This is to certify that the
dissertation entitled

EVALUATION OF THE ROLE OF PLANT ARCHITECTURE AND CUCUMBER FRUIT DEVELOPMENT IN *PHYTOPHTHORA CAPSICI* DISEASE DEVELOPMENT

presented by

KAORI ANDO

has been accepted towards fulfillment
of the requirements for the

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

Major Professor's Signature

6-10-09

Date

EVALUATION OF THE ROLE OF PLANT ARCHITECTURE AND CUCUMBER FRUIT DEVELOPMENT IN *PHYTOPHTHORA CAPSICI* DISEASE DEVELOPMENT

By

Kaori Ando

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Plant Breeding & Genetics – Horticulture

2009

ABSTRACT

EVALUATION OF THE ROLE OF PLANT ARCHITECTURE AND CUCUMBER FRUIT DEVELOPMENT IN *PHYTOPHTHORA CAPSICI* DISEASE DEVELOPMENT

By

Kaori Ando

Fruit rot caused by *Phytophthora capsici* Leonian is an increasingly serious disease affecting production of cucumber (*Cucumis sativus* L.) and other cucurbit crops in many parts of the US. The absence of genetically resistant cultivars and rapid development of fungicide resistance makes it imperative to develop integrated disease management strategies. Cucumber fruit which come in direct contact with the soil-borne pathogen are usually located under the canopy where moist and warm conditions favor disease development. Screening a collection of 150 Plant Introductions (PI) revealed variation for an array of architectural traits. One of the compact lines (PI 308916), which had a tendency to hold young fruit off the ground, exhibited lower disease occurrence which was not due to genetic resistance, suggesting that architecture which allows less contact of fruit with the soil could be useful for *P. capsici* control for cucumber. In the course of screening for resistance sources among cucumber PIs, fruit age/size was shown to be a factor in susceptibility to infection by *P. capsici.* Inoculation of greenhouse-grown fruits of known ages showed that cucumber fruits were most susceptible to *P. capsici* when they were very young and rapidly elongating, but then developed age-related resistance (ARR) as they approach full length at 10-12 days post pollination (DPP). This was observed in both the greenhouse and field and for several genotypes. Testing of seven additional cucurbit crops, zucchini, summer squash, acorn squash, pumpkin, butternut squash, melon, and watermelon also showed ARR, but to varying degrees. In

the field, infection primarily occurs at the blossom end. Inoculation of cucurbit fruits of various ages with *P. capsici* at the peduncle and blossom ends showed that as fruit age increased, the peduncle end became less susceptible sooner than the blossom end, suggesting a developmental gradient within the fruit influencing susceptibility for cucumber, zucchini, acorn, and butternut squash fruits. To understand the basis for ARR in the cucumber fruit-*P. capsici* interaction, and to increase understanding of early fruit development in cucumber, morphological and global gene expression analyses were performed. Morphological changes associated with cucumber fruit development were catalogued for hand pollinated greenhouse grown fruits at 0, 4, 8, 12, 16, 20, 26, and 32 DPP. These fruits were also collected to generate cDNA libraries for 454 pyrosequencing technology developed by Roche®. Young cucumber fruits show marked changes in fruit size, cell size, surface wax, chlorophyll content and patterns, wart formation, spine development, and placenta and seed development. 454 pyrosequencing analyses yielded 187,406 clean reads, of which 88 % could be assembled into 13,879 contigs. The number of sequences per contig, which is reflective of transcript abundance, ranged from 2-5,167. BLAST analysis of the most highly represented transcripts against the nr protein sequence NCBI database, indicated high representation of genes associated with protein synthesis, flowers, fruits or seeds of other species, latex related proteins, lipid biosynthesis, cell expansion, defense, phloem transport, and photosynthesis. Results of 454 and qRT-PCR for selected genes were comparable, indicating that the 454 transcriptome sequencing can be used for analyzing relative gene expression during fruit development. These information will contribute to the understanding of biology of cucumber fruit development and possible relationship to age-related resistance.

ACKNOWLEDGEMENTS

I would like to thank all my committee members, Drs. Mary Hausbeck, Ray Hammerschmidt, Jim Kelly, Mathieu Ngouajio, and Rebecca Grumet for their support and guidance. I want to show special appreciation to Rebecca for her patience, coaching, and providing me a great opportunity to pursuit my dream.

Great thanks to past and present members of Grumet Lab. I especially thank Sue Hammar and Holly Little for their friendship, moral support, and making my life both inside and outside of the work very stimulating. I would like to thank Zhulong Chan, a molecular technique sensei, for giving me advice. Thanks to all the undergraduates and highschool students who helped me throughout the years, especially Dan Raba who made weeding enjoyable and entertaining.

I appreciate all of fellow "joint labbers" for their helpful suggestions and discussions. I would like to acknowledge Dr. Bukovac for giving me an opportunity to work as a research assistant in his lab and for greatly inspiring me to pursue higher education. Also, I would like to thank Paolo Sabbatini for being such a fun and great friend to me. I owe special thanks to Neil Adhikari for always being there for me and for his understanding.

I would like to show gratitude to my wonderful family for being there for me despite me being more than 6,000 miles away. I love you all and am glad I have you in my life.

It's all worthwhile!

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Chapter 1

Literature Review

Introduction

Cucumber (*Cucumis sativus* L.) is cultivated in tropical and temperate regions of the world, and its origin is thought to be Africa or Central/East Asia (Miller and Wehner, 1989). Cucumber fruits are consumed fresh or processed into pickles. Michigan is the top processing (pickling) cucumber producing state in the United States. In 2008, 31,000 acres of pickling cucumber were planted in Michigan, with a gross value of 41 million dollars (United States Department of Agriculture National Agricultural Statistics Service, 2008). The pickling cucumber production acreage has remained constant in recent years, however, maintaining and increasing yield has become difficult for growers due to oomycete diseases including downy mildew caused by *Pseudoperonospora cubensis* and fruit rot disease caused by *Phytophthora capsici* (Colucci et al., 2006; Hausbeck and Lamour, 2004; Lamour and Hausbeck, 2000, 2001a,b, 2002).

Phytophthora capsici biology

Phytophthora capsici belongs to the kingdom Chromista and the class Oomycetes (Agrios, 1997). Members of the genus *Phytophthora*, which means plant destroyer in Greek, often cause disastrous diseases such as the potato leaf blight caused by *Phytophthora infestans* that led to the Irish famine in the 1840s (Agrios, 1997; Erwin and Ribiero, 1996; Judelson and Blanco, 2005). *Phytophthora capsici* was first described as a host specific pathogen of chili pepper (*Capsicum annum* L.) occurring in New Mexico

(Leonian, 1922). Since then, many crops including woody plants, ornamentals, and vegetables have been reported to be hosts (Erwin and Ribeiro, 1996). Although incidence of cucumber infection was first reported in Colorado in 1936 (Kreutzer, 1937), it has only recently become a common problem in cucumber production areas (Babadoost, 2004; Hausbeck and Lamour, 2004). Once cucumber fields are infected by *Phytophthora capsici*, severe yield loss due to infection of the fruit is frequently observed (Babadoost, 2004; Hausbeck and Lamour, 2004). *Phytophthora capsici* infected cucumber fruit typically develop a slightly discolored and depressed fruit surface which looks like water soaking, subsequently white cottony mycelium cover the infected area. Mycelium produce sporangiophores that produce distinctive lemon-shaped sporangia at their tip which lead to further disease spread.

P. capsici is a soil borne, hemibiotroph pathogen that favors warm and wet environments (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004). The optimum temperature for disease development is 25 to 28 °C. Multiple cycles of inoculation occur in a single growing season. The asexual sporangia can germinate either directly or indirectly (Katsura and Miyazaki, 1960). In direct germination, hypahe are produced from the sporangia wall and penetrate through openings, including stomata, lenticels or wounds. In indirect germination, also called zoosporogenesis, sporangia produce asexual motile zoospores, which are the primary infection source during the growing season. Temperature can influence whether sporangia undergo direct or indirect germination in *P. infestans* (Judleson and Blanco, 2005).

Zoospores, can be spread by irrigation, rain, and free surface water on plants. The wall-less zoospores swim by using two flagella and are directed chemotactially,

electrotactically, or autoaggragationally to a primary infection site of the host plant (Hardham, 2007; Latijnhouwers et al., 2003; Tyler, 2002). Chemoattractants can be non-specific such as amino acids, or specific such as the isoflavones daizen and genistein from soybean roots which only attract *Phytophthora sojae*. Electrotaxis is a response to ion exchange carried at the surface of root that creates an electrical field around the rhizosphere (Morris and Gow, 1993). Autoaggragation in which zoospores congregate to maximize infection rate, is thought to be species specific and could be related to calcium released by the cyst (Reid et al., 1995; Tyler, 2002).

Soon after contact with the plant surface, zoospores shed their flagella and encyst. Encystment can be induced by many factors including chemical and physical stimuli, temperature, calcium and phosphatic acid. However, the exact mechanisms which governs zoospore movement, taxis, and encystment are not well known. Upon encystment, zoospores secret adhesive material to help the cysts to adhere to the plant surface. In the case of *P. infestans*, the adhesive material secreted by *P. infestans* has homology to human mucins, suggesting a role in protection of the germiling from drying or physical damage (Görnhardet et al., 2000).

Encystment is followed by germination of the germ tube and appressorium formation which also directed by chemotaxic and thigmotropic growth. Periclinal or anticlinal walls on epidermis surface and stomatal complex are usually targeted for penetration (Hardham, 2007). Germ tubes often swell to form appressoria, which are smaller and not melanized compared to fungal appressroia. Whether these appresoria exert mechanical pressure on the plant surface is still unknown. *Phytophthora capsici*

can penetrate directly through the plant surface or through natural openings such as stomata (Katsura and Miyazaki, 1960).

In case of *Phytophthora palmivora,* appressorium formation *in vitro* was highly influenced by topographical signals, substrate hydrophobicity and nutrient conditions, where rough, hydrophibic, and lower nutrient artificial surface promoted appressoria formation (Bircher and Hohl, 1997). In potato leaf and *P. infestans* interaction, short germ-tubes and appressoria were associated with rapid and successful penetration whereas longer and aberrant germ-tubes were associated with unsuccessful infection or incompatible plant-pathogen interaction (Grenville-Briggs et al., 2008).

Pathogenesis related factors including cell wall degrading enzyme such as cutinases, polygalacturnonases, pectate lyases, and cellulase are thought to be produced by the germ tubes and appressoria (Judelson and Blanco, 2005; Latijnjousers et al., 2003). These enzymes are thought to help penetration of plant surface. They also can be recognized as elicitors which trigger defense response by both host and non-host plants (Hardham, 2007; Judelson and Blanco, 2005). After successful penetration of the host, hyphae grow intracellulary and develop haustoria, a specialized structure which obtain nutrients from host (Latijnhouwers et al., 2003; O'Connell and Panstruga, 2006).

In addition, *P. capsici* produces a unique thick walled sexual spore called an oospore, which enables it to overwinter. Two compatible mating types, A1 and A2, are required for oospore formation (Erwin and Ribeiro, 1996). Both mating types produce antheridium (male) and oogonium (female), and come into physical contact, antheridium fertilize oogonium, subsequently develops oospores. In case of *P. infestance*, oospore production is triggered in response to low temperature (Mosa et al., 1991).

***Phytophthora capsici* control in cucumber production**

Controlling *P. capsici* in cucumber production has been difficult. Oospores are able to survive in the soil for more than five years, making crop rotation an inadequate control method (Lamour and Hausbeck, 2001a, 2002, 2003; Hausbeck and Lamour, 2004). Also, *P. capsici* is heterothallic, therefore there is a chance of recombination to introduce new variants that carry new virulent factors, contributing to the difficulty in controlling this disease (Erwin and Ribeiro, 1996; Lamour and Hausbeck, 2003). Heavy dependence on chemical control has resulted in the development of resistance to a fungicide commonly used in Michigan, Ridomil® (active ingredient mefenoxam) (Lamour and Hausbeck, 2000). In addition, since many farms do not practice rotation with non-susceptible crops, the fields can build up high spore populations and develop resistance to fungicides sooner.

Genetic resistance would be the optimal method of disease control. Cucumber fruits are the most susceptible part of the plant (Hausbeck and Lamour, 2004). Cucumber vines will look healthy at time of harvest, however the fruits can be heavily infested by *P. capsici* (Hausbeck and Lamour, 2004). Therefore, Hausbeck et al. (2002) initiated screening for fruit resistance, but no significant resistant genotype was identified out of 238 genotypes screened. Subsequent screening, including acessions tested as part of this work (chapter 2) using a diverse collection of the cucumber germplasm selected to provide maximum genetic variance based on geographical distribution and isozyme data (Knerr et al., 1989), has not identified any resistant genotypes to date.

It is therefore important to develop an integrated system for *P. capsici* control. For example, incorporating cover crops to suppress the pathogen from spreading by

minimizing rain splash spore dispersal have been tested, however this strategy is difficult to reach an economically feasible level (Ristaino et al., 1997; Wang and Ngouajio, 2008). Modifying canopy conditions to be less favorable for *P. capsici* development may also facilitate disease control. Fruit are usually located under the canopy where the environment is moist, warm, and close to the pathogen in the soil, thus favoring conditions for disease development. Furthermore, applying fungicides to the fruits is difficult since the dense canopy is covering the fruit. Studies with trellised cucumber demonstrated reduced infection by *Rhizoctonia solani,* presumably due to higher air circulation, better fungicide distribution, and less plant parts contacting soil (Hanna et al., 1987; Konsler and Strider, 1973). However, trellising is expensive and labor intensive, making it unusable for commercial pickling cucumber production (Russo et al., 1991). Wider spacing to reduce disease pressure has been proposed, and this approach remains to be tested (Ngouajio et al., 2006). Another possible approach is to implement alternate plant architectures for a less dense canopy to allow for increased air circulation and light penetration or reduce fruit contact with the soil.

Genetic modification of plant architecture for disease control

The idea of controlling disease by reducing canopy density with altered plant architecture has been evaluated for other crops and diseases. One attempt was made by Halterlein et al. (1981) for *R. solani* control on cucumber. They sprayed herbicide over the canopy after the first flower to reduce the canopy density, and observed a significant reduction in the disease occurrence. Therefore, the authors concluded that the microclimate under the reduced canopy created unfavorable conditions for the disease

development. A reduced canopy was presumed to allow for better spray distribution, less humidity and increased light penetration, which together could contribute to disease reduction (Halterlein et al., 1981). In addition, in earlier published work, Harnish (1965) reported that continuous bright light inhibited oospore formation, while, continuous low intensity light and complete darkness did not inhibit oospore formation. While spraying herbicide on to a crop at the time of fruit development may not be a desirable production strategy, other methods such as breeding for traits that confer to reduced canopy density may be helpful. This was explored in chapter 2.

There are limited documented examples of plant breeding efforts which have used an architectural approach to control disease incidence (Ando et al., 2007). Two of the better documented examples are effect of canopy structure on white mold in bean and effect of plant height on fusarium head blight of wheat

White mold (*Sclerotinia sclerotiorum*) is a common necrotrophic and soilborne disease affecting dry bean (*Phaseolus vulgaris*) production worldwide. It favors cool and moist environment and affects stems and pods especially during the stages of flowering and pod filling stages (Fuller et al., 1984). Steadman et al. (1973) compared disease occurrence in nearly-isogenic lines differing in determinancy and in relation to plant spacing. All genotypes tested showed significantly lower disease occurrence in wider spaced plots, and also determinate lines had lower disease occurrence than indeterminate lines regardless of planting density. In addition, trellis-grown plants had higher yield and lower white mold incidence, presumably due to modified microclimate which minimized optimal conditions for white mold occurrence and increased photosynthesis by allowing sun exposure (Coyne et al., 1974).

Height and leaf distribution influenced the white mold severity even within upright growth habit beans, since taller and more open canopy upright genotypes had lower disease incidence; whereas upright genotypes with dense canopy near the soil surface had higher disease occurrence (Schwartz et al., 1987). These results suggest that plant height and canopy density are also contributing to disease occurrence. By combining protective spray application, disease control in upright growth habit genotypes can be further improved (Schwartz et al., 1987).

The relationship between agronomic traits including growth habit, days to flower, canopy height and width, branching pattern, loading, days to maturity, seed size, and yield and resistance to white mold was studied by Kolkman and Kelly (2002). Although the agronomic traits associated with disease severity differed among the locations, the most important trait was indeterminate growth habit. Based on the study, they proposed a plant ideotype that has indeterminate bush growth habit, lodging resistance, and medium canopy height, width, and branching pattern.

These results suggested the possibility of modified plant architecture as a means to promote disease avoidance mechanisms for disease control. To facilitate white mold control, bean breeders have selected for elevated canopy of an upright indeterminate bean, and the open porous canopy from determinate bean (Coyne et al., 1974; Blad et al., 1978; Fuller et al., 1984; Park, 1993). With the combination of alternating plant architecture and better cultural practice, bean breeders were able to improve disease control and increase yield.

In the case of fusarium head blight (FHB) of wheat (*Triticum aestivum*), researchers observed that tall winter wheat lines resulted in lower FHB severity than

shorter lines, presumably since the location of the ear of tall lines is further from the soil line where the inoculum is located (Mesterhazy, 1995). Hilton et al. (1999) also observed that tiller height and severity of FHB was negatively related. Near-isogenic lines with the dwarfing gene, *Rht1*, showed significantly higher disease severity compared with the lines without *Rht1*, regardless of the genetic background. Comparing microclimate differences between the tall and the short lines by measuring relative humidity at ear height revealed no significant difference in relative humidity, suggesting that the reduced disease incidence may be more directly related to distance from the soil than modified canopy conditions. F_3 families of a cross between tall and short parents co-segregated for disease resistance and tall straw, further indicating that genes controlling straw height could be linked to FHB resistant quantitative trait loci.

Tan spot caused by *Pyrenophora tritici-repentis*, is a common disease in durum wheat (*Triticum turgidum*) (De Wolf et al., 1998). The pathogen overwinters in old crop residue. Ascospores, the primary inoculum, are released from the residues under rain, high humidity, and a temperature above 10 °C. The secondary inoculum, conidia, is dispersed by wind and germinates under prolonged leaf wetness (6 hr). Tan spot severity was considered to be related to plant height as was observed for FHB. Fernandez et al. (2002) tested this hypothesis by using near isogenic lines and randomly selected lines differing in height. All the tested lines were susceptible to tan spot; however, the short lines were more diseased than the tall lines. Furthermore, the plant height and leaf area index were negatively correlated; the short lines had a denser canopy due to closer position of lower leaves of adjacent plants. The denser canopies in the short lines might

also cause more favorable environmental conditions for infection, since there was no correlation between height and disease severity under controlled inoculation.

In cucumber, a variety of growth habits exists within the cucumber germplasm (Pierce and Wehner, 1990), however these traits have not been tested for the purpose of controlling disease by architecture. There are 1,352 plant introductions (PIs) in the collection of the U.S. National Plant Germplasm System (NPGS) which might be sources of new plant architectures. In addition, other tools, such as nearly isogenic lines differing in determinancy habit and leaf size have been developed (Serce et al., 1999). The growth habit of these cucumbers might increase air circulation, light penetration, or accessibility of the applied fungicide to fruit under the canopy.

Age-related resistance

Age-related resistance (ARR), also called adult plant resistance or developmentally related resistance, is becoming increasingly recognized as an important component of plant defense against infection (Develey-Rivière and Galiana, 2007; Panter and Jones, 2002; Whallen, 2005). Increased resistance has been observed as young tissues develop in many plant-pathogen systems, including diseases caused by fungi, oomycetes, bacteria, nematodes, and viruses. ARR has been observed for *P. capsici* infection of pepper (*Capsicum annum*) plants (Kim et al., 1989). The reduced susceptibility was suggested to be related to physiological changes in root and stem tissues.

Once ARR is induced, the resistance persists for the rest of the plant life until senescence. ARR is distinct from the increase in susceptibility which occurs in

association with ripening or senescence in old, mature organs. ARR can be non-specific, increasing overall disease resistance to multiple pathogens, it can increase resistance to all strains of a particular species, or can be specific to a given pathogen strain. Little is known about the mechanisms responsible for ARR.

There is evidence that salicylic acid is important to ARR in the Arabidopsis response to *Pseudomonas syringae* (Kus et al., 2002) and *Hyaloperonospora parasitica* (McDowel et al., 2005). ARR in rice (*Oryza sativas*) to *Xanthomonas spp.* may be related to developmentally-regulated *R* gene-mediated resistance which is controlled post-transcriptionally, or by other mechanisms (Century et al., 1999; Koch and Mew, 1991; Mazzola et al., 1994). Developmentally-regulated expression of single-gene mediated resistance also has been observed for potyvirus infection of cucumber seedlings (Ullah and Grumet, 2002; Wai and Grumet, 1995).

ARR can be induced at major transitions occurring during a plant's life. For example, ARR has been observed at developmental transitions including juvenile to adult, or vegetative to flowering (O'Connell and Panstruga, 2006). Disease resistant or susceptible mutants have been identified that have altered developmental processes; conversely, mutants in developmental functions can have altered defense capability, suggesting the possibility that resistance gene products maybe evolutionary related to developmental gene products, or that developmental genes may confer resistance or induce processes that confer resistance (Whalen 2005). For example, the ARR to common rust caused by *Puccinia sorghi* is delayed in *Congrass1* (*Cg1*) maize (*Zea mays*) mutant which has an extended juvenile-vegetative phase. *Cg1* mid-whorl leaves with juvenile traits were highly susceptible, while the wild type mid-whorl leaves were

resistant (Abedon and Tracy, 1996). Also, the vegetative phase of tobacco (*Nicotiana tabacum*) was highly susceptible to *Phytophthora parasitica,* however plants become resistant at the time of the flowering phase transition (Hugot et al., 1999). Non-salicylic acid (SA) expressing transgenic (NahG) tobacco plants showed that *P. parasitica* infection of the leaves does not require SA accumulation, however, infection after floral transition requires SA accumulation. In addition, *Arabidopsis* showed ARR to cauliflower mosaic at the transition from vegetative phase to floral phase due to developmental changes affecting on the long-distance virus movement (Leisner et al., 1993).

Some plants only develop ARR in specific tissues or organs. Scab caused by *Venturia inaequalis* on apple (*Malus domestica*) leaves showed ARR, young apple leaves are more susceptible than old leaves (Li and Xu, 2002). Also, in the case of soybean (*Glycine max*), hypocotyl age influenced the susceptibility to *P. sojae* (Lazarovits et al., 1980).

ARR also has been observed in fruits. Developing grape berries (*Vitis vinifera*) showed decreased susceptibility to infection by *Unicinula necator*, causal agent of powdery mildew, and to *Plasmopara viticola*, causal agent of downy mildew (Ficke et al., 2002; Gadoury et al., 2003; Kennelly et al., 2005). Berries are highly susceptible for the first several weeks after anthesis. Developmental changes in berries, such as brix content or morphological change of stomata to lenticels were thought to be related to susceptibility changes, however, the mechanism has not been determined. In the process of screening for *P. capsici* resistance in cucumber I also observed age-related resistance in the developing fruit. This observation was studied in chapter 3.

Genomic approaches to study fruit development in cucumber

Analysis of gene expression during cucumber fruit development may provide further insight into the cucumber-*P. capsici* interaction. Cucumber genomic tools, however, have been very limited. The number of cucumber ESTs in the National Center for Biotechnology Information (NCBI) database is only approximately 8,000 (4/15/2009 searched) compared to other species such as *Arabidopsis* - 1,728,728, rice - 1,260,123, and tomato - 261,630. In the past year, however, the cucumber genome was reported to be sequenced by a group at the Chinese Academy of Agriculture Science and the Beijing Genomics Institute, Shenzhen (Huang et al., 2009). It is anticipated that the 365 Mb sequenced genome will be publicly available in the near future. Having a sequenced genome and generating more ESTs will be a tremendous advantage, not only for contig assemblies and gene annotations, but also for providing tools to study complicated biology of plants.

The development of next generation sequencing technologies has dramatically improved sequencing capability with respect to amount of data, time, and cost (Mardis, 2008; Margulies et al., 2005). Roche (Branford, CT) introduced 454 pyrosequencing technology (Margulies et al., 2005; Droege and Hill, 2008) which can sequence over 100 Mb for 7.5 hrs with average reading of 200 bp. A new version of 454, GS FLX Titanium Series reagent, was introduced in 2008 which can sequence over 400 Mb with average reading of 400 bp. The length of reads generated by 454 pyrosequencing allow for contig assemblies and gene annotations of poorly characterized genomes. In addition, one of the applications of the new sequencing technology is gene expression analysis, since the number of contig reads represents frequency of the particular gene expression (Eveland et

al., 2008; Ohtsu et al., 2007; Torres et al., 2008). 454 sequencing also has capability of loading multiple samples for a run, allowing side-by-side analysis. Gene expression analyses by 454 pyroseuquencing results have been correlated with microarrays, northern blot analysis or quantitative real time polymerase chain reaction (qRT-PCR) assays, suggesting high reproducibility and accountability for gene expression analysis (Eveland et al., 2008). It also can be used as an alternative to microarrays, especially for crops with small ESTs numbers and/or no microarray platform available, such as cucumber.

Model plants, such as *Arabidopsis thaliana* and rice (*O. sativas)* are well studied in many aspects of plant development with the aid of tremendous information generated by genomic tools. Fully sequenced genomes, microarrays, and large numbers of ESTs and mutants are available. However, these species are not well suited to address questions related to fleshy fruit development. In case of fleshy fruits, tomato (*Solanum lycopersicum*), apple, and grape (*V. vinifera*) are well studied (Carrari and Fernie, 2006; Giovannoni, 2004; Janssen et al., 2008; Lemaire-Chamley et al., 2005; Moore et al., 2002; White, 2002). The primary emphasis of fruit studies has been on late stage of development and important fruit quality attributes associated with maturation and ripening (Lee et al., 2007; Lemaire-Chamley et al., 2005).

There has been less study done on early fleshy fruit development, though this stage is essential for all fruit. For example, in tomato fruit, many new genes, and many genes with no known function were recovered from young tomato fruit cDNA libraries from tissues at ranging in 8, 12, and 15 days post anthesis (DPA). In addition, reflective of less study of these stages, 8 DPA fruit had the highest proportion of genes with no known function (Lemaire-Chamley et al., 2005). There are three main phases of early

fruit development; fruit set, cell division, and cell enlargement (Gillapsy et al., 1993). As might be expected, genes that were highly expressed in young developing tomato fruit locules and mesocarp included those with predicted functions in fruit growth, fruit protection, photosynthesis, and cell expansion (Lemaire-Chamley et al., 2005). In the case of apple, genes with functions associated with control of cellular organization, cell cycles, photosynthesis, protein synthesis had higher expression in early fruit (Janssen et al., 2008; Lee et al., 2007). Many genes had similar patterns of expression in tomato and apple, suggesting that with more increased EST data from other species it will allow better comparison and identifying common fundamental processes in fruit development (Janssen et al., 2008). Cucumber fruit development and gene expression was explored in chapter 4.

Objectives of dissertation

There are numerous difficulties limiting establishment of effective methods to control *P. capsici* in cucumber production as described above. In this dissertation, my first objective was to examine alternative cucumber architecture as a means to control *P. capsici* occurrence in cucumber production. Nearly-isogenic lines differing for determinate growth habit and leaf size were tested for disease occurrence. In addition, a sample of the cucumber germplasm, including the cucumber core collection and additional PIs that have been annotated in the NPGS database for possible short internodes or bush growing habit, were screened for additional architectural variation. Promising variants were then tested for disease occurrence in infested field plots.

I also performed additional screening for genetic sources of resistance to *P. capsici*. During screening for resistance, fruit age/size appeared to be related to susceptibility. Therefore, I examined the effect of fruit age on susceptibility to *P. capsici* in greenhouse- and field-grown cucumber and other cucurbit crops including melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), butternut squash (*Cucurbita mocshata*), and four *Cucurbita pepo* crops, zucchini, yellow summer squash, acorn squash, and pumpkin. I also examined possible roles of the cucumber fruit surface properties in age related resistance.

Lastly, to understand the basis of the age-related resistance in cucumber fruit-*P. capsici* interaction, and to increase understanding of early fruit development in cucumber, I performed morphological and global gene expression analyses. Hand-pollinated greenhouse grown cucumber fruit ranging 0-32 DPP were used to catalogue morphological changes associated with cucumber fruit development and to develop cDNA libraries for 454 pyrosequencing. Rapidly expanding 8 DPP fruit were characterized for gene expression.

Literature Cited

Abedon, B. G. and W. F. Tracy. 1996. *Congrass1* of maize (*Zea mays* L.) delays development of adult plant resistance to common rust (*Puccinia sorghi* Schw.) and European corn borer (*Ostinia nubilalis* Hubner). J. Heredity. 87:219-223.

Agrios, G. N. 1997. Plant Pathology, 4th Ed. Academic Press. London, UK.

Ando, K., R. Grumet, K. Terpstra, and J.D. Kelly. The manipulation of plant architecture to enhance crop disease control. 2007. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2:1-8.

Babadoost, M. 2004. Phytophthora blight: a serious threat to cucurbit industries. APSnet feature story Apri-May. Online publication. American Phytopathological Society, St. Paul, MN.

Biles, C. L., M. M. Wall, M. Waugh, and H. Palmer. 1993. Relationship of *Phytophthora* fruit rot to fruit maturation and cuticle thickness of New Mexican type peppers. Phytopathology. 83:607-611.

Bircher, U. and H. R. Hohl. 1997. Environmental signaling during induction of appressorium formation in *Phytophthora*. Mycol. Res. 101:395-402.

Blad, B. L., J. R. Steadman, and A. Weiss. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. Phytopathology. 68:1431-1437.

Carrari, F. and A. R. Fernie. 2006. Metabolic regulation underlying tomato fruit development. J. Exp. Bot. 57:1883-1897.

Century, K. S., R. A. Lagman, M. Adkisson, J. Morlan, R. Tobias, K. Schwartz, A. Smith, J. Love, P. C. Ronald, and M. C. Whalen. 1999. Developmental control of *Xa21*-mediated disease resistance in rice. Plant J. 20:231-236.

Colucci, S. J., T. C. Wehner, and G. J. Holmes. 2006. The downy mildew epidemic of 2004 and 2005 in the Eastern United States. Cucurbitaceae 2006. G. J. Holmes (ed).

Coyne, D. P., J. R. Steadman, and F. N. Anderson. 1974. Effect of modified plant architecture of great northern dry bean varieties (*Phaseolus vulgaris*) on white mold severity, and components of yield. Plant Dis. Rept. 58:379-382.

De Wolf, E.D., R. J. Effertz, S. Ali, and L. J. Francl. 1998. Vistas of tan spot research. Can. J. Plant Pathol. 20:349-70.

Develey-Rivière, M. P. and E. Galiana. 2007. Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytologist. 175:405-416.

Droege, M. and B. Hill. 2008. The genome sequencer FLX™ system – longer reads, more applications, straight forward bioinformatics and more complete data sets. J. Biotech. 31:136:3-10.

Erwin, D. C. and O. K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.

Eveland, A. L., D. R. McCarty, and K. E. Koch. 2008. Transcript profiling by 3'-untranslated region sequencing resolves expression of gene families. Plant Physiol. 146:32-44.

Fernandez MR. Clarke JM. DePauw RM. 2002. The effect of plant height on tan spot durum wheat in southern Saskatchewan. Crop Sci. 42:159-164.

Ficke, A., D. M. Gadoury, and R. C. Seem. 2002. Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytopathology. 92:671-675.

Fuller, P. A., J. R. Steadman, and D. P. Coyne. 1984. Enhancement of white mold avoidance and yield in dry beans by canopy elevation. HortScience 19:78-79.

Gadoury, D. M., R. C. Seem, A. Ficke, and W. F. Wilcox. 2003. Ontogenic resistance to powdery mildew in grape berries. Phytopathology. 93:547-555.

Gevens, A. J., K. Ando, K. Lamour, R. Grumet, and M. K. Hausbeck. 2006. Development of a detached cucumber fruit assay to screen for resistance and effect of fruit age on susceptibility to infection by *Phytophthora capsici*. Plant Dis. 90:1276-1282.

Gillaspy, G, H. Ben-David, and W. Gruissem. 1993. Fruits: a developmental perspective. Plant Cell. 5:1439–1451.

Giovannoni, J. J. 2004. Genetic regulation of fruit development and ripening. Plant Cell. 16:S170-180.

Görnhardt, B., I. Rouhara, and E. Schmelzer. 2000. Cyst germination proteins of the potato pathogen *Phytophthora infestans* share homology with human mucins. Mol. Plant Microbe Interact. 13:32-42.

Grenville-Briggs, L. J., V. L. Anderson, J. Fugelstad, A. O. Avrova, J. Bouzenzana, A. Williams, S. Wawra, S. C. Whisson, P.R. J. Birch, V. Bulone, and P. van West. 2008. Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato. Plant Cell 20:720-738.

Halterlein, A. J., G. L. Sciumbato, and W. L. Barrentine. 1981. Use of plant desiccants to control cucumber fruit rot. HortScience 16:189-190.

Hanna, H. Y., A. J. Adams, and R. N. Story. 1987. Increased yield in slicing cucumbers with vertical training of plants and reduced plant spacing. HortScience 22:32-34.

Hardham, A. R. 2007. Cell biology of plant-oomycete interactions. Cellular Microbiol. 9:31-39.

Harnish, W. N. 1965. Effect of light on production of oospores and sporangia in species of *Phytophthora*. Mycologia 57:85-90.

Hausbeck, M., R. Goldy, D. Fulbright, and R. Hammerschmidt. 2002. An integrated appraoch to manage phytophthora blight on Michigan's vine crops. GREEEN Project #: GR99-020. http://www.greeen.msu.edu/july02final/gr99020final.pdf.

Hausbeck, M. and K. Lamour. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Dis. 88:1292-1302.

Hilton, A.J., P. Jenkinson, T. W. Hollins, D. W. Parry. 1999. Relationship between cultivar height and severity of *Fusarium* ear blight in wheat. Plant Pathology 48:202-208.

Huang, S., Y. Du, X. Wang, X. Gu, B. Xie, Z. Zhang, J. Wang, R. Li, S. Li, Y. Ren, J. Wang, H. Yang, W. Jin, Z. Fei, A. Kilian, J. E. Staub, E. van der Vossen, and G. Li. 2009. Recent development of the cucumber genome initiative – an international effort to unlock the genetic potential of an orphan crop using novel genomic technology. PAGXVII. http://www.intlpag.org/17/abstracts/W18_PAGXVII_135.html.

Hugot, K., S. Aime, S. Conrod, A. Poupet, and E. Galiana. 1999. Developmetal regulated mechanisms affect the ability of a fungal pathogen to infect and colonize tobacco leaves. Plant J. 20:163-170.

Janssen, B. J., K. Thodey, R. J. Schaffer, R. Alba, L. Balakrishnan, R. Bishop, J. H. Bowen, R. N. Crowhurst, A. P. Gleave, S. Ledger, S. McArtney, F. B. Pichler, K. C. Snowden, and S. Ward. 2008. Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. BMC Plant Biol. 8:16.

Judelson, H. S., and Blanco, F. A. 2005. The spores of Phytophthora: weapons of the plant destroyer. Nature Reviews Microbiol. 3:47-58.

Katsura, K., and S. Miyazaki. 1960. Leaf penetration by Phytophthora capsici Leonian. Sci. Rep. Kyoto Prefect. Univ. Agric. 12:65-70.

Kennelly, M. M., D. M. Gadoury, W. F. Wilcox, P. A. Magarey, and R. C. Seem. 2005. Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. Phytopathology. 95:1445-1452.

Kim, Y. J., B. K. Hwang, and K. W. Park. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. Plant Dis. 73:745-747.

Knerr, L. D., J. E Staub, D. J. Holder, and B. P. May. 1989. Genetic diversity in *Cucumis sativus* L. assessed by variation at 18 allozyme coding loci. Theor. Appl. Genet. 78:119-128.

Koch, M. F. and T. W. Mew. 1991. Effect of plant age and leaf maturity on the quantitative resistance of rice cultivars to *Xanthomonas campestris* pv. *oryzae*. Plant Dis. 75:901-904.

Konsler, T. R. and D. L. Strider. 1973. The response of cucumber to trellis vs. ground culture. HortScience 8:220-221.

Kolkman, J. M., and J. D. Kelly. 2002. Agronomic traits affecting resistance to white mold in common bean. Crop Sci. 42:693-699.

Kus, J. V., Z. Zaton, R. Sarkar, and R. K. Cameron. 2002. Age-related resistance in Arabidopsis is a developmentally regulated defense response to *Psudomonas syrinagae*. Plant Cell. 14:479-490.

Kreutzer, W.A. 1937. A Phytophthora rot of cucumber fruit. Phytopathology 27:955.

Latijnjouwer, M., P. J. G. M. de Wit, and F. Govers. 2003. Oomycetes and fungi: similar weaponry to attack plants. Trends in Microbiol. 11:462-469.

Lazarovits, G., P. Stossel, and E. W. B. Ward. 1980. Age-related changes in specificity and glyceollin production in the hypocotyls reaction of soybeans to *Phytophthora megasperma* var. *sojae*. Phytopahtology. 71:94-97.

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.

Lamour, K. H. and M. K. Hausbeck. 2001a. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology. 91:973-980.

Lamour, K. H. and M. K. Hausbeck. 2001b. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology. 91:553-557.

Lamour, K. H. and M. K. Hausbeck. 2002. The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. Phytopathology. 92:681-684.

Lamour, K.H. and M. K. Hausbeck.2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Dis. 87:841-845.

Lee, Y. P., G. H. Yu, Y. S. Seo, S. E. Han, Y. O. Choi, D. Kim, I. G. Mok, W. T. Kim, and S. K. Sung. 2007. Microarray analysis of apple gene expression engaged in early fruit development. Plant Cell Rep. 26:917-926.

Leisner, S. M., R. Turgeon, and S. H. Howell. 1993. Effects of host plant development and genetic determinants on the long-distance movement of cauliflower mosaic virus in Arabidopsis. Plant Cell. 5:191-202.

Lemaire-Chamley, M., J. Petit, V. Garcia, D. Just, P. Baldet, V. Germain, M. Fagard, M. Mouassite, C. Cheniclet, and C. Rothan. 2005. Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. Plant Physiol. 139:750-769.

Leonin, L.H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. Phytophathology. 8:401-408.

Li, B. and X. Xu. 2002. Infection and development of apple scab (*Venturia inaequalis*) on old leaves. J. Phytopathol. 150:687-691.

Mardis, E. R. 2008. The impact of next-generation sequencing technology on genetics Trends in Genetics. 24:133-141.

Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bernben, J. Berka, M. S. Braverman, Z. Chen, S. B. Dewell et al. 2005. Genome sequencing in microfabricated high-density picrolite reactors. Nature. 437: 376-380.

Mazzola, M., J. E. Leach, R. Nelson, and F. F. White. 1994. Analysis of the interaction between *Xanthomonas oryzae* pv. *oryzae* and the rice cultivars IR24 and IRBB21. Phytopahtology. 84:392-397.

Mesterhazy, A. 1995 Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding. 114:377-86.

McDowell, J. M., S. G. Williams, N. T. Funderburg, T. Eulgem, and J. L. Dangl. 2005. Genetic analysis of developmentally regulated resistance to downy mildew (*Hyaloperonospora parasitica*) in *Arabidopsis thaliana*. Mol. Plant-Microbe Interactions. 18:1226-1234.

Miller, C. H. and T. C. Wehner. 1989. Cucumbers. pp. 245-264. In: Quality and preservation of vegetables, N. A. M. Eskin (ed.). CRC Press, Inc., Boca Raton, Fl.

Moore, S., J. Vrebalov, P. Payton, and J. Giovannoni. 2002. Use of genomics tools to isolate key ripening genes and analyze fruit maturation in tomato. J. Exp. Bot. 53:2023-2030.

Morris, B. M., and N. A. R. Gow. 1993. Mechanism of electrotaxis of zoospores of phytopathogenic fungi. Phytopathology. 83:877-882.

Mosa, A. A., K. Kobayashi, A. Ogoshi, M. Kato, and N. Sato. 1991. Formation of oospores by *Phytophthora infestans* in inoculated potato tissues. Ann. Phytopatho. Soc. Japan. 57:334-338.

Ngouajio, M., G. Wang, and M. K. Hausbeck. 2006. Changes in pickling cucumber yield and economic value in response to planting density. Crop Sci. 46:1570-1575.

O'Connell, R. J., and R. Panstruga. 2006. Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytologist. 171:699-718.

Ohtsu, K., M. B Smith, S. J. Emrich, L. A. Borsuk, R. Zhou, T. Chen, X. Zhang, M. C. P. Timmermans, J. Beck, B. Buckner, D. Janick-Buckner, D. Nettleton, M. J. Scanlon, and P. S. Schnable. 2007. Global gene expression analysis of the shoot apical meristem of maize (*Zea mays* L.). Plant J. 52:91-404.

Panter, S. N. and D. A. Jones. 2002. Age-related resistance to plant pathogens. Adv. Botanical Res. 38:251-280.

Park, S. J. 1993. Response of bush and upright plant type selections to white mold and seed yield of common beans grown in various row widths in southern Ontario. Can. J. Plant Sci. 73:265-272.

Pierce, L. K. and T. C. Wehner. 1990. Review of genes and linkage groups in cucumber. HortScience 25:605-615.

Reid, B., B. M. Morris, and N. A. R. Gow. 1995. Calcium-dependent, genus-specific, autoaggregation of zoospores of phytopaghogenic fungi. Exp. Mycol. 19:202-213.

Ristaino, J. B., G. Parra, and C. L. Campbell. 1997. Suppression of *Phytophthora* blight in bell pepper by a no-till wheat cover crop. Phytopathology. 87:242-249.

Russo, V. M., B. W. Roberts, and R. J. Schatzer. 1991. Feasibility of trellised cucumber production. HortScience. 26:1156-1158.

Schwartz, H. F, J. R. Steadman, and D. P. Coyne. 1987. Influence of *Phaseolus vulgarus* blossoming characteristics and canopy structure upon reaction to *Sclerotinia sclerotiorum*. Phytopathology. 68:465-70.

Serce, S., J. P. Navazio, A. F. Gokce, and J. E. Staub. 1999. Nearly isogenic cucumber genotypes differing in leaf size and plant habit exhibit differential response to water stress. J. Amer. Soc. Hort. Sci. 124:358-365.

Steadman, J. R., D. P. Coyne, and G. E. Cook. 1973. Reduction of severity of white mold disease on great northern bean by wider row spacing and determinate plant growth habit. Plant Dis. Rep. 57:1070-1071.

Torres, T. T., M. Metta, B. Ottenwälder, and C. Schlöttere. 2008. Gene expression profiling by massively parallel sequencing. Genome Research. 1-6.

Tyler, B. M. 2002. Molecular basis of recognition between *Phytophthora* pathogens and their hosts. Annu. Rev. Phytopathol. 40:137-167.

Ullah, Z. and R. Grumet. 2002. Localization of zucchini yellow mosaic virus to the veinal regions and role of viral coat protein in veinal chlorosis conditioned by the *zym* potyvirus resistance locus in cucumber. Physiol. Mol. Plant Pathol. 60:79-89.

Wai, T. and R. Grumet. 1995. Inheritance of watermelon mosaic virus resistance in the cucumber line TMG-1: tissue-specific expression and relationship to zucchini yellow mosaic virus resistance. Theor. Appl. Genet. 91:699-706.

Wang, G. and M. Ngouajio. 2008. Integration of cover crop, conservation tillage, and low herbicide rate for machine-harvested pickling cucumbers. HortScience. 43:1770-1774.

Whallen, M. C. 2005. Host defence in a developmental context. Mol. Plant Pathol. 6:347-360.

White, P. J. 2002. Recent advances in fruit development and ripening: an overviews. J. Exp. Bot. 53:1995-2000.

Chapter 2

Ando, K. and R. Grumet. 2006. Evaluation of altered cucumber plant architecture as a means to reduce *Phytophthora capsici* disease incidence on cucumber fruit. J. Amer. Soc. Hort. Sci. 131:491-498.

Evaluation of altered cucumber plant architecture as an approach to reduce *Phytophthora* fruit rot

Abstract

Fruit rot induced by *Phytophthora capsici* Leonian is an increasingly serious disease affecting pickling cucumber (*Cucumis sativus* L.) production in many parts of the United States. The absence of genetically resistant cultivars and rapid development of fungicide resistance makes it imperative to develop integrated disease management strategies. Cucumber fruit which come in direct contact with the soil-borne pathogen are usually located under the canopy where moist and warm conditions favor disease development. We sought to examine whether variations in plant architecture traits that influence canopy structure or fruit contact with the soil could make conditions less favorable for disease development. As an extreme test for whether an altered canopy could facilitate *P capsici* control, we tested the effect of increased row spacing and trellis culture on disease occurrence in the pickling cucumber 'Vlaspik'. Temperature under the canopy was lowest in trellis plots, intermediate in increased spacing plots, and highest in control plots. Disease occurrence in the trellis plots was significantly lower than in other treatments, indicating that preventing fruit contact with the soil reduced disease occurrence. The effect of currently available variation in plant architecture was tested using nearly-isogenic genotypes varying for indeterminate (*De*), determinate (*de*), standard leaf (*LL*), and little leaf (*ll*) traits. Plants with standard architecture had higher peak mid-day temperatures under the canopy and greater levels of *P. capsici* infection; however, levels of disease occurrence were high for all genotypes. Screening a collection of approximately to 150 diverse cucumber accessions identified to serve as a

representative sample of the germplasm, revealed variation for an array of architectural traits including main stem length, internode length, leaf length and width, and number of branches; values for 'Vlaspik' were in the middle of the distribution. Plant architectures that may allow for more open canopies, including reduced branching habit and compact growth, were tested for disease incidence. One of the compact lines (PI 308916), which had a tendency to hold young fruit off the ground, exhibited lower disease occurrence. The reduced disease occurrence was not due to genetic resistance, suggesting that architecture which allows less contact of fruit with the soil could be useful for *P capsici* control for pickling cucumber.

Introduction

Fruit rot caused by the Oomycete pathogen, *Phytophthora capsici* is currently one of the most serious diseases affecting cucumber (*Cucumis sativus* L.) production in many parts of the United States (Hausbeck and Lamour, 2004). In Michigan, the top pickling cucumber producing state, pickling cucumber acreage has increased over the past five years (Michigan Department of Agriculture, 2001), however, maintaining and increasing yield has become difficult due to losses caused by *P. capsici.*

P. capsici was first reported by Leonian (1922) as a host specific pathogen of chili pepper (*Capsicum annum* L.) occurring in New Mexico. Later, it was found to infect a wide variety of hosts ranging from woody plants and vegetable hosts (Erwin and Ribeiro, 1996). Although incidence of cucumber infection was first reported in Colorado in 1936 (Kreutzer, 1937), it has only recently become a common problem in cucumber production

areas (Babadoost, 2004; Hausbeck and Lamour, 2004). Once cucumber fields are infected by *P. capsici*, severe yield loss due to infection of the fruit is frequently observed (Babadoost, 2004; Hausbeck and Lamour, 2004). Cucumber fruit infected by *P. capsici* typically develop a depressed fruit surface with a water soaked appearance followed by white powdery mycelium covering the affected region.

Optimal growth conditions for *P. capsici* include a warm and wet environment, particularly around 25 to 30 °C (Hausbeck and Lamour, 2004). *P. capsici* sporangia have a recognizable lemon shape and produce asexual motile zoospores which are the primary cause of infection during the growing season, since they can be spread by irrigation, rain, and free surface water on plants (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004). In addition, *P. capsici* produces a unique thick walled structure called an oospore, which enables it to overwinter. Since oospores are able to survive in the soil for more than five years, crop rotation becomes impractical, contributing to the difficulty in controlling this disease (Lamour and Hausbeck, 2001a, 2002, 2003).

Heavy dependence on chemical control has resulted in the development of resistance, as has been identified with respect to mefenoxam (Ridomil Gold; Novartis, Greensboro, NC), a fungicide commonly used in Michigan (Lamour and Hausbeck, 2000, 2001a, 2001b). In addition, since many farms do not practice rotation with non-susceptible crops, spore populations can increase, and sexual recombination among mating types can allow for transfer of resistance genes (Lamour and Hausbeck, 2000, 2001a, 2001b, 2002). Gevens et al. (2006) screened 482 *C. sativus* accessions for fruit resistance, but no significant source of resistance has been identified to date. Consequently these conditions make it necessary to seek alternative control strategies.

Field observations show that cucumber fruits are susceptible to *P. capsici*, while roots or crowns are much less susceptible. Fields will frequently appear healthy at the time of harvest as judged by vegetative growth, but the fruits, which are usually located under the canopy, can be heavily diseased (Hausbeck and Lamour, 2004). The environment under the canopy is moist and warm, and the fruits are in close proximity to the soil-borne pathogen, thus favoring conditions for disease development. Also, the dense canopy covering the fruit prevents fungicides from reaching them, making control more difficult.

These observations suggested that it might be possible to reduce the problem by reducing canopy density (i.e., temperature, or humidity) by altering cultural conditions such as spacing or trellising, or with altered plant architecture. Cucumber disease control by trellis culture has been studied for *Rhizoctonia solani* Kuhn (Konsler and Strider, 1973; Hanna et al., 1987). Studies showed that trellised cucumber had less *R. solani* occurrence presumably due to higher air circulation, better fungicide distribution, and reduced contact with the soil (Hanna et al., 1987; Konsler and Strider, 1973). However, trellising is expensive and labor intensive, making it unsuitable for commercial pickling cucumber production (Russo et al., 1991).

The effect of reducing cucumber canopy density on infection by *R. solani* also was tested by herbicide treatment applied after initial flowering (Halterlein et al., 1981). The herbicide treated plots exhibited a significant reduction in disease occurrence, leading the authors to conclude that the microclimate under the reduced canopy contributed to disease reduction by allowing for better spray distribution, less humidity and increased light penetration.

Altered canopy structure may also be achieved by modified plant architecture. This strategy for disease control has been utilized in bean (*Phaseolus vulgaris* L.) breeding (Coyne, 1980). White mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] is a common disease affecting dry bean production. To facilitate white mold control, bean breeders have selected for elevated canopy of a prostrate indeterminate bean, upright canopy of indeterminate bean, and open, porous canopy from determinate bean (Blad et al., 1978; Coyne et al., 1974; Fuller et al., 1984; Kolkman and Kelly, 2002; Park, 1993). With the combination of altered plant architecture and improved cultural practices, bean breeders were able to improve disease control and increase yield.

Fortunately, a variety of growth habits exists within the cucumber germplasm (Pierce and Wehner, 1990). Some genotypes produce shorter vines, others have smaller leaves, or various fruit set positions. Determinate plants possessing the *de* gene, have shorter vines, shorter internode length, and less lateral branches (George, 1970; Grumet and Duvall, 1993; Wehner, 1989). The little-leaf trait, incorporated into breeding line H-19, is controlled by a single-gene mutantion (*ll*) and is characterized by small leaves, a highly branched growth habit with shorter vines and smaller stems (Goode et al., 1980; Schultheis et al., 1998). The compact trait, an extreme dwarf plant type, is controlled by the homozygous recessive gene *cp*, which has short internodes, poorly developed tendrils, and small flowers (Kauffman and Lower, 1976).

Four nearly isogenic pickling cucumber lines were developed by Serce et al. (1999) that differ for vine development (indeterminate and determinate), branching habit, and leaf size (standard and little-leaf). These nearly isogenic lines, which minimize other genetic influences, can be used to test effects of plant architecture. Serce et al. (1999)

tested the response of these lines to water stress; however, they have not been tested for effect on disease development. The growth habit of these cucumbers might increase air circulation, light penetration, or accessibility of the applied fungicide to fruit under the canopy.

In addition to testing existing architectural variants, identification of new plant architecture also could be valuable for influencing disease development. There are 1,352 plant introductions (PIs) in the collection of National Genebank, the U.S. National Plant Germplasm System (NPGS). Knerr et al. (1989) identified a group of 100 PIs which serve as a representative sample of the germplasm with maximum genetic variance based on geographical distribution and isozyme data.

In this study, we examined the possibility of an architectural approach to minimize *P. capsici* occurrence in pickling cucumber production. Three types of studies were performed. The first study was the evaluation of the effectiveness of trellis culture on controlling *P. capsici,* as an extreme test of the hypothesis that altered plant architecture may facilitate control of *P. capsici,* since the intended method of disease control by altered canopy structure (increased air movement, reduced humidity, or reduced fruit contact with the soil) is similar to that for trellis culture. The second study utilized the nearly isogenic lines differing for architectural traits of determinate growth habit and leaf size developed by Serce et al. (1999). The third study screened a sample of the cucumber germplasm for additional architectural variants using the collection classified by Knerr et al. (1989), and an additional 50 PIs that have been annotated in the NPGS database for possible short internodes or bush growing habit.

Materials and Methods

Trellis study

The trellis study was performed on a *P. capsici*-infested field at the Michigan State University (MSU) Muck Farm (Bath, MI) during the summer of 2003 (planting date, May 30, 2003). The commercial pickling cucumber cultivar 'Vlaspik' (Seminis Vegetable Seed Inc., Oxnard, CA) was used for all treatments and borders. Three treatments, standard spacing (0.5 m between rows), wide spacing (1.5m between rows), and trellis (1.5m between rows), were arranged in a randomized complete block design with four replications. All plots were 4.6 m wide and in-row spacing was 0.1 m. The standard spacing plots consisted of ten, 3 m-long rows, the wide spaced and trellis plots had three, 3 m-long rows. Fruits were taken from center 2.4 m x 1.2 m of the standard spacing plot and wide spacing plots, and center 2.4 m of the middle row of the trellis plot.

The trellis plots were constructed with 1.5 m high stakes placed at 1.2 m intervals within the rows. Plastic mesh was attached to braided nylon ropes which were placed parallel to the row at the top and bottom of the stakes. Cucumbers were trained to the plastic mesh with support of twine or their own tendrils. Irrigation was provided by rain or overhead sprinkler to 25 mm a week. Prior to harvest, irrigation was increased to 75-100 mm a week to stimulate infection.

Temperature under the canopy was taken every 15 minutes using WatchDog data loggers (Spectrum Technologies Inc., Plainfield, IL) from the time of first female flower bloom until second harvest for three replications. The data loggers were placed within the canopy at positions where fruits were located. The temperature data was analyzed by analysis of variance (ANOVA) using SAS proc mixed procedures (SAS Institure Inc.,

Cary, NC).

Fruits were harvested four times. Because disease frequently becomes more apparent during storage, the fruit was inspected for disease at the time of harvest and then re-evaluated after four days of storage in plastic crates in the barn. Yield and disease occurrence were analyzed by ANOVA using SAS proc mixed procedures. Samples of infected fruit were collected from each harvest to verify presence of *P. capsici* according to the method of Lamour and Hausbeck (2003). Briefly, infected fruits were sanitized with 70 % ethanol and dissected to remove 0.25 cm^3 pieces from the margin of the infected area. The epidermis was removed, pericarp pieces were placed on BARP medium (Lamour and Hausbeck, 2003), and incubated at room temperature under the light for 3 – 4 days until sporulation was observed. Fungal samples were observed by microscope for the presence of distinctive lemon shaped sporangia.

Architectural study with nearly-isogenic lines.

The architectural study was planted at the field described above on June 27, 2003, using a set of four nearly-isogenic pickling cucumber lines developed by Serce *et al.* (1999) to vary for architectural features: 1) indeterminate and standard leaf (*DeDeLL*), 2) determinate and standard leaf (*dedeLL*), 3) indeterminate and little leaf (*DeDell*), and 4) determinate and little leaf (*dedell*). Five additional lines were included: H19 (*ll*) (standard little leaf type - H19 was used as the parent to introduce the little leaf trait into the nearly isogenic lines described above); compact (*cpcp*) (J. E. Staub, personal communication); Marketmore 76 and Marketmore 86 (nearly-isogenic indeterminate (76) and determinate (86) cultivars); and Vlaspik (standard commercial cultivar, indeterminate and standard leaf).

The genotypes were planted in 1.8 m x 3.0 m plots with 0.5 m row spacing with four plants per 30 cm to approximate local commercial conditions. Each plot was paired with the standard commercial cultivar, 'Vlaspik', in order to minimize effect of potential variation in disease pressure throughout the field. The pairs of plots were planted in a randomized complete block design with four replications. Canopy data collection, harvest, disease analysis, and *P. capsici* verification were as described above.

Screening for architectural variants

The architectural screening experiment was performed in a non-replicated trial on non-*P. capsici* infested soil at the MSU Horticulture Research and Teaching Center (East Lansing, MI) during the summer of 2003 (planting date, May 20, 2003). Cucumber accessions were supplied by the North Central Regional Plant Introduction Station, Ames, Iowa. The PIs were sown in the greenhouse and transplanted to the field at 14 days. Irrigation was provided by rain or overhead sprinkler to 2.5 cm a week.

To allow for observation of architecture, plants were spaced 0.5 m apart in 3.7 m single-row long plots with 1.5 m between rows. One 'Vlaspik' plant was put at the beginning and end of each plot to separate plots. Each row included one plot of 'Vlaspik' to allow for comparison of plant architecture. All plots were visually observed weekly and plant architecture data was collected at approximately 60 days after planting for the PIs which showed obvious visual differences from 'Vlaspik'. Measurements were taken from the middle three plants from each plot. Traits evaluated were: main stem and internode length, number and position of branches, leaf size, growth habit, and fruit position relative to the soil. Main stem length was measured from the soil line to the apical bud. Internode length was measured for node positions 5 to 6 and 8 to 9 from the

base of the plant. Number of branches were counted for the main the main stem. Leaf width and length were measured for fully mature leaves at node positions 5 and 8. Correlations between traits were analyzed by SAS proc reg procedures.

Architecture study with selected PIs and reduced vine commercial lines

Several lines were selected for further evaluation based on architectural variation; these included: PI 192940 (China), 227207 (Japan), 308915 (Russia), 308916 (Russia), 358814 (Malaysia), 401734 (Puerto Rico), and 466921 (Russia). Seeds for these genotypes were multiplied in the greenhouse during spring of 2004. The above listed PIs and four additional commercial cultivars described as having short vines, 'Arabian,' 'Colt,' 'Palomino,' and 'Stallion,' were tested for their architectural effect on *P. capsici* occurrence in the summer of 2004 on infested soil at the Muck Farm as described above (planting date, June 21, 2004). 'Vlaspik' was used as control. Experimental design and data collection were performed as described above for the nearly-isogenic lines trial, except individual rather than paired plots were used, as the 2003 trial did not indicate obvious non-uniformity in disease presence in the field (data not shown). Architectural data were collected as described above.

The fruits from tested PIs and 'Vlaspik' (control) were evaluated for *P. capsici* resistance. Fruits were washed, air-dried, then inoculated with 10 day old mycelium of *P. capsici* isolate OP97 (Gevens et al., 2006) and incubated at room temperature. Initial fruit examination was made at 4 days post inoculation (DPI). Fruits were examined for presence of water soaking symptoms (visible dark discoloration on fruit surface) or sporulation (powdery mycelium on fruit surface).

Results

Trellis study

Canopy development varied among treatments in the trellis experiment. The narrow-spaced plots achieved full canopy closure one week prior to the first harvest (approximately 8 weeks post-planting). The wide-spaced plots continued to expand throughout the season, but did not fully close even at the end of the experiment. However, at the positions where the fruits were located, the canopy density above the fruit appeared to be similar for the narrow-paced and the wide-spaced plots.

The temperature under the canopy at positions where fruit were located was recorded at 15 minute intervals for approximately three weeks spanning the first and second harvest dates. The daily maximum temperature at fruit positions under the canopy was similar for the narrow-spaced and wide-spaced plots, while the trellis plots had a significantly lower temperature (Table 2-1). Minimum temperatures among the treatments followed the same trend. There was a progressive decrease in the average heat units above 25 °C moving from the narrow-spaced plot, to the wide-spaced plot, and the trellis plots.

As might be expected due to the greater number of rows, yields at the first two harvest dates were highest in the narrow-spaced plots (Figure 2-1A). Later in the season (harvests 3 and 4) as the vines increased in size, yield of the wide-spaced and trellis plots increased to match the narrow-spaced plots. Almost no fruit had obvious symptoms (sporulation) of *P. capsici* at the time of harvest on the first and second harvest dates; however, at the third and fourth harvest dates, a small portion of fruits from the narrow and wide plots showed infection at the time of harvest (Figure 2-1B). The numbers of

Table 2-1. Temperature under the canopy at cucumber fruit positions and cucumber fruit weight, number of fruit, and disease occurrence for narrow, wide, and trellis plots at harvest [0 day post harvest (DPH)] and 4 DPH.

