# TOWARDS IMPROVED MANAGEMENT OF *PHYTOPHTHORA CAPSICI*: BIOLOGY OF AND MANAGEMENT TOOLS FOR THE PATHOGEN

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Amara Ruth Dunn

May 2014

# TOWARDS IMPROVED MANAGEMENT OF *PHYTOPHTHORA CAPSICI*: BIOLOGY OF AND MANAGEMENT TOOLS FOR THE PATHOGEN

Amara Ruth Dunn

Cornell University 2014

*Phytophthora capsici* is a destructive oomycete pathogen of many vegetables, causing root, crown, and fruit rot, as well as foliar disease. Host resistance is an important management strategy in pepper, and the high levels of diversity in field populations of *P. capsici* have important implications for screening and selecting resistant cultivars. In order to better understand the changing structure of a field population, a previously-uninfested research field was inoculated with two single-spore isolates of *P. capsici* in September 2008. Isolates from this field were collected over the following four years and characterized with five microsatellite markers. A sexual population was established in the field, characterized by high genotypic diversity and no evidence of genetic drift. In addition, four novel alleles were detected, and characterization of F1 progeny from an *in vitro* cross between the same two parental isolates revealed evidence of non-Mendelian inheritance of alleles.

In order to provide practical information to New York vegetable growers, commercial bell pepper cultivars were screened for resistance to *P. capsici* using a representative local isolate, over five years. In one year, yield and fruit quality were compared among commercial cultivars at three sites (one inoculated with *P. capsici* and two uninoculated). The cultivars Aristotle, Intruder, and Paladin were consistently most resistant to the isolate used, and while total marketable yields were comparable among resistant and susceptible cultivars, these more resistant cultivars tended to have smaller fruit and more problems with silvering than the susceptible cultivars.

In order to better understand the *P. capsici*-pepper pathosystem, interactions between a *P. capsici* isolate (PcapG-a) tagged with green fluorescent protein and susceptible or resistant pepper cultivars were studied. Surprisingly, *P. capsici* zoospores attached to and germinated equally well on roots of resistant and susceptible cultivars within 120 minutes of inoculation. Differences in pathogen colonization of roots and crowns of resistant and susceptible cultivars were observed 3 days post inoculation. Although previous studies have implicated upregulation of plant defense genes in pepper host resistance to *P. capsici*, we did not see evidence of this at 8 or 24 hours post inoculation in either pepper stem or leaf tissue.

# BIOGRAPHICAL SKETCH

In 2005, Amara Ruth (Camp) Dunn received a Bachelor of Science in Biology from Juniata College in Huntingdon, PA. She then spent a year in Pittsburgh volunteering through Pittsburgh Urban Leadership Service Experience (PULSE). In 2009, she received a Master of Science in Plant Pathology from Cornell University, having been co-advised by Drs. Helene Dillard and Christine Smart. Her thesis research also focused on *Phytophthora capsici*, surveying populations of the pathogen on New York State vegetable farms, and testing the efficacy of a potential biological control organism (*Muscodor albus*). After working for a year as a lab technician, she returned to graduate school and began working on her Ph.D. with Dr. Christine Smart in 2010.

dedicated to my husband Kevin

# ACKNOWLEDGEMENTS

I would like to thank my committee members Drs. William Fry, Stephen Reiners, and Niklaus Grünwald, and especially the chairperson of my committee, Dr. Christine Smart, for all their support and advice, both in my research and in my professional development. I am very grateful for all that Chris has taught me about conducting research, communicating science, and mentoring others. She has been an outstanding role model and mentor.

I would also like to thank past and present members of the Smart Lab (Holly Lange, Maryann Herman, Lisa Jones, Carly Summers, Matthew Tancos, Zachariah Hansen, Maryn Carlson, Rachel Kreis, Ning Zhang, Tanya Taylor, Jun Gu, Mui Yun Wong, Balaji Vasudevan, Kevin Conley, Vladi Guarnanccia, and numerous undergraduate interns) as well as current and former graduate students in Cornell's Department of Plant Pathology and Plant-Microbe Biology. They have assisted me with experiments, taught me new techniques, listened and supported me when experiments were not working, and created a great atmosphere in which to learn and conduct research.

This research was supported by the Agriculture and Food Research Initiative Competitive Grant No. 2012-67011-19690 from the USDA National Institute of Food and Agriculture, and by the New York State Department of Agriculture and Markets through a New York Specialty Crops Block Grant. Support for A.R. Dunn was also provided by a fellowship from Cornell University College of Agriculture and Life Sciences, travel grants from Cornell University and the American Phytopathological Society Foundation, and by an award from the Storkan-Hanes-McCaslin Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

# Introduction

The biology and management of the plant pathogenic oomycete *Phytophthora capsici* (causal agent of Phytophthora blight) was reviewed previously in the author's Master's thesis (Camp, 2009). Although it is not known how *P. capsici* was first introduced to New York State, Wiant and Tucker (1940) reported watermelon (*Citrullus lanatus* var. *lanatus*) fruit infected with *P. capsici* in a shipment from Colorado to New York City markets in September 1935. It is possible that fruit from this or other shipments were involved in the initial introduction. The pathogen is now found on many farms in New York, and the number of affected farms continues to increase, partly due to widespread flooding in Fall 2011 and 2012 in eastern New York. While there are many steps growers can take to reduce the risk of introducing *P. capsici* into uninfested fields, the spread of *P. capsici* in flood waters highlights the fact that in spite of a grower's best efforts, it may not always be possible to keep this pathogen off of a farm. Furthermore, isolates resistant to the fungicide mefenoxam were identified for the first time in western New York in 2011 (Dunn and Smart 2012). While resistance to this fungicide has been widely reported in eastern New York, previously, all isolates collected in western New York had been sensitive (Dunn et al. 2010). Once *P. capsici* is in a field, it is not possible to completely eradicate it from the soil, so many growers will need to learn to live with this pathogen. There will likely be no "silver bullet" for controlling *P. capsici*, and successful management requires an integrated strategy that makes use of cultural practices (including crop rotation and managing soil moisture), chemicals, and host resistance. The goal of this dissertation is to increase our knowledge of the biology of *P. capsici* as well as to support and enhance the use of host resistance as part of a

successful integrated management strategy for *P. capsici*.

## *Chemical and Cultural Control*

**Chemical control.** Fungicides have an important role to play in an integrated management strategy for Phytophthora blight. Several studies have demonstrated that the combination of fungicides with resistant cultivars can improve control and yields, even when using a resistant pepper cultivar (Foster and Hausbeck 2010a; McGrath et al. 2013; Stieg et al. 2006; Wyenandt and Maxwell 2009). A variety of chemicals have been reported to effectively control Phytophthora blight on pepper (*Capsicum annuum*), either singly or in combination, including cyazofamid (Babadoost 2008), cymoxanil + famoxadone (Wyenandt and Maxwell 2009), dimethomorph (Foster and Hausbeck 2010a), fluazinam (Wyenandt and Maxwell 2009), fluopicolide (Foster and Hausbeck 2010a), mandipropamid (Babadoost 2008; Foster and Hausbeck 2010a; McGrath and Davey 2007; Wyenandt and Maxwell 2009), and (when isolates in a field are still sensitive) mefenoxam (McGrath and Davey 2007; Wyenandt and Maxwell 2009). Phosphorus acid is also recommended (Reiners and Petzoldt 2014).

In summer squash (*Cucurbita pepo*), fluopicolide (Jackson et al. 2010; Meyer and Hausbeck 2013b), mandipropamid (Meyer and Hausbeck 2013b), and dimethomorph (Meyer and Hausbeck 2013b) have provided good control of *P. capsici*. However, Meyer and Hausbeck (2013b) reported that these chemicals had to be applied as a soil drench (not a foliar spray) in order to be effective. Yandoc-Ables et al. (2007) reported similar results for phosphonates used to control crown rot in pumpkin (*C. pepo*) and zucchini. Babadoost (2013) reported that cyazofamid and mandipropamid (alternated with various other chemicals) provided the greatest reduction in pumpkin fruit rot incidence under low disease pressure from *P. capsici*. However,

Miller and Mera (2012) reported that mandipropamid did not reduce cucumber (*Cucumis sativus*) fruit rot incidence compared to the untreated control, but azoxystrobin did. Conversely, Miller et al. (2012) reported that azoxystrobin provided no control of *P. capsici* on winter squash (*C. maxima*) under high disease pressure. A preliminary test of fungicides for control of *P. capsici* on lima bean (*Phaseolus lunatus*) indicated that fluopicolide and mandipropamid significantly improved yields, while cyazofamid resulted in phytotoxicity (Enzenbacher and Hausbeck 2012b). Cyazofamid, fluopicolide, mandipropamid, and mefenoxam all provided good control of mefenoxam-sensitive *P. capsici* on snap bean (*P. vulgaris*; Strauss et al. 2012b).

Unfortunately, resistance to some of these fungicides has already been reported, with resistance to mefenoxam being most widespread (Lamour and Hausbeck 2000; Parra and Ristaino 2001; Ploetz et al. 2002; Wang et al. 2009). Jackson et al. (2012) reported that some *P. capsici* isolates collected in Georgia in 2011 showed resistance to cyazofamid, as quantified by lack of inhibition of mycelial growth or sporangia production, but zoospore germination was still inhibited in all isolates. Other studies have predicted a moderate risk of *P. capsici* developing resistance to zoxamide (Bi et al. 2011) and fluopicolide (Lu et al. 2011). Not surprisingly, a survey of *P. capsici* isolates from Florida, Georgia, Michigan, North Carolina, and South Carolina fields not previously treated with fluopicolide found no baseline resistance to this chemical, but did identify some isolates with reduced sensitivity to cymoxanil (Keinath and Kousik 2011). Jackson et al. (2010) tested *P. capsici* isolates from irrigation sources and vegetable fields in Georgia and all were sensitive to fluopicolide in culture-based assays.

**Cultural control.** The important role which water plays in the life cycle and spread of *P. capsici* within and between fields was previously reviewed, as was the role which rotation to non-susceptible crops can play in reducing the survival of oospores in the soil (Camp, 2009). Liu

et al. (2012) recently reported the isolation of *P. capsici* from castings produced by worms fed culled fruit. This finding highlights the need for careful disposal of plant material infected with *P. capsici*. If possible, infected plants should be disposed of in a sanitary landfill, buried deeply, or burned. When these options are not available, infected plant material and culled fruit should at least not be dumped in uninfested fields.

Since 2009, reports of *P. capsici* in irrigation water have increased to include not only Florida (Roberts et al. 2005) and Michigan (Gevens et al. 2007), but also Georgia (Wang et al. 2009) and New York (Jones et al. 2012). Michigan growers have been encouraged not to use any surface water sources for irrigation of vegetables susceptible to Phytophthora blight, due to the high risk of spreading *P. capsici* or increasing inoculum levels already present on vegetable farms (Granke et al. 2012). Some studies have investigated options for treating irrigation water to kill *P. capsici* propagules (Granke and Hausbeck 2010; Jones et al. 2014; Lewis Ivey and Miller 2013). With increasing emphasis on food safety and irrigation water as a source of human pathogens, there is some hope that new technologies for removing human pathogenic bacteria and viruses from irrigation water could also reduce infestations of *P. capsici* in water.

### *Host resistance*

Clearly, not all cultural practices are feasible on all farms, or under all weather conditions. Similarly, timely application of effective fungicides may also be limited by weather, time constraints, or economic considerations, and the development of fungicide resistance is likely to be a perpetual concern for this pathogen. Furthermore, biological control of this pathogen has not generally been highly effective (Camp et al. 2008; Yandoc-Ables et al. 2013). While cultural practices and the use of fungicides will always be important components of vegetable production

in the presence of *P. capsici*, the use of resistant cultivars will improve the sustainability of vegetable production, and is already a key component of Phytophthora blight management on pepper. Since 2009, additional bell pepper cultivars resistant to Phytophthora root and crown rot have been released, including Intruder (Syngenta, Greensboro, NC), Archimedes (Seminis, St. Louis, MO), and PS 09941819 (Seminis), in addition to two hot pepper cultivars (Hechicero (jalapeno) and Sequoia (ancho), both from Harris Moran, Modesto, CA).

**Peppers.** Pepper is currently the only host of *P. capsici* for which resistant cultivars are commercially available, and the host for which the genetics governing this resistance have been most studied. Studies of the inheritance of this resistance suggest that multiple genes are involved (Monroy-Barbosa and Bosland 2008), and that different genes are responsible for resistance to root rot, stem rot, and foliar lesions (Sy et al. 2005), as has been previously reviewed (Camp, 2009). Subsequent studies have classified isolates of *P. capsici* into physiological races using pepper recombinant inbred lines as differentials (da Costa Ribeiro and Bosland 2012; Monroy-Barbosa and Bosland 2011; Sy et al. 2008), but these race classifications are not universally accepted. Quantitative trait loci (QTLs) associated with resistance to root rot and foliar blight caused by *P. capsici* have been mapped to several pepper chromosomes (including chromosome 5) in several different resistant pepper lines (Ogundiwin et al. 2005; Thabuis et al. 2003; Truong et al. 2011). An early study also reported a dominant gene for Phytophthora fruit rot resistance in the commercial hot pepper cultivar Waxy Globe (Saini and Sharma 1978), and more recently Naegele et al. (2013) reported evidence of fruit rot resistance QTLs in the resistant land race CM-334.

**Cucurbits.** Although no *Phytophthora* resistant cucurbit cultivars are currently available, accessions of various cucurbit species have been screened for resistance to Phytophthora root,

crown, and fruit rot, as has been previously reviewed (Camp, 2009). Since then, a *Cucurbita* line resistant to Phytophthora crown rot has been developed, containing genetic material from *C. lundelliana* and *C. okeechobeensis* subsp. *okeechobeensis* introgressed into *C. moschata*, and this resistance appears to be controlled by three dominant genes (Padley et al. 2009). Additional screening of cucurbit accessions for Phytophthora crown rot resistance is ongoing (Chavez et al. 2011). Unfortunately, reports published so far have found little evidence of resistance to fruit rot in cucurbits (an important trait, since many cucurbit fruit are produced on vining plants grown on bare ground), although Enzenbacher and Hausbeck (2012a) reported that not all cucurbit fruit are equally susceptible. In addition, at least some cucurbit fruit are reported to become less susceptible as they age (Ando et al. 2009; Meyer and Hausbeck 2013a).

**Other susceptible vegetables.** Tomato (*Solanum lycopersicum*) and eggplant (*S. melongena*) tend to be less susceptible to *P. capsici* than cucurbits and peppers, and are more likely to develop fruit and stem lesions than for the entire plant to die (Holmes et al. 2002). Nevertheless, *Phytophthora* resistance in these crops would still be useful. Quesada-Ocampo and Hausbeck (2010) reported reduced incidence of plant death in the tomato cultivars Jolly Elf, Taladega, and Super Sweet 100 in response to four isolates of *P. capsici*. They also reported complete resistance to these isolates in one accession of the wild relative *S. habrochaites* and moderate resistance in several other *S. lycopersicum* accessions. Foster et al. (2013) screened two eggplant lines for resistance to 14 *P. capsici* isolates from Michigan and New York, and found that both were at least moderately resistant to root and crown rot caused by all isolates. As awareness of *Phaseolus* sp. as hosts of *P. capsici* has increased, currently available cultivars are being screened for resistance. While Strauss et al. (2012a) reported some differences among snap bean and dry bean (*P. vulgaris*) cultivars, no high levels of resistance were found.

## *Connection to present studies*

**Understanding changing populations of *P. capsici*.** It is well-established that individual isolates of *P. capsici* vary in their virulence on vegetable hosts, and on individual cultivars of a single host (Foster and Hausbeck 2010b; Polach and Webster 1972). Furthermore, the fact that only sexual oospores survive the winter in temperate climates suggests that different isolates of *P. capsici* (possibly with widely varying phenotypes) affect vegetable fields each year (Babadoost and Pavon 2013; Bowers et al. 1990). Ideally, new *Phytophthora*-resistant vegetable cultivars would be effective against a broad range of *P. capsici* isolates, so that these cultivars would be useful both across multiple geographic areas, and also over multiple years in a single field. Understanding the diversity of *P. capsici* field populations and how that diversity changes over time is a first step towards providing improved resistance screening methods for breeding lines and better recommendations to growers managing endemic populations of *P. capsici* in their fields.

**Pepper cultivar trials with a New York isolate of *P. capsici*.** As noted previously, isolates of *P. capsici* vary in their virulence on pepper cultivars (including some cultivars with fairly high levels of resistance to diverse isolates). Disease severity in a field can also vary from year to year, depending on weather conditions, meaning that a more susceptible cultivar grown in an infested field may still yield well in a dry year. Therefore, information about yield, fruit quality, and resistance to local *P. capsici* isolates in commercially available pepper cultivars will help growers make informed decisions about the best cultivars for their farms. While cultivars that perform well in one region may not be ideal for all production regions, such trials do provide practical information to local growers. Resistant bell pepper cultivars have previously been tested on Long Island against local *P. capsici* isolates (McGrath and Fox 2009; McGrath et al.

2013), but not in upstate New York, where the soil type and climate are very different.

**Host-pathogen interactions in pepper.** As breeders and geneticists continue to elucidate the genetics underlying host resistance to *P. capsici*, many questions remain about the interactions between pathogen and host. In order for soil borne inoculum to cause disease on a host plant, zoospores must successfully locate and attach to a root and germinate, and hyphae from germinated zoospores must successfully colonize and spread through the plant. A failure at any point in this process would result in the plant escaping infection. In the interaction between the soil borne pathogen *Phytophthora sojae* and its host (soybean), Enkerli et al. (1997) attributed host resistance in incompatible interactions to early detection of the pathogen by the plant, marked by host cell necrosis as early as 4 hours post inoculation. In contrast, during compatible interactions, *P. sojae* grew biotrophically in the host for 7 to 10 hours pi before host cell necrosis began in the outer cell layers of the root. Overall, a better understanding of the *P. capsici*-pepper pathosystem could eventually contribute to improved disease management. The fact that some individual plants of highly resistant pepper cultivars do succumb to disease under highly conducive weather conditions or high inoculum levels raises further questions about specifically what occurs in the interactions between *P. capsici* and a resistant pepper cultivar.

## *Conclusions*

Host resistance is a very promising management strategy for *P. capsici*, and is already helping pepper growers to successfully manage this pathogen. However, there is a need for new resistant pepper cultivars, as well as for resistant cucurbit, eggplant, and tomato cultivars. To support the development and deployment of host resistance against *P. capsici*, advances are needed on several fronts: (i) understanding the population biology of *P. capsici* and how it

changes over time in a field, (ii) screening existing cultivars against local *P. capsici* isolates in order to provide immediately-applicable information to growers managing this pathogen now, and (iii) understanding the physical and genetic mechanisms underlying host resistance, in order to best integrate current and future resistant cultivars with other management practices. The research presented here begins to address some of the knowledge gaps in these areas, specifically for vegetable growers in New York State. It also presents methods that may be useful in future studies, and raises new questions for others to address.

***References***


Ando, K., Hammar, S., and Grumet, R. 2009. Age-related resistance of diverse cucurbit fruit to infection by *Phytophthora capsici*. J. Amer. Soc. Hort. Sci. 134:176–182.

Babadoost, M. 2008. Fungicide efficacy for control of Phytophthora blight of bell pepper, 2007. Plant Dis. Manag. Rep. 2:V158.

Babadoost, M. 2013. Efficacy of selected fungicide for control of Phytophthora blight of processing pumpkin, 2012. Plant Dis. Manag. Rep. 7:V119.

Babadoost, M., and Pavon, C. 2013. Survival of oospores of *Phytophthora capsici* in soil. Plant Dis. 97:1478–1483.

Bi, Y., Cui, X., Lu, X., Cai, M., Liu, X., and Hao, J. J. 2011. Baseline sensitivity of natural population and resistance of mutants in *Phytophthora capsici* to zoxamide. Phytopathology. 101:1104–1111.

Bowers, J. H., Papavizas, G. C., and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Dis. 74:771–777.

Camp, A. R. 2009. Management and population structure of *Phytophthora capsici* in New York State (Master's thesis). Cornell University, Ithaca, NY.

Camp, A. R., Dillard, H. R., and Smart, C. D. 2008. Efficacy of *Muscodor albus* for the control of Phytophthora blight of sweet pepper and butternut squash. Plant Dis. 92:1488–1492.

Chavez, D. J., Kabelka, E. A., and Chaparro, J. X. 2011. Screening of *Cucurbita moschata* Duchesne germplasm for crown rot resistance to Floridian isolates of *Phytophthora capsici* Leonian. HortScience. 46:536–540.

da Costa Ribeiro, C. S., and Bosland, P. W. 2012. Physiological race characterization of *Phytophthora capsici* isolates from several host plant species in Brazil using New Mexico recombinant inbred lines of *Capsicum annuum* at two inoculum levels. J. Amer. Soc. Hort. Sci. 137:421–426.

Dunn, A. R., and Smart, C. D. 2012. Fungicide resistance found in Phytophthora blight isolates from WNY. Veg Edge. 8:10.

Dunn, A. R., Milgroom, M. G., Meitz, J. C., McLeod, A., Fry, W. E., McGrath, M. T., Dillard, H. R., and Smart, C. D. 2010. Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York State. Plant Dis. 94:1461–1468.

Enkerli, K., Hahn, M. G., and Mims, C. W. 1997. Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. Can. J. Botany. 75:1493–1508.

Enzenbacher, T. B., and Hausbeck, M. K. 2012a. An evaluation of cucurbits for susceptibility to cucurbitaceous and solanaceous *Phytophthora capsici* isolates. Plant Dis. 96:1404–1414.

Enzenbacher, T. B., and Hausbeck, M. K. 2012b. Evaluation of fungicides for crop safety and control of Phytophthora pod rot of lima bean, 2011. Plant Dis. Manag. Rep. 6:V009.

Foster, J. M., and Hausbeck, M. K. 2010a. Managing Phytophthora crown and root rot in bell pepper using fungicides and host resistance. Plant Dis. 94:697–702.

Foster, J. M., and Hausbeck, M. K. 2010b. Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24–30.

Foster, J. M., Naegele, R. P., and Hausbeck, M. 2013. Evaluation of eggplant rootstocks and pepper varieties for potential resistance to isolates of *Phytophthora capsici* from Michigan and New York. Plant Dis. 97:1037–1041.

Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology. 97:421–428.

Granke, L. L., and Hausbeck, M. K. 2010. Effects of temperature, concentration, age, and algaecides on *Phytophthora capsici* zoospore infectivity. Plant Dis. 94:54–60.

Granke, L. L., Quesada-Ocampo, L. M., Lamour, K., and Hausbeck, M. 2012. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. Plant Dis. 95:1588–1600.

Holmes, G. J., Lancaster, M. E., Rodriguez, R. J., and Redman, R. S. 2002. Relative susceptibility of cucurbit and solanaceous crops to Phytophthora blight, 2001. Biol. Cult. Tests Rep. 17:V10.

Jackson, K. L., Yin, J., Csinos, A. S., and Ji, P. 2010. Fungicidal activity of fluopicolide for suppression of *Phytophthora capsici* on squash. Crop Prot. 29:1421–1427.

Jackson, K., Yin, J., and Ji, P. 2012. Sensitivity of *Phytophthora capsici* on vegetable crops in Georgia to mandipropamid, dimethomorph and cyazofamid. Plant Dis. 96:1337–1342.

Jones, L. A., Worobo, R. W., and Smart, C. D. 2012. Oomycete and bacterial pathogens in New York surface irrigation water: Survey results and ultraviolet treatment. Phytopathology. 102:S4.60.

Jones, L. A., Worobo, R. W., and Smart, C. D. 2014. Ultraviolet light inactivation of human and plant pathogens in unfiltered surface water. Appl. Environ. Microb. 80:849-854.

Keinath, A. P., and Kousik, C. S. 2011. Sensitivity of isolates of *Phytophthora capsici* from the eastern United States to fluopicolide. Plant Dis. 95:1414–1419.

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

Lewis Ivey, M. L., and Miller, S. A. 2013. Assessing the efficacy of pre-harvest, chlorine-based sanitizers against human pathogen indicator microorganisms and *Phytophthora capsici* in non-recycled surface irrigation water. Water Res. 47:4639–4651.

Liu, B., Roos, D., Buttler, S., Richter, B., and Louws, F. J. 2012. Vegetable seedling diseases associated with earthworm castings contaminated with *Phytophthora capsici* and *Pythium attrantheridium*. Plant Health Prog. doi:10.1094/PHP-2012-0421-01-RS.

Lu, X., Hausbeck, M., Liu, X., and Hao, J. J. 2011. Wild type sensitivity and mutation analysis for resistance risk to fluopicolide in *Phytophthora capsici*. Plant Dis. 95:1535–1541.

McGrath, M. T., and Davey, J. F. 2007. Efficacy of fungicides for control of Phytophthora blight in pepper on crown rot tolerant and susceptible cultivars, 2006. Plant Disease Management Reports. 1:V131.

McGrath, M. T., and Fox, G. M. 2009. Evaluation of Phytophthora-resistant bell pepper cultivars with and without a fungicide program, 2008. Plant Dis. Manag. Rep. 3:V132.

McGrath, M. T., Menasha, S. R., and LaMarsh, K. A. 2013. Evaluation of Phytophthora-resistant bell pepper cultivars with and without a fungicide program, 2012. Plant Dis. Manag. Rep. 7:V016.

Meyer, M. D., and Hausbeck, M. K. 2013a. Age-related resistance to Phytophthora fruit rot in 'Dickenson Field' processing pumpkin and 'Golden Delicious' winter squash fruit. Plant Dis. 97:446–552.

Meyer, M. D., and Hausbeck, M. 2013b. Using soil-applied fungicides to manage Phytophthora crown and root rot on summer squash. Plant Dis. 97:107-112.

Miller, S. A., and Mera, J. R. 2012. Evaluation of fungicides for the control of downy mildew and Phytophthora fruit rot of cucumber, 2011. Plant Dis. Manag. Rep. 6:V066.

Miller, S. A., Mera, J. R., and Baysal-Gurel, F. 2012. Evaluation of Serenade Soil and Quadris 2.08SC for the management of Phytophthora blight of winter squash, 2011. Plant Dis. Manag. Rep. 6:V062.

Monroy-Barbosa, A., and Bosland, P. W. 2008. Genetic analysis of Phytophthora root rot race-specific resistance in Chile pepper. J. Amer. Soc. Hort. Sci. 133:825–829.

Monroy-Barbosa, A., and Bosland, P. W. 2011. Identification of novel physiological races of *Phytophthora capsici* causing foliar blight using the New Mexico recombinant inbred pepper lines set as a host differential. J. Amer. Soc. Hort. Sci. 136:205–210.

Naegele, R. P., Ashrafi, H., Hill, T. A., Reyes Chin-Wo, S., Van Deynze, A. E., and Hausbeck, M. K. 2013. QTL mapping of fruit rot resistance to the plant pathogen *Phytophthora capsici* Leonian in a recombinant inbred line *Capsicum annuum* L. population. Phytopathology. First look. <http://dx.doi.org/10.1094/PHYTO-05-13-0143-R>.

Ogundiwin, E. A., Berke, T. F., Massoudi, M., Black, L. L., Huestis, G., Choi, D., Lee, S., and Prince, J. P. 2005. Construction of 2 intraspecific linkage maps and identification of resistance QTLs for *Phytophthora capsici* root-rot and foliar-blight diseases of pepper (*Capsicum annuum* L.). Genome. 48:698–711.

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

Parra, G., and Ristaino, J. B. 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing Phytophthora blight of bell pepper. Plant Dis. 85:1069–1075.

Ploetz, R., Heine, G., Haynes, J., and Watson, M. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. Annals of Applied Biology. 140:61–67.

Polach, F. J., and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. Phytopathology. 62:20–26.

Quesada-Ocampo, L. M., and Hausbeck, M. K. 2010. Resistance in tomato and wild relatives to crown and root rot caused by *Phytophthora capsici*. Phytopathology. 100:619–627.

Reiners, S., and Petzoldt, C., eds. 2014. *2014 Cornell integrated crop and pest management guidelines for commercial vegetable production*. Cornell University Cooperative Extension.
Roberts, P. D., Urs, R. R., French-Monar, R. D., Hoffine, M. S., Seijo, T. E., and McGovern, R. J. 2005. Survival and recovery of *Phytophthora capsici* and oomycetes in tailwater and soil from vegetable fields in Florida. Ann. Appl. Biol. 146:351.
Saini, S. S., and Sharma, P. P. 1978. Inheritance of resistance to fruit rot (*Phytophthora capsici* Leon.) and induction of resistance in bell pepper (*Capsicum annuum* L.). Euphytica. 27:721–723.
Stieg, J. R., Walters, S. A., Bond, J. P., and Babadoost, M. 2006. Effects of fungicides and cultivar resistance for *Phytophthora capsici* control in bell pepper production. HortScience. 41:30.
Strauss, J., Carissimi, A. M., Kreis, R. A., and Dillard, H. R. 2012a. Evaluation of bean and soybean cultivar reactions to Phytophthora blight, 2011. Plant Dis. Manag. Rep. 6:V042.
Strauss, J., Carissimi, A. M., Kreis, R. A., and Dillard, H. R. 2012b. Evaluation of fungicides for management of Phytophthora blight in snap beans, 2011. Plant Dis. Manag. Rep. 6:V040.
Sy, O., Bosland, P. W., and Steiner, R. 2005. Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. J. Amer. Soc. Hort. Sci. 130:75–78.
Sy, O., Steiner, R., and Bosland, P. W. 2008. Recombinant inbred line differential identifies race-specific resistance to Phytophthora root rot in *Capsicum annuum*. Phytopathology. 98:867–870.
Thabuis, A., Palloix, A., Pflieger, S., Daubéze, A., Caranta, C., and Lefebvre, V. 2003. Comparative mapping of *Phytophthora* resistance loci in pepper germplasm: Evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. Theor. Appl. Genet. 106:1473–1485.
Truong, H. T. H., Kim, K. T., Kim, D. W., Kim, S., Chae, Y., Park, J. H., Oh, D. G., and Cho, M. C. 2011. Identification of isolate-specific resistance QTLs to Phytophthora root rot using an intraspecific recombinant inbred line population of pepper (*Capsicum annuum*). Plant Pathol. 61:48–56.
Wang, Z. Y., Langston, D. B., Csinos, A. S., Gitaitis, R. D., Walcott, R. R., and Ji, P. S. 2009. Development of an improved isolation approach and simple sequence repeat markers to characterize *Phytophthora capsici* populations in irrigation ponds in southern Georgia. Appl. Environ. Microbiol. 75:5467–5473.
Wiant, J. S., and Tucker, C. M. 1940. A rot of Winter Queen watermelons caused by *Phytophthora capsici*. J. Agr. Res. 9:73–88.
Wyenandt, C. A., and Maxwell, N. L. 2009. Evaluation of fungicides for control of the aerial phase of Phytophthora blight in bell pepper fruit, 2008. Plant Dis. Manag. Rep. 3:V025.
Yandoc-Ables, C. B., Hong, J. C., Kokalis-Burelle, N., Albano, J. P., Lamb, E. M., and Rosskopf, E. N. 2013. Evaluation of biorational products for management of Phytophthora blight of bell pepper transplants. Plant Health Prog. doi:10.1094/PHP-2013-0226-01-RS.
Yandoc-Ables, C. B., Rosskopf, E. N., and Lamb, E. M. 2007. Management of Phytophthora crown rot in pumpkin and zucchini seedlings with phosphonates. Plant Dis. 91:1651–1656.

CHAPTER 2

# Evolution of an experimental population of *Phytophthora capsici* in the field[1]


## *Abstract*

Populations of the vegetable pathogen *Phytophthora capsici* are often highly diverse, with limited gene flow between fields. To investigate the structure of a newly established, experimental population, an uninfested research field was inoculated with two single zoospore isolates of *P. capsici* in September 2008. From 2009 through 2012, approximately 50 isolates of *P. capsici* were collected from the field each year and genotyped using five microsatellite loci. The same two isolates were also crossed in the lab. High levels of diversity were detected in the research field, with 26 to 37 unique multilocus genotypes detected each year. Through 2012, genotypic diversity did not decline, and no evidence of genetic drift was observed. However, during the 2011 and 2012 growing seasons, a total of four new alleles not present in either parental isolate were observed in the field. Possible selfing (but not apomixis) was observed at low frequency among *in vitro* progeny. In addition, evidence for loss of heterozygosity was observed in half of the *in vitro* progeny. These results suggest that recombination, mutation and loss of heterozygosity can impact the genetic structure observed in *P. capsici* populations.


## *Introduction*

*Phytophthora capsici* causes Phytophthora blight on many vegetables, including cucurbits, peppers, tomatoes, eggplants, snap beans and lima beans (Davey et al. 2008; Gevens et al. 2008; Tompkins and Tucker 1941). The asexual spores of *P. capsici* (sporangia and

[1] Reprinted from Dunn, A. R., Bruening, S. R., Grünwald, N. J., and Smart, C. D. Evolution of an experimental population of *Phytophthora capsici* in the field. Phytopathology. In Press. http://dx.doi.org/10.1094/ PHYTO-12-13-0346-R.

zoospores) are relatively short-lived in the absence of susceptible plant tissue, and cannot survive in soil from one growing season to another in temperate climates (Bowers et al. 1990) . In contrast, the sexual spores (oospores), have thick walls and can remain viable in field soil for at least three years (Babadoost and Pavon 2013; Lamour and Hausbeck 2001). Because *P. capsici* is heterothallic, both mating types (designated A1 and A2) must be present for oogonia and antheridia (gametangia required for oospore formation) to be produced. Uchida and Aragaki (1980) interpreted the formation of oospores from antheridia and oogonia in a paired culture of two *P. capsici* isolates of opposite mating type separated by a membrane as evidence for selfing. However, they did not attempt to determine the viability of these oospores and confirm that they were the results of selfing. Shattock et al. (1986) grew several A1 isolates of *P. infestans* with an A2 isolate of either *P. infestans* or *P. drechsleri*, separated by a membrane, and genotyped some single-oospore progeny at two enzyme loci, concluding that at least some progeny were the products of selfing. Several studies have found no evidence of selfing in crosses of *P. capsici* (Donahoo and Lamour 2008; Hurtado-Gonzáles and Lamour 2009; Lamour et al. 2012). Throughout this paper, we will use the term 'selfing' to describe the formation of a diploid oospore from an antheridium and an oogonium produced by the same individual, following meiosis, while 'apomixis' will be used to describe the formation of an oospore through mitosis, without meiosis. Thus, selfing could involve recombination within an individual, resulting in progeny that differ from the parent, while apomixis would result in progeny genetically identical to the parent.

