sp. (1%) and *C. destructans* (<1%) (Table 8).

Table 2. Source and number of *P. cactorum* isolates recovered from ginseng in 2003 and 2004

	Gardens sampled[wx]		Isolates recovered[x]	
Location	2003	2004	2003	2004
Michigan (Upper Peninsula)	6(5)	-	23	-
Michigan State University	2(2)	1(1)	19	6
Greenhouse Seedlings[z]	-	6(6)[y]	-	56
Wisconsin	26(9)	6(6)	72	42
Total	34(16)	13(13)	114	104

[w]The number of gardens sampled is followed by the number of gardens where *P. cactorum* was recovered.
[x]Dashes represent not sampled.
[y]The number of seedling trays with disease symptoms is followed by the number of tray infected with *P. cactorum*
[z]Seedlings were obtained from stratified ginseng seeds produced in WI and germinated in research greenhouse at Michigan State University.

Table 3. In vitro mefenoxam sensitivity screening of *P. cactorum* from ginseng in 2003 and 2004

	Insensitive isolates[y]		Sensitive isolates[y]	
Location	2003	2004	2003	2004
Michigan (Upper Peninsula)	11	-	12	-
Michigan State University	15	5	4	1
Greenhouse Seedlings[z]	-	52	-	4
Wisconsin	61	37	11	5
Total	87	94	27	10

[y]Dashes represent not sampled.
[z]Seedlings were obtained from stratified ginseng seeds produced in WI and germinated in research greenhouse at Michigan State University.

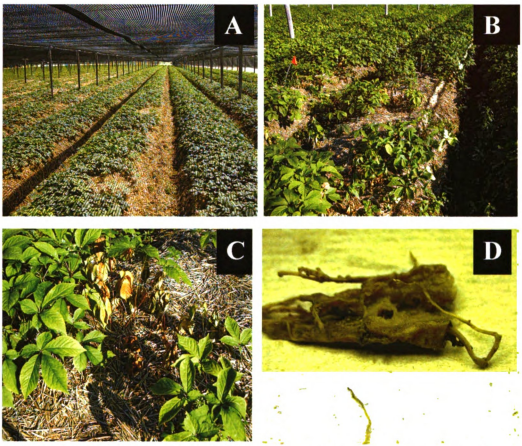

Figure 1: Ginseng plant disease symptoms caused by *Phytophthora cactorum*. Wisconsin ginseng gardens exhibiting foliar blight and plant death (A-C). Advanced stage of root rot (D). Images in this thesis are presented in color.

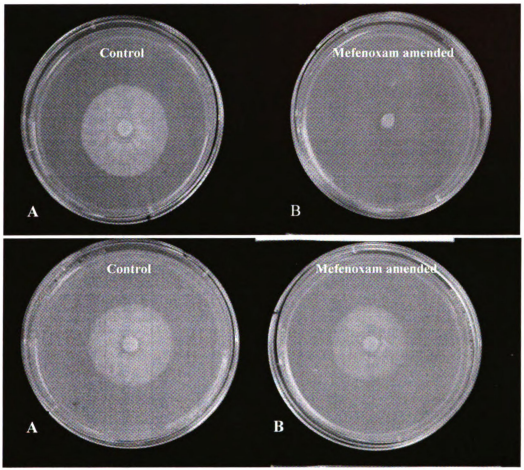

Figure 2. . Laboratory in vitro screen for mefenoxam sensitivity in *P. cactorum*. Panel 1A indicates growths on the V8 control (no agar amendment); 1B indicates limited *P. cactorum* growth of a mefenoxam-sensitive isolate on amended V8 agar. Panel 2A depicts *P. cactorum* growth on a control plate in comparison to 2B which exhibits growth of a mefenoxam insensitive isolate on mefenoxam–amended agar.

Table 4. Virulence screening of *P. cactorum* isolates from ginseng using 'McIntosh' apple fruit

	Percent surface of 'McIntosh' apple fruit blighted following inoculation with *P. cactorum* [y]					
	2003 isolates[z]			2004 isolates[z]		
Isolates sensitivity to mefenoxam	<10%	25%	50%	<10%	25%	50%
Resistant to mefenoxam	3	66	16	1	84	8
Sensitive to mefenoxam	1	22	3	0	8	1
Total no. of isolates	4	88	19	1	93	9

[y]Results presented are averaged over two experiments.
[z]Disease covering >50% of the fruit were not observed by the rating period.

Table 5. Efficacy of registered and unregistered fungicides for control of a mefenoxam-insensitive *P. cactorum* isolate using 'McIntosh' apple fruit in Experiment 1

Experiment 1 Treatments and rate/100 gal	Number of diseased fruit[x]			Lesion Diameter (cm)[yz]		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Untreated Inoculated.....	6	4	5	5.5 c	2.7 c	2.8 b
Acrobat 50 WP, 6.4 oz.....	0	0	0	0.0 a	0.0 a	0.0 a
Kocide 2000 54 DF, 3.0 lb.....	0	0	0	0.0 a	0.0 a	0.0 a
Reason 500SC, 4.0 fl oz.....	2	1	1	1.4 a	0.4 a	0.2 a
Phostrol 4.32EC, 8.0 pt[w].....	2	1	5	1.4 a	0.3 a	1.9 b
Gavel 75DF, 2.0 lb.....	5	0	1	3.0 b	0.0 a	0.6 a
Aliette80 WDG, 5.0 lb[w].....	6	2	2	5.0 c	1.3 abc	0.4 a
Ranman 400SC, 1.5 fl oz.....	6	2	2	4.4 bc	1.0 ab	0.3 a
Ridomil Gold 4EC, 0.75 pt[w].....	5	4	6	5.0 c	2.3 bc	2.8 b

[w]Registered for *P. cactorum* management on ginseng.
[x]Number of infected fruit out of 6 inoculated fruit.
[y]Diameter listed is average of 6 fruit.
[z]Column diameters followed by the same letters are not significantly different (Fisher's LSD, P=0.05).

Table 6. Efficacy of registered and unregistered fungicides for control of a mefenoxam-insensitive *P. cactorum* isolate using 'McIntosh' apple fruit in experiment 2

Experiment 2 Treatments and rate/100 gal	Number of diseased fruit[x]			Lesion Diameter (cm)[yz]		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Untreated Inoculated.....	6	5	5	5.6 e	4.3 cd	3.8 bc
Acrobat 50 WP, 6.4 oz.....	0	0	0	0.0 a	0.0 a	0.0 a
Dithane 75DF, 1.5 lb.....	0	0	5	0.0 a	0.0 a	3.9 bc
Cabrio 20WG, 4 oz.....	4	4	4	3.5 bcde	3.6 cd	3.4 bc
Pristine 38WG, 4 oz.....	1	6	4	4.9 de	3.5 cd	4.5 bc
Curzate 60 DF, 3.2 oz.....	2	4	6	3.4 bcde	5.4 d	4.5 bc
Endorse 2.5WP, 2.2 lb[v].....	3	5	5	4.0 cde	3.5 cd	4.9 c
Previcur Flex 6F, 19.2.fl oz.....	3	6	6	5.4 e	4.4 cd	5.5 c
Zoxium 80WP, 5 oz.....	3	2	5	1.2 ab	4.5 cd	3.3 bc
Quadris 2.08SC, 12.3 fl oz.....	5	4	3	3.5 bcde	4.9 d	2.2 ab
Bravo Weather Stick 6F, 3 pt.....	5	5	5	4.5 cde	4.0 cd	4.0 bc
Tanos 50DF, 10 oz.....	5	2	6	2.9 bcd	1.0 ab	4.4 bc
Omega 500 F, 8 fl oz.....	6	4	5	3.5 bcde	3.4 bcd	4.3 bc
Ridomil Gold 4EC, 0.75 pt[w].....	5	4	6	2.5 bc	2.5 bc	5.5 c

[v]Biopesticide.
[w]Registered for *P. cactorum* management on ginseng.
[x]Number of infected fruit out of 6 inoculated fruit.
[y]Diameter listed is averaged over all 6 fruit.
[z]Column diameters followed by the same letters are not significantly different (Fisher's LSD, P=0.05).

Table 7. Evaluation of registered and unregistered fungicides for control of *P. cactorum* root rot of ginseng seedlings in experiment 1

Experiment 1	Plant death (%)[yz]		
Treatments and rate/100 gal	5 May	8 May	10 May
Untreated Inoculated	75.0 bcd	75.0 bcd	75.0 bc
Kocide 2000 54 DF, 3.0 lb	0.0 a	0.0 a	0.0 a
Phostrol 4.32EC, 8.0 pt[x]	12.5 ab	12.5 ab	25.0 ab
Acrobat 50 WP, 6.4 oz	25.0 abc	25.0 abc	25.0 ab
Aliette 80 WDG, 5.0 lb[x]	25.0 abc	25.0 abc	25.0 ab
Reason 500SC, 4.0 fl oz	12.5 ab	25.0 abc	25.0 ab
Dithane DF, 1.5 lb	37.5 abcd	37.5 abcd	37.5 abc
Gavel 75DF, 2.0 lb	37.5 abcd	37.5 abcd	37.5 abc
Ranman 400SC, 1.5 fl oz	62.5 abcd	75.0 bcd	75.0 bc
Ridomil Gold 4EC, 0.75 pt[x]	75.0 bcd	75.0 bcd	75.0 bc
Omega 500F, 1.0 pt	87.5 cd	87.5 cd	87.5 bc
Zoxamide 80W, 5.0 oz	100.0 d	100.0 d	100.0 c

[x]Registered for *P. cactorum* management on ginseng.
[y]Percent of dead plants per treatment as measured for each rating date.
[z]Column means followed by the same letter are not significantly different (Student-Newman-Keuls, P=0.05).

Table 7. Continued. Evaluation of registered and unregistered fungicides for control of *P. cactorum* root rot of ginseng seedlings in experiment 2

Experiment 2 Treatment and rate/100 gal	Plant death (%)[yz] 12 May	18 May	27 May
Untreated Inoculated.....	75.0 bc	87.5 bc	87.5 bc
Kocide 2000 54 DF, 3.0 lb.....	0.0 a	0.0 a	0.0 a
Acrobat 50 WP, 6.4 oz.....	12.5 ab	25.0 ab	25.0 ab
Dithane DF, 1.5 lb.....	12.5 ab	37.5 abc	37.5 abc
Gavel 75DF, 2.0 lb.....	12.5 ab	25.0 ab	37.5 abc
Reason 500SC, 4.0 fl oz.....	37.5 abc	50.0 abc	50.0 abc
Zoxamide 80W, 5.0 oz.....	50.0 abc	50.0 abc	50.0 abc
Phostrol 4.32EC, 8.0 pt[x].....	0.0 a	62.5 abc	75.0 bc
Omega 500F, 1.0 pt.....	62.5 abc	62.5 abc	75.0 bc
Ridomil Gold 4EC, 0.75 pt[x].....	50.0 abc	75.0 bc	75.0 bc
Ranman 400SC, 1.5 fl oz.....	62.5 abc	75.0 abc	87.5 abc
Aliette80 WDG, 5.0 lb[x].....	12.5 ab	37.5 abc	87.5 bc

[x]Registered for *P. cactorum* management on ginseng.
[y]Percent of dead plants per treatment as measured for each rating date.
[z]Column means followed by the same letter are not significantly different (Student-Newman-Keuls, P=0.05)

Table 8. Incidence of fungal pathogens recovered from stratified American ginseng seed when directly plated on water agar amended with 2% ampicillin (2 ml/L)

	Incidence (%)[y]									
	Experiment 1 Seed lot sample[z]					Experiment 2 Seed lot sample[z]				
Pathogen	1	2	3	4	5	1	2	3	4	5
Pythium spp.	8.0	20.0	0.0	8.0	0.0	4.0	8.0	0.0	0.0	0.0
Fusarium spp.	52.0	72.0	44.0	76.0	76.0	52.0	16.0	44.0	40.0	32.0
Septonema sp.	8.0	24.0	36.0	20.0	0.0	28.0	8.0	4.0	8.0	8.0
Cylindrocarpon destructans	24.0	24.0	52.0	24.0	20.0	0.0	8.0	12.0	4.0	8.0
Phytophthora cactorum	12.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0

[y]Incidence of recovered pathogens from commercial ginseng gardens seed samples.
[z]Represents commercial ginseng gardens seed samples (1-5) with 25 seeds plated for each sample for both experiments.

Discussion

Phytophthora cactorum is recognized worldwide as a pathogen of numerous crops (Erwin and Ribeiro, 1996) and can be especially devastating on ginseng (Darmono et al., 1991). In 1915, Phytophthora root rot was one of the most serious diseases confronting ginseng growers (Duke, 1989). In 1984, Wisconsin growers lost up to 50% of the plants in some gardens due to *P. cactorum* (Duke, 1989).

Darmono et al., (1991) documented the presence of *P. cactorum* in Wisconsin and recovered isolates from both forest and ginseng garden soils. In our 2003 and 2004 study, 47% of all sampled symptomatic and asymptomatic plants from Wisconsin ginseng gardens yielded *P. cactorum*. Disease pressure was severe in gardens where Phytophthora was recovered.

Woods-grown ginseng is a relatively new commercial crop in Michigan, with the first seedlings (Wisconsin transplants) having been planted in 1995 (Hausbeck, 2004). This is the first report of *P. cactorum* on ginseng in Michigan. *Phytophthora cactorum* was recovered from plants in 5 of 6 gardens sampled during 2003 in the Upper Peninsula and was responsible for significant plant loss. Additional *P. cactorum* isolates were recovered from research gardens established at Michigan State University. These gardens had been established from transplants of 2- to 3-year-old roots and planting of seed from Wisconsin in 2003 and 2004. Symptoms were particularly severe during the cool, wet conditions of early spring; conditions conductive for *P. cactorum* (Pritts, 1995; Darmono et al., 1991).

Much of the ginseng sampled during this study were from 2- and 3-year old plantings collected from failing gardens. Ginseng growers prefer to harvest roots at 3 or

4 years old, as this stage is more desired for market. In addition to *P. cactorum*, other pathogens were frequently isolated from ginseng plants and include *Fusarium* spp., *C. destructans,* and *Pythium* spp. Competition among these pathogens may have hindered the recovery of *P. cactorum*.

Recovery of *P. cactorum* isolates from ginseng seedlings grown in a sterilized medium in the greenhouse and from seed directly plated on agar, suggests that *P. cactorum* may be disseminated via the seed. Hobson (1999) estimated that seed rots and seedborne diseases are responsible for much of the reduction in germination and seedling emergence of ginseng. Pathogens including *Fusarium* and *Cylindrocarpon* have been documented on ginseng seed and are thought to be early colonists of germinating and emerging seedlings (Ziezold, 1997; Hobson, 1999). It was hypothesized that quality control methods such as floating stratified ginseng seed before planting, were not effective due in part to the fact that infected seed could sink to the bottom of the float tray along with the healthy seed (Ziezold et al. 1998; Hobson, 1999). Since *P. cactorum* has only been reported on safflower seed (Zad, 1992), further research on seed transmission is needed.

All ginseng *P. cactorum* isolates collected were pathogenic to apple fruit. Pathogenicity was determined by testing each isolate on apple fruit hosts. Most *Phytophthora* spp. grow rapidly on apple tissue and the use of apples for the isolation of Phytophthora from infected plant parts has proven successful (Tucker, 1967). It has also been documented that *P. cactorum* is not host specific and is virulent on apple fruit (Tucker, 1967; Erwin and Ribeiro, 1996). Although the use of ginseng seedlings for

pathogenicity tests is relatively easy and fast (Darmono et al., 1991), healthy stratified seeds were not available during this study.

After 7 days of incubation the majority of screened isolates produced lesions that covered 25% of the apple fruit. After 14 days, many lesions expanded and covered 100% of the apple. The observed virulence in this research did not appear to be associated with mefenoxam sensitivity or origin of the isolates. Although a quarter percentage was used for determining the virulence of each isolate; five isolates (3.6 and 0.97% of isolates for 2003 and 2004 respectively) were scored as virulent to apple fruit with a maximum surface lesion of 10% of the apple fruit.

Darmono et al. (1991) reported that while apple cotyledons and ginseng leaflets could be colonized by pathogenic and non-pathogenic isolates of *P. cactorum*, the recovery of *P. cactorum* from each host was not a good predictor of pathogenicity or virulence to a ginseng seedlings. Because there was little distinction between virulence of the isolates on the apple fruit, the research findings presented should be verified on ginseng seedlings and mature plants to better estimate the disease potential of each isolate.

Phytophthora cactorum is highly destructive and fungicides are commonly required to limit plant loss. Mefenoxam is the primary registered fungicide used for *P. cactorum* control on ginseng (Hausbeck, 2004). Resistance to mefenoxam among some *Phytophthora* spp. has been documented on crops such as cucumbers, potato, and soybean (Lamour and Hausbeck, 2000; Taylor et al., 2002; Malvick, 2003). Recently, resistance to mefenoxam in *P. cactorum* was identified with isolates from strawberry roots and crowns (Jeffers and Schnabel, 2004). In this study, *P. cactorum* from ginseng

was primarily insensitive to mefenoxam. This is the first report of *P. cactorum* resistant to mefenoxam in ginseng and the second for any other crop in the United States or elsewhere.

In addition to metalaxyl and mefenoxam (Ridomil 2E or Ridomil Gold) the fungicides Aliette (aluminum tris) and Phostrol (phosphorus salts) are labeled for use on ginseng for Phytophthora control. Utkhede (1987) tested metalaxyl, aluminum tris, and mancozeb for control of *P. cactorum* crown rot of McIntosh apple trees. Utkhede concluded that metalaxyl and fosetyl - al could arrest further symptom development in 'McIntosh' apple trees naturally infected with *P. cactorum* if treated at an early stage of disease. An additional study was conducted by Utkhede and Smith (1991) verifying that metalaxyl and fosetyl - al were effective against *P. cactorum* on 'McIntosh' apple trees.

When using inoculated apple fruit to test the efficacy of different control products, the greatest lesion coverage and incidence of infection was observed with the untreated inoculated and mefenoxam-treated apples. Mefenoxam did not perform well in managing disease because of the use of a mefenoxam-insensitive *P. cactorum* isolate. Although some control was observed with phosphorus salts and fosetyl - al, the number of infected fruit and percent of lesion coverage on the apples was variable among the replicates. The most effective products in managing *P. cactorum* were dimethomorph, copper hydroxide and fenamidone. Results from this study, although on apple fruit, may provide an indication of fungicides efficacy on ginseng foliar blight.

The performance of these products were also examined when used as a soil drench to protect ginseng seedlings. As with the apple study, the greatest incidence of infection and subsequently the greatest amount of plant death was observed with the

mefenoxam- treated plants. This is again, attributed to the insensitivity of the isolate to mefenoxam. The efficacy of Phostrol (phosphorus salts) and Aliette (fosetyl - al) against *P. cactorum* was varied among the trials. The most effective products were Kocide (copper hydroxide) and Acrobat (dimethomorph).

Phytophthora cactorum (Lebert & Cohn) J. Schröt, is considered to be a devastating fungal disease on ginseng. It appears that *P. cactorum* is disseminated via seed, further hindering the ginseng industry. With only three products registered for Phytophthora control on ginseng, resistance to the fungicide standard, mefenoxam is of great concern. Additional fungicides and new cultural strategies (seed stratification and storage procedures) are needed to maintain this high value export crop.

Literature Cited

Adam, K. L. 2004. Ginseng, goldenseal and other native roots. Horticulture technical note. ATTRA publication no. IP115.

Anonymous. 2000. American ginseng: Vegetable crops production guide for the Atlantic Provinces. Atlantic Comm. on Vegetables Publications/Vegetable Production Guides. Online publication.

Baranov, A. 1966. Recent advance in our knowledge of the morphology, cultivation and uses of ginseng (*Panax ginseng* C.A. Meyer). Economic Botany 20: 403-406.

Blackwell, E. 1943. The life history of *Phytophthora cactorum* (Leb. & Cohn.) Schröt. Transactions British Mycological Society 26: 71-89.

Blackwell, E. 1949. Terminology in Phytophthora with definitions and descriptions. Commonwealth Mycological Institute Mycological papers 30:1-21 .

Beyfuss, R.L. 1998. Growing ginseng and goldenseal in your forest. Online publication. http://www.dec.state.ny.us/website/dlf/privland/forprot/ginseng/growing_guide. Html. Accessed March 16, 2004.

Brammall, R. and P. Fisher. 1993. Botrytis blight of ginseng. Ontario Ministry of Agriculture and Food, Box 587, Simcoe, Ontario N3Y 4N5, order no.: 93-071.

British Columbia Ministry of Agriculture, Food and Fisheries, Canada. 2003. Ginseng Production guide for commercial growers.

Brun, C.A. 1999. Crop profile for ginseng in Washington. USDA Regional Pest Management Centers. Online publication.

Carlson, A.W. 1986. Ginseng: America's botanical drug connection to the Orient. Economic Botany 40(2): 233-249.

Chen, Amy. 2002. Yin and Yang: Balancing health with food. Online publication. www.supermarketguru.com/page.cfm/136. Accessed 3/19/04.

Curran, D. F. 1985. The ginseng disease and pest reference guide. D.F. Curran Productions. Hamburg, WI.

Darmono, T.W. and J.L. Parke. 1990. Chlamydospores of *Phytophthora cactorum*: their production, structure, and infectivity. Canadian Journal of Botany 68: 640-645.

Darmono T.W., M.L. Owen, and J.L. Parke. 1991. Isolation and pathogenicity of *Phytophthora cactorum* from forest and ginseng gardens in Wisconsin. Plant Disease 75:610-612.

Davis, J. and P.B. Shoemarker. 1999. Ginseng disease control *Phytophthora* and *Alternaria*. Extension bulletin. North Carolina State University and A&T State University.

Department of Commerce U.S. Census Bureau, Foreign Trade Statistics - FAS Agricultural Export Commodity Aggregations. 2002.

Dekker, J. 1987. How to conduct and measure fungicide resistance. experimental Techniques in Plant Disease Epidemiology. J. Kranz and J. Rotem, (eds) New York Springer Verlag Berln Heidelberg.

Devitt, M. 2000. American ginseng may help control diabetes. www.accupuncturetoday .com/ archieves/2000/june/06ginseng.htm. Accessed March 16, 2004

Devitt, M. 2003. Ginseng shows promise in treating diabetes. www.accupuncturetoday .com/archives2003/oct/10ginsengdiabetes.gtml. Accessed March 16, 2004

Duke, J. A. 1989. Ginseng: A Concise Handbook. Reference Publications, Inc.

Erwin, D.C. and O.K. Ribeiro. 1996. Phytophthora Diseases Worldwide. St. Paul: APS Press.

Hardacre, V. 1968. Woodland nuggets of gold. Vantage Press. New York.

Harding, A.R. 1912. Revised 1936. Ginseng and other medicinal plants. A.R. Harding, Columbus, Ohio.

Harrison, H.C.,, J.L. Parke, E.A. Oelke, A.R. Kaminski, B.D. Hudelson, L.J. Martin, K.A. Kelling, and L.K. Binning. 2000. Ginseng. Alternative Field Crops Manual. University of Wisconsin-Extension, Cooperative Extension University of Minnesota Center for Alternative Plant and Animal Products and the Minnesota Extension Service.

Hausbeck, M. K. 2004. Pest management in the future. A strategic plan for the Michigan and Wisconsin ginseng industry. April 13, 2004. Michigan State University

Heffer, V., M.L. Powelson, and K.B. Johnson. 2002. Oomycetes. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2002-0225-01. St. Paul. APS Net. Online publication. http://www.apsnet.org/education/LabExercises/Oomycetes/. Accessed March 18, 2004

Hobson, J.M. 1999. Spring seeding, seed size and seed pathology of North American ginseng (*Panax quinquefolius* L.). M.S. Thesis. University of Guelph.

Hou, J.P. 1978. The myth and truth about ginseng. A.S. Barnes and Co., Inc. Cranbury, New Jersey.

Hudelson, B. (2002). Rusty root, University of Wisconsin. Extension bulletin.

Ireland, N. 2000. American ginseng reduces blood sugar. Online publication. www.newsandevents.utoronto.ca/bin/ 000410a.asp. Accessed March 16, 2004

Jeffers, S.N. and G. Schnabel, 2004. First report of resistance to mefenoxam in *Phytophthora cactorum* in the United States and elsewhere. Plant Disease 88: 576.

Keller, P. 1998. Ginseng. Southern Illinois University Carbondale. Ethnobotanical Leaflets.

Lamour, K. H. and M. K. Hausbeck. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90:396-400.

Malvick, D. 2003. *Phytophthora sojae* sensitivity to metalaxyl and mefenoxam. The Pest Management and Crop Development Bulletin. University of Illinois Extension.

Michigan Department of Agriculture. 1998. Michigan Agricultural Statistics 1997-1998. On-line publication. Kleweno and Matthews.

Mindell, E. 1992. Earl Mindell's herb bible. Simon & Schuster. New York.

Nash, G.V. 1898. American ginseng: its commercial history, protection, and cultivation. U.S. Department of Agriculture. Division of Botany. M. Kains (ed) Washington.

Natural Resources Conservation Service 2003. Plants Profile: *Panax quinquefolius* L., U.S. Department of Agriculture.

North Carolina Cooperative Extension Service. 1997. Ginseng: A Production Guide for North Carolina. North Carolina State University.

Ontario Ministry of Agriculture and Food. 2004. Ginseng Production Crop Update for May 4, 2004 Special bulletin on foliar Phytophthora. Online publication http://www.omafra.gov.

Polczinski, L.C. 1982. Ginseng (*Panax quinquefolius* L.) culture in Marathon County, Wisconsin: historical growth, distribution and soils inventory. M.S. Thesis. University of Wisconsin.

Pritts, K. D. 1995. Ginseng how to find, grow and use America's forest gold. Stackpole Books. Mechanicsburg, PA.

Proctor, J.T.A. 1996. Ginseng: old crop, new directions. J. Janick (ed.) ASHS Press.

Proctor, J.T.A., and W.G. Bailey. 1987. Ginseng: industry, botany, and culture. Horticultural Reviews 9:187-236.

Proctor, J.T.A. and Louttit, D. 1995. Stratification of American ginseng seed: Embryo growth and temperature. Korean J. Ginseng Science 19(2):171-174.

Pumphrey, D. A. 2003. Horticultural and Tropical Products Division, USDA, Foreign Agriculture Service. Online publication http://www.fas.usda.gov/htp/tropical/ 2002/ March%2020 03 %20Cover.pdf. Accessed March 15, 2004.

Putnam, M.L and J.E. Mitchell. 1984. The use of metalaxyl to control Phytophthora root rot of ginseng (*Panax quinquefolium*) (Abstr.) Phytopathology 74:854-855.

Putnam, M.L. 1984. Studies of *Phytophthora cactorum* (Leb. & Cohn) Schröet and *Rhizoctonia solani* Kühn on American ginseng (*Panax quinquefolium* L). M.S. Thesis. University of Wisconsin-Madison 124p.

Rahimian, M.K. and J.E. Mitchell. 1988. Detection and quantification of *Phytophthora cactorum* in naturally infested soils. Phytopathology 78:949-952.

Reid, T.C. 2000. Evaluation of strategies for the control of Fusarium crown and root rot of asparagus. M.S. Thesis. Michigan State University 131p.

Ribeiro, O.K. 1978. A Source Book of the Genus *Phytophthora*. J.Cramer In: der A.R. Gantner (editor). Verlag Kommandgessellschaft FL-9490 Vaduz.

Rosenbaum, J. 1915. Phytophthora disease of ginseng. N.Y. Cornell Agric. Exp. Sta. Bull. 363:65-106.

Schmitthenner, A. F. and R. G. Bhat 1994. Useful Methods for Studying Phytophthora in the Laboratory. OARDC Special Circular 143: 1-16.

Sotaniemi, E.A., E. Haapakoski and A. Rautio.1995. Ginseng therapy in non-insulin-dependent diabetic patients. Diabetes Care 18: 1373-1375, 1995.

Syngenta Crop Protection Inc. 2004. Ridomil Gold EC fungicide mode of action. Online publication. http://www.syngentacropprotection-us.com/prod/fungicide /ridomil. Accessed January 19, 2004.

Taylor, R. J., B. Salas, G. A. Secor, V. Rivera, and N. C. Gudmestad. 2002. Sensitivity of North American isolates of *Phytophthora erythroseptica* and *Pythium ultimum* to mefenoxam (metalaxyl). Plant Disease 86(7): 797-802

Tucker, C.M. 1967. The taxonomy of the genus *Phytophthora* De Bary. Verlag Von J. Cramer 3301 Lehre.

U.S. Department of Commerce Economics and Statistics Administration 1995. American Ginseng-Rooted in Wisconsin. Census of Agriculture No.2.

USDA, NRCS. 2002. The PLANTS Database, Version 3.5. Online publication. http://plants.usda.gov. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. Accessed January 29, 2004.

USDA, NASS.1997. Census of Agriculture – State Data volume 1 Online publication. http://www. nass.usda.gov/census/census97/volume1/us-51/us2_34.pdf. Accessed January 24, 2004.

USDA. 2003. Tropical products, world markets and trade.

Utkhede, R.S. 1987. Control of crown rot (*Phytophthora cactorum*) of apple trees with systemic fungicides metalaxyl and fosetyl-aluminium. Pesticide Science, 19:289-295.

Utkhede, R.S. and V.K. Gupta. 1988. In vitro selection of strains of *Phytophthora cactorum* resistant to metalaxyl. Journal of Phytopathology 122:35-44.

Utkhede, R.S. and E.M. Smith. 1991. Effects of fosetyl-al, metalaxyl and *Enterobacter aerogenes* on crown and root rot of apples trees caused by *Phytophthora cactorum* in British Columbia. Plant Disease, 75(4): 406-409.

Van Hook, J. M. 1904. Diseases of ginseng. Cornell University. Agriculture Experiment Station. Bulletin 219: 164-186.

Vuksan, V., Sievenpiper, John L. 2000. American Ginseng (*Panax quinquefolius* L) reduces postprandial glycemia in nondiabetic subjects and subjects with type 2 diabetes mellitus. Arch Intern Med 160: 1009-1013.

Zad, J. 1992. Safflower seed-borne diseases. Fac. Landbouw. Rijksuniv. Gent. Belgium 52(2a) P: 161-165.

Ziezold, A.M. 1997. Chemical control of disappearing root rot of ginseng caused by *Cylindrocarpon destrucans*, M,Sc. Thesis. University of Guelph, ON.

Ziezold, A.M., R.D. Reeleder, R. Hall, and J.T. Proctor. 1998. Seedborne, fungi and fungicide seed treatment of ginseng. Journal of Ginseng Research 22(4): 229-236.

Zhang, T., Y, Zhang, W. Chen, and C. Zhang. 1989. An analysis of seedborne fungi of imported seeds of *Panax quinquefolium.* Acta phytopathologica sinica 19 (3): 134-138.

Zhang, T. and W. Chen. 1992. A preliminary study of seed pathology of *Panax quinquefolium.* Korean Journal of Ginseng Science 16: 244-245