Treatment	Mean max Temperature[z]	Mean min temperature[z]	Mean heat unit (>25°C)[z]	Fruit wt (kg/plot)[y]	Fruit (no./plot)	Disease occurrence (%) 0 DPH[y]	4DPH[y]
Narrow	29.8b[x]	14.8b	70.1	19.9b	65.8b	1.2a	13.4b
Wide	29.1b	14.3b	58.2	12.6a	28.6a	1.3a	10.0b
Trellis	27.4a	13.1a	27.2	12.8a	36.0a	0.1a	1.4a
$LSD_{0.05}$	1.6	0.7		4.1	10.4		6.6

[z]Temperature data were taken from 16 July to 2 Aug. 2003. Each data point is the mean of three replications.

[y]Yield and disease data are averaged over 4 harvests and 4 replicate plots per treatment.

[x]Values followed by the same letter are not significantly different at $P \leq 0.05$ (LSD)

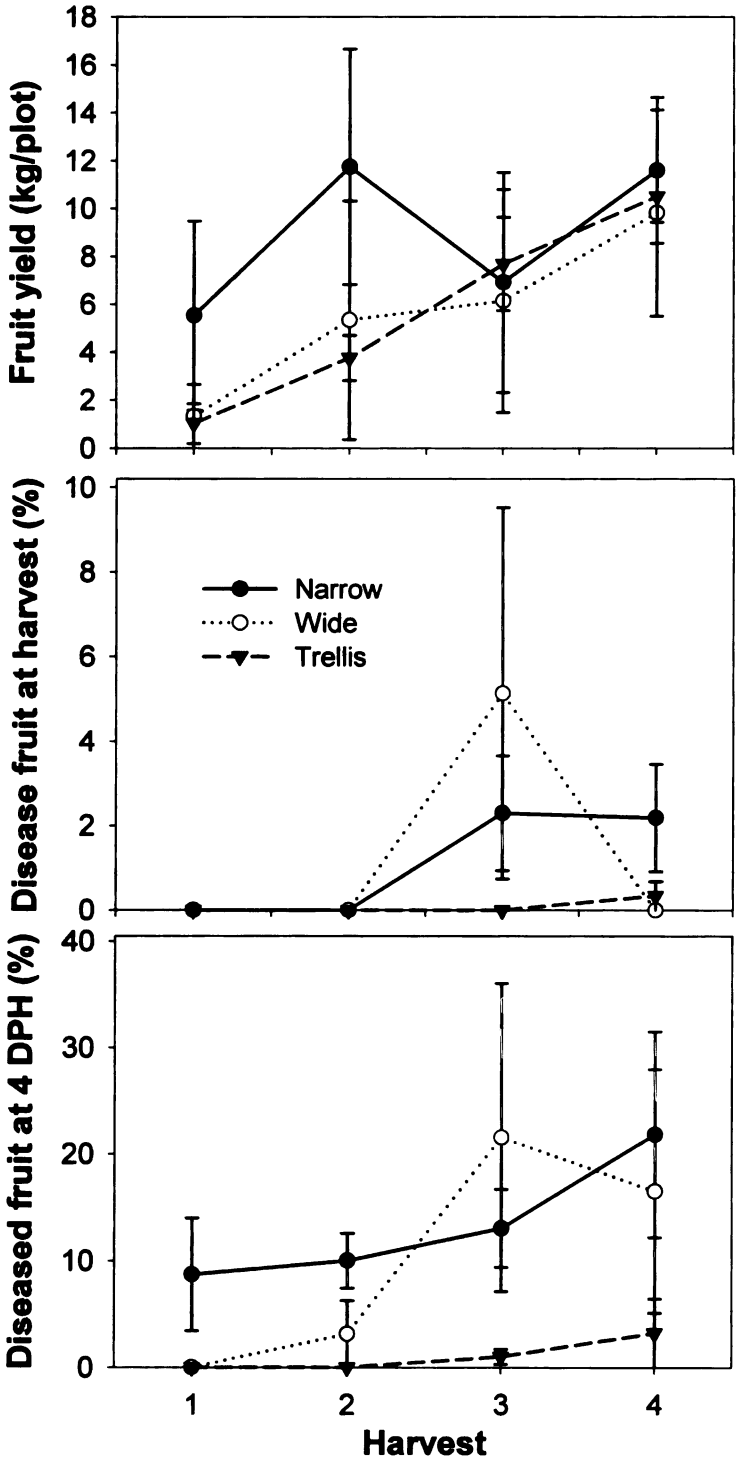


Figure 2-1. Mean cucumber fruit yield per aplot (A) and percent disease occurrence at harvest (B) and 4 days post harvest (DPH) (C) at four harvest dates for standard, narrow, and trellis plots. Each point is the mean of four replicate plots ± SE.

infected fruit increased when observed 4 days post harvest (DPH) (Figure 2-1C). Since obviously infected fruits (i.e. sporulated) were removed prior to storage, the increased percentages should reflect disease development of fruits that had been initially infected in the field. In the narrow plots, disease occurrence at 4 DPH increased as the season progressed (Figure 2-1B, C). Also, disease occurrence at 4 DPH in the wide plots increased in synchrony with expansion of the canopy (Figure 2-1C). Although total yield summed over the four harvest dates was significantly higher for the narrow than the wide plots, percent disease occurrence averaged over the four harvests was not significantly different (Table 2-1).

Disease occurrence in the trellis plots remained extremely low throughout the season, and total disease occurrence at 4 DPH in the trellis plots was only 1.4 % (Figure 2-1C, Table 2-1). Even in the third and fourth harvests, when all plots were producing similar amount of fruit (Figure 2-1A), disease incidence in the trellis plots was significantly lower (the percent infection averaged over the 3rd and 4th harvests at 4DPH was 15.2 %, 16.5 %, and 1.9 % respectively for narrow, wide spacing, and trellis plots) (Figure 2-1B,C).

Architectural study with nearly isogenic lines

There were visible differences in canopy structure and the time of canopy filling among the genotypes tested in the architectural study with near-isogenic lines differing for leaf size and determinacy. Despite smaller leaves, the *ll* genotypes (H19, *DeDell*, and *dedell*) appeared to have very dense canopy, whereas *de* genotypes generally developed a more open canopy based on visual observation. All fruits from the tested genotypes were located on the ground. The genotypes with standard commercial architecture

(indeterminate growth habit and standard leaf size, *DeDeLL*, 'Marketmore 76', and 'Vlaspik'), tended to maintain higher temperature under the canopy than those with nonstandard architecture (*dedeLL*, *DeDell*, *dedell*, 'Marketmore 86', H19 and compact). Mean peak mid-day temperatures were significantly higher for the standard types (30.9 °C) than the *de, ll,* and *cp* (26.5 °C) types (LSD = 1.4, $P \leq 0.05$), suggesting that there was an effect of the modified architecture on canopy conditions.

'Vlaspik' had significantly higher yield at the first harvest; however, the other architectural types became comparable to 'Vlaspik' at later harvests (Figure 2-2A). One of the lines, *DeDeLL* did not produce fruits and so was excluded from fruit analysis. Disease distribution in the experimental field was uniform based on disease occurrence in 'Vlaspik' plots (data not shown). Overall disease occurrence in this experiment was higher than in the trellis expreriment. This is likely due to timing of the experiments; the architecture experiment was carried out later in the season, where the higher temperatures and increased secondary inoculum population led to more optimal conditions for *P. capsici* infection. Disease occurrence at harvest increased throughout the season from 0-10 % at the first and second harvests to 15-45 % to the third harvest (Figure 2-2B).

On average 'Vlaspik' showed higher disease occurrence than the other architectural types at 4 DPH; however, disease occurrence at 4 DPH in all types was well above economically-acceptable levels (approximately 30 %) (Table 2-2, Figure 2-2C). Both 'Marketmore 76' (*DeDe*) and 86 (*dede*) had higher disease occurrence at both 0 and 4 DPH compared to other genotypes over three harvest dates, suggesting that these genotypes are more susceptible to *P. capsici* regardless of architectural effect (data not shown).

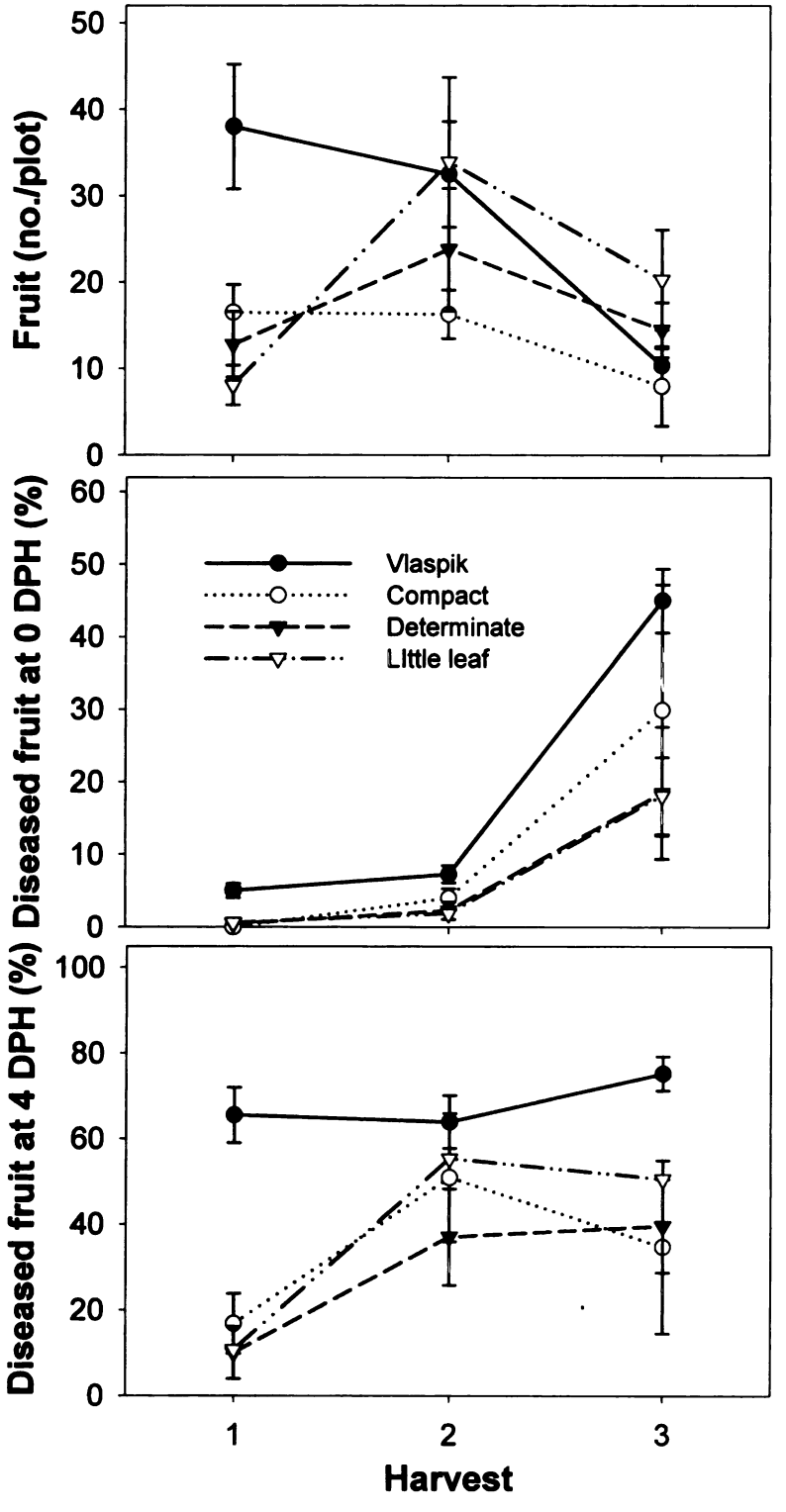


Figure 2-2. Mean fruit number per plot (A), percent disease occurrence at harvest (B) and four days post harvest (C) for 'Vlaspik', determinate (*dedeLL* and *dedell*), little leaf (*DeDell*, *dedell*, and H-19), and compact architectural types. Each point is the mean of 4-28 plots (4 replicate plots per genotype) ± SE

Table 2-2. Percent Phytophthora capsici-infected cucumber fruit rated at 4 days postharvest and averaged over three harvest dates.

Architecture type	Total disease occurrence (%)
Standard ('Vlaspik')	68.2b[z]
Little leaf (*DeDell*, *dedell*, and H-19)	38.8a
Compact	34.1a
Determinate (*dedeLL*, and *dedell*)	28.9a
$LSD_{0.05}$	17.2

[z]Values followed by same letter are not significantly different at $P \leq 0.05$ (LSD).

Screening for architectural variants

Among the 150 accessions examined in the field for architectural variation, fifty appeared to have distinctive architecture relative to 'Vlaspik' based on visual inspection. These 50 PIs were measured for main stem length, internode length, number of branches, and leaf width and length. The PIs exhibited a normal distribution for the measured traits which varied in magnitude by 2- to 10-fold (Figure 2-3). Main stem length ranged from 20 to 250 cm, average internode length from <1 to 9 cm, number of branches from <1 to 9, and leaf width from 8 to 19 cm. 'Vlaspik' was usually in the middle of the distribution. There were strong correlations between main stem length and internode length ($P < 0.001$) and between leaf width and leaf length ($P < 0.001$) as might be expected, but not between other traits (Table 2-3). An additional 110 accessions were observed in 2004, but no new architecture traits were observed relative to the 2003 sample.

Several PIs were identified for further study at commercial planting densities. PIs 192940, 227207, 249562, 358814, and 401734 which had less branching and large internode and main stem length relative to 'Vlaspik', were categorized as reduced branching type (R) (Table 2-4). The reduced branching types were selected due to their open habit suggesting they may form a more open canopy, with less canopy coverage over the fruits. PI 466921 had determinate growth habit with a short main stem (D) and no branching; therefore less canopy coverage of the fruit was expected. PI 308915 and 308916 had short internodes and upright growth habit (CP), and were similar to the compact type tested above, but with a more extreme phenotype. Compact types also had a tendency to hold young fruit off the ground, at least at the early stage of fruit development, which might help reduce disease occurrence; this trait was more

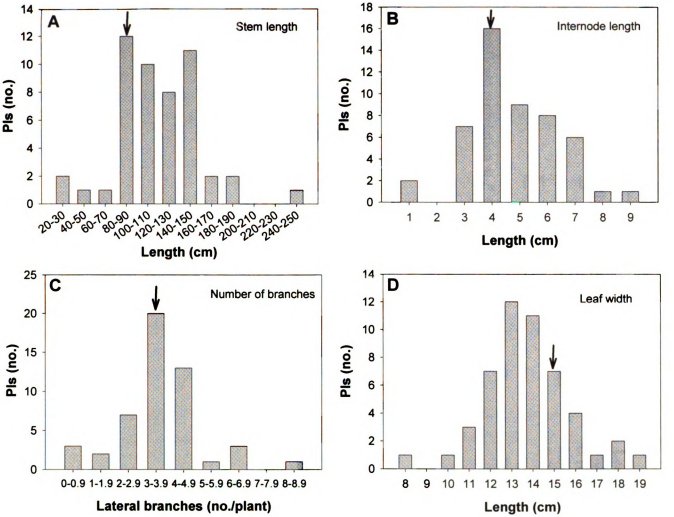


Figure 2-3. Frequency distribution of 50 cucumber PIs showing architectural variation relative to 'Vlaspik' for stem length (A), internode length (B), number of branches (C), and leaf width (D). Arrow indicates 'Vlaspik' value.

Table 2-3. Correlation (R^2 value) between stem length, internode length, leaf length and width, and number of branches.

	Main stem length	Internode length	Leaf length	Leaf width	Number of branches
Main stem length	—	0.576***	0.032	0.101	0.002
Internode	—	—	0.002	0.046	0.075
Leaf length	—	—	—	0.708***	0.053
Leaf width	—	—	—	—	0.138
Number of branches	—	—	—	—	—

***Significant correlation at $P < 0.001$.

Table 2-4. Origin, architectural type, number of branches, main stem length, fruit position, percent of infected fruit at 0 and 4 days post harvest (DPH), and susceptibility of selected Plant Introductions for architectural effect on controlling *Phytophthora capsici*.

PI	Origin	Architectural Type[z]	Branches (no.)		Main stem length (cm)		Fruit position	Infected fruit harvest 3[w] (%)		Fruit susceptibility to *P. capsici*[v]
			2003[y]	2004[x]	2003	2004		0 DPH	4 DPH	
192940	China	R	1.8	2.3	106.4	177.3	Ground	25.0b[u]	94.5b	S
227207	Japan	R	1.6	4.3	122.6	194.1	Ground	12.9ab	56.7ab	S
308915	Russia	Cp	4.8	8.9	29.7	27.3	few off ground	27.9b	72.8ab	S
308916	Russia	Cp	3.6	8.8	30.4	35.3	Frequently off ground	1.2a	25.0a	S
358814	Malaysia	R	0	2.0	159.3	157.1	Ground	35.5b	87.2b	S
401734	Puerto Rico	R	0.7	3.5	90.7	148.5	Ground	11.1ab	55.6ab	S
466921	Russia	D	0	0	52.0	48.3	Ground	91.7c	98.2b	S
Vlaspik	U.S.	S	3.7	4.4	87.7	129.4	Ground	18.4b	81.6b	S

[z]R= reduced branching, Cp-compact, D-determinate without branching, and S-standard, indeterminate

[y]Values are mean of 6 samples

[x]Values are mean of 8 samples, except PI 401734 was mean of 4 samples.

[w]2004.

[v]Greenhouse grown detached fruit were directly inoculated with *P. capsici*; S=susceptible.

[u]Numbers followed by the same letter are not significantly different from each other (LSD, $P \leq 0.05$)

pronounced for PI 308916.

Architecture study with selected PIs and commercial lines

The architecture types selected from the PI screening were tested in a *P. capsici* infested field at commercial planting densities. Four commercially available cultivars described as having small plant size (Arabian, Colt, Palomino, and Stallion) also were tested. However, in these conditions, vine length appeared to be comparable to 'Vlaspik'. We observed differences in main stem length and branching number for the selected PIs compared to the initial screen in 2003 (Table 2-4). The PIs with reduced branching habit and 'Vlaspik' generally had longer main stem length and more branching in 2004. The compact and determinate lines had equivalent stem lengths in both years, with very short vine lengths for the compact PI's. In 2004, the reduced branching lines produced more branches than had been observed in 2003 when they were more widely spaced. Similarly, the compact lines showed a marked increase in branch number in 2004.

Several of the genotypes [compact (PI 308915 and PI308916), determinate with no-branching, (PI 466921), and reduced branching (PI 224207 and PI 358814) types] did not germinate as uniformly as commercial genotypes and so these were excluded from canopy analysis. However, there were visible differences in canopy structure among the genotypes which had better germination rate. While the time of canopy filling and the density of canopy among 'Vlaspik' and the four commercial small vine types appeared to be comparable, the two reduced-branching types (PI 192940 and PI 401734), had a more open canopy compared to 'Vlaspik' and the commercial lines as assessed by amount of visible bare ground.

Earlier in the season, daily maximum temperatures under the canopy were higher in reduced-branching genotypes than commercial architecture types. The mean maximum temperatures from 27 July to 3 Aug. were 28.8 °C for reduced branching lines vs. 23.6 °C for 'Vlaspik' (LSD=1.7, $P \leq 0.05$), suggesting that the more open canopy alloweddirect sunlight penetration. As canopy filling progressed, this phenomenon diminished with mean maximum temoeratures of 21.9 °C for the reduced branching lines, and 22.7 °C for 'Vlaspik' from 8 to 17 Aug. (LSD = 1.2, $P \leq 0.05$).

For the first to third harvests, yields of 'Vlaspik' and commercial small types were very similar, whereas the two reduced branching types had significantly lower yield; however, the disease occurrence at 0 and 4 DPH was not significantly different among the architecture types (Figure 2-4A-C). The compact lines, determinate with no-laterals (PI 466921), and the rest of the reduced branching types could only be harvested at the third and fourth harvests. At 4 DPH, most of the architectural types showed increased disease occurrence compared 0 DPH; however, compact line PI 308916 remained significantly lower at both 0 and 4 DPH (Table 2-4). The difference in disease occurrence did not result from fruit resistance per se, as direct inoculation of the PI 308916 fruit showed the fruit to be comparably susceptible to 'Vlaspik' (Table 2-4). Interestingly, many fruit of PI 308916 were held above the ground throughout the experiment (Figure 2-5).

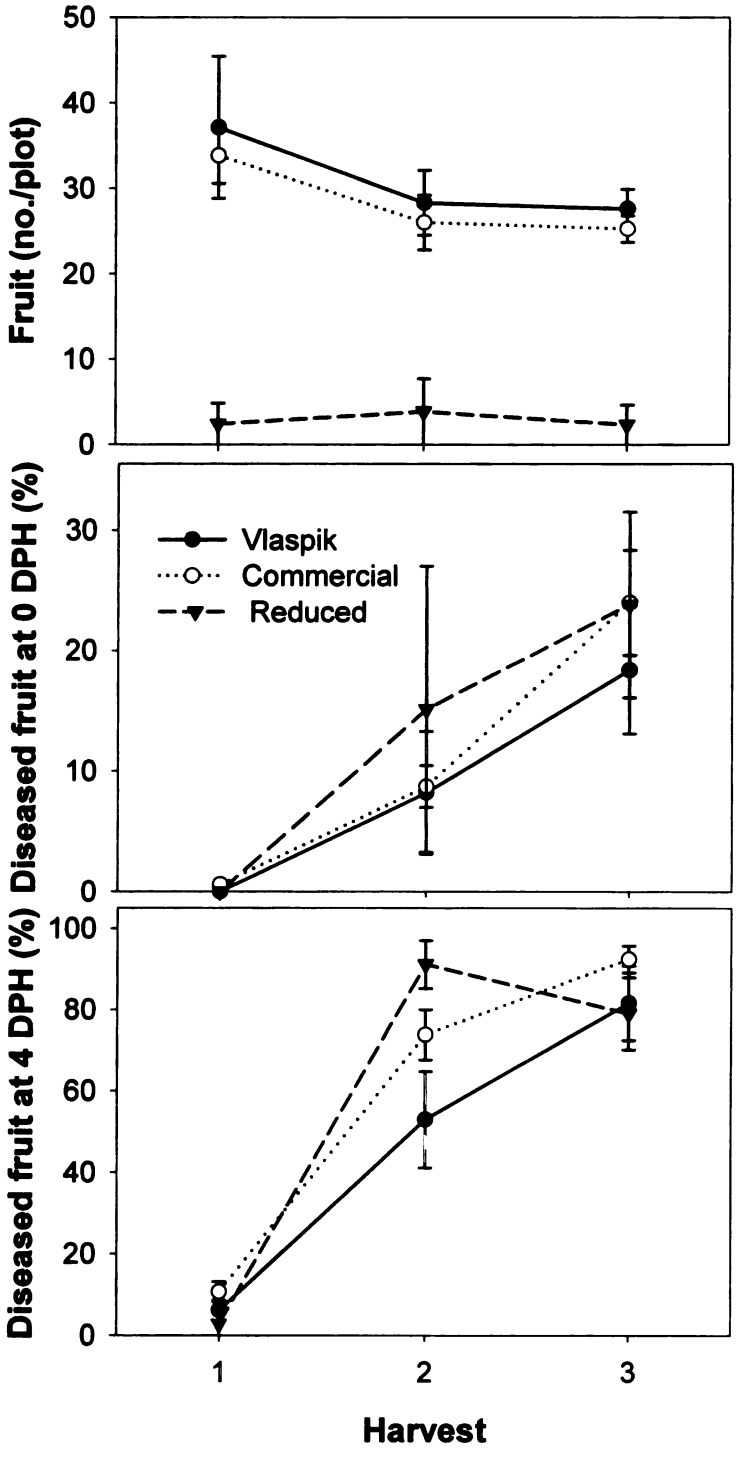


Figure 2-4. Mean fruit number per plot (A) and percent disease occurrence at harvest (B) and four days post harvest (C) for 'Vlaspik'; mean of the commercial lines 'Arabian', 'Colt', 'Palamino', and 'Stallion'; and reduced branching types (mean of PI 192940, 227207, and 401734). Each data point is the mean of 3 - 16 plots (3 replicate plots per genotype) ± SE.

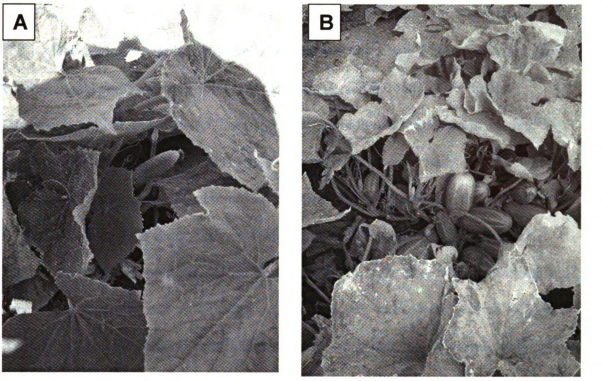


Figure2-5. Fruit position for PI 308916 early (A) and late (B) in the season.

Discussion

Developing cucumber fruit are located under the canopy where warm and moist environmental conditions are optimal for germination and growth of *P. capsici* (Hausbeck and Lamour, 2004) Therefore, we sought to determine whether using alternate plant architecture which has less canopy coverage over the fruit and allows for increased air circulation, or that removes fruit from direct contact with the soil, might be an effective strategy to reduce disease severity. A trellis study was conducted as an extreme test of this hypothesis. There were differences in the time of canopy filling and temperature under the canopy among the narrow-spaced, wide-spaced and trellis plots. As was predicted, the narrow plots had the highest temperatures under the canopy where fruit were located. The wide spaced plots had generally intermediate temperatures, and the trellis plots had lowest temperatures, likely due to increased air circulation. Although we were not able to measure humidity directly, it is likely that the more open conditions around the trellised fruit also resulted in reduced relative humidity. Even though there was open ground between rows within the wide spaced plots, the fruit were generally located under a dense canopy of the parent plant and exhibited a similar percent infection as narrow spaced plots.

The trellis plots however, had significantly lower rates of *P. capsici* infection suggesting that canopy conditions and/or removing the fruits from the ground can help reduce disease incidence. Although trellis production is not economically feasible for pickling cucumber, these results suggest that alternate cucumber architecture types which simulate trellis conditions might contribute to disease control strategies.

The effect of architectural differences on canopy development was examined using near-isogenic lines differing for leaf size and determinacy. Comparison of temperature under the canopy and other types showed that the standard type (indeterminate and standard leaf) had significantly higher daily maximum temperature than the alternate architectural types (little leaf, determinate, and compact). Goode et al. (1980) suggested that canopy differences in little leaf plots contribute to reduced occurrence of the soil-borne *R.solani* and *Pythium aphanidermatum* (Edson) Fitzp. diseases. In our study, the standard architecture lines had significantly higher occurrence of *P.capsici*-induced fruit rot than the little leaf, compact or determinate types suggesting an effect of architecture; however, disease occurrence in the severe late-season conditions still exceeded economically feasible levels.

A representative example of the cucumber germplasm was screened for other architectural variants that might be useful for reducing occurrence of *P. capsici* infection. There was a range of variation for architectural traits with up to 10-fold differences in various traits. Comparison of architectural traits showed that stem length is correlated with internode length as would be expected, although lack of perfect correlation can also reflect differences in growth pattern influencing other features that can affect vine length such as fruit and flower location, branching, and determinacy. The lack of correlation of several architectural traits indicates that different phenotypes appear to be inherited separately, and so could be genetically manipulated independently.

Possible variants which might be helpful in reducing *P. capsici* disease severity, included reduced branched types which produced a more open canopy, and compact types with initial upright fruit position that might reduce fruit contact with the soil. When

the reduced branching PIs were tested at commercial planting densities, they exhibited increased main stem length and branching number compared to the initial screening which was done with wide spacing. Schultheis et al. (1998) did not observe an effect of plant spacing on cucumber vine length. The observed differences in length may be due to different physiological age of the plants at the time of measurement or different growth conditions in the two seasons. Determinate and compact vines appear to be less affected by environmental conditions. The increase in branch number might be due to changes in planting density, since Horst and Lower (1977) noted that plant density can affect lateral number.

Of the architectural variants tested, reduction in disease occurrence was observed for only one, PI 308916. Direct fruit inoculation tests showed that the reduced infection of PI 30896 was not due to fruit resistance; therefore, the low disease incidence in PI 308916 may result from its unique architecture which allows for many of the fruit to be held above the ground. This trait may be useful for future strategies to reduce losses due to *P. capsici* infection.

In summary, alternate plant architecture could be helpful in reducing *P. capsici* infection on pickling cucumber. Varying architecture resulted in modified canopy structure as observed visually and by canopy temperature. However, disease incidence data suggest that canopy conditions are less important for disease control than removal of fruit from the soil. The trellis study and plots with the compact PI 308916, suggest that reducing contact of young cucumber fruit with the soil can be a means to alleviate *P. capsici* disease severity.

Acknowledgments

We thank the Pickle Seed Research Fund and MSU-GREEEN for support of this project. We thank Dr. Jack Staub, the Plant Introduction Station (Ames, Iowa), and Seminis Vegetable Seed Inc. for providing seed. We thank Steve Suzura and Dr. Mary Hausbeck and her lab members, R. Bounds, B. Cortright, A. Gevens, and B. Harlan, and for their help in field and fruit screening experiments and Bill Chase, Gary Winchell, and Ron Gnagney for farm assistance. We also thank Drs. Jim Kelly and Mary Hausbeck for their helpful reviews of the manuscript.

Literature Cited

Babadoost, M. 2004. Phytophthora blight: a serious threat to cucurbit industries. APSnet feature story Apri-May. Online publication. American Phytopathological Society, St. Paul, MN.

Blad, B. L., J. R. Steadman, and A. Weiss. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. Phytopathology 68:1431-1437.

Coyne, D. P. 1980. Modification of plant architecture and crop yield by breeding. HortScience 15:244-247.

Coyne, D. P., J. R. Steadman, and F. N. Anderson. 1974. Effect of modified plant architecture of great northern dry bean varieties (*Phaseolus vulgaris*) on white mold severity, and components of yield. Plant Dis. Rept. 58:379-382.

Erwin, D. C. and O. K. Ribeiro. 1996. *Phytophthora capsici*. P. 262-268. In: Phytophthora Diseases Worldwide. American Phytopathological Society, St. Paul, MN.

Fuller, P. A., J. R. Steadman, and D. P. Coyne. 1984. Enhancement of white mold avoidance and yield in dry beans by canopy elevation. HortScience 19:78-79.

George Jr., W. L. 1970. Genetic and environmental modification of determinate plant habit in cucumbers. J. Amer. Soc. Hort. Sci. 95:583-386.

Gevens A. J., K. Ando, K. Lamour, R. Grumet, and M. K. Hausbeck. 2006. Development of a detached cucumber fruit assay to screen for resistance and effect of fruit age on susceptibility to infection by *Phytophthora capsici*. Plant Dis. 90:1276-1282.

Goode, M. J., J. L. Bowers, and A. Bassi Jr. 1980. Little-leaf, a new kind of pickling cucumber plant. Arkansas Farm Research 29: 4.

Grumet, R. and R. Duvall. 1993. Testing the effect of the determinate shoot growth allele on cucumber root growth. HortScience 28:847-849.

Halterlein, A. J., G. L. Sciumbato, and W. L. Barrentine. 1981. Use of plant desiccants to control cucumber fruit rot. HortScience 16:189-190.

Hanna, H. Y., A. J. Adams, and R. N. Story. 1987. Increased yield in slicing cucumbers with vertical training of plants and reduced plant spacing. HortScience 22:32-34.

Hausbeck M. and K. Lamour. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Dis. 88:1292-1302.

Horst E. K. and R. L. Lower. 1977. Effect of plant density on lateral number and main stem length in *Cucumis sativus* L. and *Cucumis hardwickii*. HortScience 12:235-235.

Kauffman, C. S. and R. L. Lower. 1976. Inheritance of an extreme dwarf plant type in the cucumber. J. Amer. Soc. Hort. Sci. 101:150-151.

Knerr, L. D., J. E Staub, D. J. Holder, and B. P. May. 1989. Genetic diversity in *Cucumis sativus* L. assessed by variation at 18 allozyme coding loci. Theor. Appl. Genet. 78:119-128.

Kolkman, J. M. and J. D. Kelly. 2002. Agronomic traits affecting resistance to white mold in common bean. Crop Sci. 42:693-699.

Konsler, T. R. and D. L. Strider. 1973. The response of cucumber to trellis vs. ground culture. HortScience 8:220-221.

Kreutzer, W.A. 1937. A Phytophthora rot of cucumber fruit. Phytopathology 27:955.

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

Lamour, K. H. and M. K. Hausbeck. 2001a. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91:973-980.

Lamour, K. H. and M. K. Hausbeck. 2001b. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.

Lamour, K. H. and M. K. Hausbeck. 2002. The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. Phytopathology 92:681-684.

Lamour, K.H. and M. K. Hausbeck.2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Dis. 87:841-845.

Leonin, L.H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. Phytophathology 8:401-408.

Michigan Department of Agriculture, 2001. Michigan Agricultural Statistics. Lansing, Michigan

Park, S. J. 1993. Response of bush and upright plant type selections to white mold and seed yield of common beans grown in various row widths in southern Ontario. Can. J. Plant Sci. 73:265-272.

Pierce, L. K. and T. C. Wehner. 1990. Review of genes and linkage groups in cucumber. HortScience 25:605-615.

Plant Variety Protection Certificate. 1993. H-19 cucumber. PVPO no. 8900073. US Dept. Agriculture, Agriculture Marketing Service. Washington, D.C.

Russo, V. M., B. W. Roberts, and R. J. Schatzer. 1991. Feasibility of trellised cucumber production. HortScience 26:1156-1158.

Schultheis, J. R., T. C. Wehner, and S. A. Walters. 1998. Optimum planting density and harvest stage for little-leaf and normal-leaf cucumbers for once-over harvest. Can. J. Plant Sci. 78:333-340.

Serce, S., J. P. Navazio, A. F. Gokce, and J. E. Staub. 1999. Nearly isogenic cucumber genotypes differing in leaf size and plant habit exhibit differential response to water stress. J. Amer. Soc. Hort. Sci. 124:358-365.

Wehner, T. C. 1989. Breeding for improved yield in cucumber, p.323-359. In: J. Janick (ed.). Plant Breeding Reviews. vol. 6. Timber Press. Portland, Oregon.

Chapter 3

Age related resistance in cucumber and various cucurbit fruit to infection by *Phytophthora capsici*

Abstract

Cucumber fruit rot caused by *Phytophthora capsici* causes severe losses in vegetable production, including many cucurbit crops. In the course of screening for genetic sources of resistance among cucumber germplasm, fruit age/size appeared to be a factor in susceptibility to infection by *P. capsici.* To verify this observation, greenhouse grown fruits of known age were inoculated with *P. capsici.* Cucumber fruits were most susceptible to *P. capsici* when they were very young and rapidly elongating, but then developed age-related resistance (ARR) as they approach full length at 10-12 days post pollination (DPP). This was observed in both the greenhouse and field, and for several genotypes. Seven additional cucurbit crops, zucchini, yellow summer squash, acorn squash, pumpkin, butternut squash, melon, and watermelon, representing four species (*Cucurbita pepo, Cucurbita mochata*, *Cucumis melo,* and *Citrullus lanatus*) also exhibited size-related decrease in susceptibility, but to varying degrees. In the field, infection primarily occurs at the blossom end. To determine whether preferential infection of the blossom end was due to position-related differences, or was a result of earlier contact with inoculum-containing soil, greenhouse-grown cucumber fruit of known age were inoculated with *P. capsici* at the peduncle and blossom ends. The youngest fruits supported sporulation on both ends. As fruit age increased, the peduncle end became less susceptible sooner, suggesting a developmental gradient within the fruit

influencing susceptibility. This difference in susceptibility was significant for cucumber, zucchini, acorn, and butternut squash fruits. To examine whether the basis of ARR in cucumber fruit-*P. capsici* is due to changes in surface or mesocarp properties, exocarp sections from 8 DPP (susceptible) or 15 DPP (resistant) fruit were placed onto intact 8 or 15 DPP fruit, inoculated with *P. capsici* zoospore suspensions and examined for disease development. All exocarp sections from 8 DPP supported sporulation, but not 15 DPP, indicating that the exocarp plays a significant role in the transition to resistance. Microscopic analysis of zoospore inoculated fruit surface showed that short germ tubes and appressorium formation was significantly higher on 8 DPP fruit while longer and aberrant germ tubes are more prevalent on 16 DPP fruit.

Introduction

In recent years, *Phytophthora capsici* has become an increasingly severe pathogen causing disease on a wide range of vegetable crops, including cucurbit crops, where it can cause devastating yield losses (Babadoost, 2004; Hausbeck and Lamour, 2004). In some cases, losses of up to 100 % have been reported and growers have had to abandon production fields (Babadoost, 2004). *Phytophthora capsici* is a soil-borne Oomycete pathogen that can cause diseases in many plant organs and at various growth stages of the host, e.g., damping-off on processing pumpkin, and fruit rot on cucumber. It is favored by wet and warm environments and can spread with water such as rain splash or irrigation. The symptoms observed during the disease progression of *P. capsici* infected fruit is a depressed fruit surface with a water soaked appearance followed by white cottony mycelium covering the affected region.

Pickling cucumber crops in Michigan have experienced major losses to *P. capsici* in recent years (Hausbeck and Lamour, 2004). Field observations show that cucumber fruit are susceptible to *P. capsici*, while roots, vines, and leaves are much less susceptible. Fields will frequently look healthy at harvest time as judged by vegetative growth, but the fruits can be heavily diseased (Hausbeck and Lamour, 2004). Cucumber fruit are usually located under the canopy where the environment is moist, warm, and close to the pathogen in the soil, thus favoring conditions for disease development. Trellis and plant architecture studies have indicated that removal of fruit from the soil surface can reduce disease incidence (Chapter 2; Ando and Grumet 2006).

Control of the disease by chemical and cultural practices remains a challenge. Resistance to the common fungicide, mefenoxam, has emerged (Lamour and Hausbeck, 2000). Rotation to non-host crop is of limited value, since oospores of *P. capcisi* can survive in the soil for a long period of time, up to 10 years (Hausbeck and Lamour, 2004). Wider plant spacing, cover crops, trellises, or variant architectural phenotypes may help to control the disease by facilitating better fungicide coverage, modifying the microclimate, or reducing contact with the inoculum-containing soil, thereby facilitating disease avoidance (Ando and Grumet, 2006; Ngouajio et al., 2006; Ristaino et al., 1997; Wang and Ngouajio, 2008). However, these methods can be difficult to implement in a commercial setting. The most desirable solution would be to find resistant cultivars, but this has met with mixed success. Efforts are underway to identify sources of resistance in *Cucurbita pepo* (Padley et al., 2008) and to introgress resistance from the wild *Cucurbita* species, *C. lundelliana* and *C. okeechobeenesis,* into *C. mocshata* (Padley et al., 2009). To our knowledge there have not been studies to identify sources of resistance to *P.*

capsici in watermelon or melon. Screening of a diverse collection of cucumber germplasm accessions for fruit resistance to *P. capsici* has not identified any resistant genotypes to date (Gevens et al., 2006).

In the course of screening for sources of *P. capsici* resistance in cucumber germplasm, fruit used as susceptible controls did not become uniformly infected. Prior observation suggested that susceptibility of the fruit might be related to fruit size or age; the smaller fruit appeared to be more susceptible than larger fruit. Age-related resistance (ARR), also known as adult plant resistance or developmentally related resistance, in which an older plant or plant organ (e.g., leaf or fruit) becomes less susceptible to pathogens, has been observed in many plant-pathogen systems, including disease caused by fungi, oomycetes, bacteria, and viruses, and is becoming increasingly recognized as an important component of plant defense against infection (Develey-Rivière and Galiana, 2007; Panter and Jones, 2002; Whallen, 2005). If ARR is present in the cucumber fruit-*P. capsici* interaction, it would affect the methods used for accurate screening. It would also have implications for which stage of fruit is likely to become infected, possible management implications with respect to spray practices, and insight into approaches to develop more resistant fruit.

Further examination of *P. capsici* infected cucumber fruit in the field led to the observation that field-grown cucumbers are typically covered with mycelium on the blossom end of the fruit rather than the peduncle end. This observation could be either due to the tendency of the blossom end to come into contact with the inoculum containing soil sooner than the stem end, or that there is a susceptibility gradation within the fruit such that the blossom end is more susceptible than the stem end.

In this chapter, I characterized the effect of fruit age on susceptibility to *P. capsici* in cucumber and other cucurbit crops representing four species: melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), butternut squash (*Cucurbita mocshata*), and four *Cucurbita pepo* crops, zucchini, yellow summer squash, acorn squash, and pumpkin. I also examined the basis for age-related increased resistance by asking whether it is due to changes in surface or mesocarp properties, and determined the nature of the difference in occurrence of disease at the blossom and peduncle end in cucumber and other cucurbits.

Materials and methods

Cucumber screening for *P. capsici* resistance

A set of 150 cucumber accessions was supplied by the North Central Regional Plant Introduction Station, Ames, IA. The first 100 genotypes tested were selected based on isozyme data to serve as a representative sample of the cucumber germplasm with maximum genetic variance (Knerr et al., 1989). Another 50 genotypes that had been selected based on possible architectural variation were also included in the screening (Ando and Grumet, 2006). Seeds were sown in the Michigan State University (MSU) Research greenhouse, East Lansing, MI and 14-day-old seedlings were transplanted to the MSU Horticulture Research and Teaching Center (HRTC) in a nonreplicated trial on a field with no history of *P. capsici* infestation in summer 2003. Seedlings were planted 0.46 m apart in 3.67 m single-row plots with 1.52 m between rows. Plastic mulch was used to control weeds and local standard commercial production guidelines were

followed for insect control (Bird et al., 2005). Irrigation was provided by rain or overhead irrigation to 25 mm a week.

Harvested fruit from commercial standard ('Vlaspik,' Seminis Vegetable Seeds, Oxnard, CA) and test genotypes were inoculated with mycelial/sporangial plugs of *P. capsici* isolate OP97 (an isolate of *P. capsici*, provided by Dr. Mary Hausbeck Lab at Michigan State University) without wounding according to the method of Gevens et al. (2006). Briefly, a 7 mm agar plug containing 7-11 day old *P. capsici* mycelia was covered with a sterile microcentrifuge tube secured to fruit with petroleum jelly and incubated at room temperature under fluorescent lighting. Four to eight fruit were tested for each genotype. Initial fruit examination was made 4 days post inoculation (DPI). Symptom development was monitored and scored as 1 – no symptoms, 2 – water soaking, and 3 – sporulation. Fruit with no symptoms at 4 DPI were monitored daily and maintained for further observation for a minimum of 10 DPI, or until the occurrence of water-soaked lesions and sporulation.

The PIs identified in 2003 for which less than 50 % of the tested fruit exhibited pathogen sporulation at 10 DPI (PIs: 249561 Thailand, 255937 Netherlands, 263047 Russia, 288990 Hungary, and 304805 U.S.) were reevaluated using greenhouse-grown, hand-pollinated fruit in 2004. Seedlings were planted in 3.8-liter pots with sterile media (BACCTO, Michigan Peat Co., Houston, TX) under supplemental lighting (18/6 h light /dark) at 21-25 °C, fertilized with 300 $mg \cdot L^{-1}$ nitrogen from Peters Professional® 20-20-20 General Purpose water soluble fertilizer (The Scotts Company LLC, Marysville, OH) once a week. Pest control was performed according to the standard management practices in the greenhouse. 'Vlaspik' was used as a commercial standard. A minimum

of five fruit from each genotype were harvested at 7 and 14 days post pollination (DPP), and inoculated as described above. Fruit were observed initially at 4 DPI, then maintained for further evaluation until 10 DPI and scored for water-soaked lesions and pathogen sporulation.

Effect of fruit age

Cucumber cultivar 'Vlaspik', was grown in a research greenhouse at MSU during spring 2004 as described above. Flowers were hand-pollinated at 2 to 3 day intervals to ensure a range of fruit ages from 2 to 18 DPP at the day of harvest. Harvested fruit were washed with distilled water, air-dried, and measured for fruit length and diameter. The varying-aged fruit were placed at random in aluminum trays and inoculated with *P. capsici* and scored for symptoms as described above. The experiment was repeated three times with approximately 15-20 fruit per experiment.

Fruit age experiments were also conducted using fruit from field-grown plants. The slicing cucumber cultivar 'Straight Eight' was grown at the MSU HRTC in fields with no history of *P. capsici* during the summer of 2004 using standard management practices. Cucumber fruit representing a range of sizes were harvested on four dates; 15 to 20 fruit were tested per harvest date and fruit with visible wounds were not used. Fruit were prepared and inoculated as described above.

Parthenocarpic fruit experiment

Parthenocarpicly developed 'Vlaspik' fruits were grown in the MSU Research greenhouse during spring 2006 as described above. Fruits were harvested, inoculated, and scored as described above. At 10DPI, fruit were cut longitudinally to search for the

presence of seeds; data from the fruits with seeds were excluded. This experiment was repeated 3 times with a total of 58 fruit.

Fruit growth analysis

Cucumber 'Vlaspik' plants were grown in the greenhouse during the summer of 2007 under the same conditions described earlier. Fruit were marked with waterproof India ink (Sanford LP, Oak Brook, IL) with equally spaced 5 dots along the fruit length. Length between the dots which include length between the both ends and the neighboring dots were measured daily up to 34 DPP.

Fruit position experiments

'Vlaspik' fruits were grown in the greenhouse during the spring of 2004 under the same conditions as described earlier. Flowers were hand-pollinated over a period of days to allow for simultaneous harvest of fruit ranging in age from 6 to 14 DPP. Inoculation and disease screening at 4 DPI was as described earlier, except that two 6 mm diameter plugs of *P. capsici* isolate OP97 were placed approximately 2 cm from the peduncle- and blossom-end of each fruit.

Exocarp transfer experiment with zoospore

Zoospore production and inoculation preparation was performed according to Gevens et al. (2006). Concentration of the zoospore suspensions was determined with a hemacytometer and diluted to 1 x 10^6 per ml. Exocarp sections (3 cm x 3 cm x 1-2 mm) from the middle part of the fruit were excised from both 8 and 15 DPP fruit with petit knife or razor blade without introducing nicks. A sterile plastic tube (0.6 cm height, 0.8 cm diameter) was placed on the exocarp section and anchored to underlying intact 8 or 16 DPP fruit using a strip of 1 cm wide parafilm. A twenty-two gage sterile needle was used

to deliver 30 µl zoospore suspension into the tube by penetrating the parafilm; the needles did not come in contact with the fruit tissue. Intact 8 and 15 DPP fruits were similarly inoculated and included in each tray as control. Inoculation and scoring of symptom development was as described above.

Effect of fruit surface on zoospore germination

Ten greenhouse-grown cucumber fruit were harvested at 8 and 16 DPP and were inoculated with 30 µl *P. capsici* zoospore suspensions at concentration of 1×10^4, as described earlier. After 24 hours of inoculation, fruits were removed from the trays and the zoospore suspensions were allowed to air dry. A thin layer of clear nail polish (Markwins Beauty Products, Inc., City of Industry, CA) was applied on the inoculated surface of the fruits or equivalent location for non-inoculated control fruits as was described in Iwaro et al. (1997). After hardening, the nail polish was removed and mounted on the cover glass for microscope analysis. Each sample was divided into three sub-sections and twenty zoospores were counted randomly within each sub-section. Zoospores were examined for germination, length of germ tube, (short – less than 1x zoospore cyst diameter, medium – 1-5x diameter, long – greater than 5x diameter), presence of appressoria, and occurrence of aberrant germ tubes. Fruits were scored for symptom development at 6 DPI as described earlier.

Additional cucurbit fruit screening

Cucumber 'Vlaspik'; muskmelon 'Odyssey' (Rispens Seeds, Inc., Beecher, IL); butternut squash 'Waltham'(Seedway LLC., Hall, NY); watermelon 'Crimson Sweet' (Seedway); zucchini 'Black Beauty'(Seedway); yellow summer squash 'Horn of Plenty'(Seedway); acorn squash 'Royal Acorn' (Seeds of Change, Santa Fe, NM); and

pumpkin 'Baby Pam' (Seedway), were grown in a field with no history of *P. capsici* infestation at the MSU HTRC during the summer of 2006. Crops were planted in 15 m rows, 3.3 m between rows, and 50 cm spacing within rows, except cucumber which was planted at 4 cm spacing within row. Commercial pest control was applied as described earlier. Plastic mulch was used to suppress weeds. Irrigation was provided by rain or trickle irrigation to provide 25 mm per week.

Fruits were harvested on at least two dates for each crop to provide a total of 45-93 fruits per crop, representing a range of stages of development as assessed by fruit size and appearance. Harvested fruits were washed with distilled water, air-dried, and measured for length and width. The fruits were then inoculated on both ends with *P. capsici* as described earlier.

Greenhouse studies were also performed for acorn squash 'Autumn Delight' (Siegers Seeds Co., Holland, MI) butternut squash 'Waltham', and pumpkin 'Baby Pam' in the MSU Research Greenhouse during the spring and summer of 2008. Seeds were sown into 38 L plastic pots filled with BACCTO media and plants were grown under the same environmental conditions as described earlier. Flowers were hand-pollinated daily over a 45 day period to provide a set of fruit of known, different ages that could be harvested and tested simultaneously. The experiments were repeated at least twice for each crop with sixteen plants per crop per experiment to provide a total of 67-79 fruits per crop. Harvested fruits were measured for length and width, examined for color and external surface properties, then processed for inoculation on both ends and scored for symptoms as described above.

Statistical analysis

Data were analyzed by ANOVA using the SAS program 9.1 (SAS Institute Inc., Cary, NC) with mixed procedures; when appropriate, LSD was used for multiple comparisons. Regression analysis was performed using SigmaPlot 8.02 (SPSS Inc., Chicago, IL). Blossom/peduncle end comparisons of the same fruit were performed by using chi-square contingency analysis on two trials of the same subject according to Steele and Torrey (1960).

Results

Cucumber fruit exhibit age-related resistance to *P. capsici*

The majority of cucumber genotypes screened for resistance to *P. capsici* were rapidly infected and exhibited sporulation by 5 DPI (Fig 3-1A). When the evaluation was extended to 10 DPI, infection levels increased, with ca. 90 % of the PIs exhibiting sporulation on >50 % of the fruit tested (Figure 3-1A). When accessions exhibiting a low percentage of fruit with pathogen sporulation were rescreened in 2004, all were as susceptible as the commercial 'Vlaspik' standard (data not shown).

Symptom development on 'Vlaspik' fruit that were included as susceptible controls in each tray of the germplasm was inconsistent. Smaller, younger fruit tended to be more readily infected, while larger, older fruit were less frequently infected. To directly test the effect of fruit age, sets of greenhouse-grown, hand-pollinated fruits of known ages, ranging from 2 to 18 DPP were inoculated at the same time (Figure 3-2A-C). At 4 DPI, most fruit younger than 10 DPP exhibited sporulation, whereas after 12 DPP, most fruit remained symptom-free. Fruit of intermediate ages (10 to 12 DPP) often

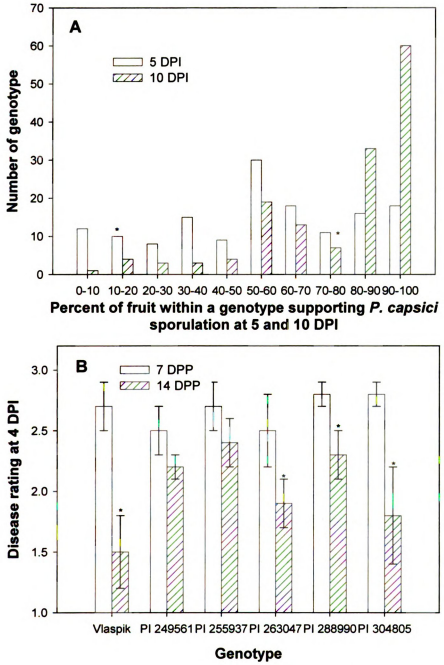


Figure 3-1. A. Frequency distribution showing number of cucumber genotypes for which sampled fruit showed the indicated sporulation (%) at 5 (blank bar) and 10 (striped bar) days post inoculation (DPI). Asterisks (*) indicate 'Vlaspik' value. B. Disease response of the candidate genotypes identified in cucumber screen 2 on greenhouse-grown hand-pollinated fruit harvested at 7 and 14 days post pollination (DPP). Fruit symptoms were scored for disease response at 4 DPI as: 0-no symptoms, 1-water soaking, and 2-sporulated. * 7 and 14 DPP fruit within a genotype were significantly different from each other, t-test ($P \leq 0.05$). Each value is the mean of minimum 5 fruit ± SE.

exhibited water-soaked regions without sporulation. Symptom-free fruit were usually oversized for pickling cucumbers (length >14 cm, width >4.5 cm).

The transition from susceptible to more resistant appeared to coincide with the transition away from the period of rapid fruit elongation (approximately 12 cm length), with a sharp decline in disease rating at approximately 10-12 DPP (Figure 3-2A, D and E). Field-grown cucumbers ('Straight Eight') harvested to include a range of fruit sizes from 2 to 22 cm in length, were also tested for response to *P. capsici* inoculation.

Although growth rate and size can be influenced by environmental conditions, the relationship between fruit size and disease susceptibility followed the same trend as observed with greenhouse grown fruit (Figure 3-2F).

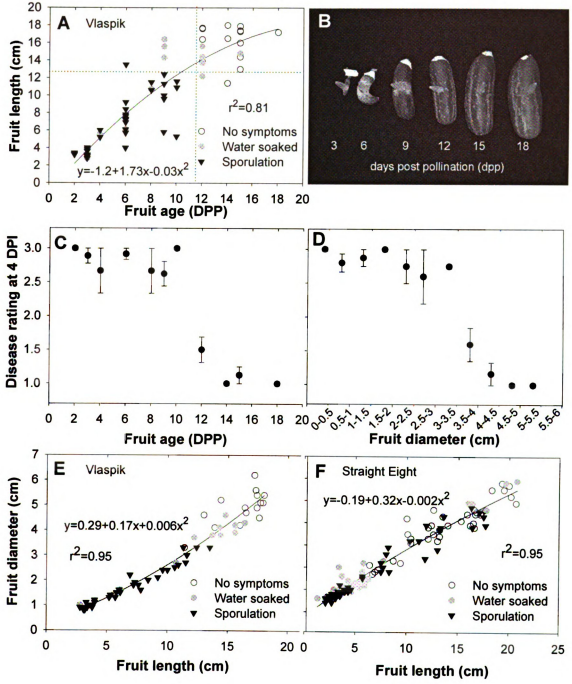
Fruit age effect was further examined with the candidate genotypes identified in the germplasm screen using both 7 and 14 DPP fruit to target pre- and post-transitional stages based on the 'Vlaspik' results. At 7 DPP, the majority of fruit from all genotypes supported sporulation; the remainder developed water-soaked regions (Fig 3-1B). At 14 DPP, fruit from three of the five candidate genotypes (PIs: 263047, 288990, and 304805) were significantly less susceptible than at 7 DPP (t-test $P \leq 0.05$), with most of these fruit showing water-soaking symptoms or no symptoms at all rather than sporulation. In no case were older fruit more susceptible than younger fruit.

Preliminary characterization of the age-related resistance in cucumber

Effect of seed formation

The time period from 12-16 DPP is associated with noticeable changes in seed development including hard seed coat formation (Chapter 4). To test whether seed

Figure 3-2. Relationship between cucumber fruit age and size, and response to inoculation with *Phytophthora capsici*. A-E. Response of greenhouse-grown hand pollinated fruit. Greenhouse fruit age experiments were repeated three times with approximately 15 to 20 fruit per experiment. The same trends were observed in each experiment. Data presented are pooled from the three experiments. A, Response of greenhouse-grown, hand-pollinated 'Vlaspik' fruit to *P. capsici* inoculation in relation to fruit age and length. B, Response of 'Vlaspik' fruit of various ages to inoculation with *P. capsici*. C. Mean disease rating (± SE) of fruit harvested at 3 to 18 days post pollination (DPP). Rating scale: 0 – no symptoms, 1 – water-soaking, and 2 – sporulated; eachpoint (with the exception of 18 DPP) is the mean of 3 to 12 fruits. D. Mean disease rating (± SE) of cucumber fruit in relation to fruit diameter . Each point is the mean of 4 to 10 fruits. E. Disease response in relation to fruit diameter and length. F. Disease response of inoculated field-grown 'Straight Eight' fruit in relation to fruit diameter and length. Symptom data were taken at 4 days post inoculation (DPI). Data are pooled from fruit sampled on four dates. r^2 values refer to quadratic binomial indicating a change in the rate of growth over time.


A
Vlaspik
Fruit length (cm)
Fruit age (DPP)
r²=0.81
y=-1.2+1.73x-0.03x²
No symptoms
Water soaked
Sporulation
B
3 6 9 12 15 18
days post pollination (dpp)
C
Disease rating at 4 DPI
Fruit age (DPP)
D
0-0.5 0.5-1 1-1.5 1.5-2 2-2.5 2.5-3 3-3.5 3.5-4 4-4.5 4.5-5 5-5.5 5.5-6
Fruit diameter (cm)
E
Vlaspik
Fruit diameter (cm)
y=0.29+0.17x+0.006x²
r²=0.95
No symptoms
Water soaked
Sporulation
Fruit length (cm)
F
Straight Eight
y=-0.19+0.32x-0.002x²
r²=0.95
No symptoms
Water soaked
Sporulation
Fruit length (cm)

development is related to the age-related resistance, parthenocarpic fruit were tested for susceptibility to infection by *P. capsici*. Various sizes of parthenocarpically developed greenhouse grown fruits also showed changes in susceptibility with fruit development (Figure 3-3A). Fruit at 1cm diameter developed either water soaking or sporulation (mean disease rating±SE, 2.6±0.1), whereas fruit with diameters greater than 3 cm did not sporulate (1.6±0.05). Disease rating of non-parthenocarpic, field grown fruits also decreased with increased with diameter, similar to parthenocarpic fruit (Figure 3-3B).

Role of fruit surface

Preliminary tests were conducted to examine whether the age-related decrease in susceptibility is associated with the fruit surface (exocarp), mesocarp or both. Fruits peeled prior to inoculation all formed sporulating lesions, whereas intact control fruit did not become infected at 4 DPI (data not shown), suggesting that the fruit surface is an important factor for the resistance of older fruits to infection by *P. capsici*. However, these experiments do not allow us to rule out possible effects of wounding due to peeling.

To further study the role of the exocarp using intact (non-wounded) fruit, exocarp exchanges were made between fruits at 8 and 15 DPP (i.e., exocarp piece from one fruit were placed on top of a second, intact fruit and inoculated with zoospores) (Figure 3-4A). The 8 DPP fruit surface pieces developed either water-soaking or sporulation regardless of fruit age underneath (mean disease rating±SE: 2.6±0.2), as did intact fruit (Figure 3-4B). Fruit surface sections from 15 DPP fruit, like intact 15 DPP fruit, generally did not sporulate regardless of fruit age underneath; however, some developed water-soaked symptoms (1.3±0.2). The fruit underneath the 8 DPP fruit surface pieces showed susceptibility similar to the fruit age study; 8 DPP fruit generally sporulated while 15

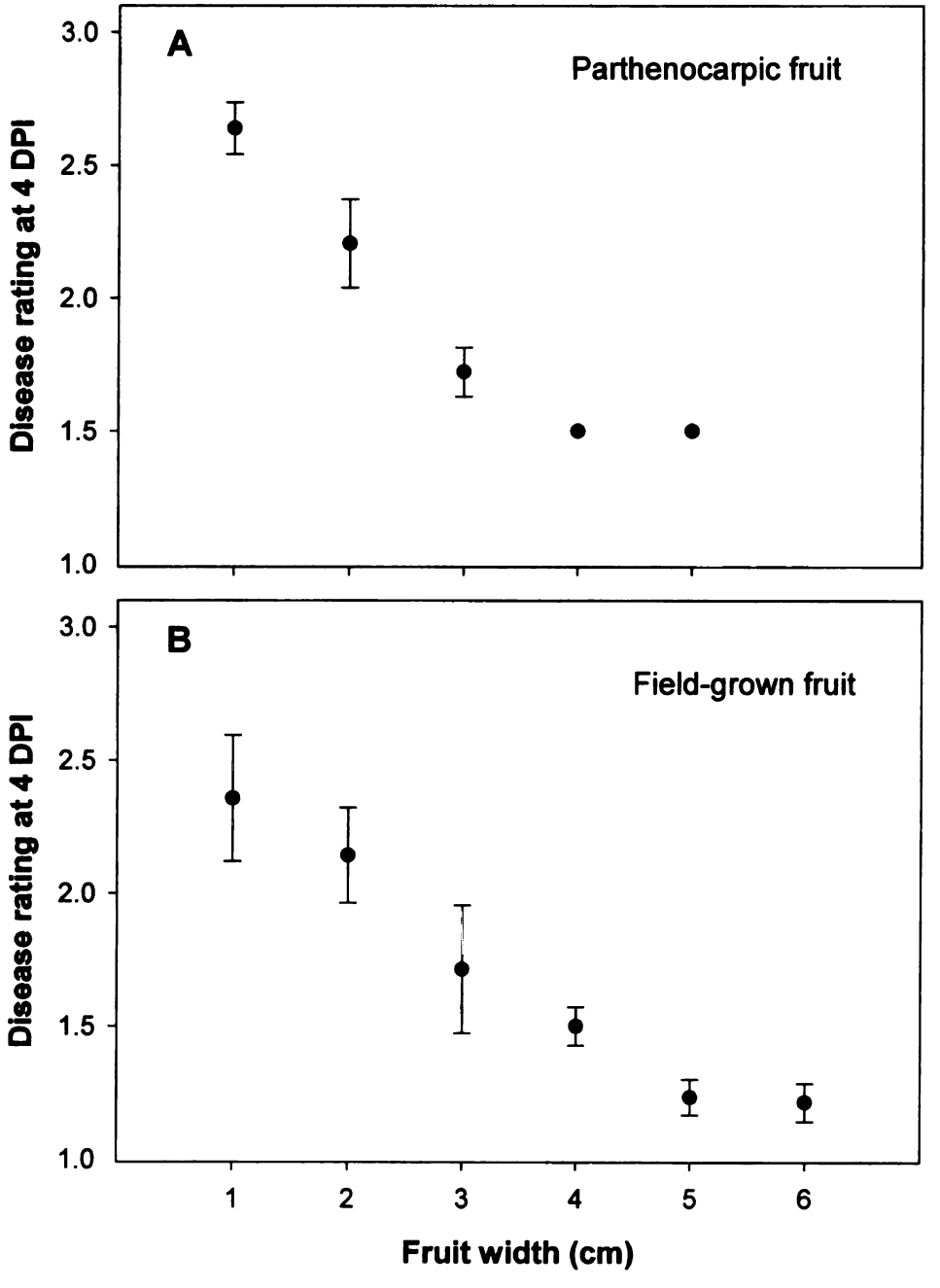


Figure 3-3. A, B. Mean disease rating at 4 days post inoculation (DPI)of parthenocarpic (A) and field-grown non-parthenocarpic (B) cucumber fruit in relation to fruit length to inoculation by *Phytophthora capsici*. Disease rating scale: 1 – no symptom, 2 – water soaking, and 3 – sporulation. Each point is the mean of at least 5 fruit ±SE.

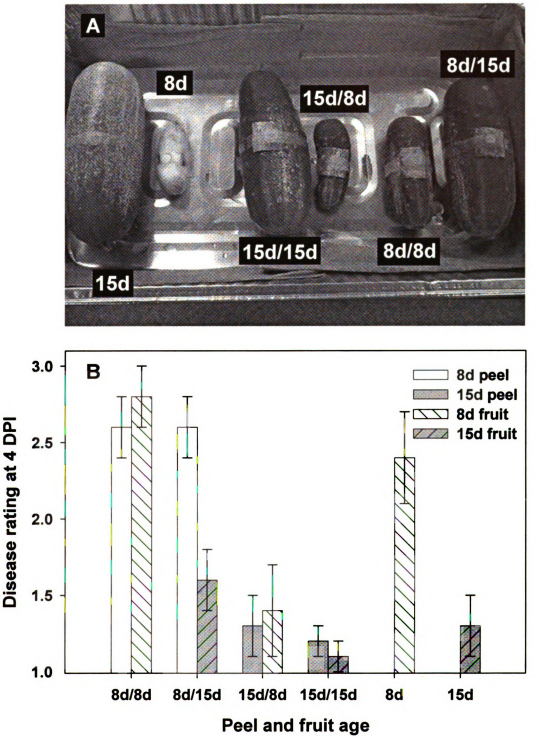


Figure 3-4. A. Picture of 8 and 15 days post pollination (DPP) exocarp section and intact fruit underneath to infection by *Phytophthora capsici* zoospore suspension. B. Disease rating at 4 days post inoculation (DPI). Disease rating: 1 – no symptoms, 2 – water soaking, and 3 – sporulation. Left bars are the exocarp section and right bars are fruit underneath. Each bar is mean of at least 9 fruit and ±SE.

DPP fruit generally did not sporulate even in the presence of sporulating 8 DPP fruit surface sections. However, when 15 DPP fruit surface pieces were inoculated, the underlying 8 DPP fruit did not sporulate, indicating that the 15 DPP fruit surface sections protected the underlying 8 DPP fruit. These results suggest that 15 DPP fruit surface section possesses properties that inhibit *P. capsici* infection. Equivalent were obtained with mycelium inoculation (data not shown).

Effect of fruit surface on zoospore germination

Since the exocarp is the first point of contact of the inoculum, it is possible that exocarp properties influence *P. capsici* development in pre-infection stage. Although, as before, the young fruit were infected while older fruit were resistant (Figure 3-5A), preliminary results showed no difference in percent zoospore germination when placed on 8 or 16 DPP fruits (Figure 3-5B). However, short germ-tubes were more prevalent on 8 DPP fruit, while medium and long germ tubes were more frequent on 16 DPP fruit. Appressoria formation was significantly higher on 8 DPP than 16 DPP fruit, whereas aberrant germ tubes were significantly higher on 16 DPP fruit.

Effect of fruit position

P. capsici infected cucumber fruit in the field are typically covered with mycelium on the blossom end of the fruit rather than the stem end (Figure 3-6A). To determine whether this phenomena is due to greater tendency of the blossom end of the fruit to touch the soil, or if there is a difference in susceptibility due to position on the fruit, hand-pollinated greenhouse grown fruit (ranging 6 to 14 DPP) were inoculated with *P. capsici* mycelium approximately 2 cm from the blossom end and the peduncle end (Figure 3-6B). When fruit were very young (6 DPP), both the blossom end and the

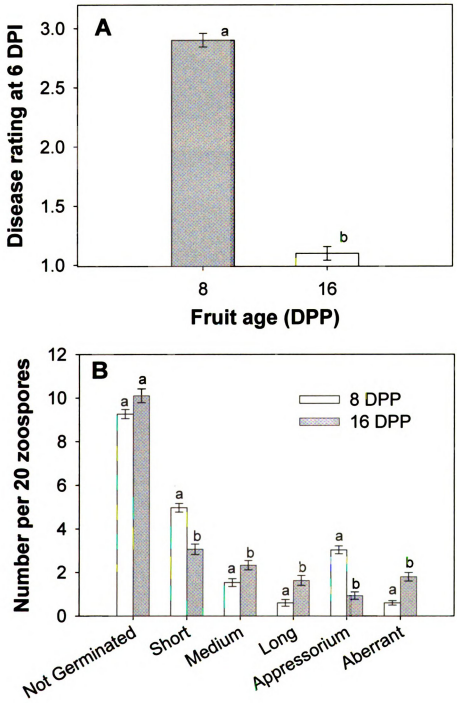
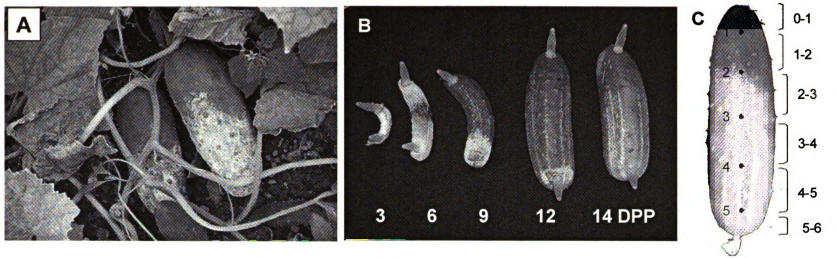


Figure 3-5. A. Mean disease rating of 8 and 16 days post pollination (DPP) to infection by *Phytophthora capsici* zoospore suspension at 6 days post inoculation (DPI). Disease rating: 1 – no symptoms, 2 – water soaking, and 3 – sporulation. B. Zoospore response on the surface of 8 and 16 DPP cucumber fruits. Each value is the mean ± SE of 10 fruit, 3 subsamples/fruit, 20 zoospores/subsample. Germ tube length: short <1x zoospore cyst diameter; medium 1-5x diameter; long >5x diameter.

Figure 3-6. A. Typical symptoms of *Phytophthora capsici* in the field - mycelium cover the blossom end of fruits. B, Mycelium inoculation of the peduncle and blossom ends of 6 to 14 days post pollination (DPP) fruit. The picture was taken at 4 days post inoculation (DPI). C. Equally spaced five dots (1 – peduncle end, 5 – blossom end) were drawn on the surface of each fruit to measure growth from various parts of fruit. D. Symptoms of fruit ranging from 6 to 14 DPP were evaluated at 4 DPI (0 – no symptom, 1 – water soaking, and 2 – sporulation). Each point is the mean of a minimum of 10 fruits ± SE. E. Fruit growth from peduncle (1-2) and blossom end (4-5) which were measured daily up to 34 DPP. Each point is a mean of 4-10 fruits.


A
B
3
6
9
12
14 DPP
C
0-1
1-2
2-3
3-4
4-5
5-6
2
3
4
5
D
Blossom-end
Stem-end
Disease rating at 4 DPI
Days post pollination (DPP)
E
1-2
4-5
Accumulative growth (cm)
Days post pollination (DPP)

peduncle end sporulated (Figure 3-6D). However, as fruit develop, the peduncle end becomes less susceptible earlier than the blossom end, so that sporulation occurs on the blossom end, but not the peduncle end (e.g. at 8-12 DPP). At 14 DPP the peduncle end did not develop symptoms whereas the blossom end sometimes developed water soaked regions. Growth analysis of developing fruit showed that the peduncle end stopped elongating earlier (10-12 DPP) than the blossom end (14-16 DPP) (Figure 3-6C, E).

Age-related resistance of fruit from other cucurbit species to *P. capsici*

Additional types of cucurbit fruit were tested to see if they also exhibit age related resistance. The fruit of different cucurbit crops exhibited variability for overall susceptibility (Figure 3-7). When fruit of all sizes were grouped together, summer squash, zucchini and melon were the most susceptible with the greatest percent producing sporulating lesions at 4 and 10 DPI and the shortest time to 50 % sporulating fruit (Figure 3-7D-F). Both summer squash and zucchini were also notable for rapid development of water soaking symptoms; more that 50 % of the tested fruit exhibited water soaking by 1 DPI (Figure 3-7A). On summer squash, water soaking symptoms occurred on 85 % of the tested fruit by 1 DPI. Watermelon, acorn squash, pumpkin, butternut squash, and cucumber were less susceptible with rare sporulation of *P. capsici* at 4 DPI (Figure 3-7E). These fruit were more likely to develop water soaking symptoms that did not progress to sporulation, even at 10 DPI (Figure 3-7B,C,F).

For almost all of the crops tested, susceptibility decreased with increasing fruit size, but to varying degrees (Figure 3-8; $P<0.01$ for all crops except melon and watermelon). Cucumber showed the most dramatic effect with near complete resistance as the fruit developed (Figure 3-8A). Butternut squash, pumpkin, and acorn squash also

Figure 3-7. Response of different cucurbit fruit to inoculation with *Phytophthora capsici.* A, D. Days after inoculation for 50 % of the fruit to exhibit water soaked (A) or sporulating lesions (D). B, C. Percent fruit showing water soaking symptoms at 4 (B) and 10 (C) days post inoculation (DPI). E, F. Percent fruit showing sporulation at 4 (E) and 10 (F) DPI. Each value is the mean of fruit from two or three replicate harvest dates (where error bars are absent, the days to symptom development on 50 % of the fruits was the same for each replicate date). Each bar is the mean ± SE of 45-93 fruit. Bars with the same letter are not significantly different at $P \leq 0.05$ (LSD).

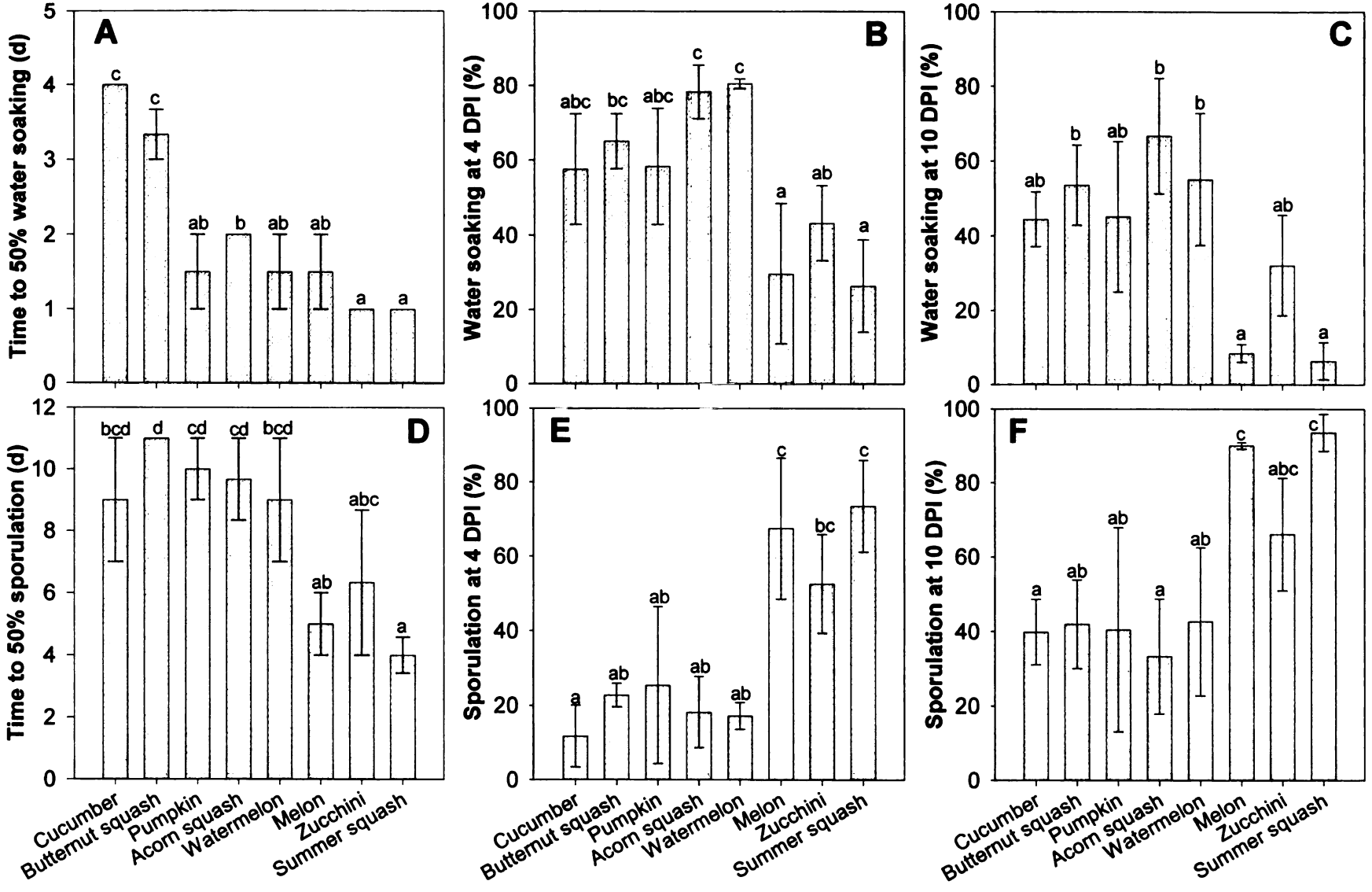

A
Time to 50% water soaking (d)
0
1
2
3
4
5
c
c
ab
b
ab
ab
a
a
B
Water soaking at 4 DPI (%)
0
20
40
60
80
100
abc
bc
abc
c
c
a
ab
a
C
Water soaking at 10 DPI (%)
0
20
40
60
80
100
ab
b
ab
b
b
a
ab
a
D
Time to 50% sporulation (d)
0
2
4
6
8
10
12
bcd
d
cd
cd
bcd
ab
abc
a
E
Sporulation at 4 DPI (%)
0
20
40
60
80
100
a
ab
ab
ab
ab
c
bc
c
F
Sporulation at 10 DPI (%)
0
20
40
60
80
100
a
ab
ab
a
ab
c
abc
c
Cucumber
Butternut squash
Pumpkin
Acorn squash
Watermelon
Melon
Zucchini
Summer squash

Figure 3-8. Fruit size of field-grown cucurbit fruit vs. response to inoculation by *Phytophthora capsici* at 4 days post-inoculation (DPI). Disease symptom scale: 1 – no symptoms; 2 – water soaking; 3 – sporulation. Each point is the mean ± SE of 2-8 fruit in a given size class. Regression lines, r^2 and *P*- values, and total number of fruits tested (n) are indicated in each panel.

A Cucumber
y=2.44-0.11x+9.96x²
r²=0.92
P<0.001
n=62
B Buttermut squash
r²=0.88
P<0.001
n=72
y=2.76-0.18x+6.08x²
C Pumpkin
r²=0.77
P=0.01
n=48
y=2.86-0.25x+9.69x²
D Acorn squash
y=2.47-0.112x+4.41x²
r²=0.73
P<0.01
n=87
E Watermelon
r²=0.46
P=0.09
n=44
y=2.50-0.11x+5.40x²
F Melon
r²=0.52
P=0.16
n=66
y=2.44-0.17x+0.03x²
G Zucchini
r²=0.87
P<0.001
n=86
y=2.70-0.18x+0.01x²
H Summer squash
r²=0.62
P<0.001
n=91
y=2.94-0.03x+0.0006x²
Disease rating at 4 DPI
Fruit length (cm)

exhibited reduced susceptibility with increasing size, but not as completely as with cucumber (i.e., fewer had no symptoms; Figure 3-8B-D). Watermelon, melon, zucchini, and summer squash remained more susceptible. There was a modest decline for zucchini and summer squash with increasing size, but the majority of fruit exhibited water soaking (disease rating above 2; Figure 3-8E-H).

To more carefully examine the effect of fruit development on disease susceptibility, greenhouse-grown, hand-pollinated butternut squash, pumpkin, and acorn squash fruit of known ages were tested for response to *P. capsici* inoculation. All three crops also showed strong age and size related decrease in susceptibility to *P. capsici* (Figure 3-9), similar to what we had observed previously in the greenhouse for cucumber (Gevens et al., 2006) and with field fruit from this study. The majority of instances of *P. capsici* sporulation occurred on fruit in the first few days post-pollination, 0-3 DPP for acorn squash, 0-5 DPP for butternut squash, and 0-8 DPP for pumpkin. Older fruit exhibited water soaking without sporulation, or did not show any symptoms of infection.

The decrease in susceptibility corresponded with visible changes in exocarp properties associated with fruit development (Table 3-1). Very young fruit of all crops were green and very waxy, virtually all fruit at this stage produced sporulating lesions. After a few days (3-4 days for acorn squash and butternut squash, 10 days for pumpkin), they lost wax but remained green. Susceptibility decreased between waxy green and green fruit stages for all three crops where fruit developed water soaked lesions that did not go on to sporulate. Each crop subsequently underwent color change/development according to their maturity type. Size did not increase significantly once color change began (Table 3-1). Butternut squash had little decline after the green non-waxy stage; the

Figure 3-9. Relationship between fruit age and size and response to inoculation by *Phytophthora capsici* at 4 days post-inocuation (DPI) for greenhouse-grown fruit. A, B, C. Response of butternut squash (A), pumpkin (B), and acorn squash (C) fruit to *P. capsici* inoculation with respect to fruit age and length. D, E, F. Mean disease rating (± SE) of butternut squash (D), pumpkin (E), and acorn squash (F) fruit in relation to fruit age. Each data point is the mean of 2-16 fruit. Disease rating scale: 1 – no symptom; 2 – water soaking; 3 – sporulation. Regression lines, r^2 and *P*- values, and total number of fruits tested (n) are indicated in each panel.

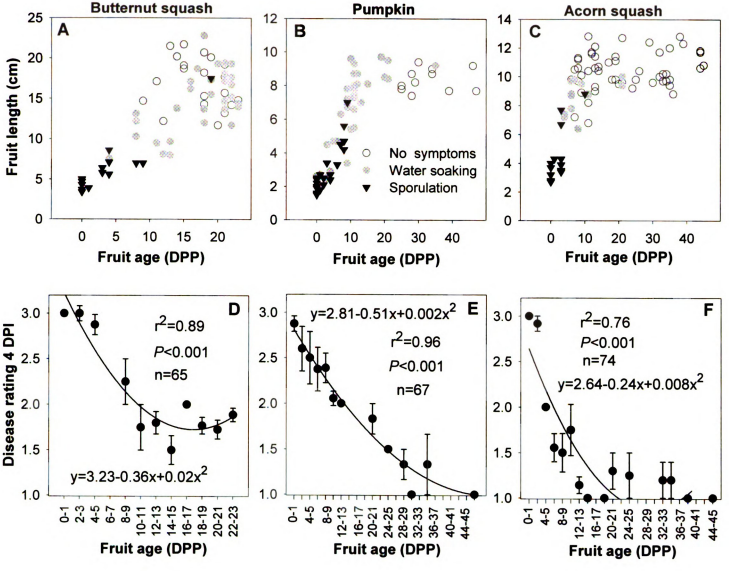

Butternut squash
Pumpkin
Acorn squash
A
B
C
Fruit length (cm)
Fruit age (DPP)
No symptoms
Water soaking
Sporulation
D
E
F
Disease rating 4 DPI
r2=0.89
P<0.001
n=65
y=3.23-0.36x+0.02x2
y=2.81-0.51x+0.002x2
r2=0.96
P<0.001
n=67
r2=0.76
P<0.001
n=74
y=2.64-0.24x+0.008x2

Table 3-1. Age, size, and response to inoculation by *Phytophthora capsici* of greenhouse grown cucurbit fruits at various stages of development as determine by visual properties.

Crop	Exocarp color	Fruit age		Fruit length	% fruit with sporulation at 4 DPI±SE[x]	% fruit with water soaking at 4 DPI±SE[x]
		[mean±SE (DPP)][z]	[range (DPP)][z]	[mean±SE(cm)]		
Butternut squash	Waxy green	1.8±0.5	0-4	5.4±0.4a[y]	100±0a[y]	0.0±0.0a[y]
	Green	12.8±1.1	8-22	13.2±1.0b	8.3±8.3b	91.7±8.3b
	Green/Tan	18.4±0.6	12-22	16.3±0.6c	1.9±1.9b	93.9±3.8b
	Tan	20.9±0.6	18-23	17.2±1.1c	0.0±0.0b	100±0.0b
Pumpkin	Waxy green	2.2±0.6	0-10	2.8±0.2a	87.5±12.5a	12.5±12.5a
	Green	10.3±2.1	5-21	6.8±1.4b	12.5±4.2b	87.5±4.2b
	Green/orange	21.3±1.3	19-25	8.9±0.4c	0.0±0.0b	100±0.0b
	Orange	34.2±1.9	25-47	8.6±0.2c	0.0±0.0b	41.7±8.3a
Acorn squash	Waxy green	1.5±0.8	0-3	4.1±0.7a	100±0.0a	0.0±0.0a
	Green	11.6±1.0	4-29	9.6±0.3b	3.3±1.7b	56.8±8.4b
	Black	27.3±2.1	7-44	10.4±0.2c	2.6±1.3b	7.9±3.9a

[z]DPP – days post pollination.
[y]Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ (LSD).
[x]DPI – days post inoculation.

majority of fruit still exhibited water soaking symptoms. For pumpkin, there was no significant difference in susceptibility between green and green/orange stage; however there was further significant decrease upon turning orange (water soaking decreased to 39 %). Acorn squash showed a further decrease in susceptibility when turning from green to black as evidenced by a decrease in the percent fruit exhibiting water soaking (59 % to 10 %).

Comparison of peduncle and blossom end susceptibility of the field-grown cucurbit fruits showed differences in susceptibility for zucchini, watermelon, butternut and cucumber (Table 3-2). Significant differences between peduncle and blossom ends were also seen for greenhouse-grown butternut and acorn squashes. Greenhouse-grown pumpkins, similar to the field-grown fruit, did not show a significant difference in susceptibility between the peduncle and blossom ends.

Table 3-2. Symptom development on peduncle and blossom ends of cucurbit crops in response to infection by *Phytophthora capsici* at 4 days post inoculation (DPI).

Species	Crop	Cultivar	Symptom comparison peduncle (p) vs. blossom (b) end at 4 DPI							
			Field				Greenhouse			
			p > b[y]	p = b	p < b	χ^{2x}	p > b[y]	p = b	p < b	χ^{2x}
Cucumis sativus	Cucumber	Vlaspik	4	28	33	22.0^{***}				
Cucurbita moschata	Butternut	Waltham Butternut	7	41	23	8.0^{***}	2	63	23	16.8^{***}
Cucurbita pepo	Pumpkin	Baby Pam	5	37	8	0.48^{NS}	8	120	10	0.13^{NS}
Cucurbita pepo	Acorn	Royal Acorn Squash/ Autumn Delight[z]	2	81	4	0.38^{NS}	4	59	13	4.25^{*}
Citrullus lanatus	Watermelon	Crimson Sweet	2	43	10	4.68^{*}				
Cucumis melo	Melon	Odyssey	22	36	12	2.65^{NS}				
Cucurbita pepo	Zucchini	Black Beauty	6	54	36	20.7^{***}				
Cucurbita pepo	Summer-squash	Horn of Plenty Premium	0	87	5	4.05^{*}				

[z]Field: 'Royal Acorn Squash', greenhouse: 'Autumn Delight.'

[y]p>b, p=b, p<b disease progression on peduncle end greater than, equal to, or less than blossom end, respectively

[x]Blossom/peduncle end comparisons of the same fruit were performed by using chi-square contingency analysis

$^{NS, *, **, ***}$ χ^2value nonsignificant of significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Discussion

Screening of approximately 150 cultigens did not identify a source of resistance superior to the partial resistance already present within many commercial cultivars. The screening process led to the observation that there are developmental differences in cucumber fruit susceptibility that can influence proper screening procedures and may also influence effective disease management strategies in the field.

Most fruits younger than 10 DPP exhibited sporulation at 4 DPI, whereas fruit older than 12 DPP generally remained symptom-free Interestingly, the transition from susceptible to resistant appeared to coincide with the end of the period of rapid fruit elongation, suggesting that physiological changes associated with fruit growth and development might explain changing susceptibility among cucumber fruit of different ages. The effect of fruit age was observed for several different cucumber cultivars and accessions. In addition, field-grown cucumbers, harvested to test a range of fruit sizes, exhibited the same trend as observed with greenhouse-grown fruits. These results indicated that there are changes in the degree of susceptibility among fruits of different ages in both greenhouse and field conditions, and that the age-related decrease in susceptibility is not genotype specific.

These observations have implications for appropriate screening procedures. If harvested fruits are too mature, resistance may be observed which is not constitutive, but is solely age-related. This maturity factor could be responsible for the failure to observe resistance upon rescreening in several cases. The lack of significant decline in susceptibility between 7- and 14-day old fruit for two of the PIs may be due to genetic

differences in susceptibility, or differences in rates of fruit development, such that 14 DPP fruit are not physiologically equivalent among all PIs.

Age-related resistance (ARR) is becoming increasingly recognized as an important component of plant defense against infection (Develey-Rivière and Galiana, 2007; Panter and Jones, 2002; Whallen, 2005). This phenomenon, which results in increased resistance as young tissues develop, is distinct from the increase in susceptibility that occurs in old, mature organs as the result of the ripening and senescence. The mechanisms of ARR are largely unknown. There is evidence that salicylic acid is important to ARR in Arabidopsis response to *Pseudomonas synringae* (Kus et al., 2002) and *Hyaloperonospora parasitica* (McDowel et al., 2005). ARR in rice (*Oryza sativa*) to *Xanthomonas spp.* (Century et al., 1999; Koch and Mew, 1991; Mazzola et al., 1994) may be related to developmentally-regulated *R* gene-mediated resistance which is controlled post-transcriptionally or by other mechanisms. Developmentally-regulated expression of single-gene mediated resistance also has been observed for potyvirus infection of cucumber seedlings (Ullah and Grumet, 2002; Wai and Grumet, 1995). Pepper (*Capsicum annuum*) plants also showed ARR to infection by *P. capsici* (Kim et al., 1989). The reduced susceptibility was suggested to be related to physiological changes in root and stem tissues.

Similar to the response of cucumber fruit to *P. capsisi*, developing grape berries (*Vitis vinifera*) also showed decreased susceptibility to infection by *Unicinula necator*, causal agent of powdery mildew, and to *Plasmopara viticola*, causal agent of downy mildew (Ficke et al., 2002; Gadoury et al., 2003; Kennelly et al., 2005). Berries are highly susceptible for the first several weeks after anthesis. Developmental changes in

berries, such as brix content or morphological change of stomata to lenticels were thought to be related to susceptibility changes, however, the mechanism has not been determined.

Examination of the age related resistance in cucumber fruit, showed that resistance was associated with the outer 1-2 mm of fruit surface. Thin sections of exocarp retained the resistance of susceptibility of the whole fruit. More short germ-tubes and appressoria, which are associated with rapid and successful penetration (Grenville-Briggs et al., 2008), were formed on the 8 DPP fruit surfaces, whereas more long germ tube and aberrant spores were found on the surface of 16 DPP fruits. This difference could be due to cuticle properties as was observed to be a physical barrier to *P. capsici* infection in New Mexican-type pepper (*C. annuum*) (Biles et al., 1993). Another possibility is a difference in frequency of stomata, since Smith et al. (1979) reported that stomata are almost absent from peduncle end compared to the blossom end, and are less frequent in larger (older) fruits relative to younger fruit. Whether the *P. capsici* zoospores primarily enter through cucumber fruit stomata remains to be determined, although that did not appear to be the case in the samples we obtained. Other possibilities include changes in cell wall properties, production of defense compounds, or induced resistance mechanisms. Further experiments will be required to determine the properties responsible for the change in susceptibility that occurs during change as fruit develop. Consistent with resistance residing in the exocarp, seed development did not influence the occurrence of ARR in cucumber.

Other cucurbit fruit exhibited variability for overall susceptibility to *P. capsici* as evidenced by number of fruits infected, time to sporulation, and extent of infection (water soaking vs. sporulation). Summer squash, zucchini, and melon were the most

susceptible, whereas watermelon, acorn squash, pumpkin, butternut squash, and cucumber were less susceptible. When comparing crops, susceptibility did not correlate with taxonomic classification. Of the four *C. pepo* crops, summer squash and zucchini were highly susceptible, whereas acorn squash and pumpkin were less susceptible. Also, thickness of rind did not correlate with the susceptibility, since muskmelon was one of the most susceptible and cucumber one of the least.

Field and greenhouse grown cucurbit fruits tested in this study also showed a general tendency of decreasing susceptibility with fruit development, but to varying degrees. Acorn squash, butternut, and pumpkin exhibited reduced susceptibility with increasing size, but not as complete as with cucumber. Despite the variations in fruit types and sizes, the transition to reduced susceptibility, appeared to correlate with the end of the rapid fruit elongation, as had been observed for cucumber fruit (Gevens et al., 2006). Similar to the differences in overall susceptibility, zucchini and summer squash remained highly susceptible even as they increased in size. The majority of the watermelon and melon fruits also developed water soaking or sporulation symptoms regardless of size.

Acorn squash, butternut squash, and pumpkin showed changes in exocarp features that corresponded with reduced susceptibility. The transition from waxy green to green exocarp was accompanied by a marked reduction in susceptibility, further suggesting that changes in exocarp properties with fruit development might influence susceptibility, although presumably many other changes are also occurring as this stage of development.

Another feature observed in cucumber, is a difference in susceptibility between the peduncle and blossom end of the fruit. In *Phytophthora* infested fields, the blossom

end is more frequently infected than the peduncle end. While more frequent or earlier contact with the soil may be a contributing factor to this observation, comparison tests of both greenhouse and field-grown fruit showed that the blossom end remained susceptible longer than the peduncle end. These results indicate that there is a gradation of susceptibility within the fruit such that the peduncle end of the fruit becomes less susceptible to *P. capsici* sooner than the blossom end. Cucumber fruit growth analyses indicated that growth ceases at the peduncle end prior to the blossom end, suggesting that differences in susceptibility may result from differences in physiological maturity between two ends and corresponds with the age-related resistance observed in whole fruit.

There also was a difference in susceptibility between peduncle and blossom ends of fruit of several of the crops. The difference was most pronounced in zucchini and butternut squash fruits. The results may reflect differences in the pattern of fruit growth and maturation for the different species. Interestingly, the three (cucumber, zucchini, and butternut squash) with the most significant differences between fruit ends, have the most elongated fruit shape, possibly allowing for a larger gradient in maturation between stem and blossom ends.

In conclusion, cucurbit fruit exhibited an age-related decrease in susceptibility to *P. capsici* where very young rapidly expanding fruit were the most highly susceptible. There was a gradation of susceptibility within elongated fruit types such that the peduncle end of the fruit became less susceptible to *P. capsici* sooner than the blossom end. In cucumber this difference in susceptibility correlated with a difference in the period of rapid elongation along the length of the fruit. Peel and zoospore germination analyses

indicate that the increased resistance in cucumber appears to be associated, at least in part, with exocarp properties.

Information about age-related resistance can be helpful in developing better integrated pest management strategies (Develey-Rivière and Galiana, 2007; Ficke et al., 2002). The above observations suggest the importance of protecting developing cucurbit fruit from anthesis through the early period of rapid longitudinal growth. Timing of chemical applications based when target tissue is most susceptible, could allow for more effective disease control and also can minimize unnecessary chemical applications.

Literature Cited

Ando, K. and R. Grumet. 2006. Evaluation of altered cucumber plant architecture as a means to reduce *Phytophthora capsici* disease incidence on cucumber fruit. J. Amer. Soc. Hort. Sci. 131:491-498.

Babadoost, M. 2004. *Phytophthora* blight: a serious threat to cucurbit industries. APSnet Feature Story April-May 2004. http://www.apsnet.org/online/feature/cucurbit/.

Biles, C. L., M. M. Wall, M. Waugh, and H. Palmer. 1993. Relationship of *Phytophthora* fruit rot to fruit maturation and cuticle thickness of New Mexican type peppers. Phytopathology. 83:607-611.

Bird, G., B. Bishop, E. Grafius, M. Hausbeck, L. Jess, W. Kirk and W. Pett. 2005. Insect, disease and nematode control for commercial vegetables. Michigan State University Ext. Bull. E-312.

Century, K. S., R. A. Lagman, M. Adkisson, J. Morlan, R. Tobias, K. Schwartz, A. Smith, J. Love, P. C. Ronald, and M. C. Whalen. 1999. Developmental control of *Xa21*-mediated disease resistance in rice. Plant J. 20:231-236.

Develey-Rivière, M. P. and E. Galiana. 2007. Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytologist. 175:405-416.

Ficke, A., D. M. Gadoury, and R. C. Seem. 2002. Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytophathology. 92:671-675.

Gadoury, D. M., R. C. Seem, A. Ficke, and W. F. Wilcox. 2003. Ontogenic resistance to powdery mildew in grape berries. Phytopathology. 93:547-555.

Gevens, A. J., K. Ando, K. Lamour, R. Grumet, and M. K. Hausbeck. 2006. Development of a detached cucumber fruit assay to screen for resistance and effect of fruit age on susceptibility to infection by *Phytophthora capsici*. Plant Dis. 90:1276-1282.

Grenville-Briggs, L. J., V. L. Anderson, J. Fugelstad, A. O. Avrova, J. Bouzenzana, A. Williams, S. Wawra, S. C. Whisson, P.R. J. Birch, V. Bulone, and P. van West. 2008. Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato. Plant Cell 20:720-738.

Hausbeck, M. and K. Lamour. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Dis. 88:1292-1302.

Iwaro, A. D., T. N. Sreenivasan, and P. Umaharan. 1997. *Phytophthora* resistance in cacao (*Theobroma cacao*): Influence of pod morphological characteristics. Plant Pathol. 46:557-565.

Kennelly, M. M., D. M. Gadoury, W. F. Wilcox, P. A. Magarey, and R. C. Seem. 2005. Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. Phytopathology. 95:1445-1452.

Kim, Y. J., B. K. Hwang, and K. W. Park. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. Plant Dis. 73:745-747.

Knerr, L. D., Staub, J. E., Holder, D. J., and May, B. P. 1989. Genetic diversity in *Cucumis sativus* L. assessed by variation at 18 allozyme coding loci. Theor. Appl. Genet. 78:119-128.

Koch, M. F. and T. W. Mew. 1991. Effect of plant age and leaf maturity on the quantitative resistance of rice cultivars to *Xanthomonas campestris* pv. *oryzae*. Plant Dis. 75:901-904.

Kus, J. V., Z. Zaton, R. Sarkar, and R. K. Cameron. 2002. Age-related resistance in Arabidopsis is a developmentally regulated defense response to *Psudomonas syrinagae*. Plant Cell. 14:479-490.

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.

Mazzola, M., J. E. Leach, R. Nelson, and F. F. White. 1994. Analysis of the interaction between *Xanthomonas oryzae* pv. *oryzae* and the rice cultivars IR24 and IRBB21. Phytopahtology. 84:392-397.

McDowell, J. M., S. G. Williams, N. T. Funderburg, T. Eulgem, and J. L. Dangl. 2005. Genetic analysis of developmentally regulated resistance to downy mildew (*Hyaloperonospora parasitica*) in *Arabidopsis thaliana*. Mol. Plant-Microbe Interactions. 18:1226-1234.

Ngouajio, M., G. Wang, and M. K. Hausbeck. 2006. Changes in pickling cucumber yield and economic value in response to planting density. Crop Sci. 46:1570-1575.

Padley, Jr., L. D., E. A. Kabelka, and P. D. Roberts. 2009. Inheritance of resistance to crown rot caused by *Phytophthora capsici* in Cucurbita. HortScience 44:211-213.

Padley, Jr., L. D., E. A. Kabelka, P. D. Roberts, and R. French. 2008. Evaluation of *Cucurbita pepo* accessions for crown rot resistance to isolates of *Phytophthora capsici*. HortScience. 43:1996-1999.

Panter, S. N. and D. A. Jones. 2002. Age-related resistance to plant pathogens. Adv. Botanical Res. 38:251-280.

Ristaino, J. B., G. Parra, and C. L. Campbell. 1997. Suppression of *Phytophthora* blight in bell pepper by a no-till wheat cover crop. Phytopathology. 87:242-249.

Smith, K. R., H. P. Fleming, C. G. van Dyke, and R. L. Lower. 1979. Scanning electron microscopy of the surface of pickling cucumber fruit. J. Amer. Soc. Hort. Sci. 104:528-533.

Steel, R.G.D and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill Book Co. New York.

Ullah, Z. and R. Grumet. 2002. Localization of zucchini yellow mosaic virus to the veinal regions and role of viral coat protein in veinal chlorosis conditioned by the *zym* potyvirus resistance locus in cucumber. Physiol. Mol. Plant Pathol. 60:79-89.

Wai, T. and R. Grumet. 1995. Inheritance of watermelon mosaic virus resistance in the cucumber line TMG-1: tissue-specific expression and relationship to zucchini yellow mosaic virus resistance. Theor. Appl. Genet. 91:699-706.

Wang, G. and M. Ngouajio. 2008. Integration of cover crop, conservation tillage, and low herbicide rate for machine-harvested pickling cucumbers. HortScience. 43:1770-1774.

Whallen, M. C. 2005. Host defence in a developmental context. Mol. Plant Pathol. 6:347-360.

Chapter 4

Development of genomic analysis tools for early developing cucumber fruit

Abstract

Cucumber fruits are usually harvested and consumed at a young, immature stage. Many studies have been published on fruit development of various species, however most focus on late stages of development and important fruit quality attributes associated with maturation and ripening, rather than early development. To increase understanding of fruit development in cucumber, morphological and global gene expression analyses were performed. Hand-pollinated greenhouse grown fruit at 0, 4, 8, 12, 16, 20, 26, and 32 days post pollination (DPP) were monitored. Young cucumber fruits (0-16 DPP) show marked changes in fruit size, cell size, surface wax, chlorophyll content and patterns, wart formation, spine development, and placenta and seed development. Pyrosequencing technology '454' analyses of rapidly growing 8 DPP fruit yielded 187,406 clean reads, of which 88 % could be assembled into 13,879 contigs, 52-2,824 nt in length. The number of sequences per contig, which is reflective of transcript abundance, ranged from 2-5,167. BLAST analysis of the most highly represented transcripts against the nr protein sequence NCBI database, indicated high representation of genes associated with protein synthesis, flowers, fruits or seeds of other species, latex related proteins, lipid biosynthesis, cell expansion, defense, phloem transport, and photosynthesis. Results of 454 and qRT-PCR for selected genes sampled at different ages were comparable,

indicating that the 454 transcriptome sequencing can be used for analyzing relative gene expression during fruit development.

Introduction

Cucumber fruit rot caused by the soil borne Oomycete pathogen, *Phytophthora capsici* results in severe yield loss that endangers the pickling cucumber industry (Hausbeck and Lamour, 2004). In the preceding chapter, existence of age related resistance (ARR) was revealed between cucumber (*Cucumis sativus*) fruit and *P. capsici*. Inoculation of greenhouse grown fruits of known age showed that young, developing cucumber fruit are highly susceptible to *P. capcisi*, while older fruit remained symptom free. There was a sharp transition from susceptible to resistant that occurs in early fruit development at around 10-12 days post pollination (DPP). The ARR appears to coincide with the end of the rapid fruit elongation (Gevens et al., 2006), both among fruits of different ages and along the length of individual fruits where the peduncle end stops elongating sooner than the blossom end. This ARR was further observed from fruit grown in the greenhouse and field, and was also observed across several genotypes and a variety of other cucurbit crops tested. Analyses of changes in gene expression during fruit development may help us to gain insights into understanding the basis for this phenomenon.

The development of next-generation sequencing technologies has dramatically improved sequencing capability with respect to amount of data, time, and cost (Mardis, 2008; Margulies et al., 2005) and has allowed for new methods for gene expression

analysis. Roche (Branford, CT) introduced 454 pyrosequencing technology (Margulies et al., 2005; Droege and Hill, 2008) which can sequence over 100 Mb in 7.5 hrs with average reading of 200 bp. A new version of 454, GS FLX Titanium Series reagent, was introduced in 2008 which can sequence over 400 Mb with average reading of 400 bp. The length of reads generated by 454 pyrosequencing allow for contig assemblies and gene annotations of poorly characterized genomes. In addition, one of the applications of the new sequencing technology is gene expression analysis, since the number of contig reads represents frequency of the particular gene expression (Eveland et al., 2008; Ohtsu et al., 2007; Torres et al., 2008). 454 sequencing also has capability of loading multiple samples for a run, allowing side-by-side analysis. Gene expression analyses by 454 pyrosequencing results have been correlated with microarrays, northern blot analysis or quantitative real time polymerase chain reaction (qRT-PCR) assays, suggesting high reproducibility and suitability for gene expression analysis (Eveland et al., 2008). It also can be used as an alternative to microarrays, especially for crops with small ESTs numbers and/or no microarray platform available, such as cucumber.

Cucumber genomic tools are limited. The number of cucumber ESTs in the National Center for Biotechnology Information (NCBI) database is only approximately 8,000 (4/15/2009 searched) compared to other species such as *Arabidopsis* - 1,728,728, rice (*Oryza sativa*) - 1,260,123, and tomato (*Solanum lycopersicum*) - 261,630. In the past year, however, the cucumber genome was reported to be sequenced by a group at the Chinese Academy of Agriculture Science and the Beijing Genomics Institute, Shenzhen (Huang et al., 2009). It is anticipated that the 365 Mb sequenced genome will be publicly available in the near future. Having sequenced genome and generating more ESTs will

be a tremendous advantage not only for contig assemblies and gene annotations, but also for providing tools to study complicated biology of plants.

Model plants, such as *Arabidopsis thaliana* and rice (*O. sativa*) are well studied in many aspects in plant development with the aid of tremendous information generated by genomic tools. Fully sequenced genomes, microarrays, and large numbers of ESTs and mutants are available. However, these species are not well suited to address questions related to fleshy fruit development. In case of fleshy fruit, tomato (*S. lycopersicum*), is a well studied model plant (Carrari and Fernie, 2006; Giovannoni, 2004; Moore et al., 2002; White, 2002). The primary emphasis of fruit studies has been on late stage of development and important fruit quality attributes associated with maturation and ripening (Lee et al., 2007; Lemaire-Chamley et al., 2005). There has been less study done on early fleshy fruit development, though this stage is essential for all fruit. Deciphering early development of fleshy fruit is important, since it influences yield and quality of all fruits. Cucumber is one of the most important vegetable crops worldwide that is harvested and consumed at a young, immature stage. Therefore, cucumber is suitable to study early fruit development.

The objectives of the research presented in this chapter are to increase understanding of early fruit development in cucumber by performing morphological and global gene expression analysis. Morphological changes associated with cucumber fruit development which could anchor the subsequent gene expression analyses were catalogued for hand pollinated greenhouse grown fruits. Different cucumber fruit growth stages (0, 4, 8, 12, 16, 20, 26, and 32 DPP) were collected to generate cDNA libraries for 454 pyrosequencing.

Materials and Methods

Cucumber fruit developmental morphological changes

Plant material and cucumber morphlogy

Three sets of 'Vlaspik' cucumber plants, planted on March 29, May 25, and June 14, 2007 were grown in the greenhouse. All sampling and data collection described below were performed on each of the three experiments. Greenhouse conditions were as described in chapter 3. To provide sufficient fruit of various ages developing under the same conditions, for each experiment 80-184 flowers from at least 80 plants were hand pollinated on a single date. Only 1-2 fruit were set per plant to limit inter-fruit competition effects on fruit development. The fruit were randomly assigned to groups of 10-20 for each harvest date. Prior to harvest, fruit were measured for length and diameter and examined for external appearances including: presence or absence of wax along the length of the fruit; wart development; color patterns (e.g., stripes); and changes in presence, color, and densities of spines.

Nine to ten fruits ranked in the middle of the size range for each age group from each experiment were harvested for further sampling for cDNA library construction. Pericarp samples consisting of exocarp, mesocarp, and placenta tissue but no seeds, were isolated from the middle of fruit by razor blade, immediately frozen by liquid nitrogen, and kept at – 80 °C until RNA was isolated as described below. The cut ends of the fruit were measured for pericarp and placenta diameter. Fruit sections adjacent to the cut ends were dissected into approximately 1 cm^3 pieces and fixed in formalin-alcohol-acetic acid (FAA) solution (Olmstead et al., 2007). Free-hand, thin sections (approximately 1 mm thick) of transverse and cross sectional tissues of fruit from five fruit of each sample age

were isolated by razor blade. Each sample was measured for five neighboring consecutive cells to obtain mean cell size and observed at three locations by light microscope at 100x magnification with acridine orange dye (0.1 mg/ml). Impressions of intact 8 and 16 DPP cucumber fruit epidermal tissue were prepared by the method described in chapter 3.

Chlorophyll measurement

An additional five 1 g fruit excocarp samples (upper 1 to 2 mm) were removed by conventional fruit peeler for chlorophyll measurement and stored at – 20 °C. Excocarp samples were subsequently thawed at room temperature and blotted on paper to remove excess water. One gram of isolated exocarp sections was immersed into *N, N*-dimethylformamide for at least 24 hours at 4 °C in dark. Total chlorophyll was calculated based on spectrophotometer absorbance measurements at 665 nm and 647 nm (Inskeep and Bloom, 1985).

Library production and 454 sequencing.

Library construction 1.

The methods for RNA and cDNA sample preparation were adapted from the procedures of Weber et al. (2007). For each age sample, RNA was pooled from 10 fruits. Total RNA was isolated using the Qiagen RNeasy Plant Mini Kit (Qiagen, Valencia, CA)or TRizol reagent (Invitrogen, Carlsbad, CA), treated with Qiagen RNase free DNAse, and assessed for quality by 2100 Bioanalyzer (Agilent, Santa Clara, CA) prior to preparation of mRNA by Qiagen oligotex kit. mRNA was quantified by fluorometer Q-bit (Invitrogen) method and 30 ng of mRNA was used for cDNA construction. First strand cDNA was synthesized using the Creator SMART cDNA library construction kit

(Clontech, Mountain View, CA) with modified CDSIII/3' primer (Schilmiller et al., unpublished) to allow for improved sequencing efficiency. Titration PCR for the second strand cDNA synthesis was performed for each sample to determine the best number of PCR cycles as assessed by 1.1 % TAE agarose gel. Once the optimum cycle was determined, second strand synthesis PCR was performed and quality assessed by 1.1 % TAE agarose gel. In order to increase the quantity of cDNA, secondary PCR was performed for 6-8 cycles. PCR products were purified by Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI). Purified PCR products were digested with *Sfi*I to remove primers and purified by Wizard SV Gel and PCR Clean-Up system. Final concentration was assessed by the nanodrop ND-1000 method (Thermo Fisher Scientific Inc., Wilmington, DE). Subsequent steps for 454 FLX pyrosequencing analysis was performed by the RTSF Genomics Facility at Michigan State University (MSU). Each sample (8 samples from 8 different DPP) was loaded on 1/16 454 Pico TiterPlate.

Library construction 2.

The same frozen 8 DPP pericarp tissues for the prior preparation were used for library constructions. Total RNA was isolated by TRizol reagent, followed by DNase treatment and clean up by RNeasy column. Quality of RNA was assessed by formaldehyde gel (Sambrook and Russell, 2001) and nanodrop method. mRNA was isolated as described earlier and was assessed with nanodrop method. 200 ng of mRNA was used for cDNA construction. First and second strand cDNA synthesis was performed as described above. Secondary PCR cycles were 10. For the 8 DPP – B sample, a modified 5' PCR primer which contains a *Sfi*I cut site was used (AGTGGCCATTACGGCCGGG). The sample preparation procedure is illustrated in

Figure 4-1. Each sample (8 DPP-A and B) was loaded on a 1/4 plate. 454 FLX pyrosequencing analysis was performed as described above.

Contig assembly and gene annotation.

Contigs of trimmed reads were assembled by the MSU bioinformatics group using The Institute for Genomic Research (TIGR) clustering tools pipeline. Reads from all samples were pooled to maximize available sequence data. Sequence data were subjected to BLAST analysis in the green plant subdivision of the NCBI nr protein database to search for homology to previously identified genes, to assist in contig assembly, and to assign possible gene functions. To compare relative abundance between samples, number of reads for a sample is divided by the total number of reads for the sample and then multiplied by 1,000.

Transcriptome analysis

To analyze gene ontology (GO), highly abundant transcripts (≥0.15% transcript frequency) were subjected to BLASTX analysis in The Arabidopsis Information Resource (TAIR V7.0) database to search for homologies in Arabidopsis. Subsequently, The Classification SuperViewer Tool w/Bootstrap web database (Provart and Zhu, 2003) was used for GO categorization.

Highly expressed transcripts (> 0.2 % frequency) also were subjected to BLAST analysis against the International Cucumber Genomic Initiative (ICuGI) EST database to search for presence or absence in current libraries from cucumber flower buds, flowers, and fruit; melon fruit; and watermelon fruit. Presence or absence data were clustered and visualized using Cluster 3.0 and TreeView 1.1.3 software (Eisen et al., 1998; de Hoon et al., 2004; Saldanha, 2004).

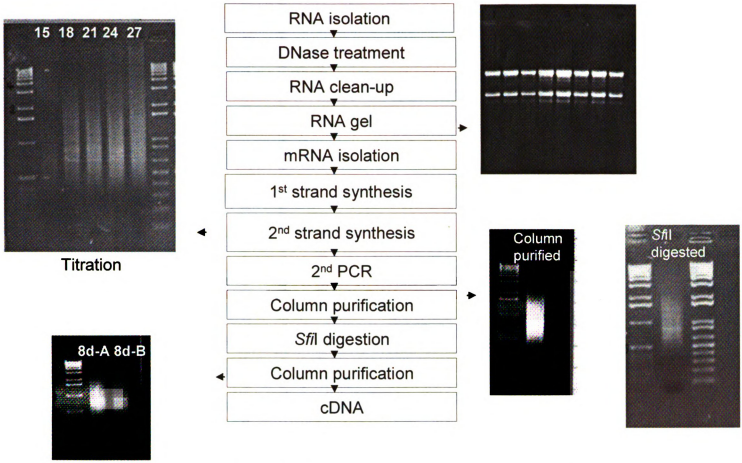


Figure 4-1. Protocol for preparation of cucumber fruit cDNA libraries for 454 pyrosequence analysis.

qRT-PCR

Total RNA was isolated from the same frozen samples described above and assessed for quality and quantity as above. RT reactions were performed using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA). Gene-specific primers were designed using Primer Express software (Applied Biosystems) ABI Prism 7900HT Sequence Detection System was used for qRT-PCR analysis. Power SYBR Green PCR Master Mix (Applied Biosystems) was used for PCR quantification. Actin from *C. sativus* (NCBI DQ641117) was used as an endogenous control for normalization. PCR products from each gene were quantified with reference to corresponding standard curves.

Results

Morphological changes during cucumber fruit development

The progression and timing of growth and developmental processes were highly reproducible across all three experiments. Fruit growth occurred rapidly after fertilization with most rapid elongation occurring between 2 and12 DPP (Figure 4-2A). Expansion of fruit diameter was observed for somewhat a longer time between 2 and 17 DPP. Microscopic analysis of cell size, regardless of their orientation, also showed rapid cell size enlargement between 4 and 12 DPP (Figure 4-2B-D). Thicker cell walls were observed from 16 DPP than 8 DPP epidermal cells (Figure 4-2E and F).

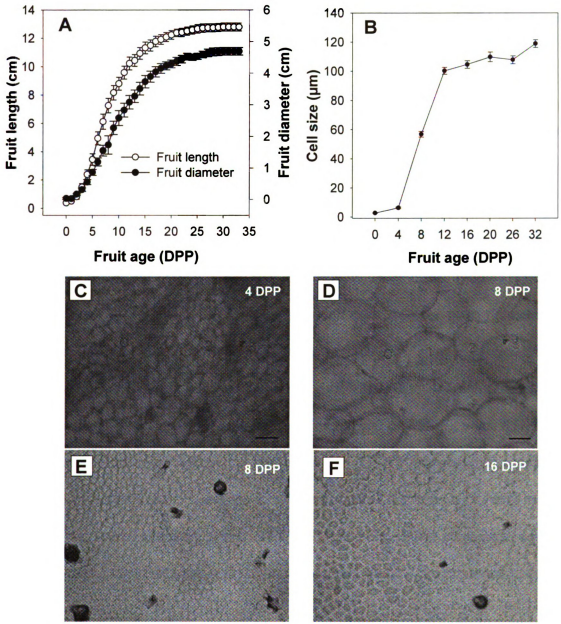


Figure 4-2. Cucumber fruit cell size in relation to fruit age. A. Fruit length and diameter measured daily up to 34 days post pollination (DPP). Each data point is mean of 4-10 fruit ± SE. B. Fruit cell size was measured from 0, 4, 8, 12, 16, 20, 26, and 32 DPP fruit. Each data point is mean ± SE of 30 fruits. Mesocarp cells from cross section of 4 (C) DPP and transverse section of 8 (D) DPP fruit were taken at 100x with acridine orange stain. Bars = 50 µm. Epidermal tissues of 8 (E) and 16 (F) DPP fruit were taken at 100x.

Morphological changes associated with fruit growth were observed for both external and internal properties. At 0 DPP, deep ridges along the length of fruit covered the surface of the fruit. Spines were randomly scattered relative to the ridges. Spine densities were the highest at 0 DPP and decreased rapidly at 4-8 DPP due to fruit expansion, then remained essentially constant after 12 DPP (Figure 4-3A). Spine color was translucent light green at 0 DPP, then started turning yellow around 8 DPP. Around 12 DPP, spines started to turn white; most were white by 16 DPP at which time many abscised from the fruit surface (Figure 4-3A). Warts, which are formed at the base of spines, were diminutive at 0 DPP, however, they rapidly developed to become highly prominent at 4 DPP (Figure 4-3A). With further fruit expansion, the warts flattened out and were completely absent by 12 DPP. At anthesis (0 DPP), fruit were covered with thick dull-looking powdery wax (i.e., bloom) (Figure 4-3B). The bloom disappeared first from the peduncle end around 4 DPP, then the blossom end by 8 DPP. Some 8 DPP fruit had lost bloom from the middle part of the fruit and by 12 DPP, the bloom disappeared from fruits completely. Stripes and specks on the surface of the fruit were absent in 0-4 DPP fruit, but started to emerge around 8 DPP (Figure 4-3B). Total chlorophyll content was the highest at 0-4 DPP, and then declined continuously until approximately 20 DPP (Figure 4-4).

There also were obvious changes in internal fruit morphology. Both placenta and pericarp rapidly expanded from 4-16 DPP. The rate and amount of expansion was very similar for both tissues (Figure 4-5A). Visible placenta changes included development of gelatinous texture and hardened seed coats (figure 4-5B). The mesocarp turned from

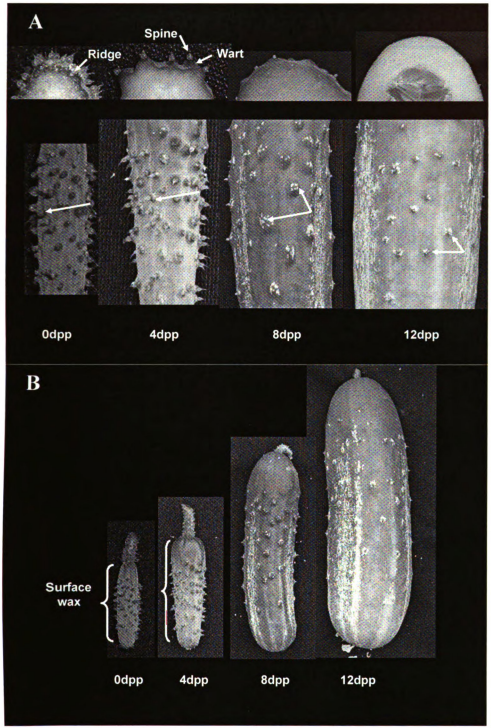


Figure 4-3. Surface property changes in early cucumber fruit development. Spine and wart development (A) and surface wax (bloom) coverage and stripe development (B) in growing cucumber fruit at 0, 4, 8, and 12 DPP fruit. Arrows indicate spine and wart.

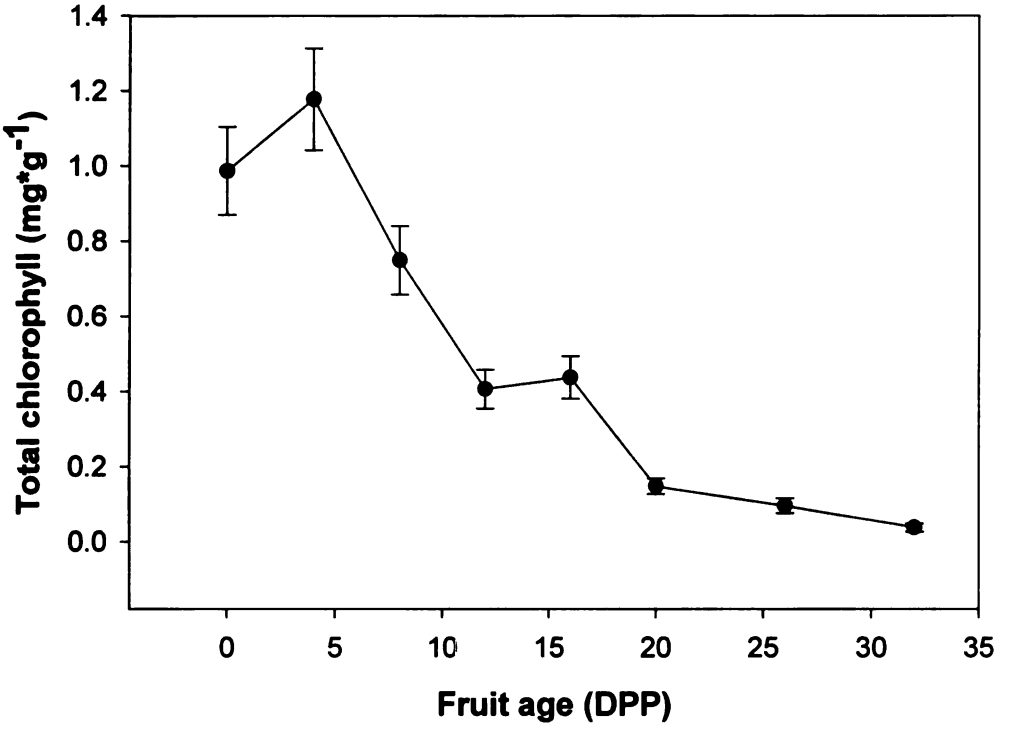


Figure 4-4. Total chlorophyll content in relation to fruit age. Each data point is mean of at least 15 fruit peel/samples ± SE.

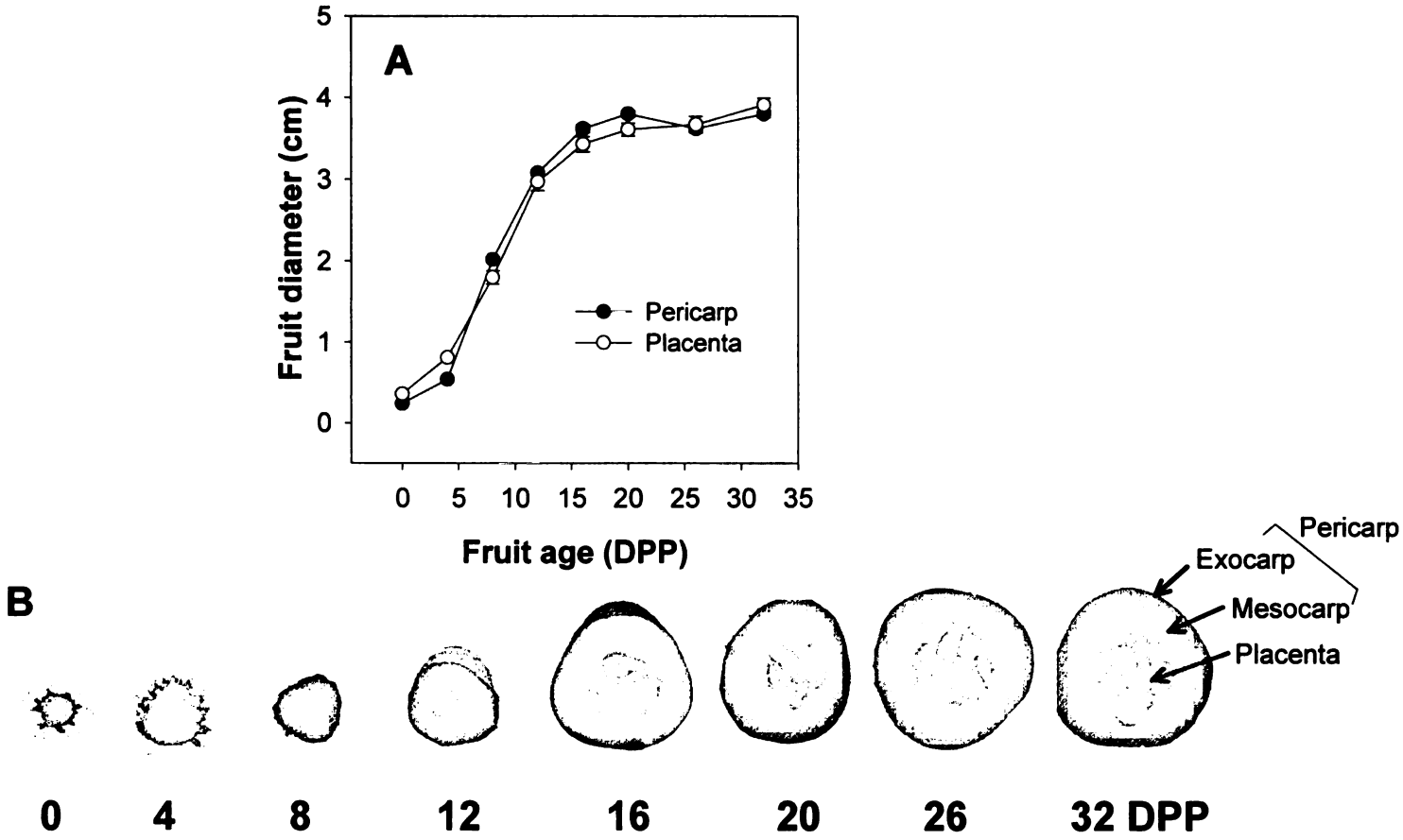


Figure 4-5. Greenhouse-grown cucumber fruit growth. A. Relative growth of the pericarp and placenta from 0, 4, 8, 12, 16, 20, 26, and 32 days post pollination (DPP) fruit. Each data point is mean of at least 25 fruit ± SE. B. Cross section of developing cucumber fruit.

green to whiter green which coincided with the development of a jelly-like placenta texture around 8-12 DPP. Seeds coats became visible around 8 DPP and turned from white to tan with thicker hard coats developing between 12 and 16 DPP.

Cucumber fruit library construction by 454 sequencing

Library construction 1

cDNA libraries from fruit samples at eight different ages (0, 4, 8, 12, 16, 20, 26, and 32 DPP) were each loaded onto a 1/16 section of Pico TiterPlate resulting in a total of 18,349 clean reads yielding approximately 4 million base pairs (bp) (Table 4-1A). The mean and median read lengths were 222-239 bp which was equivalent to the 454 FLX system standards. Each sample yielded variable results. The 20 DPP sample had 76 % clean reads which contained 8,573 reads, whereas the 32 DPP yielded only 4 % clean reads which contained 89 reads (Table 4-1B). The average percent clean reads however, was very low (33%), necessitating a revised sample preparation method. The majority of poor reads were concatemers of 5' primers used for the cDNA library constructions.

Library construction 2

Several changes were made to improve sequencing including: additional steps to obtain cleaner total RNA; higher amount of mRNA for cDNA synthesis; and re-designing a 5' primer to include a *Sfi*I cutting site. The second 454 run generated excellent overall sequence data with a total of 187,406 clean reads and 41,658,727 bp (Table 4-2) and mean and median read lengths of 221-235 bp. The two technical replications of 8 DPP samples were run side-by-side on a 1/4 plate of 454, both samples gave a high percentage of clean reads (94%), indicating very little concatemerization in this run. There was an

Table 4-1. Overview of 454 sequencing results from library construction 1.

A. Pooled samples	
Clean reads (8 x 1/16 plate sections)	18,349
Mean % clean (clean/passed)	33
Mean read length (bp)	222
Median read length (bp)	239
Total bases	4,185,656

B. Individual samples	0 DPP	4 DPP	8 DPP	12 DPP	16 DPP	20 DPP	26 DPP	32 DPP
Clean reads	2,443	3,823	412	760	1,542	8,573	707	89
% Clean (Clean/PF)	30%	38%	18%	43%	41%	76%	13%	4%
Mean read length (bp)	226	225	227	227	214	233	226	199
Median read length (bp)	244	241	241	241	229	246	241	227
Total bases	552,511	859,351	93,604	172,718	330,153	1,999,607	159,969	17,743

Table 4-2. Summary of 454 sequencing results for 8 days post pollination (DPP) cucumber fruit transcript samples

	8 DPP – A	8 DPP – B*
Clean reads (1/4 plate)	75,497	111,909
% clean (clean/passed)	93.9	94.4
Mean read length (bp)	224	221
Median read length (bp)	235	234
Total bases	16,920,666	24,738,061

*8DPP-B library was constructed by using modified 5' PCR primer for cDNA synthesis

excellent correlation in transcript frequency for contig groups between the two technical replicates indicating reproducibility of results (Figure 4-6).

Transcriptome analysis by 454 sequencing

A total of 1,818 contigs ranging 162 -1724 bp in length (average 377 bp) were assembled using 53 % of total clean reads from the first 454 run (data not shown). The number of the sequences per contig, which is reflective of transcript abundance, ranged from 2 -450 with an average of 5 transcripts per contig. After the second sequencing, both 454 runs were combined to maximize the number of sequences available for contig assembly. Of the 205,755 reads, 88.4 % could be assembled into a set of 13,878 contigs (Table 4-3). The mean contig length was 416 bp, ranging 52- 2,824 bp. The number of the reads per contig ranged from 2 to more than 5,000, with a mean of 13 reads per contig.

The 126 most highly expressed contigs (e.g. transcript frequency $\geq$ 0.15 %), which represented 0.9 % of the 13,878 total contigs, accounted for 36.8 % of total reads (Table 4-4). Approximately 10 % of the highly abundant transcripts did not have a match in the TIGR database, suggesting that these genes could be unique to cucumber fruit (Figure 4-7). Among the most highly expressed genes were latex-related proteins, and proteins associated with lipids, growth, translation, defense, phloem transport, and photosynthesis (Table 4-4).

The most highly expressed cucumber fruit gene, *Csf*-2, with more than 5,000 reads, has high homology with a major latex-like protein gene from Arabidopsis based on NCBI BLAST search (Table 4-4). Other major-latex like protein genes were also in the most frequently expressed group, including the fourth most expressed gene. In addition,

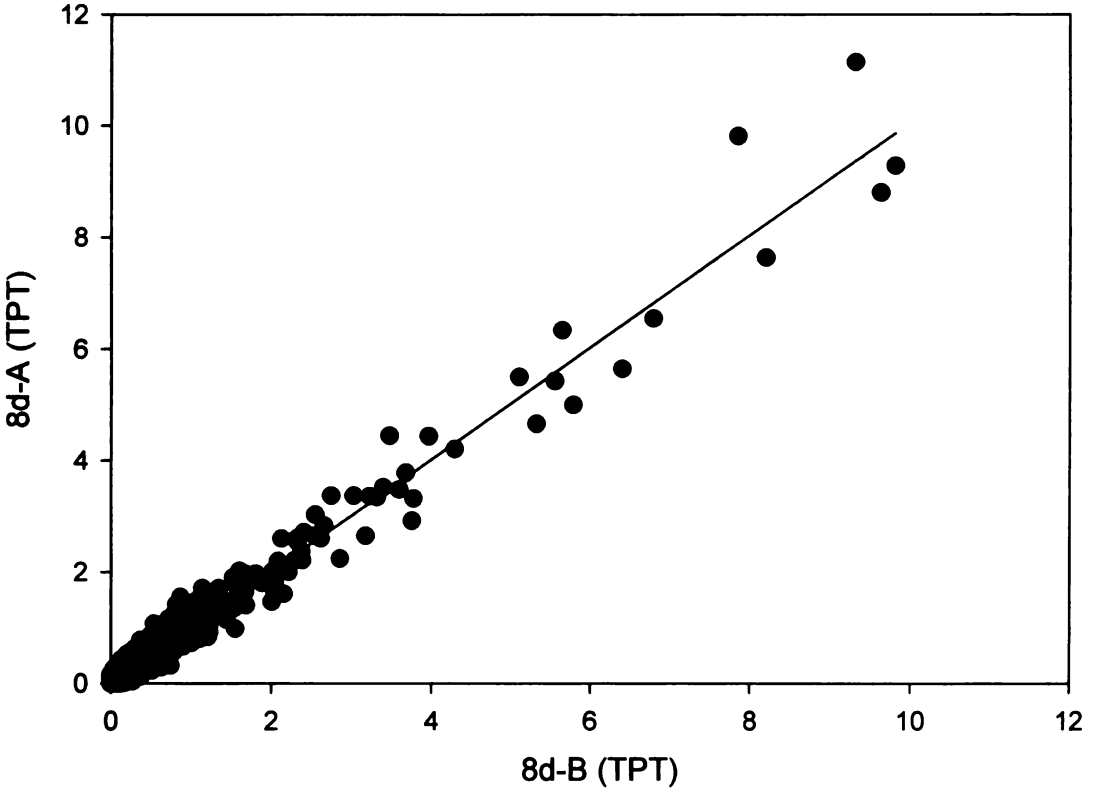


Figure 4-6. Pairwise scatter plot and regression analysis for 8 d-A and 8 d-B samples expressed in transcripts per thousand.

Table 4-3. Overall summary of contig assembly from combined 454 runs.

Total reads assembled into contigs	205,755
Number of contigs	13,878
Mean contig length (bp)	416
Range of contig length (bp)	52 - 2,824
Mean number of reads per contig	13
Range of number of reads per contig	2 - 5,167

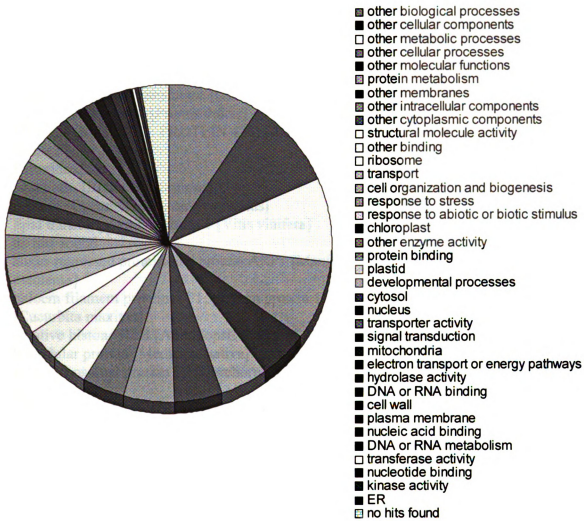


Figure 4-7. Predicted gene ontrogy (GO) classification from BLAST analysis of transcript represented at frequency of > 0.1% against TAIR 7.0 database.

Table 4-4. Predicted function (based on BLAST analysis) and number of reads for highly expressed cucumber fruit genes (≥0.15% transcript frequency)

Hit Description - Predicted function	E-value	Overall Total Reads
Csf-2 [Cucumis sativus] Latex-like protein	1.0E-87	5,167
24-sterol C-methyltransferase [Gossypium hirsutum]	1.E-165	1,992
lipid transfer protein [Prunus dulcis]	4.00E-16	1,790
MLP423 (MLP-LIKE PROTEIN 423) [Arabidopsis thaliana]	2.00E-48	1,726
Peroxidase	1.00E-169	1,716
aquaporin [Ricinus communis]	1.E-122	1,526
profilin [Cucumis melo var. reticulatus]	2.0E-69	1,300
lipid transfer protein isoform 1 [Vitis vinifera]	3.0E-30	1,197
no hits found	n/a	1,187
auxin-repressed protein-like protein ARP1 [Manihot esculenta]	1.0E-45	1,114
phloem filament protein; PP1; phloem protein 1 [Cucurbita maxima]	1.0E+00	1,079
putative histone H2B [Arabidopsis thaliana]	4.00E-16	1,059
bimodular protein [Medicago sativa]	1.0E+00	1,053
acidic ribosomal protein P3 [Corchorus olitorius]	9.00E-43	1,022
no hits found	n/a	819
SP1L1 (SPIRAL1-LIKE1) [Arabidopsis thaliana]	8.00E-32	816
no hits found	n/a	776
catalase [Cucurbita pepo]	-2018.0	711
60S ribosomal protein L21, putative, expressed [Oryza sativa (japonica cultivar-group)]	6.0E-86	702
ribosomal protein L30e [Pisum sativum]	3.0E-56	692
alpha-tubulin [Prunus dulcis]	-1800.0	663
no hits found	n/a	654
putative developmental protein [Nicotiana benthamiana]	2.00E-31	649
putative chloroplast chlorophyll a/b-binding protein [Carya cathayensis]	1.E-150	642
protein induced upon tuberization [Solanum demissum]	1.0E-24	637
type-2 metallothionein [Citrullus lanatus]	3.0E-40	585
S-adenosyl-L-methionine synthetase [Elaeagnus umbellata]	-1574.0	573
no hits found	n/a	563
translation elongation factor 1A-2 [Gossypium hirsutum]	883	541
vacuolar H+-ATPase c subunit [Citrus unshiu]	316	538
ribosomal protein L15 [Elaeis guineensis]	1.00E-109	518
Unknown protein [Arabidopsis thaliana]	1.0E-25	501
LTCOR11 [Lavatera thuringiaca]	8.0E-34	498
ADP ribosylation factor 002 [Daucus carota]	1.E-100	498

Table 4-4 (Continued from previous page.) Hit Description - Predicted function	E-value	Overall Total Reads
no hits found	n/a	487
proteasome maturation factor UMP1 family protein [Arabidopsis thaliana]	9.00E-53	451
DNA-binding protein, putative [Arabidopsis thaliana]	9.00E-53	439
thioredoxin h [Hevea brasiliensis]	2.0E-44	430
latex allergen	9.0E-11	418
lipase [Gossypium hirsutum]	1.00E-150	406
calmodulin [Prunus avium]	3.00E-79	392
chlorophyll a/b binding protein [Cicer arietinum]	1.00E-106	386
14-3-3 protein homolog [Maackia amurensis]	1.E+00	372
60S ribosomal protein L19 [Capsicum annuum]	1.00E-101	371
plastidic cysteine synthase 1 [Solanum tuberosum]	1.00E-101	370
60s acidic ribosomal protein [Prunus dulcis]	8.00E-46	368
tonoplast intrinsic protein bobTIP26-1 [Brassica oleracea var. botrytis]	1.E-106	366
aquaporin 1 [Gossypium hirsutum]	1.E-150	365
ribulose bisphosphate carboxylase/oxygenase precursor peptide	1.E-101	361
unnamed protein product [Vitis vinifera]	5.0E-89	359
26 kDa phloem protein [Cucumis sativus]	1.E-129	351
actin depolymerizing factor-like protein [Arachis hypogaea]	3.0E-66	349
putative copper chaperone [Citrus cv. Shiranuhi]	8.0E-29	345
integral membrane family protein [Arabidopsis thaliana]	4.00E-36	343
major latex-like protein 1 [Plantago major]	1.00E-18	341
snakin-1 [Solanum tuberosum]	6.00E-18	338
translationally controlled tumor protein-related protein [Cucumis melo]	5.0E-89	337
lipid transfer like protein [Vigna unguiculata]	6.0E-24	329
arginosuccinate synthase family [Arabidopsis thaliana]	0	324
no hits found	n/a	319
40S ribosomal protein S23 [Elaeis guineensis]	4.00E-76	311
chlorophyll a/b-binding protein	1.E-149	305
60S ribosomal protein L32A [Cucumis melo]	2.0E-48	299
four F5 protein-related / 4F5 protein-related [Arabidopsis thaliana]	2.00E-20	297
chloroplast photosystem II PsbR [Prosopis juliflora]	2.0E-63	296
unnamed protein product [Vitis vinifera]	1.E-143	296
histone H1C [Nicotiana tabacum]	4.0E-60	290
putative ribosomal protein S29 [Oryza sativa (japonica cultivar-group)]	1.28E-26	287
cyclophilin [Cucumis sativus]	2.0E-96	286
unnamed protein product [Vitis vinifera]	5.0E-57	283

many lipid transfer protein genes and many genes encoding ribosomal proteins were in the most highly expressed group.

Transcripts were compared to entries in the ICuGI EST database including cucumber, melon, and watermelon ESTs. The majority of the most highly expressed cucumber fruit genes were also expressed in melon or watermelon fruit (Table 4-5). Many genes were also found in current cucumber fruit and flower bud EST libraries, but less in cucumber leaf. The cases where expression was not observed in the current cucumber EST library likely reflect the very small number of deposits of cucumber fruit sequence data. In most cases the genes not present in the ICuGI database were somewhat less frequently expressed in the 454 sequencing result, and so perhaps less likely to be included in a small set of transcripts.

Verification of transcritome analysis by 454 sequencing by qRT-PCR

Selected highly expressed transcripts, which had 20 reads or more and differential expression in rapidly growing young (4 DPP) fruit versus older, post-rapid expansion (20 DPP) fruit from the first 454 run, were subjected to qRT-PCR analysis to verify transcript frequency difference from fruit samples of different ages. Transcripts such as aquaporin and chlorophyll a/b binding protein showed high expression (TTP) in younger fruit; while, transcripts such as metallothionein, auxin-repressed protein-like protein (ARP1), and catalase showed higher expression at later stage of development (Figure 4-8).

qRT-PCR results for the selected genes showed expression patterns similar to 454 sequencing results (Figure 4-8). Alpha tubulin, chlorophyll a/b binding protein, and aquaporin genes were highly expressed in early fruit development (4 DPP).

Table 4-5. Presence/absence of the top 0.2 % cucumber fruit transcripts in the International Cucurbit Genomic Initiative (ICuGI) EST libraries of cucumber fruit, leaf, male and female flower buds, melon fruit, and watermelon fruit.

Hit Description - Predicted function	cuucmber leaf	cucumber flower buds	ICuGI cucumber fruit	454 cucumber fruit	melon fruit	watermelon fruit
Csf-2 [Cucumis sativus]	-	-	+	+	-	-
peroxidase	-	-	+	+	-	-
MLP423 (MLP-LIKE PROTEIN 423) [Arabidopsis thaliana]	+	-	+	+	-	+
ribosomal protein L30e [Pisum sativum]	+	-	+	+	-	-
no hits found	-	-	+	+	+	-
ribosomal protein L15 [Elaeis guineensis]	-	+	+	+	+	-
bimodular protein [Medicago sativa]	-	+	+	+	+	+
acidic ribosomal protein P3 [Corchorus olitorius]	-	+	+	+	+	+
Unknown protein [Arabidopsis thaliana]	-	+	+	+	+	+
no hits found	-	+	+	+	+	+
no hits found	-	-	+	+	+	+
24-sterol C-methyltransferase [Gossypium hirsutum]	-	-	+	+	+	+
auxin-repressed protein-like protein ARP1 [Manihot esculenta]	-	-	+	+	+	+
putative histone H2B [Arabidopsis thaliana]	-	-	+	+	+	+
latex allergen	-	-	+	+	+	+
thioredoxin h [Hevea brasiliensis]	-	-	+	+	+	+
no hits found	-	-	+	+	+	+
protein induced upon tuberization [Solanum	-	-	+	+	+	+
putative developmental protein [Nicotiana benthamiana]	-	-	+	+	+	+
putative chloroplast chlorophyll a/b-binding protein [Carya cathayensis]	+	+	+	+	+	-
60S ribosomal protein L21, putative, expressed [Oryza sativa (japonica cultivar-group)]	+	-	+	+	+	-
chlorophyll a/b binding protein [Cicer arietinum]	+	-	+	+	+	-
aquaporin [Ricinus communis]	+	+	+	+	-	+
alpha-tubulin [Prunus dulcis]	+	+	+	+	+	+
lipid transfer protein isoform 1 [Vitis vinifera]	+	+	+	+	+	+
translation elongation factor 1A-2 [Gossypium hirsutum]	+	+	+	+	+	+
type-2 metallothionein [Citrullus lanatus]	+	+	+	+	+	+
lipid transfer protein [Prunus dulcis]	-	+	+	+	-	+
profilin [Cucumis melo var. reticulatus]	-	+	+	+	-	+
catalase [Cucurbita pepo]	-	+	+	+	-	+
no hits found	-	+	-	+	-	+
proteasome maturation factor UMP1 family protein [Arabido.	-	+	-	+	-	+
DNA-binding protein, putative [Arabidopsis thaliana]	-	+	-	+	-	+
vacuolar H+-ATPase c subunit [Citrus unshiu]	+	-	-	+	-	+
calmodulin [Prunus avium]	+	-	-	+	+	+
SP1L1 (SPIRAL1-LIKE1) [Arabidopsis thaliana]	-	-	-	+	+	-
no hits found	-	+	-	+	+	-
S-adenosyl-L-methionine synthetase [Elaeagnus umbellata]	-	+	-	+	+	-
phloem filament protein, PP1; phloem protein 1 [Cucurbita maxima]	-	-	-	+	+	+
LTCOR11 [Lavatera thuringiaca]	-	-	-	+	+	+
ADP ribosylation factor 002 [Daucus carota]	-	+	-	+	+	+
CBL-interacting protein kinase 9 [Populus trichocarpa]	-	-	-	+	-	-
lipase [Gossypium hirsutum]	-	-	-	+	-	-

*+ = EST was present in the library, - = EST was absent.

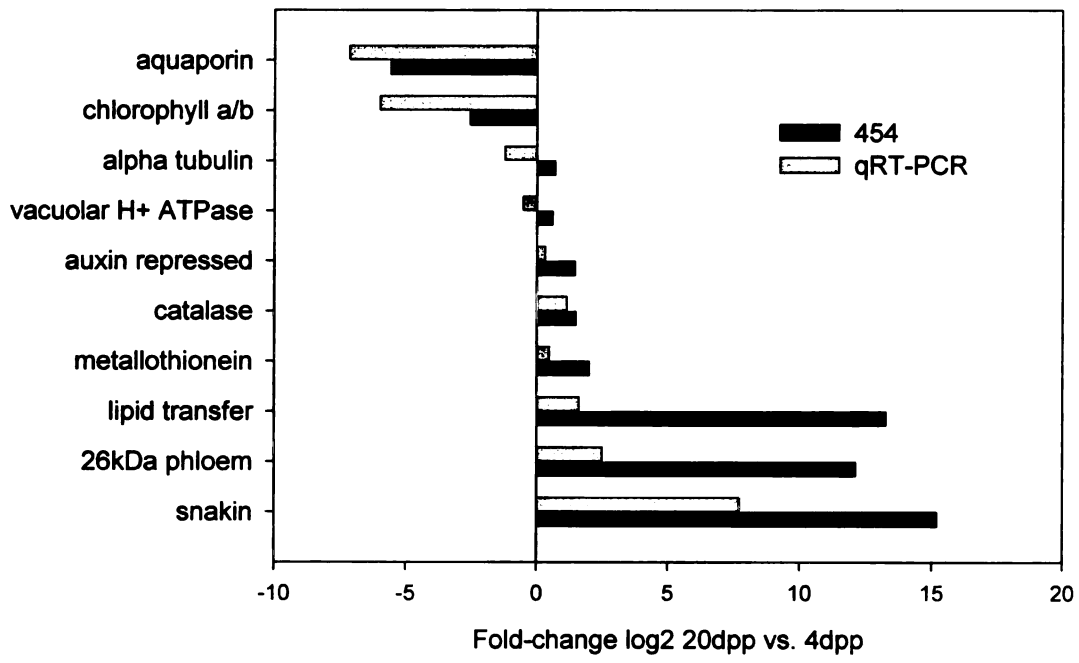


Figure 4-8. Relative transcript levels of selected highly expressed genes in 20 vs. 4 DPP fruit measured by 454 sequencing and quantitative PCR.

Auxin-repressed protein, catalase, and metallothionein showed somewhat greater expression at 20 DPP vs. 4 DPP, while lipid transfer protein, 26 kDa phloem protein, and snakin genes were much more highly expressed in later fruit development (20 DPP) (Figure 4-8).

The selected genes were further examined by qRT-PCR for fruit at 4, 12, 20, and 32 DPP (Figure 4-9). Alpha tubulin, chlorophyll a/b binding protein, and aquaporin showed high expression in younger fruit and decreased as fruit aged. Auxin-repressed protein, catalase, metallothionein, and snakin showed low expression in young fruit and increased as fruit aged. 26 kDa phloem protein, lipid transfer protein, and vacuolar H+ ATPase showed more variable patterns of expression.

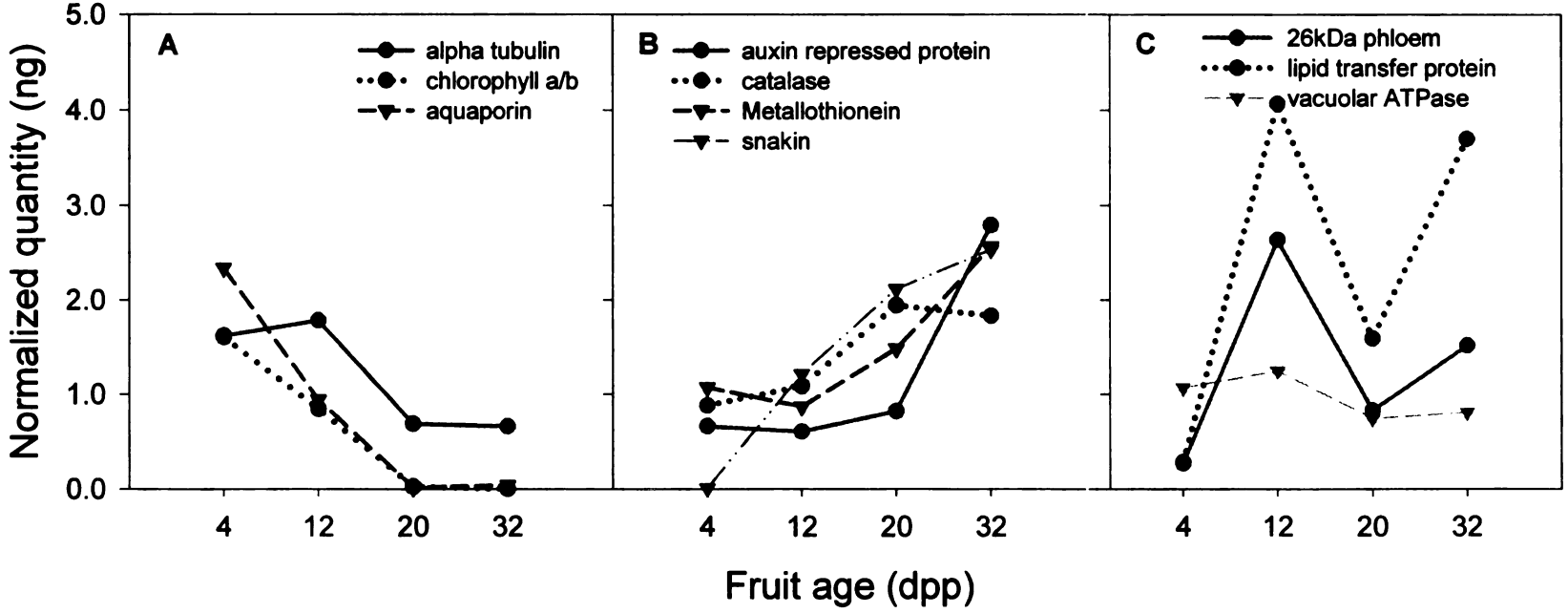


Figure 4-9. qRT-PCR analysis of the selected highly expressed genes in 4, 12, 20 and 32 DPP fruit. Each gene expression was calculated based on standard curve method and normalized with actin expression level. A. Transcripts expressed higher in early developing fruit (alpha tubulin, chlorophyll a/b binding protein, and aquaporin). B. Transcripts expressed higher in later development (auxin-repressed protein, catalase, metallothionein, and snaking). C. Transcripts expressed in variable patterns (26kDa phloem loading protein, lipid transfer protein, and vacuolar ATPase).

Discussion

Cucumber is a non-climacteric fruit consumed at an immature stage of development while most fruits are consumed after maturation and ripening. For instance, pickling cucumbers are typically harvested approximately 8 to 10 DPP when the fruit attain desirable size for market, whereas tomato fruit are harvested usually at 35 DPP (mature green stage) or later when the fruit start ripening (Janssen et al., 2008; Kader et al., 1977; Miller and Wehner, 1989). Many studies of fruit development have been directed toward later development and quality, however, there is increased interest to study early fruit development since it sets a foundation for final yield and quality (Lee, et al., 2007; Mounet et al., 2009).

The early stages of cucumber fruit growth are marked not only by cell division and rapid cell enlargement typical of fruit development in general (Gillapsy et al., 1993), but also by other visible changes in morphology and development which highlight dynamic developmental processes, especially during the period from anthesis (0 DPP) to 12 DPP (Figure 4-10). Early developing cucumber fruit undergo loss of spines, warts, and surface wax, chlorophyll degradation, and placenta and seed development. These morphological and physiological changes provide context for gene expression analysis during early fruit development. This time period also coincides with the stage at which fruit are susceptible to infection by *P. capsici* (Ando et al., 2009; Gevens et al., 2006). Fewer obvious external changes were observed later ages (after 12 DPP).

Challenges were encountered when constructing cucumber fruit cDNA libraries for 454 sequencing. Poor recoveries of clean reads were obtained from the first set of 454 samples. This has been commonly observed among cDNA libraries constructed for

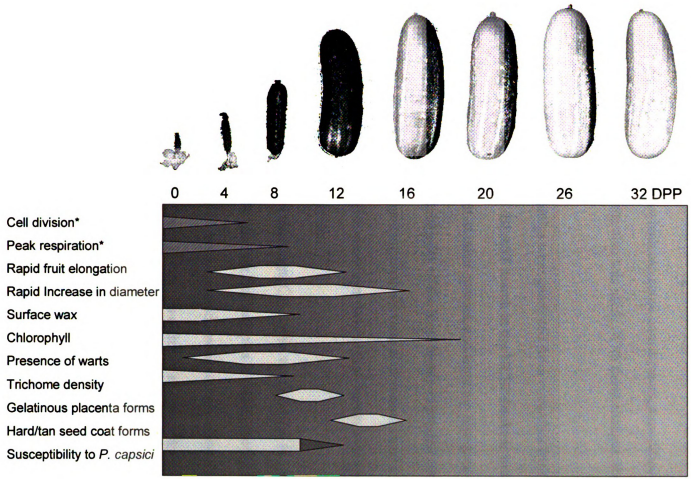


Figure 4-10. External and internal development of cucumber fruit at 0, 4, 8, 12, 16, 20, 26, 32 days post pollination (DPP) fruit. *Marcelis and Hofman-Eijer, 1993.

454 pyrosequencing by similar methods due to concatemerization of 5' primers caused by unknown mechanism(s) (Ali et al., 2009). Modifications were made to improve the second 454 run. To obtain cleaner total RNA, additional steps were taken to remove polysaccharides. Also, the quantity of total RNA was increased in order to increase mRNA yield, and three-fold increased (30 ng to 200 ng) mRNA was used for cDNA synthesis. To remove the region which can cause concatemers, a modified 5' primer which contains a *Sfi*I restriction site also was used. With these changes, the second 454 run yielded an excellent result.

Approximately 14,000 contigs were generated from cucumber fruit cDNA libraries by 454 pyrosequencing. BLAST search in the NCBI database indicate that many genes are also found in fruit of other species, and there are also genes that appear to be unique to cucumber fruit. The majority of the most highly expressed cucumber fruit genes obtained from the 454 sequencing analysis were also present in other cucurbit fruits. There was more overlap with cucumber flowers than leaves as might be expected since fruits develop from floral tissues.

The most highly abundant transcript, *Csf-2*, also was found to be one of three highly expressed genes in young cucumber fruit samples at 3 days post pollination (Suyama et al., 1999). *Csf-2* was highly expressed throughout the cucumber fruit development as indicated by the first 454 run. Its deduced sequence has high homology to major latex-like proteins (MLP), and to a family of flower and fruit specific genes. MLP homologues were observed from fruit of other plants including opium poppy, bell pepper, raspberry, strawberry, and tomato (Jones et al., 1998; Nam et al., 1999; Nessler et al., 1994; Pouzeta-Romero et al., 1995; Ruperti et al.; 2002; Tsugane et al., 2005). MLP

was found to be localized to membrane bound vesicles in the laticifer of opium and to be associated with small vacuoles close to the plasma membrane in bell pepper fruit (Pouzeta-Romero et al., 1995; Griffing and Nessler, 1989; Strömvik et al., 1999). Cucumber, peach, and soybean MLP homologues were highly expressed in immature fruit, while musk melon, strawberry, and raspberry homologues were expressed in ripening fruit, and tobacco and pepper homologues were expressed upon wounding (Ruperti et al., 2002). The function of MLP is largely unknown, however there was a linear correlation between peach MLP homologue (*Pp-MLP1*) mRNA accumulation and fruit relative growth rate, suggesting that peach MLP is associated with fruit cell expansion. Expression in fruits at various stages of development suggests possible additional function of MLPs. MLP also has high homology to intracellular pathogenesis-related protein (IPR or PR-10) and major pollen allergen proteins, suggesting a role in pathogen defense (Osmark et al., 1998; Strömvik et al., 1999).

Interestingly, *Csf-2* was not seen in melon or watermelon fruit according to the ICuGI database. It should be noted though that genes may not have been present in the ICuGI database due to limited EST collection sizes, although if it were expressed at a comparable level as in cucumber, we would expect it to be present in the melon and watermelon databases. Other MLP genes were expressed in melon fruit (Aggelis et al., 1997; Hadfield et al., 2000), suggesting that related genes may play similar functions in the different cucurbit fruits. Furthermore the majority of MLP homologues in melon, including the Csf2 homolog, are expressed in root or phloem tissue (Aggelis et al., 1997; Hadfield et al., 2000; ICuGI database).

One emerging application of 454 sequencing studies is gene expression analysis as an alternative to methods such as microarrays, northern blots and subtraction cDNA libraries (Shendure, 2009; Vera et al., 2008). 454 pyrosequencing has many advantages including reduced cost relative to developing microarrays for non-sequenced species, recovery of rare transcripts, lack of necessity to clone into vectors, and massive number of gene sequences which can be analyzed side-by-side from multiple samples (Shendure, 2008; Vera et al., 2008).

Analysis of a selected set of transcripts over the time period from 4-32 DPP fruits by qRT-PCR showed that genes can be grouped by patterns of expression which may provide information about the function and the importance of various gene products at different stages of fruit development. For example, alpha tubulin, chlorophyll a/b binding protein, and aquaporin genes are highly expressed in early fruit development (4 DPP) but not in older fruit. These three genes were frequently observed in early developing fruit of other species and all belong to gene families that appear to be associated with rapid growth (Janssen et al., 2008; Lee et al., 2007; Lemaire-Chamley et al., 2005; Schlosser et al., 2008; Wechter et al., 2008). Alpha tubulin which is related to formation of cytoskeleton was highly expressed in developing watermelon and apple (Janssen et al., 2008; Wechter et al., 2008). Chlorophyll a/b binding protein which has function in photosynthesis was highly expressed in early developing apple (Lee et al., 2007) as would be expected for young green fruit rather than mature fruit. Aquaporin, which mediates water flow across plant membranes during cell expansion, was highly expressed in young grape berry and tomato fruit (Lemaire-Chamley et al., 2005; Schlosser et al., 2008).

In contrast, auxin-repressed protein, catalase, metallothionein, and snakin genes were more highly expressed in later fruit development (20 DPP). Auxin-repressed protein was found in ripening apples and cassava root, however the function is unknown (Mir et al., 2001; Reiley et al., 2007). Catalase is a detoxifying enzyme which scavenges reactive oxygen species; it has been observed in apple and tomato fruit (Esaka et al., 1997; Janssen et al., 2008). Metallothionein has been observed in many fruit at ripening stage, including pineapple, banana, grape, and raspberry and is thought to have copper tolerance and homeostasis function (Cobbett and Goldsbrough, 2002; Goes da Silva et al., 2005; Jone et al., 1998; Liu et al., 2002; Moyle et al., 2005). Snakin, an antimicrobial protein, was expressed in fully developed petals and carpels of potato flowers as well as tuber cork and medulla, and tomato exocarp (Lemarie-Chamley et al., 2005; Segura et al., 1999).

Expression patterns of vacuolar H+ ATPase, lipid transfer protein, and 26 kDa phloem protein were variable. Vacuolar ATPase expression was relatively constant throughout the cucumber fruit development. Its function is thought to be involved in cellular pH regulation and expression was induced upon ripening in tomato fruit (Moore et al., 2005). Lipid transfer protein and 26 kDa phloem protein had a similar expression pattern, high in 12 and 32 DPP and low in 4 and 20 DPP. Lipid transfer protein was also expressed in fruits of pear, strawberry, grape, and tomato, and in most of the cases, increased expression was observed in later development (Fonseca et al., 2004; Janssen, et al., 2008; Tomassen et al., 2007; Yubero-Serrano et al., 2003). In tomato fruit, it was isolated as one of the components of the polygaractonase (PG) multicomplex involved in fruit cell softening, and was suggested to have a possible role as a modulator of the

activity and stability of cell wall based enzymes (Tomassen et al., 2007). The 26 kDa phloem protein has a possible function in long distance translocation of protein (Gomez et al., 2004), and was commonly observed in three genenra of cucurbits, *Cucurbita, Cucumis*, and *Citrullus* (Read and Northcote, 1983).

The cucumber transcriptome data obtained from the 454 runs could be considered as an 8 DPP pericarp transcriptome profile (rapid growth stage), since the majority of transcripts were sequenced from 8 DPP libraries from the second 454 run. In a tomato transcriptional profile from early fruit development (3, 6, 8, and 15 DPP), many of the genes identified were absent from the previous tomato EST database including that early fruit development genes were less well characterized (Lemaire-Chamley et al., 2005). Genes with functions in fruit growth, such as those associated with accumulation of soluble sugars and acids in vacuoles (e g., vacuolar invertase and phosphoenolypyruvate carboxylase) and modifying cell walls (e.g., pectate lyase, pectinesterase, extensions, and expansins) were highly expressed in the exocarp of developing tomato fruit. Genes with functions in fruit protection or protection from pathogens (e.g., lipid transfer protein, chitinase and glucanase), also were highly expressed in the exocarp of young tomato fruit which provides a barrier to the fruit. Genes involved in cell expansion by controlling the flux of water across the plasma membrane or vacuolar membrane (e.g., the plasma membrane instrinsic protein, aquaporins), photosynthesis-related genes, and auxin- and giberellin-controlled genes were highly observed in locules of early developing tomato fruit. Many of these genes observed in young developing tomato fruit were also found in the 8 DPP cucumber fruit trancriptome, suggesting fundamental similarities in early fleshy fruit development.

In summary, theses results indicate that sequences obtained by 454 pyrosequencing will contribute to our understanding cucumber fruit genes in developing fruit. Comparative transcriptome analysis can be used to examine changes in gene expression associated with different stages of development. Further analysis of different ages and fruit tissues also may help to elucidate underlying mechanism in cucumber fruit-*P. capsici* age related resistance.

Literature Cited

Aggelis, A., I. John, Z. Karvouni, and D. Grierson. 1997. Characterization of two cDNA clones for mRNAs expressed during ripening of melon (*Cucumis melo* L.) fruits. Plant Mol. Biol. 33:313-322.

Ali, S., C. L. Wright, A. Lane, E. Vlach, L. Hetrick, J. Thimmapuram, J. Drnevich, D. R. Vullaganti, M. Band, A.G. Hernandez, A. Avivi, M. A. Mikel, H. Bohnert. 2009. Development of adapter free transcriptome sequencing procedure in Roche/454 GSFLX. PAGXVII. http://www.intl-ag.org/17/abstracts/P01_PAGXVII_044.html.

Ando, K., S. Hammar, and R. Grumet. 2009. Age-related resistance of diverse cucurbit fruits to infection by *Phytophthora capsici.* J. Amer. Soc. Hort. Sci. In press.

Carrari, F. and A. R. Fernie. 2006. Metabolic regulation underlying tomato fruit development. J. Exp. Bot. 57:1883-1897.

Cobbett, C. and P. Goldsbrough. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. 53:159–82

de Hoon, M. J. L., S. Imoto, J. Nolan, and S. Miyano. 2004. Open source clustering software. Bioinformatics, 20:1453-1454.

Droege, M. and B. Hill. 2008. The genome sequencer FLX™ system – longer reads, more applications, straight forward bioinformatics and more complete data sets. J. Biotech. 31:136:3-10.

Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. PNAS 95:14863.

Esaka, M., N. Yamada, M. Kitabayashi, Y. Setoguchi, R. Tsugeki, M. Kondo, and M. Nishimura. 1997. cDNA cloning and differential gene expression of three catalases in pumpkin. Plant Mol. Biol. 33:141–155.

Eveland, A. L., D. R. McCarty, and K. E. Koch. 2008. Transcript profiling by 3'-untranslated region sequencing resolves expression of gene families. Plant Physiol. 146:32-44.

Fonseca, S., L. Hackler, Jr., Á. Zvara, S. Ferreira, A. Baldé, D. Dudits, M. S. Pais, and L. G. Puskás. 2004. Monitoring gene expression along pear fruit development, ripening and senescence using cDNA microarrays. Plant Sci. 167:457–469.

Gevens, A. J., K. Ando, K. Lamour, R. Grumet, and M. K. Hausbeck. 2006. Development of a detached cucumber fruit assay to screen for resistance and effect of fruit age on susceptibility to infection by *Phytophthora capsici.* Plant Dis. 90:1276-1282.

Gillaspy, G., H. Ben-David, and W. Gruissem. 1993. Fruits: a developmental perspective. Plant Cell 5:1439–1451.

Giovannoni, J. J. 2004. Genetic regulation of fruit development and ripening. Plant Cell. 16:S170-180.

Goes da Silva, F., A. Iandolino, F. Al-Kayal, M. C. Bohlmann, M. A. Cushman, H. Lim, A. Ergul, R. Figueroa, E. K. Kabuloglu, C. Osborne, J. Rowe, E. Tattersall, A. Leslie, J. Xu, J. Baek, G. R. Cramer, J. C. Cushman, and D. R. Cook. 2005. Characterizing the grape transcriptome. Analysis of expressed sequence tags from multiple *vitis* species and development of a compendium of gene expression during berry development. Plant Physiol. 139:574-597.

Gómez, G. and V. Pallá. 2004. A long-distance translocatable phloem protein from cucumber forms a ribonucleoprotein complex in vivo with *Hop Stunt Viroid* RNA. J. Virology. 78:10104–10110.

Griffing, L. R. and C. L. Nessler. 1989. Immunlocalization of the major latex proteins in developing laticifers of opium poppy (*Papaver somniferum* L.). J. Plant Physiol. 134:357-363.

Hadfield, K. A., T. Dang, M. Guis, J. Pech, M. Bouzayen, and A. B. Bennett. 2000. Characterization of ripening-regulated cDNAs and their expression in ethylene-suppressed charentais melon fruit. Plant Physiol. 122:977-983.

Hausbeck, M. and K. Lamour. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Dis. 88:1292-1302.

Huang, S., Y. Du, X. Wang, X. Gu, B. Xie, Z. Zhang, J. Wang, R. Li, S. Li, Y. Ren, J. Wang, H. Yang, W. Jin, Z. Fei, A. Kilian, J. E. Staub, E. van der Vossen, and G. Li. 2009. Recent development of the cucumber genome intiative – an international effort to unlock the genetic potential of an orphan crop using novel genomic technology. PAGXVII. http://www.intlpag.org/17/abstracts/W18_PAGXVII_135.html.

Inskeep, W. P. and P. R. Bloom. 1985. Extinction coefficients of chlorophyll *a* and *b* in *N*, *N*-dimethylformanide and 80 % acetone. Plant Physiol. 77:483-485.

Janssen, B. J., K. Thodey, R. J. Schaffer, R. Alba, L. Balakrishnan, R. Bishop, J. H. Bowen, R. N. Crowhurst, A. P. Gleave, S. Ledger, S. McArtney, F. B. Pichler, K. C. Snowden, and S. Ward. 2008. Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. BMC Plant Biol. 8:16.

Jones, C. S., R. J. McNicol, H. V. Davies, M. A. Taylor. 1998. Analysis of genes differentially expressed during fruit ripening in raspberry (*Rubus idaeus* cv. glen clova). Acta Hort. 505:401-402.

Kader, A. A., M. A. Stevens, M. Albright-Holton, L. L. Morris, and M. Alazi. 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. 102:724-731.

Lee, Y. P., G. H. Yu, Y. S. Seo, S. E. Han, Y. O. Choi, D. Kim, I. G. Mok, W. T. Kim, and S. K. Sung. 2007. Microarray analysis of apple gene expression engaged in early fruit development. Plant Cell Rep. 26:917-926.

Lemaire-Chamley, M., J. Petit, V. Garcia, D. Just, P. Baldet, V. Germain, M. Fagard, M. Mouassite, C. Cheniclet, and C. Rothan. 2005. Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. Plant Physiol. 139:750-769.

Liu, P., C. Goh, C. Loh, and E. Pua. 2002. Differential expression and characterization of three metallothioneinlike genes in Cavendish banana (*Musa acuminata).* Physiologia Plantarum. 114: 241–250.

Marcelis, L. F. M. and L. R. B. Hofman-Eijer. 1993. Cell division and expansion in the cucumber fruit. J. Hort. Sci. 68:665–671.

Mardis, E. R. 2008. The impact of next-generation sequencing technology on genetics Trends in Genetics. 24:133-141.

Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bernben, J. Berka, M. S. Braverman, Z. Chen, and S. B. Dewell et al. 2005. Genome sequencing in microfabricated high-density picrolite reactors. Nature. 437:376-380.

Miller, C. H., and T. C. Wehner. 1989. Cucumbers. pp. 245-264. In: Quality and preservation of vegetables, N. A. M. Eskin (ed.). CRC Press, Inc., Boca Raton, Fl.

Mir, N. A., M. Whitaker, S. Mekhedov, and R. M. Beaudry. 2001. AF336307 *Malus* x *domestica* auxin-repressed protein like-protein mRNA. Direct Submission. NCBI.

Moore, S., J. Vrebalov, P. Payton, and J. Giovannoni. 2002. Use of genomics tools to isolate key ripening genes and analyze fruit maturation in tomato. J. Exp. Bot. 53:2023-2030.

Moore, S., P. Payton, M. Wright, S. Tanksley, and J. Giovannoni. 2005. Utilization of tomato microarrays for comparative gene expression analysis in the Solanaceae. J. Exp. Bot. 56:2885-2895.

Mounet, F., A. Moing, V. Garcia, J. Petit, M. Maucourt, C. Deborde, S. Bernillon, G. Le Gall, I. Colquhoun, M. Defernez, J. Giraudel, D. Rolin, C. Rothan, and M. Lemarie-Chamley. 2009. Gene and metabolite regulatory network analysis of early developing fruit tissues highlight new candidate genes for the control of tomato fruit composition and development. Plant Physiol. 149:1505-1528.

Moyle, R., D. J. Fairbairn, J. Ripi, M. Crowe, and J. R. Botella. 2005. Developing pineapple fruit has a small transcriptome dominated by metallothionein. J. Exp. Bot. 56:101–112.

Nam, Y., L. Tichit, M. Leperlier, B. Cuerq, I. Marty, and J. Lelièvre. 1999. Isolation and characterization of mRNAs differentially expressed during ripening of wild strawberry (*Fragaria vesca* L.) fruits. Plant Mol. Biol. 39:629–636.

Nessler, C. L. 1994. Sequence analysis of two new members of the major latex protein gene family supports the triploid-hybrid origin of the opium poppy (Laticifer; *Pupauer somniferum).* Gene. 139:207-209.

Ohtsu, K., M. B Smith, S. J. Emrich, L. A. Borsuk, R. Zhou, T. Chen, X. Zhang, M. C. P. Timmermans, J. Beck, B. Buckner, D. Janick-Buckner, D. Nettleton, M. J. Scanlon, and P. S. Schnable. 2007. Global gene expression analysis of the shoot apical meristem of maize (*Zea mays* L.). Plant J. 52:91-404.

Olmstead, J.W., A.F. Iezzoni, and M.D. Whiting. 2007. Genotypic differences in sweet cherry (*Prunus avium* L.) fruit size are primarily a function of cell number. J. Amer. Soc. Hort. Sci. 132:697-703.

Osmark, P., B. Boyle, and N. Brisson. 1998. Sequential and structural homology between intracellular pathogenesis-related proteins and a group of latex proteins. Plant Mol. Biol. 38:1243–1246, 1998.

Pozueta-Romero, J., M. Klein, G. Houln, M. Schantz, B. Meyer, and R. Schantz. 1995. Characterization of a family of genes encoding a fruit-specific wound-stimulated protein of bell pepper *(Capsicum annuum):* identification of a new family of transposable elements. Plant Mol. Biol. 28: 1011-1025

Provart, N., and T. Zhu. 2003. A browser-based functional classification SuperViewer for Arabidopsis genomics. Currents in Computational Mol. Biol. 271-272.

Read, S. and D. Northcote. 1983. Chemical and immunological similarities between the phloem proteins of three genera of Cucurbitaceae plants. Planta. 158:119-127.

Reilly., K., D. Bernal, D. F. Cortés, R. Gómez-Vásquez, J. Tohme, and J. R. Beeching. 2007. Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. Plant Mol. Biol. 64:187–203.

Ruperti, B., C. Bonghi, F. Ziliotto, S. Pagni, A. Rasori, S. Varotto, P. Tonutti, J. J. Giovannoni, and A. Ramina. 2002. Characterization of a major latex protein (MLP) by ethylene during peach fruitlet abscission. Plant Sci. 163:265-272.

Saldanha, A. J. 2004. Java Treeview—extensible visualization of microarray data. Bioinformatics. 20:3246-3248.

Sambrook, J. and D. W. Russell. 2001. Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schlosser, J., N. Olsson, M. Weis, K. Reid, F. Peng, S. Lund, and P. Bowen. 2008. Cellular expansion and gene expression in the developing grape (*Vitis vinifera* L.). Protoplasma. 232:255-265.

Segura, A., M. Moreno, F. Mo, A. Molina, and F. Garcia-Olmedo. 1999. Snakin-1, a peptide from potato that is active against plant pathogens. Mol. Plant Microbe Interact. 12:16-23.

Shendure, J. 2008. The beginning of the end for microarrays? Nature Methods. 5:585-587.

Strömvik, M. V., V. P. Sundararaman, and L. O. Vodkin. 1999. A novel promoter from soybean that is active in a complex developmental pattern with and without its proximal 650 base pairs. Plant Mol. Biol. 41:217-231.

Suyama, T., K. Yamada, H. Mori, K. Takeno, and S. Yamaki. 1999. Cloning cDNAs for genes preferentially expressed during fruit growth in cucumber. J. Am. Soc. Hortic. Sci. 124:136-139.

Tomassen, M. M. M., D. M. Barrett, H. C. P. M. van der Valk, and E. J. Woltering. 2007. Isolation and characterization of a tomato non-specific lipid transfer protein involved in polygalacturonase-mediated pectin degradation. J. Exp. Bot. 58:1151–1160.

Torres, T. T., M. Metta, B. Ottenwälder, and C. Schlöttere. 2008. Gene expression profiling by massively parallel sequencing. Genome Research. 1-6.

Tsugane, T., M. Watanabe, K. Yano, N. Sakurai, H. Suzuki, and D. Shibata. 2005. Expressed sequence tags of full-length cDNA clones from the miniature tomato (*Lycopersicon esculentum*) cultivar Micro-Tom. Plant Biotech. 22:161–165.

Vera, J. C., C. W. Wheat, H. W. Fescemyer, M. J. Frilander, D. L. Crawford, I. Hanski, and J. H. Marden. 2008. Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. Mol. Ecology. 17:1636-1647.

Weber, A., K. L. Weber, K. Carr, C. Wilkerson, and J. B. Ohlrogge. 2007. Sampling the Arabidopsis transcriptome with massively parallel pyrosequencing. Plant Physiol. 144:32-42.

Wechter, W. P., A. Levi, K. R. Harris, A. R. Davis, Z. Fei, N. Katzir, J. J. Giovannoni, A. Salman-Minkov, A. Hernandez, J. Thimmapuram, Y. Tadmor, V. Portnoy, and T. Trebitsh. 2008. Gene expression in developing watermelon fruit. BMC Genomics 9:275.

White, P. J. 2002. Recent advances in fruit development and ripening: an overviews. J. Exp. Bot. 53:1995-2000.

Yubero-Serrano, E., E. Moyano, N. Medina-Escobar, J. Muñoz-Blanco, and J. Caballero. 2003. Identification of a strawberry gene encoding a non-specific lipid transfer protein that responds to ABA, wounding and cold stress. J. Exp. Bot. 54:1865-1877.

Conclusions and future work

Cucumber fruit rot caused by the soil borne Oomycete pathogen, *Phytophthora capsici,* is a serious problem in many parts of the U.S. Finding genetic resistance would be the optimal method of disease control, however, no resistant cultivar or strong source of resistance for breeding is available to date. Therefore, establishing alternative methods to control the disease incidence, and understand *P. capsici*-cucumber fruit interaction could provide insight into effective disease management.

In Chapter 1, I tested whether an alternate plant architecture could be helpful in limiting *P. capsici* infection on cucumber. Based on my results, alternative cucumber architecture resulted in changes in the canopy structure and temperature under the canopy. However, the incidence of disease was significantly higher than economically acceptable level in many alternate architectures I tested. In other experiments, fruit from trellised vines and the compact PI 308916, which holds young fruit off the ground had significantly lower disease incidence than other architecture types, suggesting that reducing the contact of young cucumber fruit with soil can used as a tool to prevent *P. capsici* infection. Thus, canopy conditions including temperatures under the canopy or canopy structure are less important for disease control than preventing contact of the fruit with the soil.

When screening fruit for resistance to infection by *P. capsici*, it was observed that age/size appeared to greatly influence fruit susceptibility. To study this aspect more carefully, greenhouse-grown, hand-pollinated cucumber fruit were inoculated with *P. capsici*. Young and rapidly elongating cucumber fruit were most susceptible to *P.*

capsici, but when they ceased elongation [approximately 10-12 days post pollination (DPP)] they develop age-related resistance (ARR). ARR was also observed in field-grown cucumber, other cucumber genotypes, other cucurbit fruit (acorn squash, butternut squash, pumpkin, zucchini, watermelon, melon, yellow summer squash), and parthenocarpic cucumber fruit. The peduncle end of the fruit became less susceptible to *P. capsici* earlier than the blossom end, thus showing a gradation of susceptibility within elongated fruit types. In cucumber, this difference in susceptibility correlated with differences in the period of rapid elongation along the length of the fruit. Exocarp properties at least partially appear to be responsible for the increased resistance in cucumber, as was evidenced by zoospore germination analyses on exocarp section (peel).

Transcriptome samples from developing cucumber fruit (0-32 DPP) were analyzed by 454 pyrosequencing as a starting point to decipher underlying mechanisms of age-related resistance in the *P. capsici*-cucumber-fruit interaction and to fill the gap in knowledge of early development of fleshy fruit. Additionally, these same fruit used for making cDNA libraries for 454 sequencing were used to catalogue morphological changes in order to anchor changes in gene expression relative to developmental processes. Dramatic morphological changes were observed both internally and externally during early cucumber fruit growth. Developing cDNA libraries for 454 pyrosequencing was challenging, however the modified method greatly improved quality of the result. Approximately 14,000 cucumber fruit ESTs were obtained by 454 pyrosequencing. Many of the genes highly expressed in developing cucumber fruit were also observed in the developing stages of other fruit such as tomato, apple, and grape. The cucumber fruit transcriptomes obtained from chapter 4 and the recently sequenced cucumber genome,

can complement each other and can be used as a tool to gain insights in biology of developing fruit, and to explore the basis of cucumber fruit and *P. capsici* age related resistance.

These findings provided both short- and long-term applications for cucumber production. For immediate application, protecting fruit from early development by spraying fungicides can minimize disease incidence and may be applicable for many cucurbit crops. Constructing cucumber fruit transcriptome libraries will be a tremendous asset for not only understanding for immediate questions I presented here, but for future questions.

In the future, more transcriptome data from other cucumber fruit development stages (0, 4, 12, and 16 DPP) and other tissues including exocarp and mesocarp will be needed for further analysis to understand the underlying age related resistance mechanism and aspects in fruit development. Also, comparison between cucumber fruit transcriptomes with other fruit ESTs will provide more insights into fruit development.

Cucumber fruit transcriptomes from fruit highly susceptible (8 DPP) and resistant (16 DPP) to infection by *P. capsici* can be compared. Transcripts that are uniquely expressed in 16 DPP, including these that are absent or only present in 16 DPP, and down- or up-regulated compared to 8 DPP will be candidate genes for further analysis. In addition, since exocarp properties might be the sources for ARR in cucumber fruit to *P. capsici* infection, the exocarp transcriptome of the peduncle end of 12 DPP fruit can be compared to the exocarp transcriptome of the blossom end of 12 DPP fruit. 12 DPP will be of use since it showed intermediate response to infection by *P. capsici*, and also showed difference in susceptibility between the peduncle and stem end. The mesocarp

transcriptome from 12 DPP can be subtracted from the 12 DPP exocarp transcriptome to gain information specific to exocarp. Literature available from oomycete- or *Phytophthora*-plant interaction will be referred to in selecting possible candidate genes.

A complete set of cucumber transcriptome data from 0 to 16 DPP will be a useful tool to study early fruit development since these time points cover the period from cell division to cell enlargement which is a hallmark of early fruit development (Gillarpsy et al., 1993). Comparison to each DPP transcriptome data as described above will be implemented. Further, transcriptomes from other early developing fleshy fruit such as tomato, grape, and apple will be compared to cucumber transcriptomes. Morphological markers which were already recorded in chapter 4 will also be anchored to gene expression.

Literature Cited

Gillaspy, G., H. Ben-David, and W. Gruissem. 1993. Fruits: a developmental perspective. Plant Cell. 5:1439–1451.