Phytophthora blight epidemics in temperate climates are initiated by the germination of oospores and can be sustained by mixed sexual (oospores) and asexual (sporangia and zoospores) reproduction. While studies have shown that the percent of viable *P. capsici* oospores

recovered from field soil declines over time (Babadoost and Pavon 2013; Bowers et al. 1990), it is unknown what proportion of oospores germinate within a year of formation. In a field that has been infested with *P. capsici* for some time, and where susceptible crops have been present for multiple years, the oospores that germinate in any given season might have been formed two or more years earlier. In addition, while Satour and Butler reported that oospore germination improved after a resting period of at least one month (1968), to the best of our knowledge, no published studies have investigated how many sexual cycles of *P. capsici* can take place during a growing season. If oospores can mature and germinate within a month of formation, then theoretically progeny from oospores formed early in the season could produce more oospores by the end of the growing season, resulting in more than one generation of the pathogen per season.

Based on studies conducted in Peru (Hurtado-Gonzáles et al. 2008) and Argentina (Gobena et al. 2012), populations of *P. capsici* can be predominantly clonal. A study of isolates collected from pepper in the Gansu province of China found evidence for three distinct clonal lineages (with genetic diversity within each lineage), in spite of the fact that both mating types were recovered from single fields (Hu et al. 2013). Surveys of *P. capsici* in the United States have also reported the frequent presence of both mating types in a single field, but also high levels of diversity, low gene flow between fields, and no evidence that clonal lineages persist over time (Dunn et al. 2010; Lamour and Hausbeck 2001). The fact that recombination occurs, particularly when oospores serve as a means of surviving the winter, might explain the high levels of diversity observed in US populations, in spite of limited gene flow between fields. In addition, *P. capsici* has been described as a very "plastic" organism, capable of changing and adapting rapidly, and studies investigating the potential for development of fungicide resistance in *P. capsici* are consistent with this observation (Bi et al. 2011; Lu et al. 2011).

There are several likely mechanisms by which *P. capsici* could first be introduced into a new field. *Phytophthora capsici* has frequently been isolated from surface water used for irrigation (Gevens et al. 2007; Jones et al. 2012; Roberts et al. 2005; Wang et al. 2009), so irrigating with infested water could certainly move the pathogen between fields. In New York, new outbreaks of Phytophthora blight in previously unaffected fields have occurred following major flooding events, and the introduction of *P. capsici* has also been traced to the movement of tractors or trucks between infested and uninfested fields (C.D. Smart, *unpublished*). A single infected fruit may contain numerous oospores of both A1 and A2 mating type (Lamour and Hausbeck 2000), so the dumping of a few infected fruit in a previously uninfested field could also establish a permanent, sexually-reproducing population. In one instance, *P. capsici* was brought onto a farm in earthworm castings used as fertilizer (Liu et al. 2012).

The establishment of a sexual population of *P. capsici* in a new field requires the introduction of at least two genetically distinct isolates where one is of each mating type, but the genotypic diversity resulting from a founding population depends on the allelic diversity introduced. Based on the high levels of differentiation reported between populations in even nearby fields (Dunn et al. 2010; Lamour and Hausbeck 2001), gene flow between fields is likely to be low, once a new population is established. The present study was undertaken to establish a sexually reproducing experimental population of *P. capsici* by introduction of two parental genotypes characterized by microsatellite markers, and to monitor evolution of this population over subsequent years. Such a scenario is similar to what could occur in a commercial vegetable field when *P. capsici* is first introduced, and the grower successfully prevents subsequent re-introductions. The objectives of this study were to determine whether, during the first four years following the founding of the population, (i) a sexual population would establish after

introduction of strains of opposite mating type, (ii) genetic drift would occur given the relatively small population size found in a single field, and (iii) new alleles would be identified in the population.

***Materials and Methods***

*Field inoculation.* Pumpkins (17 cultivars of *Cucurbita maxima* and *C. moschata*) were direct seeded on 25 Jun 2008 into a 0.8-hectare field at the New York State Agricultural Experiment Station in Geneva, NY. Approximately 200 seeds were sown in rows on approximately 0.05 hectares of the field with 1.8 m between rows, and 1.2 m between plants in a row. The field had no history of *P. capsici* infestation, and was located on a research farm with no history of *P. capsici*, and where only non-hosts (shrub willow, tree fruit, grapes, other small fruit) are grown. Nearly all farm equipment is used exclusively in this field, and not shared with other research farms at the New York State Agricultural Experiment Station, none of which have a history of *P. capsici*. Pumpkins (all highly susceptible to *P. capsici*) remained free of *P. capsici* symptoms through Jul and Aug 2008, until after they were inoculated on 8 Sept 2008, although there was adequate rainfall (8.76, 12.67, and 10.57 cm in Jun, July, and Aug, respectively) during the season. The single-zoospore isolates used for inoculation were NY 0664-1 (A1 mating type, isolated from sweet pepper) and NY 06180-4 (A2 mating type, isolated from butternut squash). Both were collected in 2006 from farms in central New York, are sensitive to the fungicide mefenoxam, and were previously characterized (Dunn et al. 2010) using published microsatellite markers (Meitz et al. 2010; Wang et al. 2009; Table 2.1). To produce inoculum for the field, each isolate was grown separately for 6 to 12 weeks on a sterile mixture of V8 juice and vermiculite, as described previously (Camp et al. 2008). Equal volumes of vermiculite

colonized with each isolate were mixed and spread throughout the pumpkin field (especially around the ripening fruit) at an approximate rate of 1 L per 7.3 m of row. Decaying plant debris was left undisturbed in the field throughout the winter.

**Table 2.1** Multilocus genotypes of two single zoospore isolates of *Phytophthora capsici* used to inoculate a research field and crossed in the laboratory. Mating type (MT) of each isolate and alleles (designated by length in base pairs) present at each of five microsatellite loci are shown.

| | | Allele sizes (bp) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MT | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC-26 | |
| NY 0664-1 | A1 | 241 | 241 | 434 | 446 | 277 | 277 | 377 | 386 | 186 | 186 |
| NY 06180-4 | A2 | 241 | 241 | 434 | 446 | 274 | 291 | 359 | 359 | 186 | 189 |

*Collection of P. capsici isolates from field and characterization with microsatellite markers.* Beginning with the 2009 field season, the *P. capsici* population in the research field described above was sampled annually by collecting isolates from symptomatic plants in the field. In the spring of 2009, the entire field was tilled and raised beds covered with plastic mulch were prepared in a section (approximately 0.02 hectares) of the area of the field where the pumpkins had been inoculated with *P. capsici* the previous fall. On 16 June 2009, approximately 150 summer squash (3-4 weeks old, cultivar Zucchini Elite) were transplanted into these beds, and plants with symptoms of Phytophthora blight (e.g., wilting, shriveled crown lesions) were collected over the next month. *Phytophthora capsici* was isolated from symptomatic plants and single zoospore isolates were obtained and stored in sterile distilled water for further characterization including mating type determination as previously described (Dunn et al. 2010). DNA extraction was performed as previously described (Dunn et al. 2010) or by using a modified CTAB method (Keb-Llanes et al. 2002) in which polyvinylpyrrolidone was excluded from the EBA buffer. Isolates were confirmed as *P. capsici* by species-specific PCR, as previously described (Dunn et al. 2010) and microsatellite genotypes were determined as

described below. Similar protocols were followed to collect isolates of *P. capsici* from the same field in the summers of 2010 through 2012, with additional susceptible vegetables being planted in the field in subsequent years, either on black plastic or on bare ground (Table 2.2). In 2010 and 2011, the entire 0.8-hectare field was planted with various susceptible vegetables, and in 2012, approximately 0.13 hectares were planted with susceptible vegetables. No subsequent inoculations of *P. capsici* were made in the field.

**Table 2.2** Number of *Phytophthora capsici* isolates and hosts from which they were collected. Population samples were either from an *in vitro* cross or collected over time from infected plants in a research field after inoculation with parental isolates NY 0664-1 and NY 06180-4 in 2008.

| | Number of Isolates | | | | |
|---|---|---|---|---|---|
| | *In vitro* cross[a] | Field 2009 | Field 2010 | Field 2011 | Field 2012 |
| Host plant | | | | | |
| Pumpkin | -- | --[b] | 32 | 10 | -- |
| Tomato | -- | -- | 23 | 18 | 18 |
| Summer squash | -- | 47 | 5 | 1 | 10 |
| Cucumber | -- | -- | 0 | 7 | -- |
| Pepper | -- | -- | 0 | 18 | 18 |
| Eggplant | -- | -- | 0 | 4 | 0 |
| Total isolates | 53 | 47 | 60 | 58 | 46 |

[a] *In vitro* cross isolates were single oospore progeny from a cross between isolates NY 0664-1 and NY 06180-4 on V8 agar, and were not isolated from plants.
[b] The symbol -- indicates that this host was not planted in the field in this season.

*In vitro cross between parental isolates.* The same single-zoospore isolates used in the field (NY 0664-1 and NY 06180-4) were crossed in the lab, and single germinating oospore isolates were obtained using protocols modified from Donahoo and Lamour (2008). Agar containing mature oospores was homogenized in a round-bottom 2 ml microcentrifuge tube with a 4.5 mm sterile zinc-plated steel BB (Daisy Outdoor Products, Rogers, AR) and sterile distilled water by shaking in a TissueLyser (Qiagen, Valencia, CA) at 30 Hz for 30 s, twice. Several layers of cheesecloth were then used to filter out agar chunks and mycelial debris. Following the treatment with *Trichoderma harzianum* lysing enzyme (Sigma Aldrich, St. Louis, MO), the

oospores were also treated with 0.1% $KMnO_4$ (Sigma Aldrich), as described by Pavon et al. (2008), then incubated in sterile water at 4°C for 3 days, then at room temperature for 3-4 days, before being spread on water agar plates. Single germinating oospores were picked within 24 hrs with the aid of a dissecting microscope and transferred to PARP (Schmitthenner and Bhat 1994; with 0.025 g rather than 0.1 g pentachloronitrobenzene). For each single-oospore isolate, mating type was determined; DNA was extracted from mycelia; isolates were confirmed as *P. capsici* by species-specific PCR; and alleles present at five microsatellite loci were determined as with isolates from the research field.

*On-farm population.* A naturally occurring reference population from a farm near Albany, NY was previously sampled for *P. capsici* in 2007 (Dunn et al. 2010) and was utilized for comparison with the experimental population. A total of 23 isolates were collected from this farm in a single pumpkin field and characterized for microsatellite genotype as described below. This field had the highest level of diversity observed among fields sampled in New York (Dunn et al. 2010). Alleles from locus SSRPC26 were also amplified from DNA previously extracted from these isolates (as described below). Isolates from this farm are hereafter referred to as sample CD-4.

*SSR analysis.* For all isolates, alleles present at microsatellite loci Pcap1, Pcap3, Pcap5, and Pcap7 were determined as previously described (Dunn et al. 2010). These loci are not in coding regions of the genome, nor are they under selective pressure (Meitz et al. 2010). In addition, alleles present at another microsatellite locus (SSRPC26) were determined using primers and amplification conditions described by Wang et al. (2009), with the modification that the 5' end of the forward primer was labeled with 6-FAM (Applied Biosystems, Carlsbad, CA). Primers for this locus were designed from a database of expressed sequence tags (Wang et al.

2009). Based on the published genetic map of *P. capsici* (Lamour et al. 2012) and a search of the *P. capsici* genome (available at: http://genome.jgi.doe.gov/Phyca11/Phyca11.home.html), loci Pcap3 and Pcap5 appear to be located on linkage groups 14 and 1, respectively. The locations of loci Pcap1, Pcap7, and SSRPC26 cannot be determined based on the current assembly of the genome.

Presence of novel alleles not expected given parental strains was confirmed by sequencing. If the isolate was homozygous at the locus in question, the PCR amplicon of the locus was directly sequenced on a 3730XL (Sanger) DNA Analyzer (Applied Biosystems) at the Cornell University Life Sciences Core Laboratories Center. If the isolate was heterozygous at the locus, the PCR amplicons were cloned using a TOPO TA cloning kit (Invitrogen Life Technologies, Grand Island, NY) and sequenced as described above. Approximately 10 clones were sequenced per isolate.

*Statistical analysis.* For the purpose of analysis, isolates collected in a single season from the research field (or all isolates from the *in vitro* cross) were considered to be a population sample, for a total of six samples (isolates from the *in vitro* cross, isolates collected from the field in 2009 through 2012, and isolates from the naturally infested field CD-4). Unless otherwise noted, all analyses were conducted on both clone-corrected and total data (Grünwald and Hoheisel 2006). When calculating the number of unique multilocus genotypes (MLGs) and clone correcting data, mating type was treated as a sixth locus, but mating type data was not used in the calculation of other parameters (either prior to or following clone correction.)

Population differentiation was determined in Arlequin version 3.5 (Excoffier and Lischer 2010), without assuming a step-wise mutation model, because preliminary analysis indicated that mutations in the research field population did not involve the addition or subtraction of a single

copy of the repeat motif. In order to test for significant differentiation between samples or between host plants from which field isolates were obtained (pumpkin, summer squash, and tomato, only) pairwise $F_{ST}$ values were calculated and their significance tested using 1,000 permutations. These three hosts were chosen because similar and comparatively large (42 to 63) numbers of isolates were collected from each of these hosts over the four growing seasons. Fixation indices ($F_{IS}$) were calculated for each of the six samples, and significance of these values was tested using 5,040 permutations. If calculated $F_{IS}$ values were negative, then *P*-values were subtracted from 1 because Arlequin performs a one-sided test (ie, is $F_{IS}$ significantly greater than 0). Both $F_{ST}$ and $F_{IS}$ values were calculated based on differences in allele frequencies (not allele sizes). Whether each polymorphic locus was in Hardy-Weinberg equilibrium was tested using a Markov chain with 1,000,000 steps and 100,000 dememorization steps, on clone corrected data, only.

The 'poppr' package (Kamvar et al. 2014) in R version 3.0.1 (R Development Core Team 2013) was used to identify the number of MLGs in each sample (based on alleles and mating type of each isolate), and to generate a clone-corrected data set. Clone correction was performed within each of the six samples (not across all samples). This program was also used to calculate the following values for each sample: Stoddart and Taylor's index of MLG diversity (Stoddart and Taylor 1988), the index of association ($I_A$; Brown et al. 1980), evenness ($E_5$; Grünwald et al. 2003), and the expected number of MLGs at the smallest common sample size, using rarefaction (Hurlbert 1971). Significance was tested with 999 permutations. The poppr package was also used to draw dendrograms for all isolates together, and for each of the six population samples based on Bruvo's genetic distance (Bruvo et al. 2004) using the unweighted pair-group method with arithmetic averages (UPGMA) with 999 permutations and a bootstrap value cutoff of 70%.

Minimum spanning networks were also drawn based on Bruvo's genetic distance. Both minimum spanning networks and dendrograms were drawn only using clone corrected data.

To test whether the ratio of isolates with A1 mating type to isolates with A2 mating type differed significantly from the expected 1:1 ratio, a Chi-squared goodness-of-fit test was performed (Sheskin 1996). GenAlEx version 6.5 (Peakall and Smouse 2006; 2012) was used to calculate allele frequencies following clone correction.

***Results***

*Isolate collection from the field and amplification of alleles at microsatellite loci.* Within two weeks of inoculation, pumpkin vines began to wilt, and by 9 Oct 2008 most vines were dead and many fruit had developed lesions typical of infection by *P. capsici* (Fig. 2.1). Beginning in 2009, between 46 and 60 single-zoospore isolates of *P. capsici* were collected from the inoculated research field per year from various hosts (Table 2.2). In eggplant, *P. capsici* was only isolated from symptomatic fruit on plants that appeared otherwise healthy, while *P. capsici* was isolated from crown, stem, and fruit tissue of other hosts.


**Figure 2.1** Successful establishment of a recombining, experimental population of *Phytophthora capsici.* Pumpkins were grown in a research field with no history of *P. capsici* infection until ripening fruit were inoculated with two single-spore isolates of *P. capsici* (NY 0664-1 and NY 06180-4) on 8 Sept 2008. (A) Healthy vines prior to inoculation, and (B) symptoms on pumpkins one month after inoculation.

*Genotypic diversity observed in research field population.* The *P. capsici* population in our experimental field was characterized by high genotypic diversity, high turnover of genotypes, mixed reproduction, sexuality (after clone correction), and Hardy-Weinberg equilibrium. Between 26 and 37 unique multilocus genotypes (MLGs) were identified among isolates collected from the field per year (Fig. 2.2). In each year, multiple isolates were collected from the research field with identical MLGs, but the most frequently detected MLG was not identical across years. In addition, the number of MLGs detected in multiple isolates varied among years. In 2009 each MLG was identified in five or fewer isolates, while in 2012 one MLG was predominant, with most of the remaining MLGs only detected in one isolate (Fig. 2.2). Out of 81 unique MLGs identified in the four research field samples, 52 were only detected in one year. Nineteen and ten unique MLGs were detected in two or three years, respectively, and no MLG was detected in all four years. No isolates collected from the research field were identical to either parental isolate. Prior to clone correction, values for Stoddart and Taylor's index ($G$) were similar, except for the 2012 field sample, which had much lower genotypic diversity. A similar trend was seen in the expected number of MLGs calculated for each sample using rarefaction (Table 2.3). Prior to clone correction, evenness ($E_5$) was fairly close to one in all field samples except 2012. In sample CD-4, 22 unique MLGs were identified among 23 isolates, and no MLGs were detected in both CD-4 and the research field. The value of $G$ for sample CD-4 was similar to values observed in the research field samples from 2009 through 2011, prior to clone correction, but the number of expected MLGs was higher. Evenness in field CD-4 was nearly one prior to clone correction (Table 2.3).

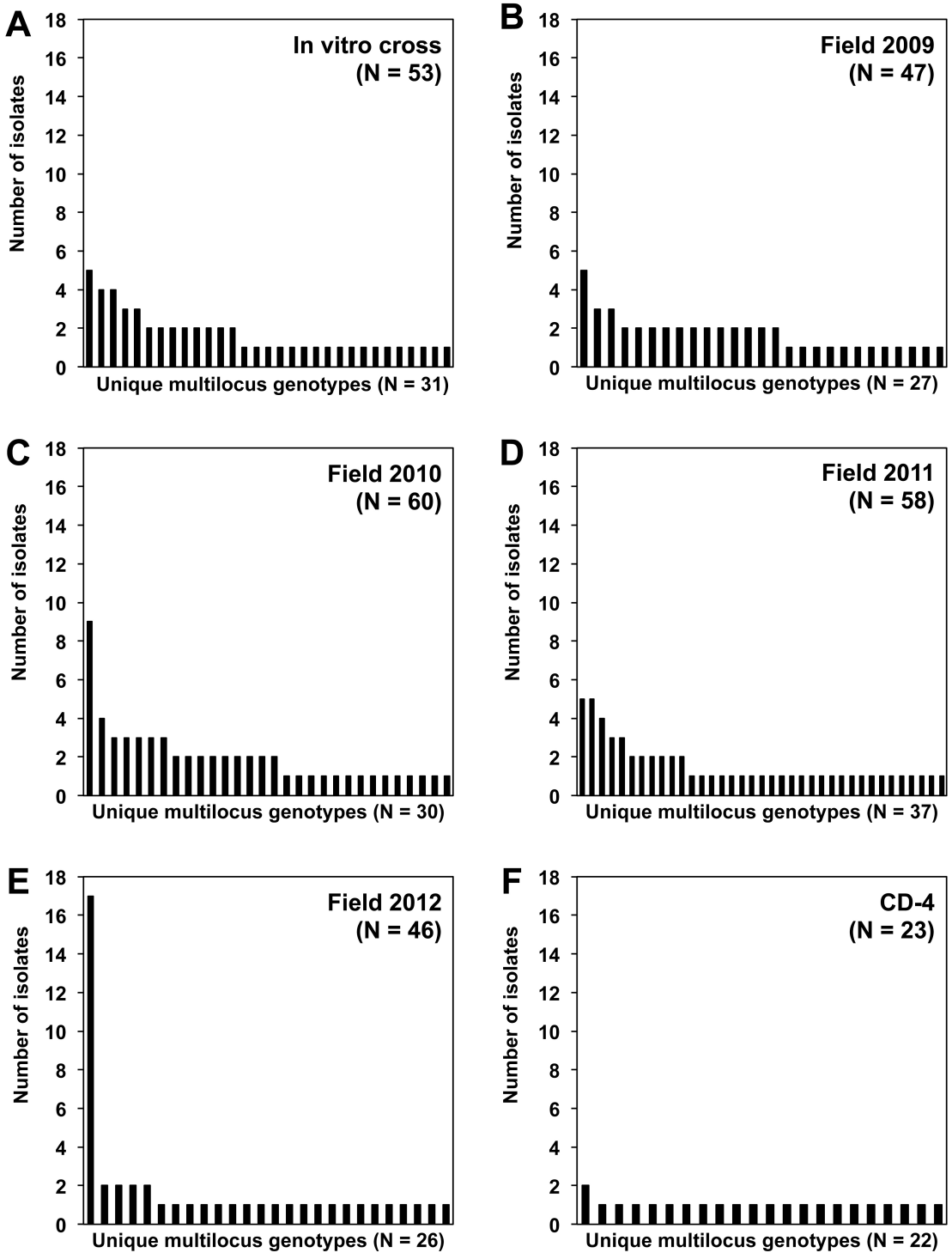



**Figure 2.2** Number of multilocus genotypes (MLGs) in six population samples. Isolates are single-oospore progeny from an *in vitro* cross between *Phytophthora capsici* isolates NY 0664-1 and NY 06180-4, from an experimental research field inoculated only with these two isolates in 2008 and sampled over four growing seasons (2009-2012), and from a commercial vegetable farm sampled in 2007 (CD-4). MLGs are defined by alleles present at five microsatellite loci and mating type of each isolate. No clone correction was conducted prior to this analysis.

**Table 2.3** Parameters of genetic diversity calculated for six population samples of *Phytophthora capsici* without clone-correction. Samples were from an *in vitro* cross between isolates NY 0664-1 and NY 06180-4, from an experimental research field inoculated only with these two isolates in 2008 and sampled over four growing seasons (2009-2012), and from a commercial vegetable farm sampled in 2007 (CD-4).

| | Sample | | | | | |
|---|---|---|---|---|---|---|
| | *In vitro* cross | Field 2009 | Field 2010 | Field 2011 | Field 2012 | CD-4 |
| MT | | | | | | |
| A1 | 25 | 17 | 22 | 21 | 16 | 9 |
| A2 | 28 | 30 | 38 | 37 | 30 | 14 |
| $\chi^2$ *P*-value[a] | NS | NS | 0.039 | 0.036 | 0.039 | NS |
| $F_{IS}$[b] | -0.102 | -0.127 | -0.041 | -0.031 | 0.219 | 0.265 |
| *P* value | 0.042 | 0.020 | NS | NS | <0.001 | <0.001 |
| $I_A$[c] | 0.065 | -0.002 | 0.141 | 0.106 | 0.803 | 0.096 |
| *P* value | 0.047 | NS | 0.001 | 0.007 | 0.001 | NS |
| *G*[d] | 22.12 | 21.45 | 18.75 | 24.03 | 6.49 | 21.16 |
| $E_5$[e] | 0.84 | 0.88 | 0.76 | 0.81 | 0.41 | 0.98 |
| eMLG[f] | 18 | 18 | 17 | 18 | 15 | 22 |
| Unique MLGs[g] | 31 | 27 | 30 | 37 | 26 | 22 |
| Total isolates | 53 | 47 | 60 | 58 | 46 | 23 |

[a] A Chi-squared test was performed to determine if the observed numbers of A1 and A2 isolates differed significantly from a 1:1 ratio. NS = not significant at $P = 0.05$.
[b] Whether $F_{IS}$ values are significantly different from zero was tested in Arlequin version 3.5 (Excoffier and Lischer 2010) with 5040 permutations. NS = not significant at $P = 0.05$.
[c] Index of association ($I_A$) was calculated in R using the 'poppr' package (Kamvar et al. 2014), with significance tested by 999 permutations. *P*-values shown here were calculated using the multilocus permutation method (Agapow and Burt 2001). NS = not significant at $P = 0.05$.
[d] Stoddart and Taylor's index (*G*) of MLG diversity calculated in R using the 'poppr' package.
[e] Evenness calculated in R using the 'poppr' package.
[f] Number of expected MLGs based on rarefaction if each sample contained 23 individuals was calculated in R using the 'poppr' package.
[g] Number of unique multilocus genotypes identified in each population using mating type and alleles present at five microsatellite loci.

Mating type ratios and the index of association indicate that the research field population was sexual after clone correction. The mating type ratio in most years (with the exception of 2011) did not deviate significantly from the expected 1:1 ratio, and the fixation index ($F_{IS}$) was not significantly different from zero ($P = 0.05$; Table 2.4). The observed index of association was not significantly different from randomly permuted samples indicating that the population is

sexual (Table 2.4). Following clone correction, approximately half of the polymorphic loci were in Hardy-Weinberg equilibrium, except in the 2012 field sample, when four out of five loci were in Hardy-Weinberg equilibrium.

**Table 2.4** Parameters of genetic diversity calculated for six population samples of *Phytophthora capsici* following clone-correction. Samples were from an *in vitro* cross between isolates NY 0664-1 and NY 06180-4, from an experimental research field inoculated only with these two isolates in 2008 and sampled over four growing seasons (2009-2012), and from a commercial vegetable farm sampled in 2007 (CD-4).

| | Sample | | | | | |
|---|---|---|---|---|---|---|
| | *In vitro* cross | Field 2009 | Field 2010 | Field 2011 | Field 2012 | CD-4 |
| MT | | | | | | |
| A1 | 17 | 11 | 16 | 11 | 14 | 9 |
| A2 | 14 | 16 | 14 | 26 | 12 | 13 |
| $\chi^2$ *P*-value[a] | NS | NS | NS | 0.014 | NS | NS |
| $F_{IS}$[b] | -0.058 | -0.094 | -0.104 | 0.015 | 0.135 | 0.275 |
| *P* value | NS | NS | NS | NS | NS | <0.001 |
| $I_A$[c] | -0.052 | -0.094 | -0.072 | 0.006 | 0.061 | 0.068 |
| *P* value | NS | NS | NS | NS | NS | NS |
| *G*[d] | 29.12 | 27 | 30 | 33.39 | 26 | 22 |
| $E_5$[e] | 0.98 | 1 | 1 | 0.97 | 1 | 1 |
| eMLG[f] | 22 | 22 | 22 | 21 | 22 | 22 |
| Total isolates | 31 | 27 | 30 | 37 | 26 | 22 |

[a] A Chi-squared test was performed to determine if the observed numbers of A1 and A2 isolates differed significantly from a 1:1 ratio. NS = not significant at $P = 0.05$.
[b] Whether $F_{IS}$ values are significantly different from zero was tested in Arlequin version 3.5 (Excoffier and Lischer 2010) with 5040 permutations. NS = not significant at $P = 0.05$.
[c] Index of association ($I_A$) was calculated in R using the 'poppr' package (Kamvar et al. 2014), with significance tested by 999 permutations. *P*-values shown were calculated using the multilocus permutation method (Agapow and Burt 2001). NS = not significant at $P = 0.05$.
[d] Stoddart and Taylor's index (*G*) of MLG diversity calculated in R using the 'poppr' package.
[e] Evenness calculated in R using the 'poppr' package.
[f] Number of expected MLGs based on rarefaction if each sample contained 22 individuals was calculated in R using the 'poppr' package.

*Differentiation and divergence in the research field population.* Pairwise $F_{ST}$ values comparing field isolates from 2009 through 2012 ranged from -0.012 to 0.001 following clone correction, and none were significantly different from zero at $P = 0.05$ (data not shown). Pairwise $F_{ST}$ values comparing sample CD-4 to all other samples were significantly different from zero ($P < 0.01$) with $F_{ST}$ values ranging from 0.156 to 0.216, following clone correction. Among pairwise $F_{ST}$ values comparing isolates collected from different hosts in 2009 through 2012 (pumpkin, summer squash, and tomato, only), no values were significantly different from zero at $P = 0.05$, following clone correction.

The dendrogram of all isolates showed little structure (Fig. 2.3), with only one bootstrap value exceeding the 70% cutoff. Many isolates from the CD-4 sample tended to cluster together, but not in a single clade. Isolates from the four field samples did not cluster together by year, nor did progeny from the *in vitro* cross. Similarly, when dendrograms were drawn for each sample individually, few bootstrap values exceeded the 70% cutoff, and showed few changes between years in samples from the research field (Fig. 2.4-2.9). In the minimum spanning network, MLGs from sample CD-4 generally clustered together, although not on a single branch of the network (Fig. 2.10). Similar to the dendrogram, MLGs from the *in vitro* progeny or each of the research field samples did not consistently cluster together.

**Figure 2.3** Dendrogram for all six population samples of *Phytophthora capsici*. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

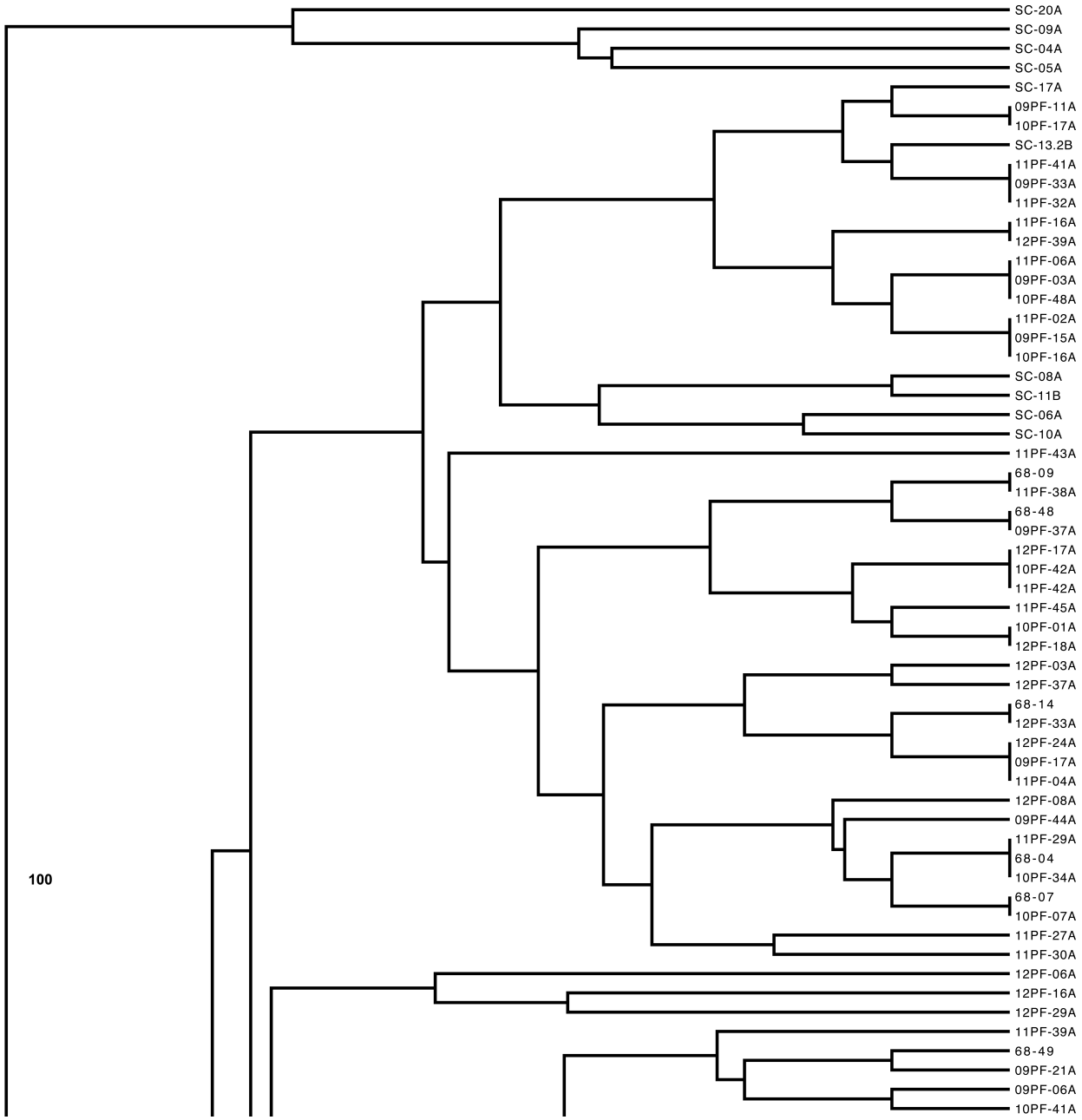


**Figure 2.3 (continued)**

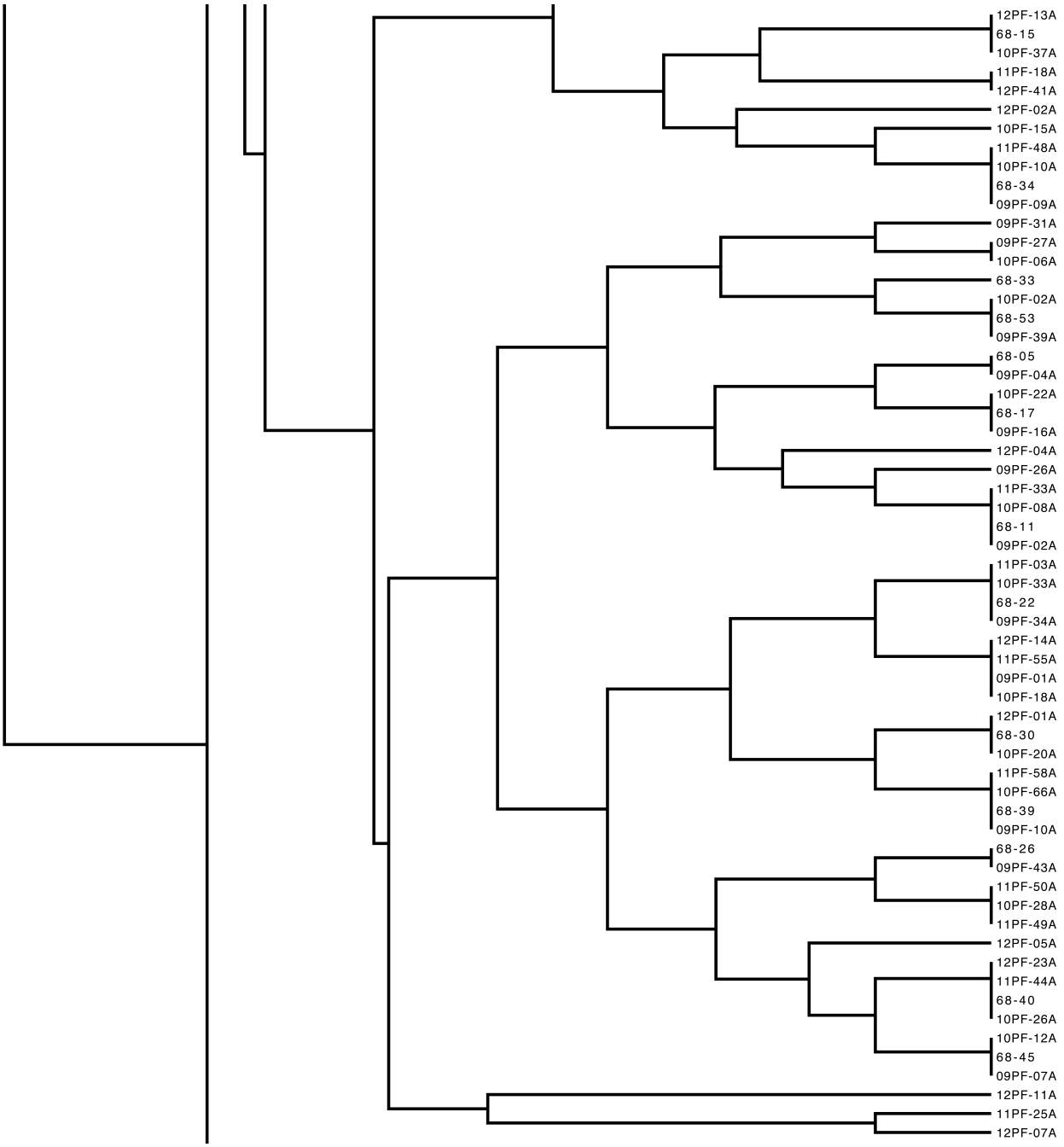
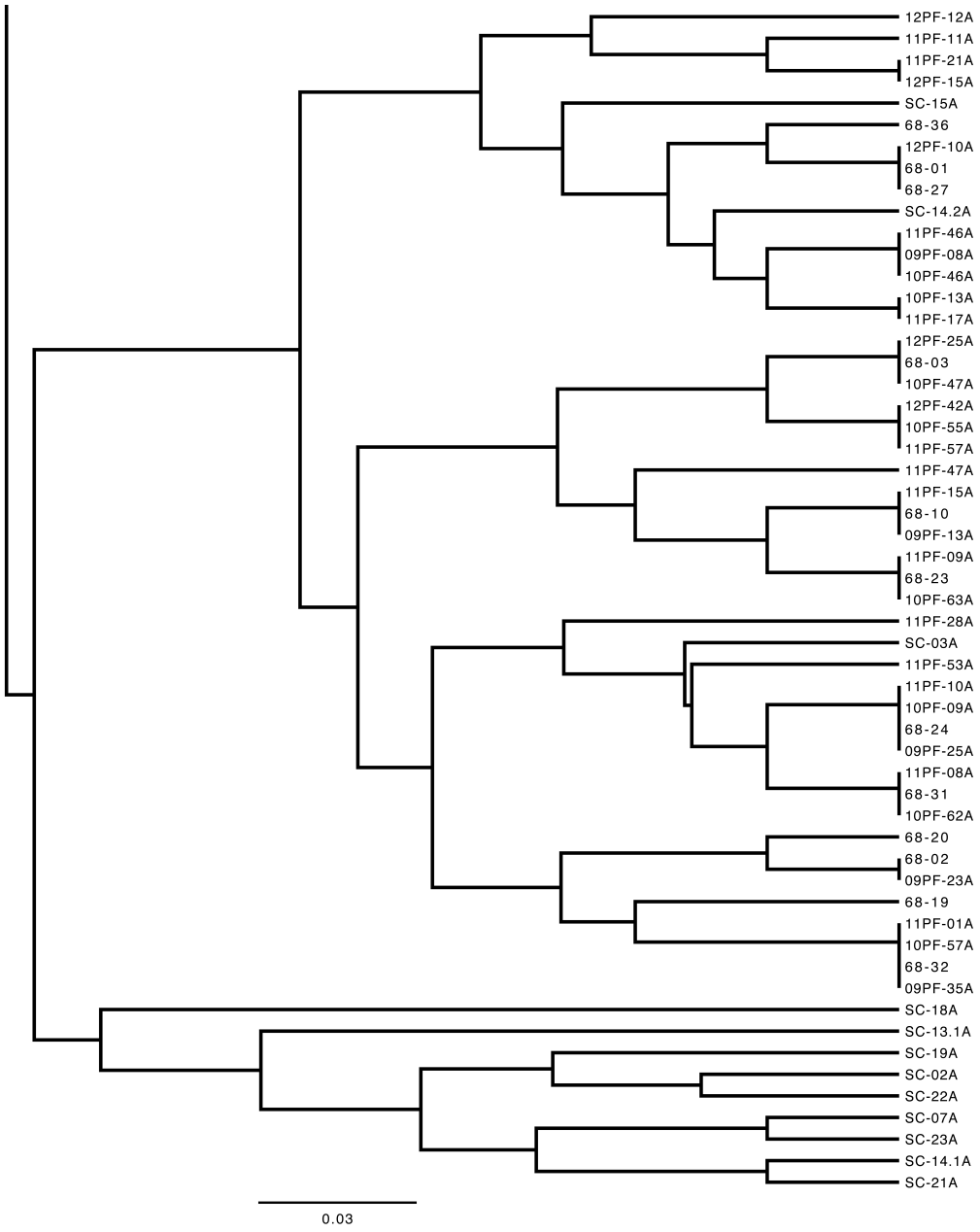


**Figure 2.3 (concluded)**



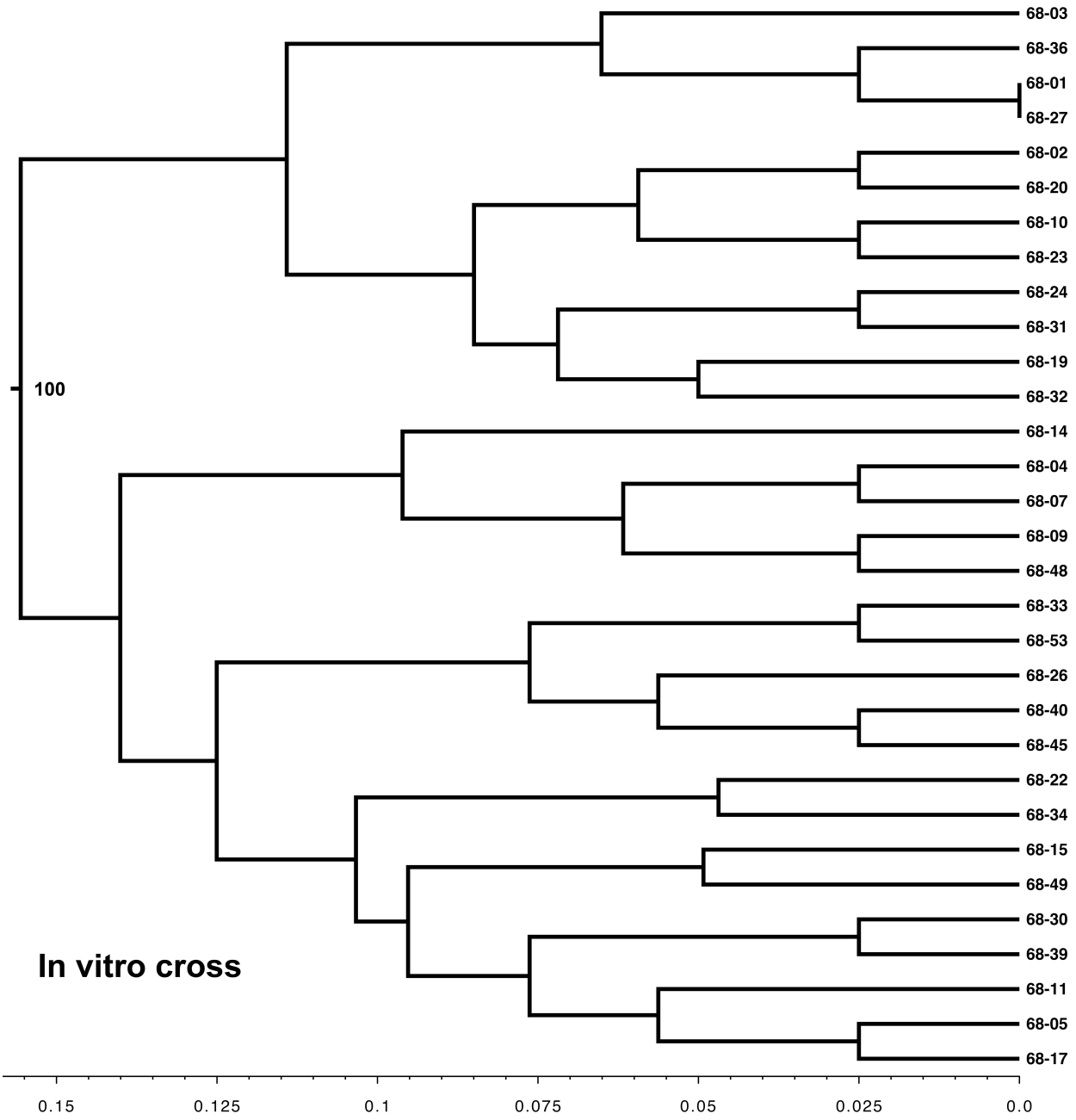



**Figure 2.4** Dendrogram for the *in vitro* cross population sample. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

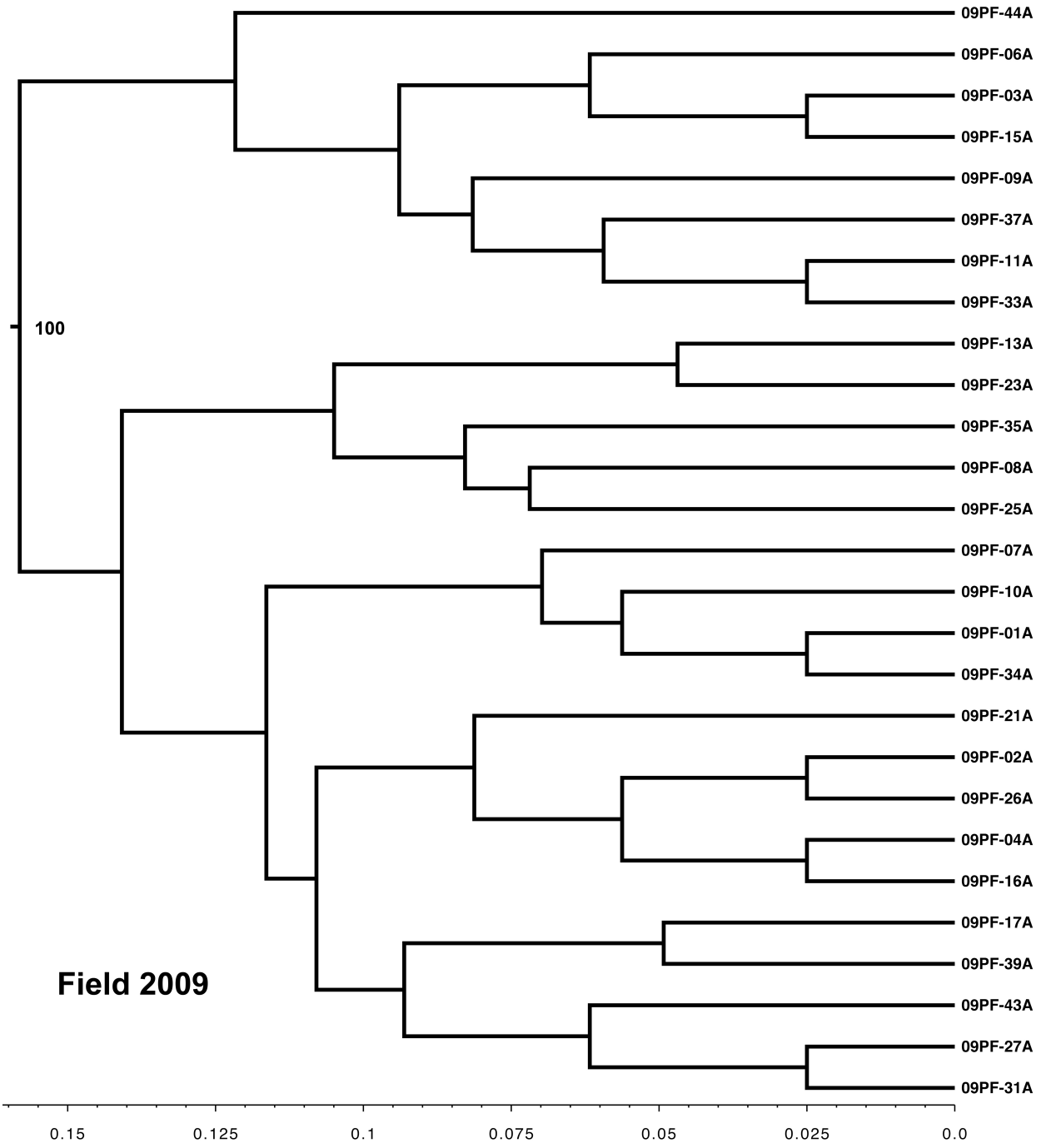



**Figure 2.5** Dendrogram for the 2009 research field population sample. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

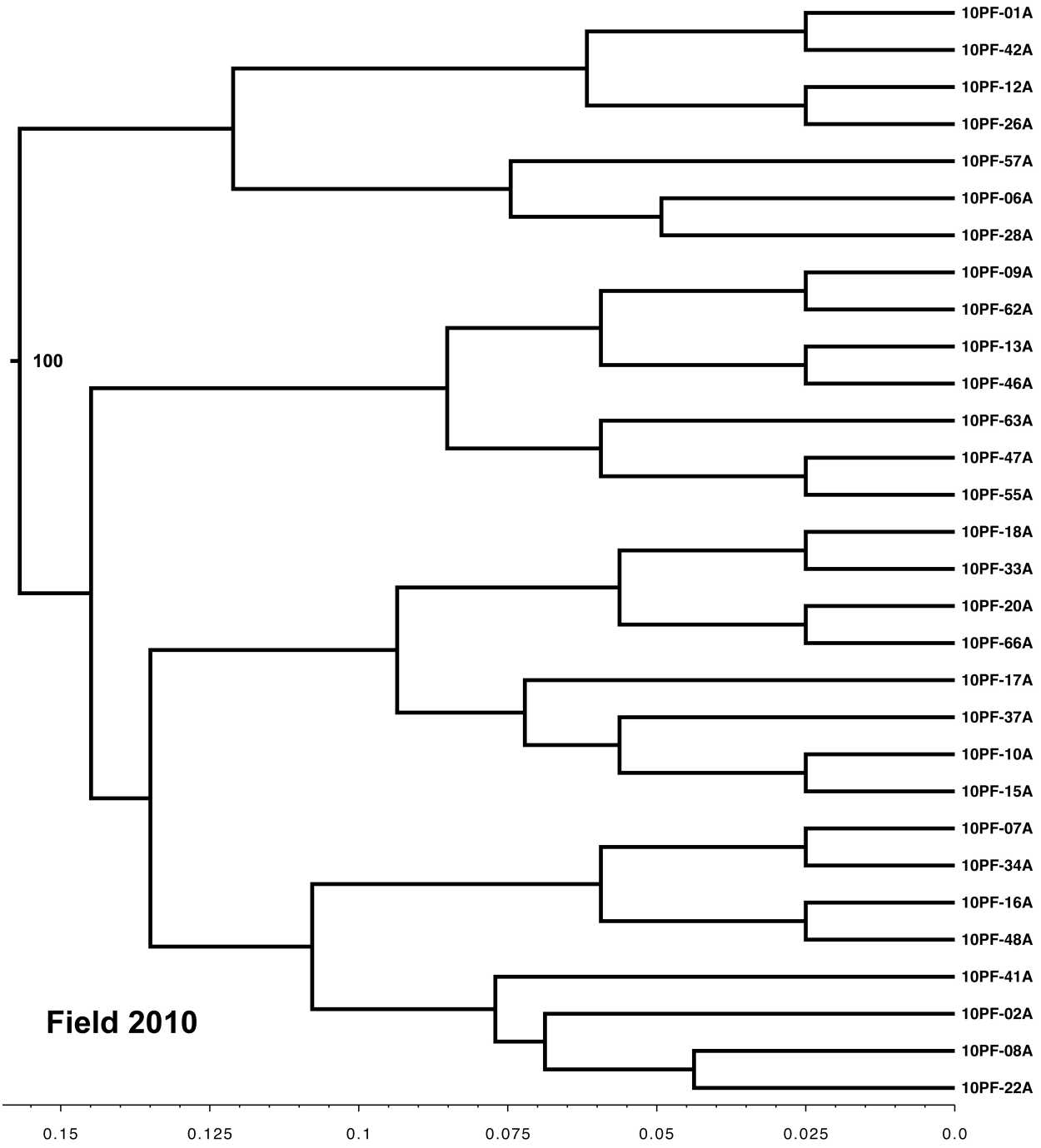



**Figure 2.6** Dendrogram for the 2010 research field population sample. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

**Figure 2.7** Dendrogram for the 2011 research field population sample. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

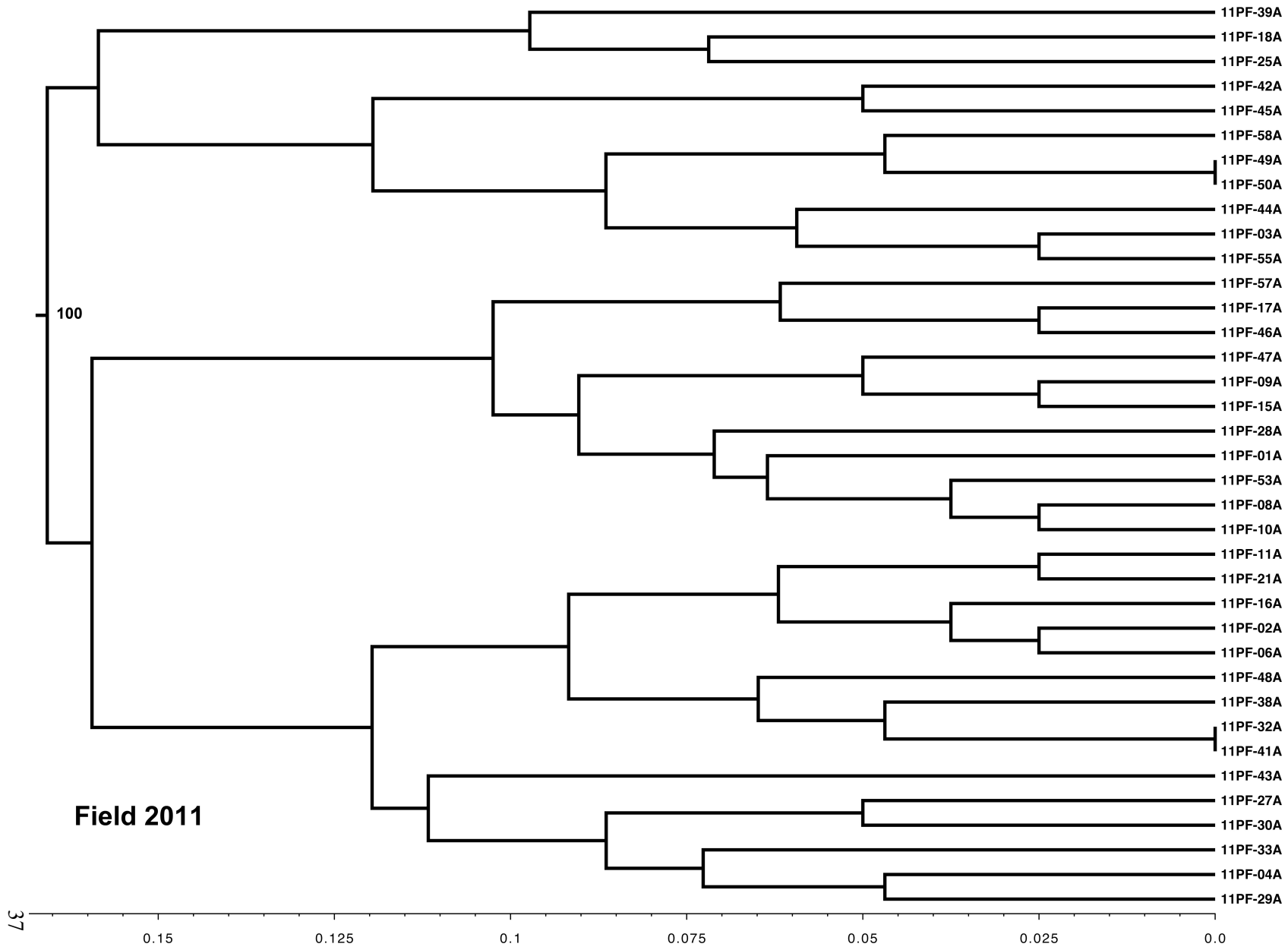
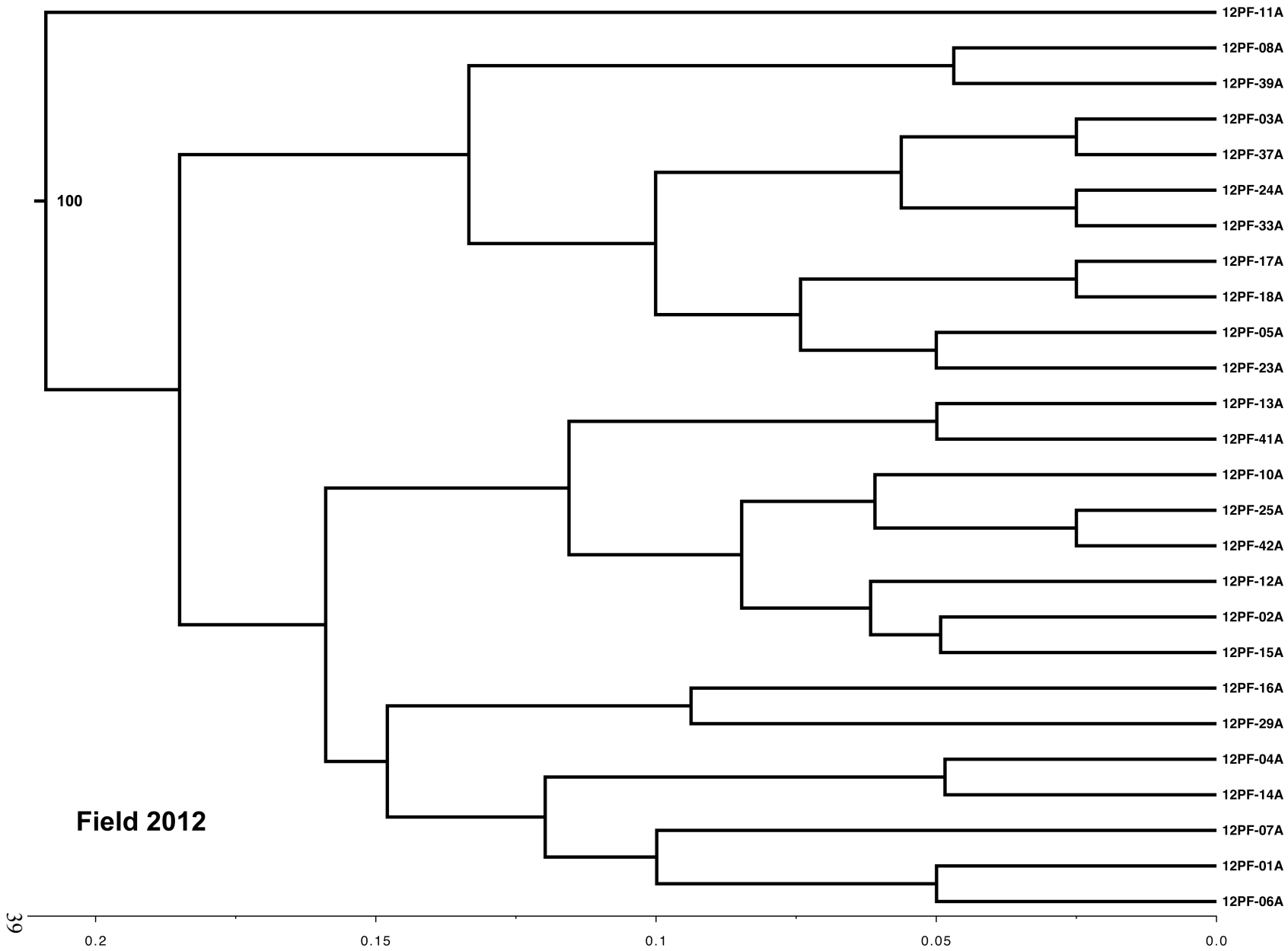

11PF-39A
11PF-18A
11PF-25A
11PF-42A
11PF-45A
11PF-58A
11PF-49A
11PF-50A
11PF-44A
11PF-03A
11PF-55A
11PF-57A
11PF-17A
11PF-46A
11PF-47A
11PF-09A
11PF-15A
11PF-28A
11PF-01A
11PF-53A
11PF-08A
11PF-10A
11PF-11A
11PF-21A
11PF-16A
11PF-02A
11PF-06A
11PF-48A
11PF-38A
11PF-32A
11PF-41A
11PF-43A
11PF-27A
11PF-30A
11PF-33A
11PF-04A
11PF-29A
100
Field 2011
37
0.15
0.125
0.1
0.075
0.05
0.025
0.0

**Figure 2.8** Dendrogram for the 2012 research field population sample. Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.


12PF-11A
12PF-08A
12PF-39A
12PF-03A
12PF-37A
12PF-24A
12PF-33A
12PF-17A
12PF-18A
12PF-05A
12PF-23A
12PF-13A
12PF-41A
12PF-10A
12PF-25A
12PF-42A
12PF-12A
12PF-02A
12PF-15A
12PF-16A
12PF-29A
12PF-04A
12PF-14A
12PF-07A
12PF-01A
12PF-06A
100
Field 2012
39
0.2
0.15
0.1
0.05
0.0

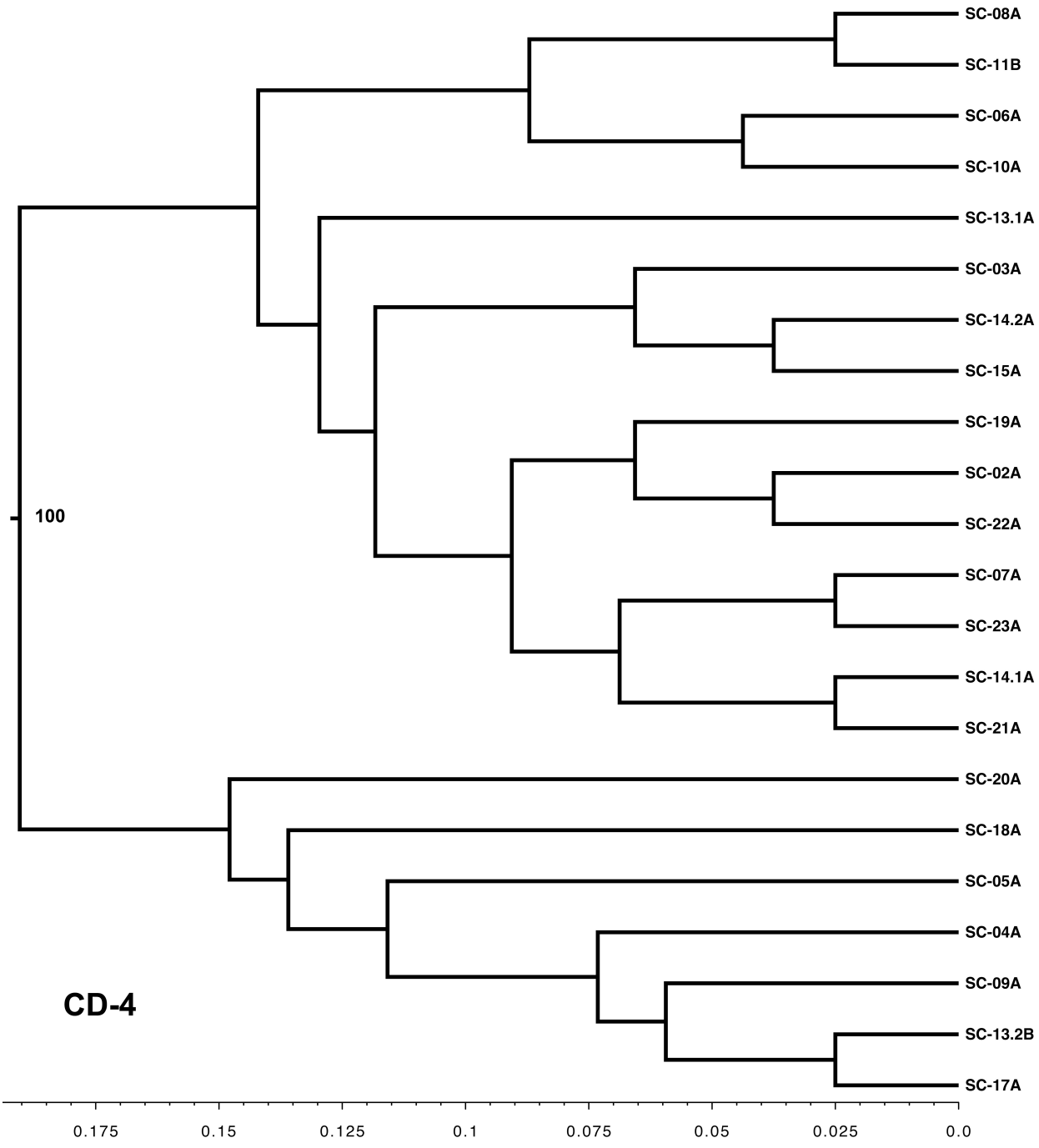



**Figure 2.9** Dendrogram for the CD-4 population sample (from a commercial farm). Bruvo's genetic distance was used to draw the dendrogram (following clone correction), employing the unweighted pair-group method with arithmetic averages and 999 permutations. Only bootstrap values greater than 70% are shown.

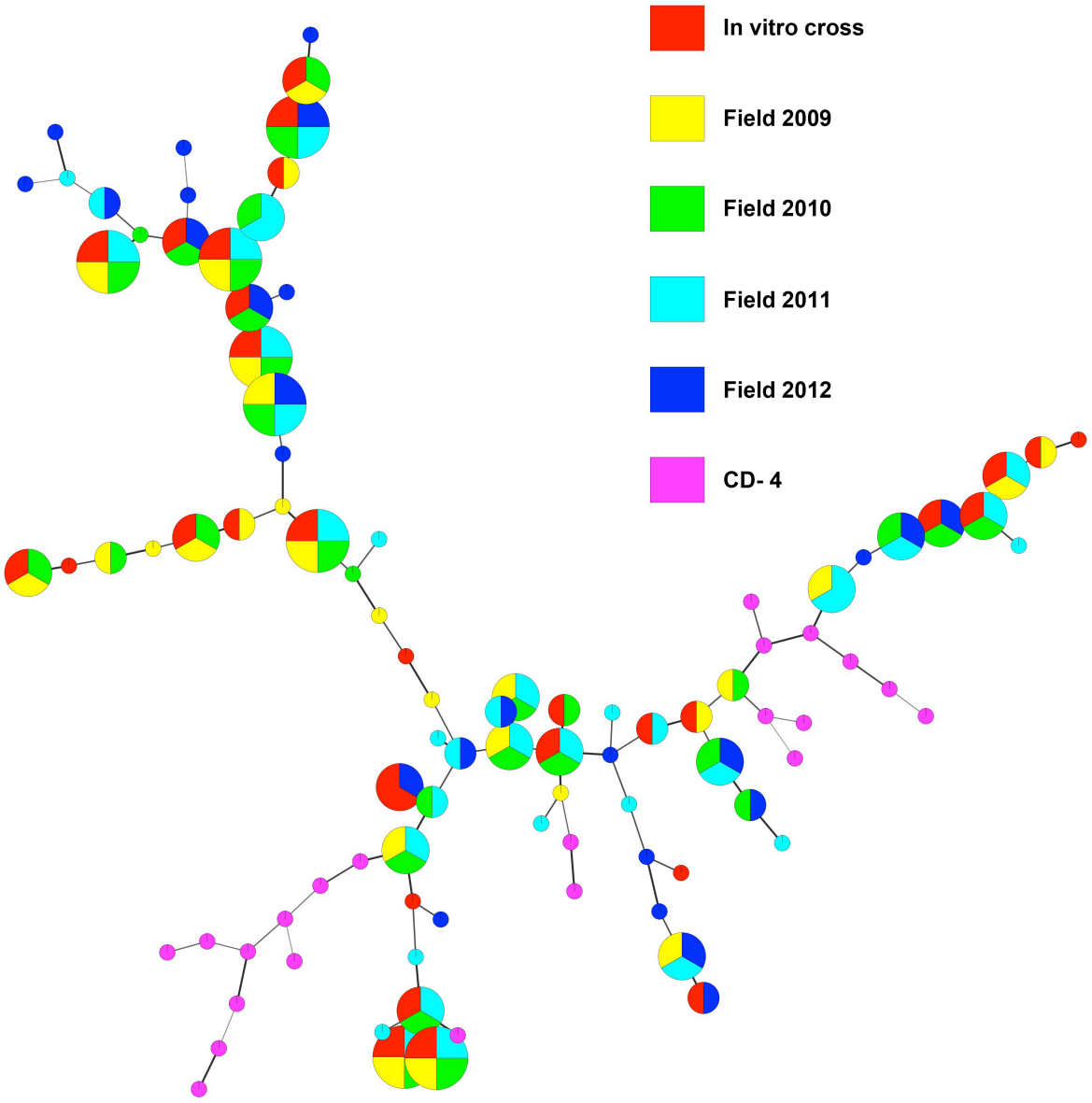



**Figure 2.10** Minimum spanning network for all six population samples of *Phytophthora capsici*. Network was drawn using Bruvo's genetic distance, following clone correction of microsatellite genotype data from six samples of *P. capsici* isolates. Isolates are single-oospore progeny from an *in vitro* cross between NY 0664-1 and NY 06180-4, from an experimental research field inoculated only with these two isolates in 2008 and sampled over four growing seasons (2009-2012), and from a commercial vegetable farm sampled in 2007 (CD-4). Each circle represents a unique multilocus genotype (MLG), the colors indicate the samples in which each MLG was detected, and the size of the circle corresponds to the number of individuals identified with that MLG. Where more than one isolate per year was identified with the same MLG, these isolates differed only in mating type. Thicker and darker lines represent MLGs that are more closely related (smaller Bruvo's distances).

*Allele frequencies in field samples.* All alleles present in the parental isolates were observed each year in the research field. At loci Pcap1, Pcap3, Pacp5, and SSRPC26, allele frequencies in the 2009 field sample did not differ markedly from those expected in the F1 generation based on the genotypes of the parent isolates (Table 2.5).

**Table 2.5** Frequencies of alleles observed at five microsatellite loci in *Phytophthora capsici* isolates from six population samples. Prior to calculating allele frequencies, data were clone corrected so that only one representative from each multilocus genotype was included.

| | | Allele frequencies | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Locus | Size (bp)[a] | Expected[b] | *In vitro* cross | 2009 | 2010 | 2011 | 2012 | CD-4 |
| Pcap1 | 233 | 0 | 0 | 0 | 0 | 0.01 | 0.02 | 0.05 |
| | 239 | | 0 | 0 | 0 | 0 | 0 | 0.11 |
| | 241 | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 | 0.96 | 0.84 |
| | 260 | 0 | 0 | 0 | 0 | 0.01 | 0.02 | 0 |
| Pcap3 | 434 | 0.50 | 0.45 | 0.50 | 0.55 | 0.58 | 0.52 | 0.70 |
| | 440 | | 0 | 0 | 0 | 0 | 0 | 0.23 |
| | 446 | 0.50 | 0.55 | 0.50 | 0.45 | 0.42 | 0.48 | 0.07 |
| Pcap5 | 274 | 0.25 | 0.18 | 0.31 | 0.23 | 0.24 | 0.10 | 0.70 |
| | 277 | 0.50 | 0.58 | 0.48 | 0.50 | 0.51 | 0.54 | 0 |
| | 291 | 0.25 | 0.24 | 0.20 | 0.27 | 0.24 | 0.37 | 0.23 |
| | 299 | | 0 | 0 | 0 | 0 | 0 | 0.05 |
| | 302 | | 0 | 0 | 0 | 0 | 0 | 0.02 |
| Pcap7 | 359 | 0.50 | 0.23 | 0.28 | 0.25 | 0.19 | 0.29 | 0 |
| | 374 | | 0 | 0 | 0 | 0 | 0 | 0.02 |
| | 377 | 0.25 | 0.27 | 0.41 | 0.38 | 0.42 | 0.40 | 0.39 |
| | 380 | | 0 | 0 | 0 | 0 | 0 | 0.16 |
| | 383 | | 0 | 0 | 0 | 0 | 0 | 0.27 |
| | 386 | 0.25 | 0.50 | 0.31 | 0.37 | 0.36 | 0.31 | 0.16 |
| | 389 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 |
| | 392 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 |
| SSRPC26 | 183 | | 0 | 0 | 0 | 0 | 0 | 0.11 |
| | 186 | 0.75 | 0.77 | 0.78 | 0.73 | 0.80 | 0.83 | 0.61 |
| | 189 | 0.25 | 0.23 | 0.22 | 0.27 | 0.20 | 0.17 | 0.27 |

[a] Size of each observed allele in base pairs.
[b] Expected allele frequencies based on alleles observed in parent isolates.

However, at locus Pcap7, the frequency of the 359 bp allele was substantially less than expected, and the frequencies of the 377 bp allele (and to a lesser extent the 386 bp allele) were greater than expected in the 2009 field sample, compared to expected allele frequencies in an F1 generation from this cross. Between 2009 and 2011, allele frequencies at all loci remained relatively constant, with the exception that in 2012, the frequency of the 274 bp allele at locus Pcap5 decreased, while the frequency of the 291 bp allele increased (Table 2.5).

*Detection of rare, novel alleles at loci Pcap1 and Pcap7.* At locus Pcap1, the parental isolates used to inoculate the field were both homozygous for a 241 bp allele. However, two alleles (233 bp and 260 bp) that were not present in either parental isolate or in field isolates from previous years were identified in the 2011 field sample. Each new allele was observed in one isolate that was heterozygous at this locus with the 241 bp allele from the parental isolates. Based on sequencing, the 233 bp allele had an 8 bp deletion outside of the repeat region of the locus, compared to the 241 bp allele present in NY 0664-1 and NY 06180-4. The 260 bp allele had three additional copies of the AC repeat motif in addition to a string of 13 cytosine bases, amounting to a 19 bp addition in the repeat region of the locus, compared to the parental allele. These alleles were also found in the 2012 field sample, observed in one isolate each as heterozygotes with the parental allele. The respective deletions and insertions occurred in the same positions in the locus as those observed in 2011. These alleles appear to be the result of insertions and do not follow the stepwise mutation model expected for microsatellites.

At locus Pcap7, two alleles (389 bp and 392 bp) were also identified in the 2011 field sample, but were not present in either parental isolate. Each new allele was observed in one isolate that was heterozygous at this locus, and also contained an allele observed in one of the parental isolates. Sequencing of these new alleles confirmed that they contained one or two

additional copies of the CTT repeat motif in the repeat region of the locus, compared to the largest allele observed in the two parental isolates (386 bp in isolate NY 0664-1). Neither of these new alleles was observed in isolates collected from the field in 2012. These alleles appear to have arisen by following a stepwise mutation model.

*Oospore progeny from an in vitro cross behaves like the field population.* Germinating oospores collected *in vitro* were the F1 progeny of a cross between *P. capsici* isolates NY 0664-1 and NY 06180-4. A total of 53 single-oospore isolates were collected, and 22 of these were removed from analysis following clone correction. Among the 53 progeny, 31 unique MLGs were identified, with up to five progeny sharing a MLG (Fig. 2.2). Based on values for Stoddart and Taylor's Index ($G$), evenness ($E_5$), and eMLG, genotypic diversity of the *in vitro* progeny was similar to that observed in field samples, following clone correction (Table 2.4). Genotypic evenness was relatively close to one, even prior to clone correction (Table 2.3). Among the *in vitro* progeny, the ratio of A1 isolates to A2 isolates did not differ significantly from 1:1 at $P$ = 0.05 after clone correction, and the fixation index ($F_{IS}$) was not significantly different from zero (Table 2.4). The index of association was not significantly different from randomly permuted samples (Table 2.4). Similar to the research field population, two of the four polymorphic loci were in Hardy-Weinberg equilibrium among the *in vitro* progeny, following clone correction. No pairwise $F_{ST}$ values comparing clone-corrected progeny from the *in vitro* cross to research field samples from 2009 through 2012 were significantly different from 0 at $P$ = 0.05 (data not shown). However, pairwise $F_{ST}$ values were significantly different from 0 when comparing the *in vitro* progeny to the CD-4 sample whether or not data were clone corrected.

All alleles present in the parent isolates were also observed among *in vitro* progeny, and at loci Pcap1, Pcap3, Pacp5, and SSRPC26, allele frequencies (following clone correction) did

not differ markedly from expected frequencies based on the genotypes of the parent isolates. However, at locus Pcap7, the frequency of the 359 bp allele was substantially less than expected, and the frequency of the 386 bp allele was substantially greater than expected, as was seen in the 2009 research field sample (Table 2.5). No *in vitro* progeny were identical to either parent isolate, and no progeny shared the same MLG with isolates from sample CD-4.

*Unusual patterns of inheritance.* In the parent isolates NY 0664-1 and NY 06180-4, alleles at loci Pcap5 and Pcap7 were unique to either one parent or the other (Table 2.6). Thus, the A1 parent (NY 0664-1) could only contribute the 277 bp allele at locus Pcap5, and the 377 bp or 386 bp alleles at locus Pcap7 to oospore progeny in the F1 generation. Similarly, the A2 parent (NY 06180-4) could only contribute the 274 bp or 291 bp alleles at Pcap5, and the 359 bp allele at locus Pcap7. Of the 53 single-oospore progeny from the *in vitro* cross, only 21 had an allele from each parent at both loci (Pcap5 and Pcap7). The remaining 32 progeny appeared to inherit two alleles from one parent at one or both of these loci. In only a few of these progeny (n = 4) were alleles at both loci Pcap5 and Pcap7 from the same parent (always NY 0664-1), which could be consistent with selfing. No progeny had alleles only from parental isolate NY 06180-4. Many oospore progeny (n = 28) appeared to inherit alleles from both parents at locus Pcap5 (or in one case, SSRPC26), but only an allele from NY 0664-1 at locus Pcap7 (either 377 bp or 386 bp; Table 2.6). Over more than 5 years and 12 transfers in the lab, as well as inoculation and re-isolation from hosts in the greenhouse, alleles at all five microsatellite loci have remained unchanged in isolates NY 0664-1 and NY 06180-4.

**Table 2.6** Evidence of unusual recombination in *Phytophthora capsici.* All isolates are single-oospore progeny of an *in vitro* cross between isolates NY 0664-1 (A1 mating type) and NY 06180-4 (A2 mating type).

| | | | Loci | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | Pcap3 | Pcap5 | | Pcap7 | | SSRPC26 | |
| Type[a] | N[b] | MT | Alleles | Alleles | Alleles | Parent[c] | Alleles | Parent[c] | Alleles | Parent[c] |
| NY 0664-1 | | A1 | 241 / 241 | 434 / 446 | 277 / 277 | | 377 / 386 | | 186 / 186 | |
| NY 06180-4 | | A2 | 241 / 241 | 434 / 446 | 274 / 291 | | 359 / 359 | | 186 / 189 | |
| possible self | 1 | A1 | 241 / 241 | 434 / 434 | 277 / 277 | NY 0664-1 | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| possible self | 1 | A2 | 241 / 241 | 434 / 434 | 277 / 277 | NY 0664-1 | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| possible self | 1 | A1 | 241 / 241 | 446 / 446 | 277 / 277 | NY 0664-1 | 377 / 377 | NY 0664-1 | 186 / 186 | unknown |
| possible self | 1 | A1 | 241 / 241 | 446 / 446 | 277 / 277 | NY 0664-1 | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| other | 1 | A2 | 241 / 241 | 434 / 434 | 277 / 277 | NY 0664-1 | 386 / 386 | NY 0664-1 | 186 / 189 | both |
| other | 3 | A1 | 241 / 241 | 434 / 434 | 277 / 291 | both | 386 / 386 | NY 0664-1 | 186 / 189 | both |
| other | 5 | A2 | 241 / 241 | 434 / 446 | 274 / 277 | both | 377 / 377 | NY 0664-1 | 186 / 186 | unknown |
| other | 1 | A1 | 241 / 241 | 434 / 446 | 274 / 277 | both | 377 / 377 | NY 0664-1 | 186 / 189 | both |
| other | 1 | A2 | 241 / 241 | 434 / 446 | 274 / 277 | both | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| other | 1 | A1 | 241 / 241 | 434 / 446 | 274 / 277 | both | 386 / 386 | NY 0664-1 | 186 / 189 | both |
| other | 4 | A2 | 241 / 241 | 434 / 446 | 277 / 291 | both | 377 / 377 | NY 0664-1 | 186 / 186 | unknown |
| other | 2 | A1 | 241 / 241 | 434 / 446 | 277 / 291 | both | 377 / 377 | NY 0664-1 | 186 / 189 | both |
| other | 2 | A2 | 241 / 241 | 434 / 446 | 277 / 291 | both | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| other | 3 | A1 | 241 / 241 | 434 / 446 | 277 / 291 | both | 386 / 386 | NY 0664-1 | 186 / 189 | both |
| other | 1 | A1 | 241 / 241 | 446 / 446 | 274 / 277 | both | 386 / 386 | NY 0664-1 | 186 / 189 | both |
| other | 2 | A2 | 241 / 241 | 446 / 446 | 277 / 291 | both | 386 / 386 | NY 0664-1 | 186 / 186 | unknown |
| other | 2 | A1 | 241 / 241 | 446 / 446 | 277 / 291 | both | 386 / 386 | NY 0664-1 | 186 / 189 | both |

[a] Type of unusual recombination observed. Single-oospore progeny from this cross which did not receive one allele from each parent isolate at these loci either received only alleles from one parent (consistent with possible selfing, and designated 'possible self"), or received an allele from each parent at locus Pcap5 or SSRPC26, but only alleles from NY 0664-1 at locus Pcap7 ('other').

[b] Number of single-oospore progeny with the designated multilocus genotype.

[c] Parent isolate (NY 0664-1, NY 06180-4, or both) from which alleles at each locus were derived. At locus SSRPC26, the 186 bp allele could have been inherited from either parent, hence the designation 'unknown'

In the 2009 research field sample (the first time the field was sampled following inoculation, but not necessarily the F1 generation), 20 of the isolates (representing 12 unique MLGs) collected from the field contained an allele from each parent at locus Pcap5, but only an allele from NY 0664-1 at locus Pcap7. These included some isolates collected early in the season on 22 Jun, only a week after the squash were transplanted into the field. One unique MLG (represented by two isolates collected on the same date) contained only the 274 bp allele at locus Pcap5 (from NY 06180-4) and only the 377 bp allele at locus Pcap7 (from NY 0664-1). No isolates collected from the field in 2009 contained only alleles from one parent or the other at both loci Pcap5 and Pcap7.

***Discussion***

We successfully established a field population *P. capsici* that reproduced both sexually and clonally, but that appeared sexual (after clone correction) with mating types in the expected 1:1 ratio and no detectable linkage among microsatellite loci. In spite of the small number of loci being studied, substantial genotypic diversity was observed in this isolated research population of *P. capsici*. Furthermore, this diversity appears to be maintained over time (at least over four years following inoculation), and novel, rare alleles were identified in the population during this time. It is likely that greater diversity, and possibly additional novel alleles, could be detected using additional loci or different genotyping methods. The fact that not all single-oospore progeny from the *in vitro* cross had unique MLGs is consistent with the hypothesis that this study underestimates diversity in the population. Therefore detection of many MLGs in two or three years is not evidence for survival of asexual propagules of *P. capsici* over the winter in New York State. The fact that no isolates identical to either parent (even based on the small number of

loci used in this study) were sampled from the field in 2009 is strong evidence that asexual propagules cannot survive the winter.

Previous studies have found evidence of apomixis, but not selfing, in crosses between *P. capsici* isolates, or between *P. capsici* and the closely-related *P. tropicalis* (Donahoo and Lamour 2008; Hurtado-Gonzáles and Lamour 2009). It seems likely that the potential for viable progeny to be produced via selfing or apomixis would vary among isolates, since Donahoo et al. (2008) reported that proportions of apomictic compared to hybrid progeny varied between two separate crosses, and Judelson (1997) made similar observations of selfed versus hybrid progeny in *P. infestans* crosses. While the presence of only alleles from isolate NY 0664-1 at two polymorphic loci in four progeny from the in vitro cross could be consistent with selfing, these genotypes could also have resulted from either a cross between the two parents or apomixis, followed (in either case) by loss of heterozygosity via gene conversion. Because both parental isolates had at least one allele in common at the other three loci studied, patterns of inheritance at these other loci are not informative in distinguishing among these possibilities. Alternative genotyping techniques would be needed to clarify which mechanism was responsible for the genotypes we observed. Similarly, with the microsatellite markers used in this study, it is not possible to determine whether selfing or apomixis occurred in the research field population. While selfing may occur in the research field, it is likely less frequent than outcrossing, based on the lack of linkage observed among loci. This is in contrast to evidence of frequent selfing in field populations of the homothallic oomycete *Aphanomyces euteiches* (Grünwald and Hoheisel 2006).

Results presented here suggest that genotypic diversity remained high over four growing seasons in this isolated research population of *P. capsici*. Based on the high levels of diversity

detected in on-farm populations of *P. capsici* in New York State, this was not surprising (Dunn et al. 2010). The lower values of *G* and the expected numbers of MLGs (prior to clone correction) in the 2012 research field sample are probably related to oversampling of a single dominant genotype, which spread asexually throughout the research field in 2012 as the season progressed, rather than to a decline in genotypic diversity by the fourth year after inoculation. While values for *G* were similar between the CD-4 population and the research field samples, the higher number of expected MLGs in the CD-4 population compared to the research field samples prior to clone correction suggests that diversity in this commercial field is higher than in the research field.

Because the overwintering spores of *P. capsici* are sexual (unless apomixis is occurring), it is not surprising that the data presented here are consistent with a sexual population in this research field. Since diversity in the founding population was low (two single-zoospore isolates), all progeny from these two parental isolates are relatively closely related, and mating among close relatives can result in higher $F_{IS}$ values (Goodwin 1997). However, this was not observed in this research population. Although the CD-4 population had a larger $F_{IS}$ compared to annual samples from the research field, the value is still consistent with random mating, and is similar to $F_{IS}$ values observed for other heterothallic oomycetes (Goodwin 1997).

Based on calculated pairwise $F_{ST}$ values and similar allele frequencies observed in the research field over time, little or no genetic drift has occurred in this research population, to date. However, it is difficult to quantify drift because the number of generations since the founding of the population is unknown. In agreement with the calculated pairwise $F_{ST}$ values, neither dendrograms nor minimum spanning networks provided evidence of high differentiation among research field samples from different years, or progeny from the *in vitro* cross.

The 'plasticity' of the genus *Phytophthora* has been noted previously (Judelson 2007; Lamour et al. 2012). This report of four novel alleles at two of the five loci studied (two alleles at each locus) suggests that high rates of mutation may contribute to this plasticity in *P. capsici*. It is likely that new alleles were not detected among the *in vitro* progeny, or in the first two years of the field study due to sample size, since novel alleles are relatively rare (based on their infrequent detection in the 2011 and 2012 field samples). We cannot be certain how many generations occur annually in this field, however, attempts to germinate oospores of *Phytophthora* sp. in the lab suggest that a maturation time of approximately one month is required, and germination of oospores formed during the *in vitro* cross in this study are consistent with this observation. Assuming a four-month growing season when host tissue is in the field and temperatures are conducive to growth of *P. capsici*, a maximum of 17 generations might have occurred between September 2008 and the end of the 2012 growing season. If this assumption is correct, then the mutations observed in this population occurred at a rate of at least 0.047 mutations per locus per sexual generation, although this number could be inflated if mutations occurred during asexual reproduction (Ellegren 2000). While no new alleles were observed in the two parental isolates over more than five years of asexual propagation in the lab (data not shown), we cannot rule out the possibility that somatic mutations are occurring at these loci in the field. Mutation rates are generally expected to be higher at microsatellite loci than in other parts of the genome, and this mutation rate is in line with some of the higher microsatellite mutation rates observed in some animals (Ellegren 2000), and in chickpea (*Cicer arietinum*; Udupa and Baum 2001). Lamour et al. (2012) compared nine *P. capsici* isolates using restriction-site-associated DNA sequencing, and found a high density of single nucleotide variants among *P. capsici* isolates (23.4/kb). They also speculated that the relatively high

proportion of *P. capsici* single nucleotide variants in coding regions of the genome could be evidence for overall high mutation rates in *P. capsici*.

Since a small number of loci were used in this study, we cannot conclude that *P. capsici* has an inherently high mutation rate at all microsatellite loci, or in all parts of the genome. Loci Pcap1 and Pcap7 could simply be highly-mutable. A study of microsatellite mutations in humans found that mutations occurred at some loci but not others, and the authors observed more mutations at loci with a tetranucleotide repeat, than at loci with a dinucleotide repeat (Weber and Wong 1993). Udupa and Baum (2001) also observed variation in mutation rates at chickpea loci, and found a positive correlation between the number or repeats in the ancestral microsatellite allele at a locus and the number of mutations. The number of loci and mutations in the present study were too small to determine whether a similar pattern exists in *P. capsici*. Both Weber and Wong (1993) and Udupa and Baum (2001) reported that the most frequent mutation was gain or loss of a single copy of the repeat motif, with gain or loss of more than one copy of the repeat still occurring, but at a much lower rate. These observations are consistent with the stepwise mutation model, as are the novel alleles observed at locus Pcap7 in this study. The 392 bp allele at this locus could have resulted from two sequential mutations, each adding a single repeat unit. However, mutations at the Pcap1 locus do not fit this model, suggesting that the stepwise mutation model may not fully describe the occurrence of mutations in *P. capsici* at the microsatellite loci described here.

The unusual patterns of inheritance observed in the F1 progeny from the *in vitro* cross are similar to what has been previously reported for *P. cinnamomi* (Dobrowolski et al. 2002), *P. infestans* (Carter et al. 1999), and *P. parasitica* (Forster and Coffey 1990). Mechanisms proposed in these studies include nondisjunction and the initial presence of heterokaryotic nuclei in

oospores. Vercauteren et al. (2011) also reported the inheritance of an allele from just one parent in crosses between isolates of *P. ramorum*, and speculated that it could be attributed to anomalous meiosis. Our observations are also similar to what Lamour et al. (2012) described as 'loss of heterozygosity,' which was observed in 23 of 65 progeny from an *in vitro* cross between isolates of *P. capsici*. In some of these progeny, loss of heterozygosity was associated with a change in mating type or with loss of pathogenicity on cucumber and pepper fruit over 6 years of storage and culturing in the lab. The authors speculated that the cause could be deletion of one allele at a locus, translocation, homologous recombination, or gene conversion. Chamnanpunt et al. (2001) also detected conversion to homozygosity in a linkage group that included the *Avr1b* avirulence gene in *P. sojae*, with rates between $5x10^{-4}$ and $3x10^{-2}$ per locus per nucleus per generation. The authors attributed their observations to gene conversion without crossing over, resulting in heterokaryotic progeny. They observed this phenomenon in both F1 progeny, and also in single zoospore lines vegetatively propagated in the lab, but saw higher rates of conversion to homozygosity following sexual reproduction. Conversion rates also differed between crosses, and appeared to favor certain alleles over others. Abu-El Samen et al. (2003) reported genetic variation in *P. infestans* among single zoospore progeny from a single hyphal tip isolate, attributing it to some combination of spontaneous mutation, gene conversion, mitotic crossing over, or extra-chromosomal elements. It should be noted that in our study the observation of an allele from only one parent at locus Pcap7 was interpreted as presence of two copies of that allele in the isolate. However, we cannot rule out the possibility of a null allele at this locus, because homozygosity cannot be distinguished from the presence of a null allele in our data set.

Previous reports of loss of heterozygosity in *P. capsici* have been in progeny from crosses made in the lab. The observation of numerous isolates collected from the field in 2009 with the same allele patterns observed in the *in vitro* progeny could be the first evidence that loss of heterozygosity also occurs in the field. The number of generations occurring between the inoculation of the field on 8 Sept 2008 and 22 Jun 2009 (when the first isolates were collected from the field) cannot be determined with certainty. However, the fact that this unusual recombination was observed early in the 2009 growing season is consistent with the hypothesis that some of these field isolates are in fact F1 progeny. Because alleles at these five loci were invariable in the parent isolates over numerous transfers in the lab, we did not investigate the possibility that genetic variation observed in the progeny could be occurring during asexual, as well as sexual, reproduction, as Abu-El Samen et al. (2003) and Chamnanpunt et al. (2001) reported in *P. infestans*, and *P. sojae*, respectively.

Previous work has demonstrated that once *P. capsici* is introduced into a field, gene flow between fields is limited (Dunn et al. 2010; Lamour and Hausbeck 2001). Furthermore, depending on when and how *P. capsici* was introduced into a grower's field, the gene pool of the founding population could be quite small. However, in commercial fields it is not always known exactly when the pathogen was first introduced. It would be virtually impossible to be certain of the identity of the founding individuals, or to know that no subsequent introductions had occurred. For these reasons, the research field described here presents a unique opportunity to study the population genetics of *P. capsici*, and to investigate how such high levels of diversity are maintained in fields where gene flow is limited. In this study, high levels of diversity were maintained over time, little evidence of genetic drift and four new alleles were observed, using only five microsatellite loci and mating type to characterize isolates. It is likely that other

genotyping methods would provide additional information about this isolated population. The fact that *P. capsici* is heterothallic and therefore sexual recombination is required to form overwintering structures, as well as the lack of aerial dispersal have long been assumed to impact the genetic structure of field populations. The results of this study suggest that spontaneous mutation, as well as mechanisms such as loss of heterozygosity or abnormal meiosis also play important roles.

***Acknowledgements***

This project was supported by the Agriculture and Food Research Initiative Competitive Grant No. 2012-67011-19690 from the USDA National Institute of Food and Agriculture and by the New York State Department of Agriculture and Markets through a NY Specialty Crops Block Grant. Support for A. R. Dunn was also provided by a fellowship from Cornell University College of Agriculture and Life Sciences, and by an award from the Storkan-Hanes-McCaslin Foundation. The authors thank H.W. Lange, S. Reiners, B. Nesin, M. Day, E. Wyckoff, and E. Call for technical assistance.

***References***

Abu-El Samen, F. M., Secor, G. A., and Gudmestad, N. C. 2003. Genetic variation among asexual progeny of *Phytophthora infestans* detected with RAPD and AFLP markers. Plant Pathol. 52:314–325.

Agapow, P. M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes 1:101–102.

Babadoost, M. and Pavon, C. 2013. Survival of oospores of *Phytophthora capsici* in soil. Plant Dis. 97:1478-1483.

Bi, Y., Cui, X., Lu, X., Cai, M., Liu, X., and Hao, J. J. 2011. Baseline sensitivity of natural population and resistance of mutants in *Phytophthora capsici* to zoxamide. Phytopathology 101:1104–1111.

Bowers, J. H., Papavizas, G. C., and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Dis. 74:771–777.

Brown, A., Feldman, M. W., and Nevo, E. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. Genetics 96:523–536.

Bruvo, R. R., Michiels, N. K. N., D'Souza, T. G. T., and Schulenburg, H. H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Mol. Ecol. 13:2101–2106.

Camp, A. R., Dillard, H. R., and Smart, C. D. 2008. Efficacy of *Muscodor albus* for the control of Phytophthora blight of sweet pepper and butternut squash. Plant Dis. 92:1488–1492.

Carter, D. A., Buck, K. W., Archer, S. A., Van der Lee, T., Shattock, R. C., and Shaw, D. S. 1999. The detection of nonhybrid, trisomic, and triploid offspring in sexual progeny of a mating of *Phytophthora infestans*. Fungal Genet. Biol. 26:198–208.

Chamnanpunt, J., Shan, W., and Tyler, B. 2001. High frequency mitotic gene conversion in genetic hybrids of the oomycete *Phytophthora sojae*. P. Natl. Acad. Sci. 98:14530–14535.

Davey, J. F., Gregory, N. F., Mulrooney, R. P., Evans, T. A., and Carroll, R. B. 2008. First report of mefenoxam-resistant isolates of *Phytophthora capsici* from lima bean pods in the mid-Atlantic Region. Plant Dis. 92:656.

Dobrowolski, M. P., Tommerup, I. C., Blakeman, H. D., and O'Brien, P. A. 2002. Non-mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. Fungal Genet. Biol. 35:197–212.

Donahoo, R. S., and Lamour, K. H. 2008. Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. Mycologia 100:911–920.

Dunn, A. R., Milgroom, M. G., Meitz, J. C., McLeod, A., Fry, W. E., McGrath, M. T., Dillard, H. R., and Smart, C. D. 2010. Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York State. Plant Dis. 94:1461–1468.

Ellegren, H. H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet. 16:551–558.

Excoffier, L. L., and Lischer, H. E. L. H. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10:564–567.

Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Forster, H., and Coffey, M. 1990. Mating behavior of *Phytophthora parasitica*: Evidence for sexual recombination in oospores using DNA restriction fragment length polymorphisms as genetic markers. Exp. Mycol. 14:351–359.

Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology 97:421–428.

Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. Plant Dis. 92:201–209.

Gobena, D., Roig, J., Galmarini, C., Hulvey, J., and Lamour, K. 2012. Genetic diversity of *Phytophthora capsici* isolates from pepper and pumpkin in Argentina. Mycologia 104:102–107.

Goodwin, S. B. 1997. The population genetics of *Phytophthora*. Phytopathology 87:462–473.

Grünwald, N. J., and Hoheisel, G.-A. 2006. Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the Oomycete *Aphanomyces euteiches*. Phytopathology 96:1134–1141.
Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93:738–746.
Hu, J., Pang, Z., Bi, Y., Shao, J., Diao, Y., Guo, J., Liu, Y., Lu, H., Lamour, K., and Liu, X. L. 2013. Genetically diverse long-lived clonal lineages of *Phytophthora capsici* from pepper in Gansu, China. Phytopathology 103:920–926.
Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577–586.
Hurtado-Gonzáles, O. P., and Lamour, K. H. 2009. Evidence for inbreeding and apomixis in close crosses of *Phytophthora capsici*. Plant Pathol. 58:715–722.
Hurtado-Gonzáles, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R., and Lamour, K. 2008. Survival and spread of *Phytophthora capsici* in coastal Peru. Phytopathology 98:688–694.
Jones, L. A., Worobo, R. W., and Smart, C. D. 2012. Oomycete and bacterial pathogens in New York surface irrigation water: Survey results and ultraviolet treatment. Phytopathology 102:S4.60.
Judelson, H. S. 1997. Expression and inheritance of sexual preference and selfing potential in *Phytophthora infestans*. Fungal Genet. Biol. 21:188–197.
Judelson, H. S. 2007. Genomics of the plant pathogenic oomycete *Phytophthora*: Insights into biology and evolution. In Fungal Genomics, Advances in Genetics, ed. Dunlap, JC. p. 97–141.
Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281 <http://dx.doi.org/10.7717/peerj.281>.
Keb-Llanes, M., González, G., Chi-Manzanero, B., and Infante, D. 2002. A rapid and simple method for small-scale DNA extraction in *Agavaceae* and other tropical plants. Plant Mol. Biol. Rep. 20:299.
Lamour, K. H., and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90:396–400.
Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91:973–980.
Lamour, K. H., Mudge, J., Gobena, D., Hurtado-Gonzales, O. P., Schmutz, J., Kuo, A., Miller, N. A., Rice, B. J., Raffaele, S., Cano, L. M., Bharti, A. K., Donahoo, R. S., Finley, S., Huitema, E., Hulvey, J., Platt, D., Salamov, A., Savidor, A., Sharma, R., Stam, R., Storey, D., Thines, M., Win, J., Hass, B. J., Dinwiddie, D. L., Jenkins, J., Knight, J. R., Affourtit, J. P., Han, C. S., Chertkov, O., Lindquist, E. A., Detter, C., Grigoriev, I. V., Kamoun, S., and Kingsmore, S. F. 2012. Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. Mol. Plant-Microb Interact. 25:1350–1360.
Liu, B., Roos, D., Buttler, S., Richter, B., and Louws, F. J. 2012. Vegetable seedling diseases associated with earthworm castings contaminated with *Phytophthora capsici* and *Pythium attrantheridium*. Plant Health Prog. doi:10.1094/PHP-2012-0421-01-RS.
Lu, X., Hausbeck, M., Liu, X., and Hao, J. J. 2011. Wild type sensitivity and mutation analysis for resistance risk to fluopicolide in *Phytophthora capsici*. Plant Dis. 95:1535–1541.

Meitz, J. C., Linde, C. C., Thompson, A., Langenhoven, S., and McLeod, A. 2010. *Phytophthora capsici* on vegetable hosts in South Africa: distribution, host range and genetic diversity. Australas. Plant Pathol. 39:431–439.

Pavon, C. F., Babadoost, M., and Lambert, K. N. 2008. Quantification of *Phytophthora capsici* oospores in soil by sieving-centrifugation and real-time polymerase chain reaction. Plant Dis. 92:143–149.

Peakall, R., and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295.

Peakall, R., and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28:2537–2539.

R Development Core Team. 2013. R: A language and environment for statistical computing. <http://www.R-project.org/>.

Roberts, P. D., Urs, R. R., French-Monar, R. D., Hoffine, M. S., Seijo, T. E., and McGovern, R. J. 2005. Survival and recovery of *Phytophthora capsici* and oomycetes in tailwater and soil from vegetable fields in Florida. Ann. Appl. Biol. 146:351.

Satour, M. M., and Butler, E. E. 1968. Comparative morphological and physiological studies of the progenies from intraspecic matings of *Phytophthora capsici*. Phytopathology 58:183–192.

Schmitthenner, A. F., and Bhat, R. G. 1994. Useful methods for studying *Phytophthora* in the laboratory. Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.

Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986. Genetics of *Phytophthora infestans*: Characterization of single-oospore cultures from A1 isolates induced to self by intraspecific stimulation. Phytopathology 76:407–410.

Sheskin, D. J. 1996. Handbook of parametric and nonparametric statistical procedures. Boca Raton: CRC Press.

Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: Estimation and prediction in samples. Genetics 118:705–711.

Tompkins, C. M., and Tucker, C. M. 1941. Root rot of pepper and pumpkin caused by *Phytophthora capsici*. J. Agr. Res. 63:417–426.

Uchida, J. