IDENTIFICATION AND CHARACTERIZATION OF RESISTANCE TO PHYTOPHTHORA CAPSICI WITHIN SQUASH (CUCURBITA SPP.)

By

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To my wife, Michelle Cook Padley; my son, Jonathan Edwin Padley; and both of my families I would not have accomplished so much or be where I am today without your love and guidance. Thank You All.

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Abstract of Dissertation Presented to the Graduate School
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By

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Phytophthora capsici Leonian causes several disease syndromes on squash (*Cucurbita* spp.) including crown rot, foliar blight, and fruit rot, which can lead up to 100% crop loss. Currently, there are no summer or winter squash cultivars resistant to this pathogen which can aid in disease management strategies.

I evaluated 115 summer squash (*C. pepo*) accessions for their response to crown inoculation with a suspension of *P. capsici* isolates. Replicates of each accession were rated on a scale ranging from 0 (no symptoms) to 5 (plant death). Mean disease rating scores (DRS) and standard deviations ranged from 1.3 to 5.0 and 0 to 2.0, respectively. Accessions with the lowest mean DRS were rescreened and paralleled those of the initial study with PI 181761 exhibiting the lowest mean DRS at 0.5. Further screening and selection from the *C. pepo* germplasm collection will aid in the development of summer squash cultivars with *P. capsici* crown rot resistance.

A series of interspecific hybridizations of two wild *Cucurbita* species with winter squash (*C. moschata*) led to the development of *Cucurbita* breeding line #394 which segregated for resistance to *P. capsici* crown rot. Additional selections of #394 for resistance to *P. capsici* crown rot were performed. Breeding line #394-1-27-12 was created and is homozygous resistant

to *P. capsici* crown rot. The inheritance of resistance to *P. capsici* found within #394-1-27-12 was determined through pollination with 'Butterbush' a susceptible butternut-type winter squash (*C. moschata*). Segregation ratios of the F_2 and BC progeny of this cross support a model in which resistance to *P. capsici* crown rot, within #394-1-27-12, is conferred by three dominant genes. Introgression of *P. capsici* crown rot resistance from #394-1-27-12 into the morphologically diverse domesticates within *Cucurbita* is currently underway.

My research identified sources of resistance to *P. capsici* within summer squash (*C. pepo*), developed a *Cucurbita* breeding line with *P. capsici* crown rot resistance, and determined the inheritance of *P. capsici* crown rot resistance introgressed from two *Cucurbita* wild species. Summer and winter squash breeding material developed from this project will aid in the disease management of *P. capsici*.

CHAPTER 1
LITERATURE REVIEW

Introduction

Squash, pumpkins and gourds (*Cucurbita* spp.) rank among the top producing vegetable crops in the world (FAOSTAT, 2007a). From this worldwide production, the United States ranked 5th, in 2006, producing 861,000 metric tons of which squash represented 474,100 metric tons (FAOSTAT, 2007b). This 474,100 metric tons of yield grossed over $229 million in sales for the United States squash industry making this vegetable crop a multimillion dollar business (USDA, 2007a).

Squash is affected by many pathogens and pests (Zitter et al., 1996). One of the most devastating is the oomycetous pathogen, *Phytophthora capsici*. The incidence of disease caused by *P. capsici* on cucurbits has increased with reported yield loss as high as 100% (Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). *Phytophthora capsici* can infect cucurbits at any growth stage and is capable of causing crown rot, foliar blight, and fruit rot (Zitter et al., 1996; Roberts et al., 2001). Given optimal conditions an entire field of cucurbits can be devastated by *P. capsici* in a matter of days (Babadoost, 2004; Hausbeck and Lamour, 2004; Lee et al., 2001; Roberts et al., 2001). With the increased occurrence and severity of *P. capsici*, research for management alternatives, including breeding cucurbits for resistance, is key in effectively managing this pathogen (Hausbeck and Lamour, 2004; French-Monar et al., 2005; Keinath, 2007). Currently, there are no summer or winter squash cultivars resistant to the various disease syndromes caused by *P. capsici*.

Germplasm collections, representing wild and exotic material, are valuable sources of beneficial genes and have been used to identify sources of resistance to numerous plant pathogens (Herrington et al., 2001; Paris and Cohen, 2000; Stephens, 2003; USDA, 2006c,d).

For summer squash, a *C. pepo* germplasm collection, maintained at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, has more than 900 *C. pepo* accessions available for evaluation. For winter squash, resistance to *P. capsici* derived from two *Cucurbita* wild species, *C. lundelliana* and *C. okeechobeenesis* sbsp. *okeechobeenesis* has recently been introgressed into a *C. moschata* genetic background (Kabelka et al., 2007). One particular breeding line, designated #394, exhibits heterozygosity for resistance to the crown rot syndrome of *P. capsici* and is of particular interest for continued selection to produce a homozygous crown rot resistant winter squash breeding line.

This research was initiated to identify sources of resistance to *P. capsici* for introgression into summer squash (*C. pepo*) and to better understand the nature of *P. capsici* resistance recently introgressed into winter squash (*C. moschata*). While there are several disease syndromes of *P. capsici*, this research focuses on crown rot with the long-term goal of developing advanced breeding material with resistance.

The specific objectives of this research project were: (1) identify sources of resistance to *P. capsici* crown rot within the *C. pepo* germplasm collection; (2) develop a homozygous *P. capsici* crown rot resistant *Cucurbita* breeding line; and (3) characterize the resistance to *P. capsici* crown rot found within *Cucurbita* breeding line #394. The successful completion of this research will not only aid in the disease management of *P. capsici* within squash but will result in continued productivity and profitability of both summer and winter squash.

Squash (*Cucurbita* spp.)

Taxonomy

The genera *Cucurbita*, within the family *Cucurbitaceae*, consists of 27 species native to the Western hemisphere (Wilson, 1990). Of these 27 species, five are cultivated and include *C. pepo*, *C. moschata*, *C. maxima*, *C. argyrosperma* (formerly *C. mixta*) and *C. ficifolia*

(Robinson and Decker-Walters, 1999). *Cucurbita* cultivars are categorized as summer squash or winter squash. Summer squash are eaten immature when tender and seeds are small and soft. Winter squash are generally eaten when rind and seeds are fully mature. Summer squash cultivars are *C. pepo* while winter squash cultivars can be *C. pepo*, *C. maxima*, *C. moschata*, or *C. argyrosperma*.

Fruit shape within *C. pepo* is the basis for ten horticultural classifications of this species (Paris, 1986; 1996). These include eight edible-fruited cultivar groups designated pumpkin, vegetable marrow, cocozelle, zucchini, acorn, scallop, crookneck, and straightneck and two non-edible-fruited ornamental cultivar groups designated orange gourd and ovifera gourd. There is considerable morphological diversity of *C. moschata* and horticultural groupings, based on market-type cultivars, include neck, cheese, tropical, and japonica (Bates et al., 1990; Robinson and Decker-Walters, 1999). *Cucurbita maxima* exhibits greater diversity of fruit types than *C. pepo* (Bates et al., 1990; Robinson and Decker-Walters, 1999). Several horticultural groups based on market-type describe this species and include Australian blue, banana, buttercup, hubbard, mammoth, and turban. Cultivars of *C. argyrosperma* and *C. ficifolia* are produced primarily for their seed which provides a considerable amount of oil and protein.

Production

In 2006, squash, pumpkins and gourds were ranked 11th in terms of production among worldwide vegetable crops with a total yield of 21,003,464 tons (FAOSSTAT, 2007b). The top producing countries for squash, pumpkins and gourds were China (6,060,250 tons), India (3,678,413 tons), Russian Federation (1,184,670 tons), Ukraine (1,064,000 tons) and the United States (861,870 tons). From the 861,870 tons of squash, pumpkins and gourds produced in the United States, squash composed 55% (474,100 tons) of this total with a value of approximately $229 million. Of this $229 million industry, Florida ranked second in the nation earning 38

million dollars in revenue. Other top states, in terms of revenue from squash production, include Georgia ($49,920,000), California ($37,929,000), New York ($28,274,000) and Michigan ($14,994,000) (USDA, 2007b).

Cultivation

Squash are annual herbaceous bushes or vines that are typically grown in the fall or spring when the threat of freeze or frost is over (Peet, 2001a). Seeds are planted in bare ground, or raised beds, with a soil high in organic matter and a ph between 6 and 6.5. Optimum growing temperature for squash is between 24 to 29ºC during the day and 16 to 21ºC at night. Temperatures below 4.4ºC for several days can cause severe damage to the plants, and exposure to temperatures above 29ºC can cause fruit drop along with small fruit size (Peet, 2001b). Like other cucurbits, squash can be transplanted from greenhouse to field to increase earliness and decrease the chance of frost damage. Flowers on squash plants are monoecious, produced just above the axil of the leaf, and are conspicuously bright yellow to orange in color (Swiader & Ware, 2002). Female flowers are easily distinguished from male flowers due to the developing ovary at the base of the flower. Typically male flowers are produced 3-4 days before the female flowers with a ratio of 3:1 male to female flowers, developing as the plant grows. Changes in photoperiod and temperature can affect the flower ratio and blooming time (Stephens, 2003).

Winter squash are grown for a period of 80 to 140 days until fruit reach full maturity (Swiader & Ware, 2002). Summer squash are grown for 40-60 days before producing marketable fruit after the first pollinations. Sunny dry weather is needed throughout the growing season for optimum fruit production.

For pollination, honey bees are required for squash due to the large pollen size and the short pollination window, 8-10 a.m., in which the female flowers are open. The size and shape of the fruit, along with the quantity and thickness of the seed is directly proportional to the

amount of pollen placed onto the stigma. If adequate pollen is not deposited on the female flower the fruit will become misshapen or possibly abort. One honey bee hive per acre is recommended to insure fruit set (Peet, 2001b; Swiader and Ware, 2002).

High levels of fertilization are required to grow squash. The amount and frequency of fertilizer used is based on soil type, climate, plant spacing and cultural management of the crop.

Postharvest Practices

Maintaining the postharvest quality of summer and winter squash from harvest to the retail level has few similarities and many differences (Kader, 2002). Both summer and winter squash are non-climacteric fruit, meaning they do not undergo a final ripening caused by the release of ethylene. This lack of color change and conversion of starch to sugars during final ripening allows squash fruit to be at horticultural mature when harvested. Once harvested the fruit of summer and winter squash are ready to eat. Beyond these similarities summer and winter squash differ from harvest through retail level in the maintenance of postharvest quality.

Summer squash are harvested several times throughout the season at an immature stage 40-60 days after planting (Swiader and Ware, 2002). Since the fruit are harvested at a young stage the rind is still very soft making the fruit more susceptible to damage and deterioration. Due to their soft rinds and immature state, summer squash are harvested by hand and the fruit are cooled immediately after harvest. Fruit are stored in a cooler at 7°C - 10°C with a relative humidity (RH) of 95% (Kader, 2002). The high humidity in storage is used to compensate for the soft rind of the fruit that allows for greater water loss. Under these storage conditions summer squash can be kept at commercial standards for a maximum of 2 weeks (Kader, 2002). Chilling injury can occur if the fruit are stored below 5°C or they undergo one light freeze. Summer squash are displayed for retail sale as fresh or cut fruit in a refrigerated area due to their high perishability (McCollum, 2004).

Winter squash are harvested once, by hand, at physiological maturity, usually between 80 to 140 days after planting (Brecht, 2004). At physiological maturity, the fruit have a strong flesh color with a hard, non-glossy, rind that is resistant to damage and deterioration (Olson et al., 2007). After harvest the fruit may be cured for 10 to 20 days at a temperature of 24°C to 27°C with a RH of 80%. This process allows for wounds to heal, the rind to harden, and any immature fruit to ripen for a longer storage life (Swiader and Ware, 2002). Fruit are room cooled and stored at 12°C - 15°C with a RH of 50% - 70% (Kader, 2002). Winter squash are less chilling sensitive then summer squash and are able to withstand one to two light freezes without injury. At the retail level winter squash are displayed at room temperature (Brecht, 2004).

Phytophthora capsici

Phytophthora capsici Leonian is a devastating plant pathogen in the Phylum oomycete (Babadoost, 2004). This fungal-like pathogen produces mycelium that branch at 90 degree angles and reproduces asexually by means of sporangium and zoospores and sexually by means of oospores. *Phytophthora capsici* was first discovered in the fall of 1918 by Leon H. Leonian at the New Mexico Agricultural Research Station in Les Cruces. In a report published in 1922, he described a new species of phytophthora as the cause of considerable damage to a field of chili peppers in 1918 which then reappeared in the same field and surrounding farms the next year (Leonian, 1922). Since its discovery, *P. capsici* has caused severe epidemics of many vegetable crops in Central and South America, Europe, Asia, Korea and the United States (Roberts et al., 2001).

In the United States, *P. capsici* has been identified in many of the vegetable producing states including California, North and South Carolina, Florida, Georgia, Illinois, New Jersey, Michigan and Texas (Babadoost and Islam, 2003; Café-Filho et al., 1995; Isaleit, 2007; Lamour and Hausbeck, 2003; Ristaino, 1990). In the state of Florida, *P. capsici* has been known to cause

severe outbreaks during unusually wet and warm weather. During one of the last outbreaks, a survey of growers in Manatee County showed losses from *P. capsici* ranging from 35% in tomato, 65% in cantaloupe, 42% in bell and jalapeno peppers, 100% in squash, and 36% in watermelon (Roberts et al., 2001).

Host Range

Phytophthora capsici has a wide host range that includes a minimum of 53 susceptible species in 24 different families (Table 1-1). The families *Cucurbitaceae* and *Solanaceae* contain many horticulturally significant crops worldwide that are susceptible to *P. capsici* (Babadoost, 2004; Hausbeck and Lamour, 2004). The level of virulence an isolate has on a host plant can vary greatly depending on the pathogenicity of the isolate and the host/isolate interaction (Hausbeck and Lamour, 2004). Determining the virulence among *P. capsici* isolates is the key to developing an effective way of controlling this pathogen in the field. Host-specific isolates have been found in tomato and pepper (Babadoost et al., 2008; Hausbeck and Lamour, 2004; Lee et al., 2001; Ristaino, 1990). Isolates also exist that can infect multiple hosts allowing them to survive from one growing season to another.

Disease Symptoms

Phytophthora capsici can cause disease on susceptible plants at any growth stage; although immature plants of some species are more susceptible thin mature plants (Roberts et al., 1999; Tiam and Babadoost, 2004; Lee et al., 2001). Symptoms caused by *P. capsici* include seed rot (pre-emergence damping-off), seedling blight (post-emergence damping-off), root rot, and crown rot which can cause plant stunting, wilting and/or death of the entire plant in a very short period of time. Stem lesions can occur along any part of the stem in a host plant and appear as dark brown, water-soaked lesions that can girdle the plant. Leaf spots develop when infected water lands on a leaf causing dark brown spots to appear that will range from one-half to several inches

in diameter. If these leaf spots coalesce, foliar blight can occur. Symptoms of fruit infection on a susceptible host are easily recognized. It begins as a dark sunken lesion that can develop into a fine white powder-like layer of spores which can cover the entire surface of the fruit. Fruit infection can occur several days before the symptoms are visible allowing fruit rot to develop postharvest.

Reproduction

Phytophthora capsici can reproduce through means of sexual or asexual spores (Babadoost, 2004). There are two types of asexual spores; sporangium and zoospores. Sporangia are lemon-shaped spores that are produced on the surface of the plant or fruit. These spores can be spread through rain water, irrigation water or wind blown rain. In a moist environment, the sporangium can either directly germinated to infect a host or release smaller biflagellate swimming spores called zoospores. Only one zoospore is needed to infect a plant. Zoospores of this pathogen are attracted to the root exudates of a host and can travel for several hours through water in search of new host to infect (Babadoost, 2004). The zoospore's ability to move through water, along with multiple infection cycles in one season, allows *P. capsici* to begin as a small infection that can expand to a large epidemic in a relatively short period of time (Hausbeck and Lamour, 2004).

The sexual spores of *P. capsici* are called oospores (Babadoost, 2004). Oospores are produced when two compatible mating types, A1 and A2, come together and undergo sexual recombination. Oospores are formed when a male gametangium, called an antheridium, and a female gametangium, called an oogomium, undergo meiosis and fuse through plasmogamy and karyogamy to produce an oospore with half the genetic material from each parent. The oospores of *P. capsici* are thick-walled, resistant to desiccation and cold temperatures, and can survive in the soil for many years (Keinath, 2004). Once triggered by the proper environmental condition,

the oospore can germinate to produce a germ tube, sporangium, and/or zoospores to infect new plants (Babadoost, 2004).

Disease Management

Phytophthora capsici has been controlled mainly by use of fungicides. However, this practice has changed as A1 and A2 mating types have been introduced into fields allowing for mutation and recombination of the parental types which creates resistance to the various fungicides. Isolates of *P. capsici* from many states are now resistant to mefenoxam, the active ingredient in a common fungicide used to control oomycetes (Hausbeck and Lamour, 2004; Roberts et al., 1999). A combination of factors, including water management, crop rotation, cultural practices, fumagation and resistant/tolerant crop varieties, along with fungicides are needed to properly control the level and spread of this pathogen (Tian and Babadoost, 2004; Keinath, 2004).

Proper drainage in the field is a key factor for controlling *P. capsici* in the field (Ristaino and Johnston, 1999). A level, well drained, field with no low lying areas will help prevent focal points for the development of epidemics. A proper irrigation plan will also help reduce the incidence of disease in the field. A study conducted by Café-Filho et al. (1995) showed that avoiding excessive irrigation reduced the loss in yield due to *P. capsici* in a furrow field. Furrow irrigation should also be limited due to the easy spread of *P. capsici* in water from the point of origin down the field to non-infected plants (Café-Filho et al., 1995)

Crop rotation is used to decrease the level of *P. capsici* in the field between planting of susceptible crops (Tian and Babadoost, 2004). A minimum three years crop rotation of plants not susceptible to *P. capsici* is recommended to decrease the level of oospores in the field (Hausbeck and Lamour, 2004). Rotating a crop between two susceptible hosts, such as pepper and cucurbits, can result in severe disease problems in the field (Ristaino, 1990)

There are several cultural practices that will help manage *P. capsici* in the field (Babadoost, 2004). These include: growing susceptible crops on raised beds (6 inch minimum) in fields with no history of the disease; selecting fields that are isolated from known *P. capsici* infected fields; scouting fields for symptoms of *P. capsici*; plowing under the parts of a field with diseased plants including the healthy plants that border the diseased area; removing diseased fruit from the field; and cleaning farm equipment of soil when traveling between fields. The newest tool in disease scouting is the use of PCR based methods to identify *P. capsici* at the early stage of infection (Babadoost, 2004; Tian and Babadoost, 2004; Hausbeck and Lamour, 2004).

A proper fungicide rotation can be effective in controlling *P. capsici* under normal field conditions (Café-Filho, et al., 1995). However, in a conducive environment, these fungicides have been proven inadequate in controlling this pathogen. Mefenoxam, a systemic phenylamide chemical used in many fungicides by growers, has become ineffective in controlling *P. capsici* due to a possible single gene mutation in the pathogen (Lamour and Hausbeck, 2003). The fumigant, methyl bromide, has been used extensively to control *P. capsici* throughout the United States but due to its deleterious effects on the ozone it is being phased out (USDA, 2006a). Alternative fungicides are being tested to manage *P. capsici*; however, efficancy is limited under conductive conditions, nor does there appear to be a simple broad-spectrum fumigant to replace mefenoxam and methyl bromide.

Host Resistance

Resistance to *P. capsici* exists within certain plant species. In pepper, resistance to *P. capsici* comes from two different sources: PI 201232 which has intermediate levels of resistance and the Mexican land race called ‘Criollo de Morelos 334’ which has resistance to foliar blight, stem blight, and root rot (Alcantara and Bosland, 1994; Ortega et al., 1995; Sy et

al., 2005). In watermelon, Lee et al. (2001) screened nine Korean and Japanese pumpkin cultivars which showed a quantitative level of resistance to *P. capsici*, with variety 'Danmatmaetdol' being the most resistant. In squash, there are no known public sources of resistance to *P. capsici*.

Table 1-1. Plant species susceptible to *Phytophthora capsici*.

Family	Common Name	*Scientific Name*
Aloaceae	Aloe	*Aloe* sp.
Apiaceae	Carrot	*Daucus carota*
Araceae	Flamingo lily; oilcloth flower	*Antherium andreanum*
Asteraceae	Cosmos	*Cosmos Cav.* sp.
	Safflower	*Carthamus tinctorius* L.
Brassicaceae	Cauliflower	*Brassica oleracea* L.
	Radish	*Raphanus sativus*
	Turnip	*Brassica rapa*
Cactaceae	Indian Fig	*Opuntia ficus-indica* Mill.
Caryophyllaceae	Carnation	*Dianthus barbathus* L.
Chenopodiaceae	Beet	*Beta vulgaris*
	Spinach	*Spinacia oleracea*
	Swiss-chard	*Beta vulgaris* var. *cicla*
Cucurbitaceae	Acorn squash	*Cucurbita moschata*
	Blue Hubbard squash	*Cucurbita pepo*
	Cantaloupe	*Cucumis melo*
	Cucumber	*Cucumis sativus*
	Gourd	*Cucurbita pepo*
	Honeydew Melon	*Cucumis melo*
	Melon	*Pisum melo*
	Muskmelon	*Cucumis melo*
	Pumpkin	*Cucurbita maxima*
	Red Bryony; wild hop	*Bryonia dioica Jacq.*
	Watermelon	*Citrullus lanatus*
	Yellow squash	*Cucurbita pepo*
	Zucchini squash	*Cucurbita pepo*
Ebenaceae	Persimmon	*Diospyros kaki* L.
Fabaceae	Alfalfa; lucerne	*Medicago sativa* L.
	Broadbean	*Vicia faba* L.
	Butter or civet bean	*Phaseolus lunatus* L.
	Green bean	*Phaseolus vulgaris*
	Lima bean	*Phaseolus lunatus*
	Snow Pea	*Pisum sativus*
Lauraceae	Avocado	*Persea americana* Mill.
Liliaceae	Carolina geranium	*Geranium carolinianum*
	Onion	*Allium cepa* L.
Linaceae	Flax	*Linum* sp.
Malvaceae	Cotton	*Gossypium hirsutum* L.

Table 1-1. Continued.

Family	Common Name	*Scientific Name*
Malvaceae	Okra	*Abelmoschus esculentus*
	Velvet-leaf	*Abutilon theophrasti*
Moraceae	Fig	*Ficus carica*
Orchidaceae	Vanilla	*Vanilla planifolia Andr.*
Piperaceae	Betle	*Piper betle* L.
	Black pepper	*Piper nigrum*
Portulacaceae	Common Purslane	*Portulaca oleracea*
Proteaceae	Macadamia nut	*Macadamia integrifolia*
	Pincushion flower	*Leucospermum* R. Br.
Rosaceae	Apple	*Malus pumila* Mill.
	Hawthorn	*Crataegus oxyacantha* L.
	Peach	*Prunus persica* (L.) *Batsch*
Rutaceae	Citrus	*Citrus* sp.
Solanaceae	American Black Nightshade	*Solanum americanum*
	Bell pepper	*Capsicum annuum* L.
	Eggplant	*Solanum melongena*
	Hot pepper	*Capsicum annuum* & *Capsicum frutescens*
	Jimson weed	*Datura stramonium* L.
	Tobacco	*Nicotiana tabacum*
	Tomato	*Lycopersicon esculentum*
Sterculiaceae	Cocoa	*Theobroma cacao*

(Bittenbender et al., 1992; Hausbeck and Lamour, 2004; Holmes et al., 2001; Kellam and Zentmyer, 1986; Lamour and Hausbeck, 2003; Lee et al., 2001; Roberts et al., 2001; French-Moroa et al., 2006; Tian and Babadoost, 2004; Zentmyer, 1983).

CHAPTER 2
EVALUATION OF CUCURBITA PEPO ACCESSIONS FOR CROWN ROT RESISTANCE TO SQUASH ISOLATES OF PHYTOPHTHORA CAPSICI

Introduction

The oomycetous pathogen, *Phytophthora capsici* Leonian, infects a wide range of plant taxa involving more than 49 species (Erwin and Ribeiro, 1996). Oospores, the sexual stage of *P. capsici*, can survive in the soil, in crop debris, and in certain weeds for long periods of time (Zitter et al., 1996; Hausbeck and Lamour, 2004; French-Monar et al., 2006). The asexual zoospores of *P. capsici* contained in sporangia can be dispersed across a field by rain drops and irrigation water in a relatively short period of time. Given optimal conditions an entire field of crops can be devastated by *P. capsici* in a matter of days (Zitter et al., 1996; Roberts et al., 2001).

The incidence of disease caused by *P. capsici* on cucurbits has increased in vegetable production regions of the U.S. with reported yield loss as high as 100% (Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). The increased occurrence and severity of *P. capsici* has prompted research for fungicide management alternatives and interest in breeding cucurbits for resistance or tolerance (Babadoost, 2000; Stevenson et al., 2000; 2001; Seebold and Horten, 2003; Hausbeck and Lamour, 2004; McGrath, 2004; Tian and Babadoost, 2004; Waldenmaier, 2004; French-Monar et al., 2005; Keinath, 2007).

Cucurbita pepo L. (pumpkin, squash, and gourd) is an economically important group of the *Cucurbitaceae* (Paris et al., 2003). Eight cultivar-groups of edible-fruited domesticates of *C. pepo* have been described (Paris, 1986) which includes pumpkin, cocozelle, vegetable marrow, zucchini, acorn, scallop, crookneck, and straightneck. *Phytophthora capsici* can infect *C. pepo* at any growth stage and is capable of causing crown rot, foliar blight and fruit rot (Zitter et al., 1996; Roberts et al., 2001). Crown rot appears at the soil line causing stems to turn dark

brown, become water-soaked, and quickly collapse causing plant death. Foliar symptoms appear as rapidly expanding, water-soaked lesions. Dieback of shoot tips, wilting, shoot rot, and plant death quickly follows initial infection. Fruit, which can be infected at any stage of maturity, may exhibit sunken, brown, water-soaked areas which are rapidly covered by white sporangial growth under moist environmental conditions. Currently, there are no *C. pepo* cultivars resistant or tolerant to *P. capsici* (Hausbeck and Lamour, 2004).

Germplasm collections are valuable sources of beneficial genes including resistance or tolerance to numerous plant pathogens. The *C. pepo* germplasm collection maintained at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, has more than 900 *C. pepo* accessions available for evaluation (USDA, 2006b). While *P. capsici* causes several disease syndromes on *C. pepo*, the objective of this study was to evaluate a select group of *C. pepo* accessions for resistance to the crown rot syndrome of *P. capsici*.

Materials and Methods

Plant Material

Because no core collection representing *C. pepo* has been established to date, accessions selected for this study were based on two criteria: fruit type and geographic location. Based on NCRPIS descriptors, accessions with oblong yellow fruit were chosen. In addition, randomly chosen representatives from each geographic location of the collection with at least two accessions were selected. Overall, the 115 accessions selected represented 24 countries (Fig. 2-1). Susceptible controls used in this study were two open pollinated commercial *C. pepo* cultivars ‘Early Prolific Straightneck’ and ‘Yellow Summer Squash’.

Phytophthora capsici Isolates and Inoculum Preparation

Three highly virulent *P. capsici* mating type A1 isolates (01-1938A, RJM98-730 and RJM98-805) collected from squash were obtained from Dr. P. Roberts (University of Florida,

Southwest Florida Research and Education Center, Immokalee, FL). Inoculum was prepared using a modified procedure based on Mitchell, 1978, Mitchell et al., 1978, and Mitchell and Kannwischer-Mitchell, 1992. For each *P. capsici* isolate, one 5-mm mycelial plug from cornmeal agar was transferred to a 20% clarified V8 agar plate. After 7 days of growth at room temperature, ten 5-mmV8 agar mycelial plugs from each plate were placed into a 20% clarified V8 broth plate to grow for an additional 7 days in a 28°C incubator. The V8 broth was then drained and each plate was washed two times with sterilized distilled water. Sterilized distilled water was added to cover mycelial growth in all plates which were then placed under incandescent lights at 28-30°C to induce sporangial development. After 24 h, sporangia were chilled at 4°C for 45 min to induce zoospore release. The mycelia from each plate were strained through cheesecloth and a 1-ml encysted zoospore sample was counted using a hemacytometer. A suspension of the three isolates, containing equal portions of each, was prepared at a concentration of $2x10^4$ zoospores/ml.

Greenhouse Studies, Inoculation, and Scoring for Response to Inoculation

The selected *C. pepo* accessions were evaluated in two separate studies. The first study evaluated accessions based on fruit type (71 accessions). The second study evaluated accessions based on geographic location (44 accessions). For each study, a randomized complete block design was used. Eight blocks containing a single seed of each accession and the two susceptible controls were sown in standard 18 cell flats containing Fafard #3S potting mix (Fafard Inc., Agawam, MA). Not all of the 115 accessions germinated in all eight replications. Greenhouse temperatures were maintained between 19°C to 34°C. Seedlings were watered daily and at the cotyledon stage each received 1 g of slow-release fertilizer (Osmocote 14-14-14 NPK, Grace Sierra Horticulture Products, Milpitas, CA). At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Prior

to inoculation, the potting mix was watered and remained saturated for 24-36 hours to optimize the zoospore infection process. Fourteen days after inoculation, the plants were visually rated based on a scale ranging from 0 to 5; where 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death (Fig. 2-2). A mean disease rating score (DRS), calculated as a weighted average, and standard deviations (SD) were calculated for each accession and the susceptible controls. A third test was performed to rescreen accessions from the first two studies exhibiting a mean DRS of less than 2. Eight replications consisting of one plant of each of these accessions and the susceptible control 'Yellow Summer Squash' were planted in a randomized complete block design, inoculated, and visually rated for their response to *P. capsici* as above. Mean DRSs and SDs were calculated for each of the rescreened accessions and the susceptible control.

Results and Discussion

Mean DRSs for crown inoculation with *P. capsici* among the 115 accessions ranged from 1.3 to 5 (Table 2-1). Average standard deviation of DRSs within accessions was 1.1 and ranged from 0 to 2. Thirteen accessions (11.3%) had at least 50% of their replicates with a DRS of 0 or 1. Eight of these had a mean DRS of less than 2. These eight accessions were chosen for rescreening (Table 2-2). Results of the rescreen study paralleled those of the initial study in that the mean DRSs among the accessions remained less than 2 and the average SD was 1.4. PI 181761 exhibited the lowest mean DRS at 0.5 with all plants in this accession rated as either 0 or 1. These findings suggest that accessions within the *C. pepo* collection are potential sources of resistance to *P. capsici*.

In this study, all accessions collected in the United States were susceptible to the suspension of *P. capsici* isolates from Florida. Based on the origin of the eight accessions chosen for rescreening, four were obtained from Germany and Turkey. Evaluating additional accessions from Asia, Europe, and Mexico in the future might be worthwhile.

The findings from these tests suggest that many of the C. pepo accessions exhibited ssegergation for resistance to *P. capsici* crown rot. Many cucurbit accessions are maintained by open or sib-pollination, therefore, segergation for a particular trait may occur. Screening and continuous selection of individuals originating from cucurbit accessions can lead to breeding lines homogeneous for particular trait(s). This approach was used to develop the melon race one powdery mildew [*Podosphaera xanthii* (syn. *Sphaerotheca fuliginea* auct. p.p.)] resistant watermelon (*Citrullus lanatus* var. *lanatus*) line PI 525088-PMR (Davis et al., 2006).

Phytophthora capsici can cause disease on all plant tissue of susceptible hosts. Disease on each of these tissues can be considered a separate disease syndrome, i.e., crown rot, root rot, foliar blight, and fruit rot. Different genetic mechanisms may be responsible for host resistance to the various syndromes. This is the case with root rot and foliar blight resistance in pepper (*Capsicum annuum* var. *annuum*) (Walker and Bosland, 1999). A similar situation exists in the host-pathogen interaction of *P. infestans* and potato (*Solanum tuberosum* L.). Different genes are responsible for the resistances in tubers, vines, and foliage of the potato plant (Budin et al., 1978; Howard, 1978).

Physiological races within the *P. capsici-C. annuum* interaction have been identified (Oelke et al., 2003; Glosier et al., 2008). Pathogen races are important in pepper breeding as cultivars resistant to *P. capsici* isolates found in specific growing regions continue to be developed. While we have tentatively identified resistance to isolates of *P. capsici* from Florida

in *C. pepo*, additional studies will be performed to evaluate the Phytophthora crown rot resistant lines against isolates from around the world. If physiological races within the *P. capsici*-*C. pepo* interaction are identified, it will play an important role in breeding for Phytophthora resistance of the edible-fruited domesticates of *C. pepo*.

Results from this study indicate that there is potential resistance to *P. capsici* crown rot within *C. pepo* accessions. Through screening and selection, the development of *C. pepo* lines homozygous for *P. capsici* resistance will allow us to study the inheritance of resistance, evaluate the *P. capsici*-*C. pepo* interaction, and create Phytophthora crown rot resistant cultivars to aid in disease management of this pathogen. Further studies are also necessary to evaluate the Phytophthora crown rot resistant *C. pepo* breeding lines developed from this study for their response to Phytophthora foliar blight and fruit rot.

Table 2-1. Response of *Cucurbita pepo* accessions to a suspension of *Phytophthora capsici* squash isolates from Florida. Accessions are ranked according to their mean disease rating score (DRS).

Accession or Cultivar[z]	Mean DRS (0-5 scale)	SD	% plants with disease rating ≤ 1	Species[y]	Seed origin
PI 181761	1.3	0.9	75	Cp	Lebanon
PI 615132	1.3	0.5	75	Cp	Mexico
PI 174185	1.4	0.5	63	Cp	Turkey
PI 615142	1.4	0.5	63	Cp	Kazakhstan
PI 169417	1.6	1.6	71	Cp	Turkey
PI 266925	1.8	1.5	75	Cp	Germany
PI 209783	1.9	1.4	50	Cp	Germany
PI 512709	1.9	2.0	75	Cp	Spain
PI 169450	2.1	1.4	38	Cp	Turkey
PI 181944	2.1	1.8	63	Cp	Syria
PI 167053	2.3	1.8	38	Cp	Turkey
PI 169476	2.3	1.9	50	Cp	Turkey
PI 179267	2.3	1.9	50	Cp	Turkey
PI 181878	2.3	1.3	25	Cp	Syria
PI 288240	2.3	1.8	50	Cp	Egypt
PI 299574	2.5	1.9	50	Cp	South Africa
PI 234252	2.6	1.7	38	Cp	Argentina
PI 173684	2.7	1.6	14	Cp	Turkey
PI 136448	2.8	1.6	25	Cp	China
PI 285611	2.8	1.4	0	Cp	Poland
PI 169469	2.9	2.0	43	Cp	Turkey
PI 177377	2.9	1.9	38	Cp	Syria
PI 193501	2.9	1.4	0	Cp	Ethiopia
PI 368592	2.9	1.5	13	Cp	Macedonia
PI 458731	2.9	1.6	25	Cp	Argentina
PI 507885	2.9	1.6	25	Cp	Hungary
PI 163232	3.0	1.9	38	Cp	India
PI 165018	3.0	1.5	25	Cp	Turkey
PI 169448	3.0	1.9	29	Cp	Turkey
PI 222721	3.0	1.7	13	Cp	Iran
PI 311102	3.0	1.2	0	Cp	Guatemala
PI 311741	3.0	1.6	25	Cp	Poland
PI 532355	3.0	1.9	20	Cpf	Mexico
PI 167199	3.1	1.5	13	Cp	Turkey
PI 193502	3.1	1.6	25	Cp	Ethiopia

Table 2-1. Continued.

Accession or Cultivar[z]	Mean DRS (0-5 scale)	SD	% plants with disease rating ≤ 1	Species[y]	Seed origin
PI 304061	3.1	1.7	25	Cp	Pakistan
PI 355054	3.1	1.6	13	Cp	Iran
PI 169418	3.2	2.0	33	Cp	Greece
PI 169442	3.2	1.6	0	Cp	Turkey
PI 169443	3.3	1.9	25	Cp	Turkey
PI 257287	3.3	1.3	0	Cp	Spain
PI 234615	3.3	1.7	14	Cp	South Africa
PI 169425	3.4	1.8	25	Cp	Turkey
PI 318826	3.4	1.3	13	Cp	Mexico
PI 169426	3.5	1.7	13	Cp	Turkey
PI 181760	3.5	1.9	25	Cp	Lebanon
PI 274787	3.5	1.2	0	Cp	India
PI 357940	3.5	1.7	13	Cp	Yugoslavia
PI 175705	3.6	1.9	29	Cp	Turkey
PI 169472	3.6	1.9	25	Cp	Turkey
PI 212060	3.6	1.7	13	Cp	Greece
PI 269483	3.6	1.5	13	Cp	Pakistan
PI 364241	3.6	1.9	25	Cp	Hungary
PI 379307	3.6	1.6	13	Cp	Yugoslavia
PI 176964	3.7	1.7	14	Cp	Turkey
PI 183678	3.8	1.8	13	Cp	Turkey
PI 169475	3.8	1.8	20	Cp	Turkey
PI 175704	3.8	1.8	17	Cp	Turkey
PI 532354	3.9	1.5	0	Cpf	Mexico
Ames 26619	3.9	1.2	0	Cpo	United States
PI 169461	3.9	1.6	13	Cp	Turkey
PI 172860	3.9	1.6	13	Cp	Turkey
PI 183232	3.9	1.8	25	Cp	Egypt
PI 385970	3.9	1.6	0	Cp	Kenya
PI 532356	3.9	1.8	13	Cpf	Mexico
PI 93458	3.9	1.5	14	Cp	China
PI 135394	4.0	1.3	0	Cp	Afghanistan
PI 169429	4.0	1.9	14	Cp	Turkey
PI 169453	4.0	1.5	0	Cp	Turkey
PI 357929	4.0	1.4	13	Cp	Macedonia
Ames 26871	4.1	1.6	13	Cpo	United States
PI 169477	4.1	1.2	0	Cp	Turkey

Table 2-1. Continued.

Accession or Cultivar[z]	Mean DRS (0-5 scale)	SD	% plants with disease rating ≤ 1	Species[y]	Seed origin
PI 614683	4.1	1.6	13	Cpf	Mexico
PI 167042	4.3	1.4	0	Cp	Turkey
PI 171631	4.3	1.5	13	Cp	Turkey
PI 181763	4.3	1.2	0	Cp	Lebanon
PI 234616	4.3	1.3	0	Cp	South Africa
PI 169435	4.4	1.0	0	Cp	Turkey
PI 169445	4.5	1.0	0	Cp	Turkey
PI 169458	4.5	0.9	0	Cp	Turkey
PI 169478	4.5	0.9	0	Cp	Turkey
PI 181758	4.5	1.1	0	Cp	Lebanon
Ames 26873	4.8	0.7	0	Cpo	United States
Ames 26882	4.8	0.7	0	Cpo	United States
PI 167084	4.8	0.7	0	Cp	Turkey
PI 172868	4.8	0.7	0	Cp	Turkey
PI 274336	4.8	0.7	0	Cp	Guatemala
Ames 26608	4.9	0.4	0	Cpo	United States
Ames 26622	4.9	0.4	0	Cpo	United States
PI 135398	5.0	0.0	0	Cp	Afghanistan
PI 379314	5.0	0.0	0	Cp	Macedonia
PI 615141	5.0	0.0	0	Cp	Kazakhstan
Ames 26607	5.0	0.0	0	Cpo	United States
Ames 26609	5.0	0.0	0	Cpo	United States
Ames 26610	5.0	0.0	0	Cpo	United States
Ames 26612	5.0	0.0	0	Cpo	United States
Ames 26616	5.0	0.0	0	Cpo	United States
Ames 26617	5.0	0.0	0	Cpo	United States
Ames 26620	5.0	0.0	0	Cpo	United States
Ames 26624	5.0	0.0	0	Cpo	United States
Ames 26833	5.0	0.0	0	Cpo	United States
Ames 26872	5.0	0.0	0	Cpo	United States
Ames 26875	5.0	0.0	0	Cpo	United States
Ames 26876	5.0	0.0	0	Cpo	United States
Ames 26877	5.0	0.0	0	Cpo	United States
Ames 26879	5.0	0.0	0	Cpo	United States
Ames 26883	5.0	0.0	0	Cpo	United States
Ames 26884	5.0	0.0	0	Cpo	United States
Ames 26885	5.0	0.0	0	Cpo	United States

Table 2-1. Continued.

Accession or Cultivar[z]	Mean DRS (0-5 scale)	SD	% plants with disease rating ≤ 1	Species[y]	Seed origin
Ames 26887	5.0	0.0	0	Cpo	United States
Ames 26888	5.0	0.0	0	Cpo	United States
Ames 26890	5.0	0.0	0	Cpo	United States
Ames 26891	5.0	0.0	0	Cpt	United States
Ames 26892	5.0	0.0	0	Cpt	United States
Ames 26893	5.0	0.0	0	Cpt	United States
EPS	5.0	0.0	0	Cp	United States
YSS	5.0	0.0	0	Cp	United States

[z]Susceptible *C. pepo* controls EPS, 'Early Prolific Straightneck' and YSS, 'Yellow Summer Squash'.

[y]Cp, *Cucurbita pepo*; Cpf, *Cucurbita pepo* subsp. *fraterna*; Cpo, *Cucurbita pepo* var. *ozarkana*; Cpt, *Cucurbita pepo* var. *texana*.

Table 2-2. Response of eight selected accessions of *Cucurbita pepo* to a suspension of three *Phytophthora capsici* isolates from Florida.

Accession or Cultivar[z]	Disease Rating Scale (DRS)[y] Number of plants within each category						Mean DRS	SD
	0	1	2	3	4	5		
PI 181761	4	4	0	0	0	0	0.5	0.5
PI 615132	4	1	0	2	1	0	1.4	1.7
PI 174185	2	3	3	0	0	0	1.1	0.8
PI 615142	1	2	4	1	0	0	1.3	0.9
PI 169417	5	0	1	1	0	1	1.3	1.9
PI 266925	2	4	0	1	0	1	1.5	1.7
PI 209783	3	1	2	0	0	2	1.9	2.1
PI 512709	2	0	5	0	0	1	1.9	1.6
YSS	0	0	0	0	0	8	5.0	0.0

[z]Susceptible *C. pepo* control YSS, 'Yellow Summer Squash'.

[y]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death).

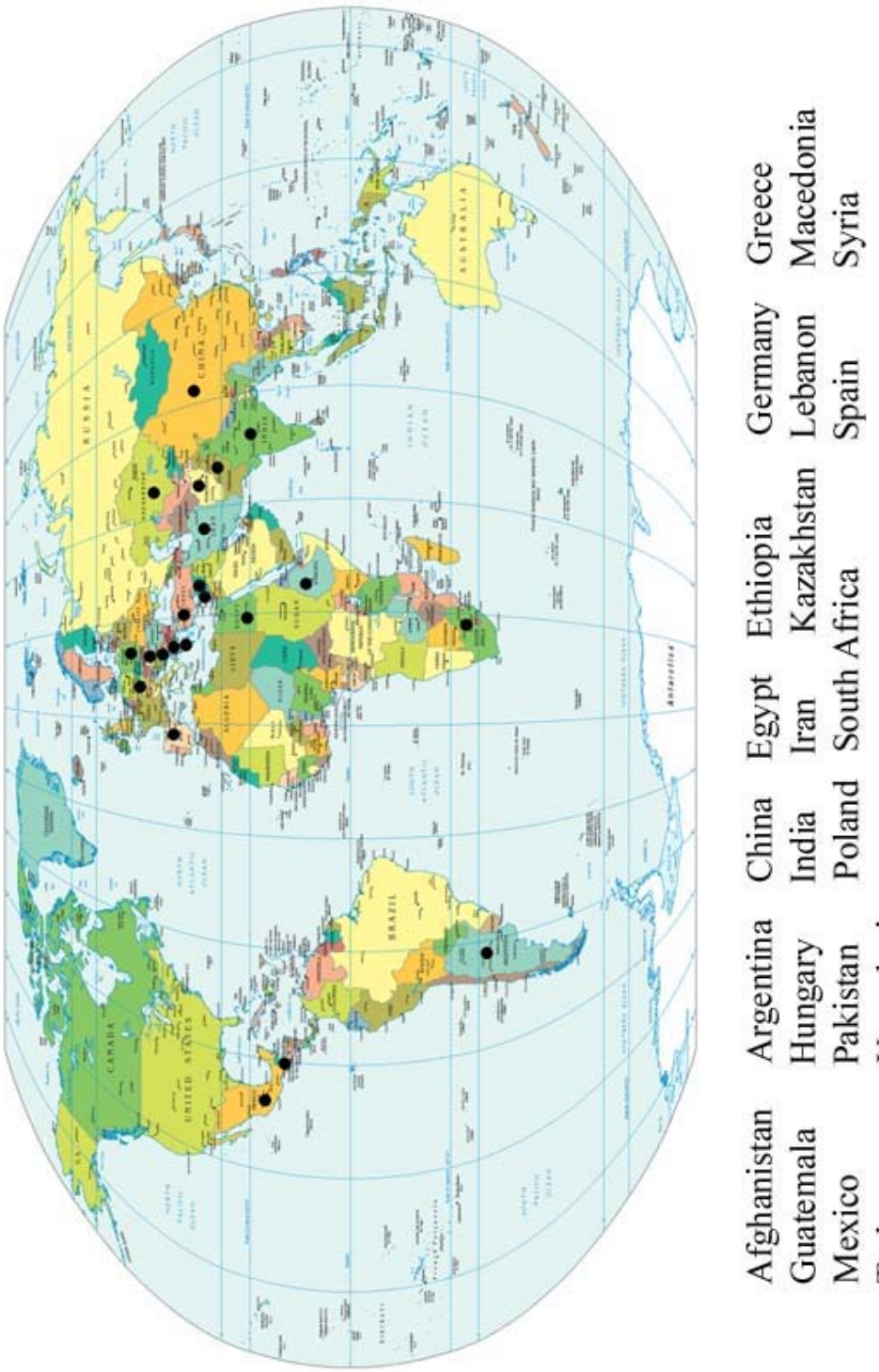

Figure 2-1. Geographic location of 115 *Cucurbita pepo* accessions representing 24 countries (USDA, 2006b).

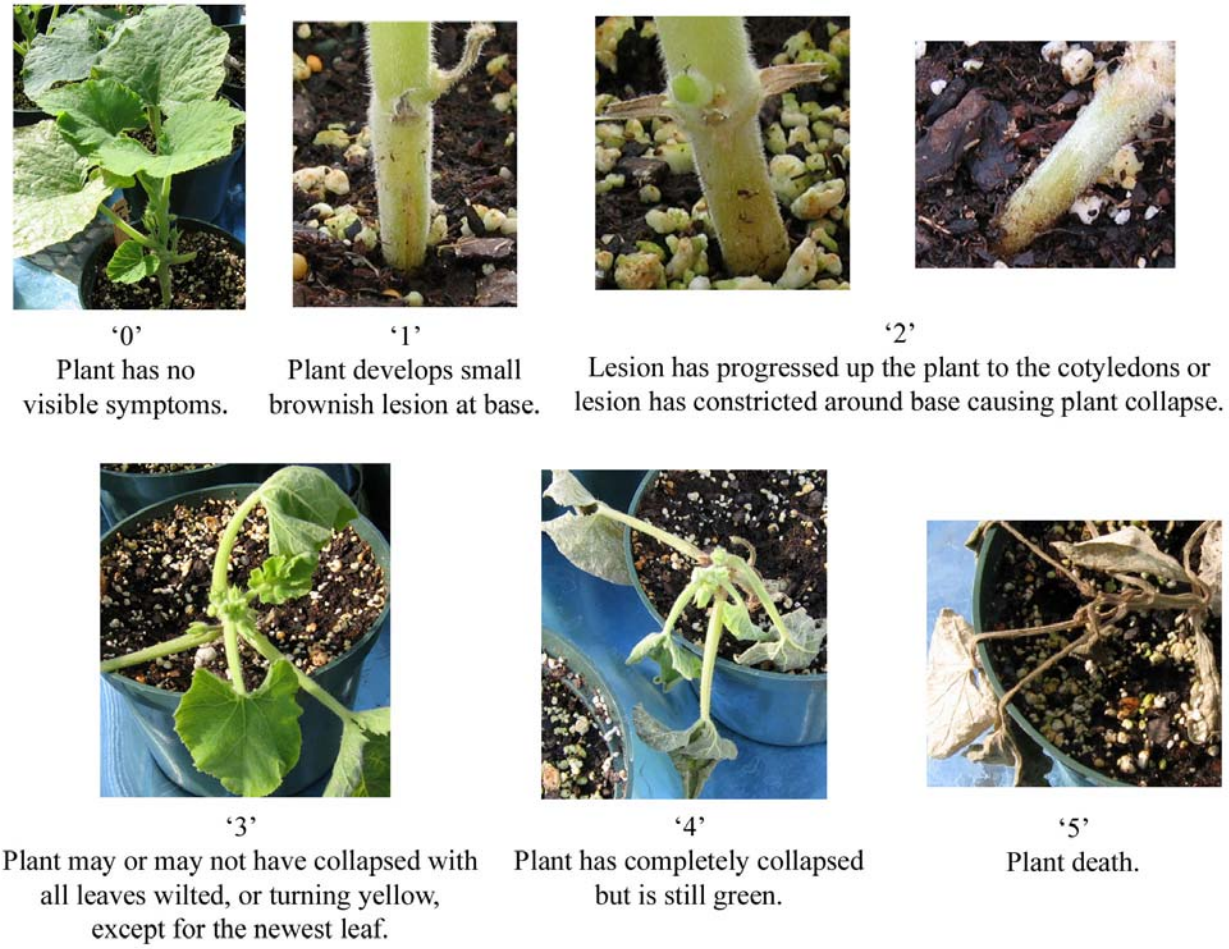

Figure 2-2. Disease rating scale (0-5) for *Phytophthora capsici* crown inoculation on *Cucurbita pepo*.

CHAPTER 3
A CUCURBITA BREEDING LINE WITH CROWN ROT RESISTANCE TO PHYTOPHTHORA CAPSICI DERIVED FROM WILD CUCURBITA SPECIES.

Introduction

Phytophthora capsici Leonin is a devastating oomycetous pathogen that affects many vegetable crops. Since its discovery in 1918, the incidence of disease caused by *P. capsici* has increased throughout the United States with reports of yield loss from many vegetable producing states including California, North and South Carolina, Florida, Georgia, Illinois, New Jersey, Michigan and Texas (Babadoost and Islam, 2003; Café-Filho et al., 1995; Ristaino, 1990; Hausbeck and Lamour, 2004; Isaleit, 2007; Lamour and Hausbeck, 2003; Leonian, 1922; Tian and Babadoost, 2004). With the increased occurrence and severity of *P. capsici* within the U.S., researchers have begun looking into alternative forms of managing this pathogen, including breeding for resistance (Hausbeck and Lamour, 2004; French-Monar et al., 2005; Keinath, 2007).

In cucurbits, *P. capsici* can infect at any growth stage and given optimum conditions an entire field can be destroyed in a matter of days (Roberts et al., 2001). The disease syndromes of *P. capsici* on cucurbits includes crown rot, foliar blight and fruit rot (Zitter et al., 1996; Roberts et al., 2001). Crown rot appears at the soil line where stems turn dark brown, become water-soaked, and quickly collapse causing plant death. Foliar symptoms appear as rapidly expanding, water-soaked lesions. Dieback of shoot tips, wilting, shoot rot, and plant death quickly follows initial infection. Fruit, which can be infected at any stage of maturity, may exhibit sunken, brown, water-soaked areas, rapidly covered by white sporangial growth under moist environmental conditions.

In squash (*Cucurbita* spp.), resistance to *P. capsici* had recently been found within two wild gourd species, *C. lundelliana* PI 438542 and *C. okeechobeenesis* sbsp. *okeechobeenesis*

(Kabelka et al., 2007). Resistance to the crown rot syndrome caused by *P. capsici*, derived from the two wild species, was introgressed through a series of hybridizations providing breeding material 62.5% *C. moschata*, 25% *C. lundelliana* PI 438542 and 12.5% *C. okeechobeenesis* sbsp. *okeechobeenesis*. From this series, 19 lines were tested for response to *P. capsici* and all were found to be segregating for resistance (Kabelka et al., 2007). The objective of this study was to develop, from this material, a *Cucurbita* breeding line homozygous resistant to the crown rot syndrome of *P. capsici*.

Materials and Methods

Plant Material

The development of *Cucurbita* breeding material, with *P. capsici* resistance, was accomplished through a series of interspecific hybridizations of *C. lundelliana*, *C. okeechobeensis* sbsp. *okeechobeenesis* and *C. moschata*. At each hybridization event, selections for horticultural characteristics of fruit shape and rind and flesh color were made. Nineteen lines, exhibiting desirable horticultural traits, were selected and evaluated for response to *P. capsici* inoculation and all segregated for resistance based on replicated greenhouse trials (Table 3-1). Of the lines evaluated, #394, which has pear-shaped fruit and dark orange flesh color, was chosen for further evaluation and selection for resistance to crown rot caused by *P. capsici*.

Greenhouse Studies and Single Plant Selections for Homozygosity to *P. capsici* Crown Rot Resistance

A series of greenhouse studies, with selections for resistance to *P. capsici* crown rot inoculation, were performed to develop a homozygous *P. capsici* crown rot resistant *Cucurbita* breeding line. Throughout all studies, greenhouse temperatures were maintained between 19°C to 34°C, plants were watered daily, and at the cotyledon stage the plants received 1 g of slow-

release fertilizer (Osmocote, 14-14-14 NPK, Grace Sierra Horticulture Products, Milpitas, CA). Fafard #3S potting mix (Fafard Inc., Agawam, MA) was used throughout. *Phytophthora capsici* isolates and inoculum preparation, the crown inoculation protocol, scoring for response to inoculation, and analysis of data are described below. Upon completion of each study, and for the purpose of developing next generation progeny, selected asymptomatic plants were transplanted to 9.5 L plastic pots for further growth and development, self-pollination, fruit harvest and seed extraction. Each transplant received an additional 5 g of slow release fertilizer (Osmocote, 14-14-14 NPK).

The first of the series of greenhouse studies evaluated breeding line #394 (F_4) and progeny from the self-pollination of an asymptomatic single plant selection from #394, designated #394-1 (F_5). Using a completely randomized design, 10 seed of #394, 20 seed of #394-1, and 5 seed of the susceptible control 'Early Prolific Straightneck' (*C. pepo*) were sown into standard 18 cell flats. At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Twenty one days after inoculation, plants were visually rated for response and asymptomatic individuals of #394-1 were transplanted for the development of F_6 generation seed.

The second greenhouse study evaluated progeny from an asymptomatic individual, designated #394-1-27 (F_6), using a randomized complete block design. Eight replications consisting of one plant each of #391-1-27 and of the susceptible control, an open pollinated commercial cultivar 'Butterbush' (*C. moschata*), were sown into 152.4 mm diameter plastic azalea pots. At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Twenty one days after inoculation,

plants were visually rated for response and asymptomatic individuals of #394-1-27 were transplanted for the development of F_7 generation seed.

A third greenhouse study was performed to test the accuracy of my *P. capsici* crown inoculation protocol was in identifying asymptomatic individuals and to rule out possible escapes. For this test, I utilized rooted cuttings made from the seven asymptomatic #394-1-27 (F_6) transplants, from the second greenhouse study, including a non-inoculated susceptible control 'Butterbush' plant. The stem end of three to four cuttings from each of the seven asymptomatic plants, for a total of 26, and four cuttings from 'Butterbush' were dipped into indole-3-butyric acid (0.1%) to enhance root development and planted into 152.4 mm diameter plastic azalea pots arranged in a randomized complete block design. All cuttings were watered daily and at root development each cutting received 1 g of slow-release fertilizer (Osmocote, 14-14-14 NPK). Two weeks later, each rooted cutting was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Scoring for response and data analyses were performed as described below.

A final greenhouse study evaluated seed of #394-1-27 (F_6) and #394-1-27-12 (F_7), progeny from an asymptomatic selection from the second greenhouse study. Eight replications consisting of one plant of each and the susceptible control 'Butterbush' were planted in a randomized complete block design. At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Twenty one days after inoculation, plants were visually rated for their response to *P. capsici*.

Phytophthora capsici Isolates and Inoculum Preparation

Three highly virulent *P. capsici* mating type A1 isolates (01-1938A, RJM98-730 and RJM98-805), collected from squash, were obtained from Dr. Pamela Roberts (Southwest Florida Research and Education Center, Immokalee, FL). A suspension of the three isolates, containing

equal portions of each, was prepared as described in Chapter 2 of this dissertation, at a concentration of $2x10^4$ zoospores/ml.

Phytophthora capsici Crown Inoculation, Scoring for Response and Data Analysis

At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Prior to inoculation, the potting mix was watered and remained saturated for 24-36 hours to optimize the zoospore infection process. Twenty one days after inoculation, the plants were visually rated based on a scale ranging from 0 to 5; where 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death (Fig. 3-1). A mean disease rating score (DRS), calculated as a weighted average, and standard deviations (SD) were calculated for each line and the susceptible controls.

Results and Discussion

Evaluation of breeding line #349 (F_4) and progeny from the self-pollination of an asymptomatic single plant selection from #394, designated #394-1 (F_5), revealed each to be segregating for resistance to crown rot caused by *P. capsici* (Table 3-2). By day 21, both lines had greater than 50% of their progeny symptomatic with disease ratings of greater than 1. Breeding line #394 had a mean DRS of 1.0 and a SD of 1.5 while #394-1 had a mean DRS and SD of 0.7. All replications of the susceptible open pollinated commercial control, 'Early Prolific Straightneck', rapidly developed tan-brown water-soaked lesions at their crowns which rapidly collapsed and caused plant death.

Evaluation of breeding line #394-1-27 (F_6), an asymptomatic individual selected from the previous study, revealed seven out of eight of its progeny to be asymptomatic 21 days after *P. capsici* crown inoculation, with a mean DRS of 0.1 and SD of 0.4 (Table 3-3). In this study,

all replications of the susceptible control, 'Butterbush', developed tan-brown water-soaked lesions at their crowns which rapidly caused plant death.

The next study tested the accuracy of my *P. capsici* crown inoculation protocol in identifying asymptomatic individuals. For this test, I utilized rooted cuttings made from the seven asymptomatic #394-1-27 (F_6) transplants including a non-inoculated susceptible control 'Butterbush' plant. Evaluation of 26 rooted cuttings revealed all to be asymptomatic post *P. capsici* crown inoculation (Table 3-3). All rooted cuttings from a non-inoculated susceptible control 'Butterbush' plant quickly died following *P. capsici* crown inoculation. This study reveals that my crown inoculation protocol is accurate in determining response to crown rot caused by *P. capsici* in *Cucurbita* breeding material.

A final study evaluated breeding lines #394-1-27 (F_6) and #394-1-27-12 (F_7) and revealed all plants within each to be asymptomatic to crown inoculation with *P. capsici* (Table 3-4). As above, all plants of the susceptible control 'Butterbush' died following *P. capsici* crown inoculation. This final study suggests I have successfully developed a *Cucurbita* breeding line homozygous resistant to *P. capsici* crown rot.

Resistance to *P. capsici* has been found in other vegetable crops. In pepper (*Capsicum annuum* L.), resistance to *P. capsici* comes from two different sources; PI 201232 and a Mexican landrace called 'Criollo de Morelos 334' (Alcantara and Bosland, 1994; Ortega et al., 1995; Sy et al., 2005). In watermelon (*Citrullus lanatus* L.), a screen of nine Korean and Japanese cultivars by Lee et al. (2001), showed a variety named 'Danmatmaetdol' as having the highest level of resistance to *P. capsici*. With potential resistance to *P. capsici* within *C. pepo* identified in Chapter 1 of this dissertation and with the germplasm developed in this Chapter, breeding for resistance to *P. capsici* within *Cucurbita* will aid in the disease management of this pathogen.

A *Cucurbita* breeding line, designated #394-1-27-12, was developed with resistance to the crown rot syndrome caused by *P. capsici* (see Figure 3-2 for its pedigree). At maturity, #394-1-27-12 produces smooth, medium-green, striped, obovate-shaped fruit, with medium orange flesh color. Growth habit is vine; leaves are shallow-lobed and mottled. *Cucurbita* breeding line #394-1-27-12 will be a useful source of *P. capsici* crown rot resistance for introgression into the morphologically diverse edible-fruited domesticates within *Cucurbita*. Further studies are needed to evaluate #394-1-27-12 for its response to foliar blight and fruit rot and to determine the inheritance of resistance to crown rot caused by *P. capsici*.

Table 3-1. Description and response of *Cucurbita lundelliana* PI 438542, *C. okeechobeeness* sbsp. *okeechobeenesis*, 19 *Cucurbita* breeding lines, and susceptible controls 'Yellow Summer Squash' (*C. pepo*) and 'Early Prolific Straightneck' (*C. pepo*) to a suspension of *Phytophthora capsici* isolates.[z]

Line	Fruit Shape	Fruit Color	Flesh Color	No. of Plants[y]	
				R	S
C. lundelliana PI 438542	Oval	Dark Green w/ stripes	Light Yellow	8	0
C. okeechobeeness sbsp. *okeechobeenesis*[w]	Oval	Dark Green w/ stripes	Light Yellow	6	0
Breeding line #322	Elongate	Green w/ stripes	Orange	11	7
Breeding line #381	Oblate	Green	Orange	20	9
Breeding line #382	Round	Dark Green	Orange	9	9
Breeding line #383	Oblate	Green w/ stripes	Orange	15	6
Breeding line #384	Round	Green	Orange	7	2
Breeding line #385	Round	Dark Green	Dark Orange	10	18
Breeding line #387	Pear	Green	Orange	12	6
Breeding line #388	Oblate	Green	Orange	13	3
Breeding line #389	Oblate	Green	Light Yellow	10	1
Breeding line #390	Oblate	Green	Dark Orange	20	6
Breeding line #391	Oblate	Green	Orange	7	4
Breeding line #393	Round	Green	Orange	22	3
Breeding line #394	**Pear**	**Green w/ stripes**	**Dark Orange**	**19**	**9**
Breeding line #395	Round	Green	Orange	11	5
Breeding line #396	Round	Green	Orange	14	10
Breeding line #397	Round	Dark Green	Dark Orange	9	18
Breeding line #398	Round	Dark Green	Orange	16	4
Breeding line #399	Oblate	Green w/stripes	Orange	11	8
Breeding line #400	Oblate	Dark Green	Orange	14	10
'Yellow Summer Squash'	-	-	-	0	10
'Early Prolific Straightneck'	-	-	-	0	10

[z]Data from Kabelka et al., 2007.

[y]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death). Plants scored as 0 were classified as resistant (R) while those scored 1-5 were classified as susceptible (S).

[w]Seed source collected from Torrey Island, Okeechobee, FL and provided by T.W. Walters and D.S. Decker-Walters, Fairchild Tropical Garden, Miami, FL.

Table 3-2. Response of *Cucurbita* breeding line #394, #394-1, and susceptible control 'EarlyProlific Straightneck' (*C. pepo*) to a suspension of *Phytophthora capsici* isolates.

Line	Generation	Disease Rating Scale (DRS)[z] No. of plants within each category						Mean DRS	SD
		0	1	2	3	4	5		
#394	F4	4	5	0	0	0	1	1.0	1.5
#394-1	F5	8	10	2	0	0	0	0.7	0.7
'Early Prolific Straightneck'	-	0	0	0	0	0	5	5.0	0.0

[z]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death).

Table 3-3. Response of *Cucurbita* breeding line #394-1-27, rooted cuttings of #394-1-27, and susceptible control 'Butterbush' (*C. moschata*) to a suspension of *Phytophthora capsici* isolates.

Line	Generation	Disease Rating Scale (DRS)[z] No. of plants within each category						Mean DRS	SD
		0	1	2	3	4	5		
#394-1-27	F6	7	1	0	0	0	0	0.1	0.4
'Butterbush'	-	0	0	0	0	0	8	5.0	0.0
#394-1-27 rooted cuttings[y]	F6	26	0	0	0	0	0	0.0	0.0
'Butterbush' rooted cuttings[x]	-	0	0	0	0	0	4	5.0	0.0

[z]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death).
[y]Three to four cuttings taken from each of seven asymptomatic mature plants of #394-1-27 (F_6).
[x]Cuttings taken from an asymptomatic mature plant of 'Butterbush'.

Table 3-4. Response of *Cucurbita* breeding lines #394-1-27, #394-1-27-12 and susceptible control 'Butterbush' (*C. moschata*) to a suspension of *Phytophthora capsici* isolates.

Line	Generation	Disease Rating Scale (DRS)[z] No. of plants within each category						Mean DRS	SD
		0	1	2	3	4	5		
#394-1-27	F6	8	0	0	0	0	0	0.0	0.0
#394-1-27-12	F7	8	0	0	0	0	0	0.0	0.0
'Butterbush'	-	0	0	1	0	0	7	4.6	1.1

[z]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death).

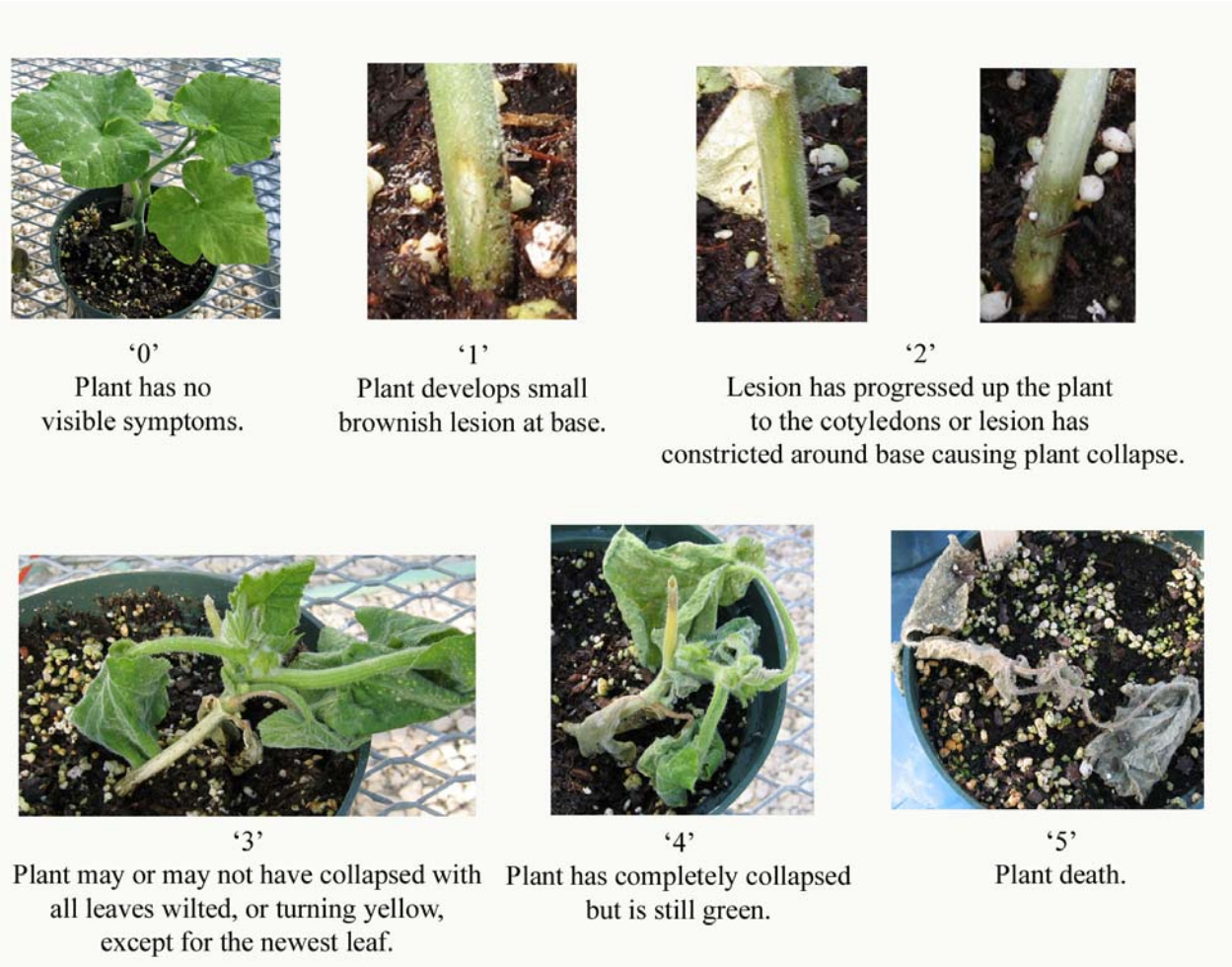

Figure 3-1. Disease rating scale (0-5) for *Phytophthora capsici* crown inoculation on *Cucurbita moschata*.

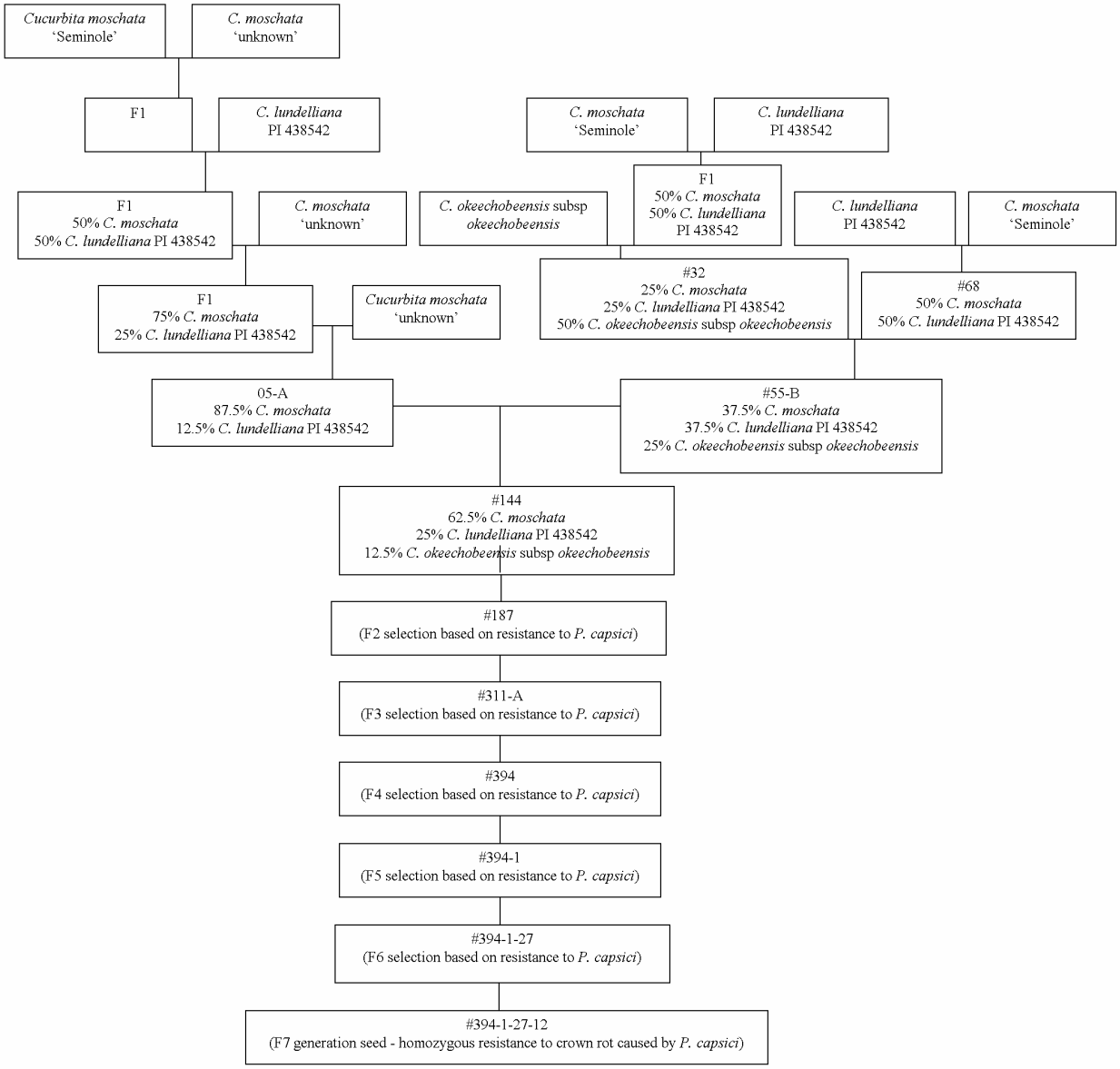

Figure 3-2. Pedigree of *Phytophthora capsici* crown rot resistant *Cucurbita* breeding line #394-1-27-12.

CHAPTER 4
INHERITANCE OF RESISTANCE TO CROWN ROT CAUSED BY PHYTOPHTHORA CAPSICI IN CUCURBITA.

Introduction

The oomycetous pathogen, *Phytophthora capsici* Leonian, is capable of causing several disease syndromes in cucurbits including crown rot, foliar blight and fruit rot (Zitter et al., 1996; Roberts et al., 2001). Crown rot appears at the soil line on the plant as a dark brown, water-soaked lesion that quickly collapses the stem causing plant death. Foliar blight appears as rapidly expanding, water-soaked lesions on the leaves that eventually causes dieback of shoot tips, wilting, shoot rot, and plant death. Fruit rot appears as sunken, brown, water-soaked areas which are rapidly covered by white sporangial growth under moist environmental conditions.

The incidence of disease caused by *P. capsici* in cucurbit production areas of the United States has increased with reported yield loss as high as 100% (Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). Given optimal environmental conditions, an entire field of cucurbits can be destroyed by *P. capsici* in a matter of days (Zitter et al., 1996; Roberts et al., 2001). The increased occurrence and severity of *P. capsici* has prompted research for fungicide management alternatives and interest in breeding cucurbits for resistance (Babadoost, 2000; Stevenson et al., 2000, 2001; Seebold and Horten, 2003; Hausbeck and Lamour, 2004; McGrath, 2004; Tian and Babadoost, 2004; Waldenmaier, 2004; French-Monar et al., 2005; Keinath, 2007).

Cucurbita are considered to be one of the most morphologically variable genera in the plant kingdom (Whitaker and Robinson, 1986; Robinson and Decker-Walters, 1999). There are 22 wild and five cultivated species of *Cucurbita*. The cultivated species, grown around the world, include *C. pepo*, *C. moschata*, *C. maxima, C. argyrosperma* (formerly C. *mixta*) and *C. ficifolia*. *Cucurbita* cultivars are categorized as summer or winter squash. Summer squash are eaten immature when tender and seeds are small and soft. Winter squash are generally eaten

when rind and seeds are fully mature. Summer squash cultivars are *C. pepo* while winter squash cultivars can be *C. pepo*, *C. maxima*, *C. moschata*, or *C. argyrosperma*.

A search for sources of resistance within *Cucurbita*, to the various syndromes of *P. capsici*, had been performed and included representatives from *C. maxima*, *C. moschata*, *C. pepo*, and three wild species, *C. ecuadorensis*, *C. lundelliana*, and *C. okeechobeensis* (Kabelka et al., 2007). From this screen, resistance to the crown rot syndrome of *P. capsici* was identified in the wild species, *C. lundelliana* PI 438542 and *C. okeechobeenesis* subsp. *okeechobeenesis*. This resistance was introgressed, through a series of hybridizations, self-pollinations, and single plant selections, into a winter squash (*C. moschata*) background. One line, designated #394-1-27-12, was advanced to the F_7 generation and is homozygous for *P. capsici* crown rot resistance. The objective of this study was to characterize the inheritance of resistance to crown rot caused by *P. capsici* within the *Cucurbita* breeding line #394-1-27-12.

Materials and Methods

Plant Material

The *Cucurbita* breeding line #394-1-27-12, resistant to crown rot caused by *P. capsici*, was crossed with 'Butterbush' (BB), a butternut-type winter squash (*C. moschata*) highly susceptible to *P. capsici*. Controlled pollinations were carried out in the greenhouse to generate F_1 (BB x 394-1-27-12), F_2 and reciprocal backcross (BC) progenies. The susceptible control used in all studies was an open pollinated commercial cultivar 'Butterbush'.

Phytophthora capsici Isolates and Inoculum Preparation

Three highly virulent *P. capsici* mating type A1 isolates (01-1938A, RJM98-730 and RJM98-805), collected from squash, were obtained from Dr. Pamela Roberts (Southwest Florida Research and Education Center, Immokalee, FL). A suspension of the three isolates, containing

equal portions of each, was prepared as described in Chapter 2 of this dissertation, at a concentration of $2x10^4$ zoospores/ml.

Experimental Design and Data Analysis

Evaluation of #394-1-27-12, 'Butterbush', F_1, F_2 and BC progenies for response to *P. capsici* crown inoculation was performed in greenhouse studies using completely randomized designs. Seed were sown in 15.2 cm azalea plastic pots containing Fafard #3S potting mix (Fafard Inc., Agawam, MA). Seedlings were watered daily and greenhouse temperatures were maintained between 19°C to 34°C. At the cotyledon stage, each seedling received 1 g of slow-release fertilizer (14-14-14 NPK, Grace Sierra Horticulture Products, Milpitas, CA). The F_2 progeny test consisted of 200 individuals plus 10 replicates of both parents. The four BC progeny tests, performed separately, consisted of 100 individuals each of BB x F_1 and F_1 x BB plus 10 replicates of both parents and 50 individuals each of 394-1-27-12 x F_1 and F_1 x 394-1-27-12 plus 8 replicates of both parents and the F_1.

At the second to third true-leaf-stage, each seedling was inoculated at its crown with 5 ml of the $2x10^4$ zoospores/ml suspension of *P. capsici*. Prior to inoculation, the potting mix was watered and remained saturated for 24-36 hours to optimize the zoospore infection process. Twenty-one days after inoculation, the plants were visually rated based on a scale ranging from 0 to 5; where 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death. In all studies, plants scored as 0 were classified as resistant while those scored 1-5 were classified as susceptible. Segregation ratios were analyzed by Chi-square analysis.

Results and Discussion

The *Cucurbita* breeding line #394-1-27-12 exhibited no symptoms of crown rot caused by *P. capsici* under the conditions of this study (Fig. 4-1). However, five days post-inoculation, the susceptible cultivar Butterbush developed a tan-brown water-soaked lesion at its crown that rapidly expanded, caused stem collapse, and plant death. The F_1 of the cross between 'Butterbush' and #394-1-27-12 reacted similarly to that of the resistant parent, #394-1-27-12, remaining asymptomatic (Table 4-1). The F_2 progeny segregated in 27:37 [resistant (R):susceptible (S)] ratio while the backcrosses to the susceptible parent, 'Butterbush' segregated in a 1:7 (R:S) ratio. Progeny of the backcrosses to the parent #394-1-27-12 were all resistant. Collectively, the segregation ratios support a model in which resistance to the crown rot syndrome caused by *P. capsici* is conferred by three dominant genes.

In pepper (*Capsicum annuum* L.), it has been shown that resistance to root rot, stem blight, and foliar blight, caused by *P. capsici*, are under different genetic mechanisms (Sy et al., 2005). This is also suggested for potato (*Solanum tuberosum* L.) where tuber, vine, and foliage resistance to another Phytophthora species, *P. infestans* (Mont.) de Bary, are controlled by separate genes (Bonde et al., 1940; Rudorf et al., 1950). Recently, #394-1-27-12 has been found to possess resistance to the foliar blight syndrome of *P. capsici*. Studies are currently underway to determine if the genetic mechanisms for crown rot and foliar blight resistance within #394-1-27-12 are the same or are different (Kabelka, personal communication, 2008).

Physiological races of *P. capsici* have been identified within the *P. capsici*-*C. annuum* interaction (Oelke et al., 2003; Glosier et al., 2008). This plays an important role in developing pepper cultivars with resistance to *P. capsici* isolates found in specific growing regions. The resistance in #394-1-27-12 is currently being tested against *P. capsici* isolates from different regions of the United States and Europe to test for specificity. If physiological races within the

P. capsici-*Cucurbita* interaction are identified, it will play an important role in breeding for *P. capsici* resistance within *Cucurbita*.

Different screening methods have been developed to examine plants for their response to the various disease syndromes caused by *P. capsici*. The greenhouse assay used in this study allowed for precise observations of plant response to crown inoculation and for the determination of inheritance of resistance to crown rot caused by *P. capsici*. This assay provides a standardized test environment. It also allows for the screening of test material using defined *P. capsici* inoculum sources. This assay will aide in the introgression of *P. capsici* crown rot resistance from #394-1-27-12 into the morphologically diverse edible-fruited domesticates within *Cucurbita*.

Molecular linkage maps are useful tools to facilitate breeding efforts providing molecular markers for marker-assisted-selection and to increase our knowledge of *Cucurbita* genetics. Using molecular markers, instead of phenotypic assays, can increase the precision and efficiency of subsequent selection steps applied in plant breeding. Co-dominant PCR-based molecular markers tightly linked (<5 cM) to *P. capsici* crown rot resistance would provide the most benefit allowing distinction between homozygous resistant and heterozygous resistant individuals. Studies are currently underway to create a molecular linkage map of the segregating progeny developed in this study to identify markers linked to *P. capsici* crown rot resistance. As the genes for resistance to *P. capsici* crown rot within #394-1-27-12 may be from either *C. lundelliana* PI 438542 or *C. okeechobeenesis*, or both, molecular analysis may also shed light as to the contributor of this resistance.

Table 4-1. Segregation for resistance to *Phytophthora capsici* crown inoculation in *Cucurbita* breeding line #394-1-27-12, 'Butterbush', F_1, F_2 and BC progeny.

	No. of plants[z]		Genetic Models					
Generation	R	S	One-gene Expected ratio (R:S)[y]	X^2	Two-gene Expected ratio (R:S)[y]	X^2	Three-gene Expected ratio (R:S)[y]	X^2
#394-1-27-12	28	0	-	-	-	-	-	-
Butterbush (BB)	0	38	-	-	-	-	-	-
F_1 (BB x 394-1-27-12)	8	0	-	-	-	-	-	-
F_2 (BB x 394-1-27-12)	92	108	3:1	89.7***	9:7	8.5***	27:37	1.2^{ns}
BC_1 (BB x F_1)	17	83	1:1	43.6***	1:3	3.4^{ns}	1:7	1.9^{ns}
BC_1 (F_1 x BB)	10	85	1:1	59.2***	1:3	10.6***	1:7	0.3^{ns}
BC_1 (394-1-27-12 x F_1)	50	0	1:0	-	1:0	-	1:0	-
BC_1 (F_1 x 394-1-27-12)	50	0	1:0	-	1:0	-	1:0	-

[z]R=resistant, S=susceptible.
[y]Ratio based on data classified as either resistant (0) or susceptible (1,2,3,4,5).
[ns]X^2 value not significant $P \leq 0.05$.
[***]Significant at 0.0001 probability level.

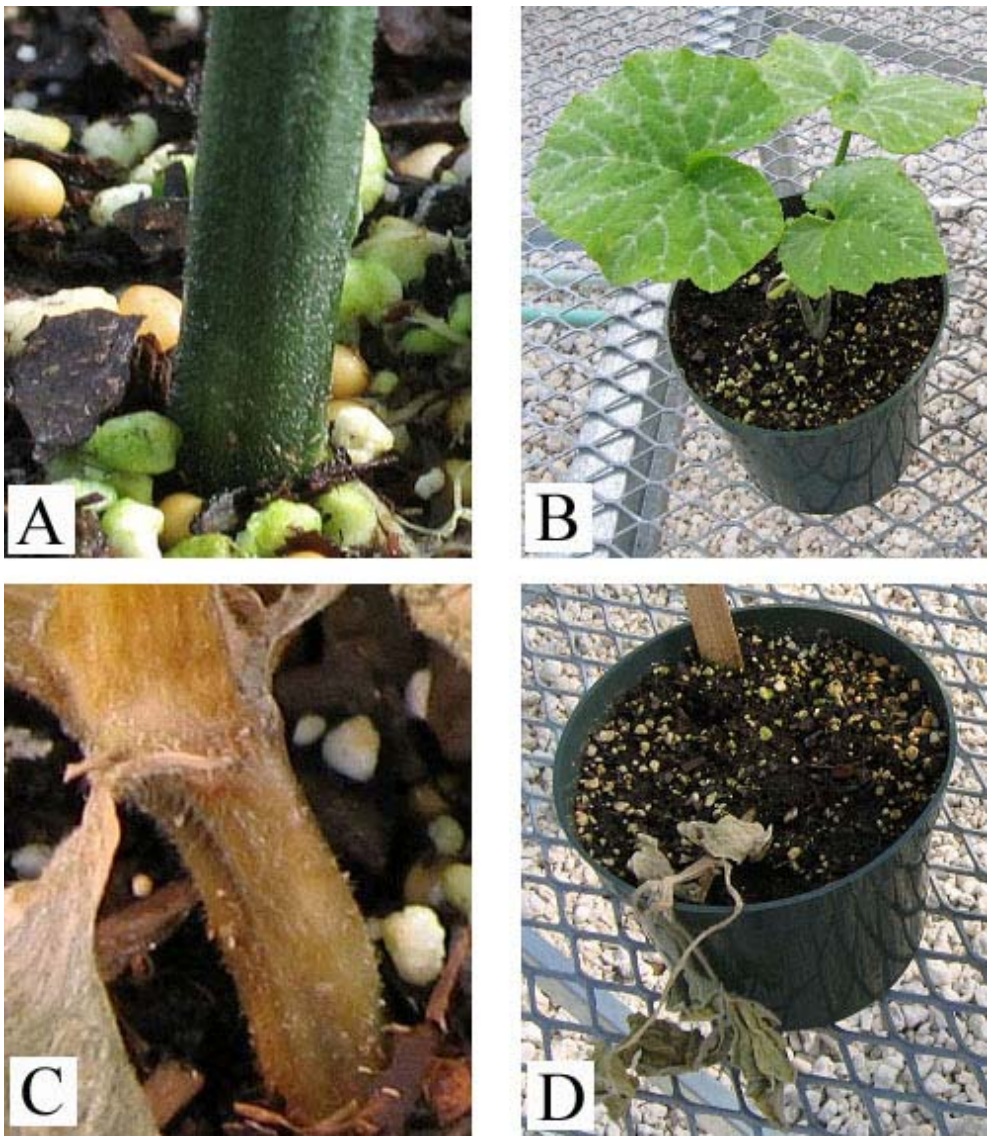

Figure 4-1. Response of *Cucurbita* breeding line #394-1-27-12 and 'Butterbush' to crown inoculation with a suspension of *Phytophthora capsici* isolates. A & B) Breeding line #394-1-27-12 remained asymptomatic post crown inoculation. C) 'Butterbush' develops a tan-brown water-soaked lesion at its crown that rapidly expands causing stem collapse. D) plant death.

CHAPTER 5
OVERALL CONCLUSIONS

Squash (*Cucurbita* spp.) is a multimillion dollar industry in the United states producing over $229 million in sales for the year 2006 alone (USDA, 2007b). *Phytophthora capsici* Leonian is a fungal-like pathogen that has causes several disease syndromes in squash including crown rot, foliar blight and fruit rot. In the United States, *P. capsici* has been identified in many of the vegetable producing states including California, North and South Carolina, Florida, Georgia, Illinois, New Jersey, Michigan, and Texas (Babadoost and Islam, 2003; Café-Filho et al., 1995; Isaleit, 2007; Lamour and Hausbeck, 2003; Ristaino, 1990). The objectives of this research project were: (1) identify sources of resistance to *P. capsici* crown rot within the *C. pepo* germplasm collection; (2) develop a homozygous *P. capsici* crown rot resistant *Cucurbita* breeding line; and (3) characterize the resistance to *P. capsici* crown rot found within *Cucurbita* breeding line #394.

For the first objective, 150 accessions from 24 different countries were chosen from the United States *C. pepo* germplasm collection and screened for their response to a three isolate suspension of *Phytophthora capsici* in three independent studies. From the 115 accessions thirteen (11.3%) had at least 50% of their replicates with a DRS of 0 or 1. Eight of these accessions had a mean DRS of less than 2 and were rescreened. Results of the rescreen study paralleled those of the initial study in that the mean DRSs among the accessions remained less than 2 and the average SD was 1.4. PI 181761 exhibited the lowest mean DRS at 0.5 with all plants in this accession rated as either 0 or 1. The findings from these screens suggest that accessions within the *C. pepo* collection are potential sources of resistance to *P. capsici*. Through screening and selection, the development of *C. pepo* lines homozygous for *P. capsici* resistance will allow us to study the inheritance of resistance, evaluate the *P. capsici*-*C. pepo*

interaction, and create Phytophthora crown rot resistant cultivars to aid in disease management of this pathogen. Based on the origin of the eight accessions chosen, evaluating additional accessions from Asia, Europe, and Mexico in the future might be worth considering. Further studies are also necessary to evaluate the Phytophthora crown rot resistant *C. pepo* breeding lines developed from this study for their response to Phytophthora foliar blight and fruit rot.

In the second objective, resistance to *P. capsici* was found within two wild gourd species, *C. lundelliana* PI 438542 and *C. okeechobeenesis sbsp. okeechobeenesis* (Kabelka et al., 2007). Resistance to the crown rot syndrome caused by *P. capsici*, derived from the two wild species, was introgressed through a series of hybridizations providing breeding material 62.5% *C. moschata*, 25% *C. lundelliana* PI 438542 and 12.5% *C. okeechobeenesis sbsp. okeechobeenesis*. From this series, breeding line #394 was tested for response to *P. capsici* and was found to be segregating for resistance (Kabelka et al., 2007). The objective of this study was to develop, from this material, a Cucurbita breeding line homozygous resistant to the crown rot syndrome of *P. capsici*.

Breeding line #349 (F4) and three generations of progeny from the self-pollination of an asymptomatic single plant selection were screened for resistance to the *P. capsici*. Results from these screened revealed the mean DRS and SD of each generation decrease with selection. Breeding line #394 had a mean DRS of 1.0 and a SD of 1.5 while #394-1 had a mean DRS and SD of 0.7. Evaluation of breeding line #394-1-27 (F6) revealed seven out of eight of its progeny to be asymptomatic with a mean DRS of 0.1 and SD of 0.4. A final study evaluating breeding lines #394-1-27-12 (F7) revealed all plants to be asymptomatic to crown inoculation with *P. capsici. Cucurbita* breeding line #394-1-27-12 was determined to be homozygous resistant to the crown rot syndrome caused by *P. capsici*. At maturity, #394-1-27-12 produces smooth,

medium-green, striped, obovate-shaped fruit, with medium orange flesh color. Growth habit is vine; leaves are shallow-lobed and mottled. *Cucurbita* breeding line #394-1-27-12 will be a useful source of *P. capsici* crown rot resistance for introgression into the morphologically diverse edible-fruited domesticates within *Cucurbita*. Further studies are needed to evaluate #394-1-27-12 for its response to foliar blight and fruit rot and to determine the inheritance of resistance to crown rot caused by *P. capsici*.

The third objective of my research was to characterize the inheritance of resistance to crown rot caused by *P. capsici* within the *Cucurbita* breeding line #394-1-27-12. The *Cucurbita* breeding line #394-1-27-12 was crossed with 'Butterbush' (BB), a butternut-type winter squash (*C. moschata*) highly susceptible to *P. capsici*. Controlled pollinations were carried out in the greenhouse to generate F1 (BB x 394-1-27-12), F2 and reciprocal backcross (BC) progenies. Twenty-eight plants of *Cucurbita* breeding Line #394-1-27-12, 38 plants of 'Butterbush', eight F1s, 200 F2s, 100 plants each of BB x F1 and F1 x BB and 50 plants each of 394-1-27-12 x F1 and F1 x 394-1-27-12 were screened for their response to a three isolate suspension of *Phytophthora capsici*. The *Cucurbita* breeding line #394-1-27-12 and the F1 exhibited no symptoms of crown rot caused by *P. capsici* under these conditions. The F2 progeny segregated in 27:37 [resistant (R):susceptible (S)] ratio while the backcrosses to the susceptible parent, 'Butterbush' segregated in a 1:7 (R:S) ratio. Progeny of the backcrosses to the parent #394-1-27-12 were all resistant. Collectively, the segregation ratios support a model in which resistance to the crown rot syndrome caused by *P. capsici* is conferred by three dominant genes. The resistance in #394-1-27-12 is now currently being tested against *P. capsici* isolates from different regions of the United States and Europe to test for specificity. Studies are also being conducted

to create a molecular linkage map of the segregating progeny developed in this study to identify markers linked to *P. capsici* crown rot resistance.

APPENDIX A
RESPONSE OF CUCURBITA PEPO ACCESSIONS TO CROWN INOCULATION WITH SUSPENSIONS OF PHYTOPHTHORA CAPSICI ISOLATED FROM TOMATO AND PEPPER.

Previous studies have shown that *P. capsici* from one host, such as tomato, can be pathogenic on other susceptible hosts, such as pepper (Ristaino, 1990). Two common susceptible hosts that are grown in ratation with squash in Florida are tomatoes and peppers. I evaluated 46 accessions from the *C. pepo* germplasm collection for their response, in separate studies, to crown inoculation using suspensions of *P. capsici* isolated from tomato and pepper. *Phytophthora capsici* isolates from tomato (M29, Cp-27 and Cp-36) and pepper (Cp-32, Cp-30 and Imm018) were obtained from Dr. Pamela Roberts (Southwest Florida Research and Education Center, Immokalee, FL). Inoculum preparation, experimental design, the crown inoculation protocol, scoring for response to inoculation, and analysis of data were followed as described in Chapter 2 of this dissertation.

Mean disease rating scores (DRSs) to crown inoculation with *P. capsici* isolated from tomato among the 46 accessions ranged from 1.5 to 5 (Table A-1). Average standard deviation of DRSs within accessions was 1.3 and ranged from 0 to 2. Eight accessions (17.4%) had at least 50% of their replicates with disease ratings of 0 or 1. Six of these had a mean DRS of less than 2. Mean disease rating scores (DRSs) to crown inoculation with *P. capsici* isolated from pepper among the 46 accessions ranged from 1.5 to 5 (Table A-2). Average standard deviation of DRSs within accessions was 1.3 and ranged from 0 to 2.2. Four accessions (8.7%) had at least 50% of their replicates with disease ratings of 0 or 1. Three of these had a mean DRS of less than 2. These findings suggest that accessions within the *C. pepo* collection are worth considering as potential host differentials to the isolates of *P. capsici* collected from tomato and pepper.

Table A-1. Response of *Cucurbita pepo* accessions to a suspension of *Phytophthora capsici* isolated from tomato in Florida. Accessions are ranked according to their mean disease rating score (DRS).

Accession or Cultivar[z]	Mean DRS (0-5 scale)[y]	SD	% plants with disease rating ≤1	Seed Origin
PI 209783	1.5	0.8	63	Germany
PI 299574	1.5	0.8	63	South Africa
PI 512709	1.5	1.5	63	Spain
PI 174185	1.8	0.5	25	Turkey
PI 266925	1.9	1.1	50	Germany
PI 355054	1.9	0.6	25	Iran
PI 163232	2.0	1.4	50	India
PI 181761	2.0	1.3	38	Lebanon
PI 318826	2.0	1.3	38	Mexico
PI 179267	2.1	1.5	38	Turkey
PI 458731	2.1	1.6	50	Argentina
PI 285611	2.3	1.3	38	Poland
PI 136448	2.4	1.7	38	China
PI 173684	2.4	1.7	38	Turkey
PI 181944	2.4	1.8	50	Syria
PI 311741	2.4	1.7	38	Poland
PI 304061	2.6	1.6	25	Pakistan
PI 181763	2.8	1.8	38	Lebanon
PI 183232	2.8	1.4	25	Egypt
PI 288240	2.8	1.7	33	Egypt
PI 615142	2.8	1.3	13	Kazakhstan
PI 257287	2.9	1.6	14	Spain
PI 222721	2.9	1.8	25	Iran
PI 311102	2.9	1.6	25	Guatemala
PI 379307	3.0	1.7	50	Yugoslavia
PI 507885	3.0	2.0	25	Hungary
PI 615132	3.0	1.7	13	Mexico
PI 234615	3.2	1.5	0	South Africa
PI 274787	3.3	1.5	13	India
PI 269483	3.4	1.8	25	Pakistan
PI 234252	3.5	1.6	13	Argentina
PI 193502	3.6	1.7	13	Ethiopia
PI 357940	3.6	1.9	25	Yugoslavia
PI 357929	3.8	1.8	13	Macedonia
PI 177377	3.9	1.6	13	Syria
PI 364241	3.9	1.2	0	Hungary
PI 212060	4.0	1.7	17	Greece

Table A-1 Continued.

Accession or Cultivar[z]	Mean DRS (0-5 scale)[y]	SD	% plants with disease rating ≤1	Seed Origin
PI 368592	4.0	1.5	13	Macedonia
PI 193501	4.1	1.4	0	Ethiopia
PI 169418	4.3	1.5	14	Greece
PI 093458	4.6	0.7	0	China
PI 135394	4.8	0.7	0	Afghanistan
PI 135398	5.0	0.0	0	Afghanistan
PI 181758	5.0	0.0	0	Lebanon
PI 274336	5.0	0.0	0	Guatemala
PI 615141	5.0	0.0	0	Kazakhstan
EPS	5.0	0.0	0	United States
YSS	5.0	0.0	0	United States

[z]Susceptible *C. pepo* controls EPS, 'Early Prolific Straightneck' and YSS, 'Yellow Summer Squash'.

[Y]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (dead plant).

Table A-2. Response of *Cucurbita pepo* accessions to a suspension of *Phytophthora capsici* isolated from pepper in Florida. Accessions are ranked according to their mean disease rating score (DRS).

Accession or Cultivar[z]	Mean DRS (0-5 scale)[y]	SD	% plants with disease rating ≤1	Seed origin
PI 181763	1.5	0.5	50	Lebanon
PI 355054	1.9	1.5	63	Iran
PI 364241	1.9	1.4	50	Hungary
PI 181944	2.0	1.4	50	Syria
PI 181761	2.1	1.9	38	Lebanon
PI 368592	2.2	1.5	33	Macedonia
PI 311102	2.4	1.4	25	Guatemala
PI 615142	2.4	1.1	0	Kazakhstan
PI 512709	2.5	1.7	38	Spain
PI 318826	2.6	1.6	25	Mexico
PI 458731	2.6	0.7	0	Argentina
PI 169418	2.9	2.1	14	Greece
PI 135398	2.9	1.8	25	Afghanistan
PI 173684	2.9	1.4	0	Turkey
PI 274336	2.9	1.4	13	Guatemala
PI 174185	3.0	1.8	25	Turkey
PI 179267	3.0	1.8	25	Turkey
PI 266925	3.0	1.7	25	Germany
PI 209783	3.1	2.1	38	Germany
PI 357929	3.1	1.6	13	Macedonia
PI 234252	3.3	1.9	25	Argentina
PI 288240	3.3	1.9	25	Egypt
PI 615132	3.3	2.2	25	Mexico
PI 234615	3.4	1.8	13	South Africa
PI 136448	3.5	1.5	13	China
PI 379307	3.5	1.7	13	Yugoslavia
PI 212060	3.8	1.8	13	Greece
PI 257287	4.0	1.5	14	Spain
PI 163232	4.1	1.4	0	India
PI 222721	4.3	1.5	13	Iran
PI 285611	4.3	1.2	0	Poland
PI 311741	4.3	1.4	13	Poland
PI 181758	4.4	1.4	13	Lebanon
PI 299574	4.4	1.4	13	South Africa
PI 177377	4.5	1.4	13	Syria
PI 615141	4.5	0.8	0	Kazakhstan
PI 093458	4.6	1.1	0	China
PI 357940	4.6	0.7	0	Yugoslavia
PI 507885	4.6	1.1	0	Hungary
PI 193502	4.9	0.4	0	Ethiopia

Table A-2. Continued.

Accession or Cultivar[z]	Mean DRS (0-5 scale)[y]	SD	% plants with disease rating ≤1	Seed origin
PI 135394	5.0	0.0	0	Afghanistan
PI 183232	5.0	0.0	0	Egypt
PI 193501	5.0	0.0	0	Ethiopia
PI 269483	5.0	0.0	0	Pakistan
PI 274787	5.0	0.0	0	India
PI 304061	5.0	0.0	0	Pakistan
EPS	5.0	0.0	0	United States
YSS	5.0	0.0	0	United States

[z]Susceptible *Cucurbita pepo* controls EPS, 'Early Prolific Straightneck' and YSS, 'Yellow Summer Squash'.

[y]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (dead plant).

APPENDIX B
RESPONSE TO CROWN INOCULATION USING THREE DIFFERENT PHYTOPHTHORA CAPSICI ZOOSPORE CONCENTRATIONS.

Many *P. capsici* crown inoculation studies have been conducted at different zoospore concentrations with no standard inoculum level determined (Alcantara and Bosland, 1994; Ortega et al., 1995; Lee et al., 2001). A study was conducted to compare the response of *C. pepo* to crown inoculation using a suspension of *P. capsici* squash isolates at 100,000, 50,000 and 25,000 zoospore concentrations. Two *C. pepo* accessions, PI 179267 and PI 181761, progeny from previously selected asymptomatic plants from these two accessions, 179267-17 and 181761-18, and susceptible controls 'Early Prolific Straightneck' and 'Yellow Summer Squash' were used. *Phytophthora capsici* type A1 isolates (01-1938A, RJM98-730 and RJM98-805) collected from squash were obtained from Dr. P. Roberts (University of Florida, Southwest Florida Research and Education Center, Immokalee, FL). Inoculum preparation, experimental design, the crown inoculation protocol, scoring for response to inoculation, and analysis of data were followed as described in Chapter 2 of this dissertation. Results of this study showed no significant difference in the response of each PI, their progeny, or susceptible controls to the three different concentrations of *P. capsici* zoospores.

Table B-1. Response of *Cucurbita pepo* PI 179267, PI 181761 and their selfed progeny to a suspension of *Phytophthora capsici* squash isolates at 100,000, 50,000 and 25,000 zoospore concentrations.

PI 179267

Zoospore Concentration	Disease Rating Scale (DRS)[y]						Mean DRS[x]
	0	1	2	3	4	5	
100,000	1	4	2	0	0	0	1.1[a]
50,000	1	4	1	0	1	0	1.4[a]
25,000	0	4	2	0	0	1	1.9[a]
Pooled	2	12	5	0	1	1	1.5

179267-17[z]

Zoospore Concentration	Disease Rating Scale (DRS)						Mean DRS
	0	1	2	3	4	5	
100,000	1	5	0	0	1	1	1.8[a]
50,000	1	6	0	1	0	0	1.1[a]
25,000	0	6	1	0	0	1	1.6[a]
Pooled	2	17	1	1	1	2	1.5

PI 181761

Zoospore Concentration	Disease Rating Scale (DRS)						Mean DRS
	0	1	2	3	4	5	
100,000	0	3	2	0	0	3	2.8[a]
50,000	0	0	6	1	1	0	2.4[a]
25,000	0	2	1	1	0	3	3.1[a]
Pooled	0	5	9	2	1	6	2.8

181761-16[z]

Zoospore Concentration	Disease Rating Scale (DRS)						Mean DRS
	0	1	2	3	4	5	
100,000	4	2	1	0	0	1	1.1[a]
50,000	3	3	1	0	0	1	1.3[a]
25,000	5	2	0	0	1	0	0.8[a]
Pooled	12	7	2	0	1	2	1.1

Early Prolific Straightneck[w]

Zoospore Concentration	Disease Rating Scale (DRS)						Mean DRS
	0	1	2	3	4	5	
100,000	0	0	0	0	0	8	5.0
50,000	0	0	0	0	0	8	5.0
25,000	0	0	0	0	0	8	5.0
Pooled	0	0	0	0	0	24	5.0

Yellow Summer Squash[w]

Zoospore Concentration	Disease Rating Scale (DRS)						Mean DRS
	0	1	2	3	4	5	
100,000	0	0	0	0	0	8	5.0
50,000	0	0	0	0	0	8	5.0
25,000	0	0	0	0	0	8	5.0
Pooled	0	0	0	0	0	24	5.0

[z]Progeny of a self-pollinated plant from PI with disease rating score of 0.

[y]Disease rating based on a scale ranging from 0 (no symptoms) to 5 (dead plant). Number of plants scored within each category listed.

[x]Means followed by the same letter are not significantly different at p=0.05.

[w]*Cucurbita pepo* susceptible controls.

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BIOGRAPHICAL SKETCH

Les Padley Jr. was born in Largo, Florida, in January 9, 1980, to Les and Pam Padley. He graduated high school from the Center for Advanced Technologies in 1998, and received his Bachelor of Science degree from Florida Southern College in environmental horticultural and business. In 2005 he obtained his Master of Science degree in plant breeding from the University of Florida. Currently, he is now working on his Doctor of Philosophy in plant breeding under Dr. Eileen Kabelka and has begun training as the NAFTA squash breeder for Syngenta.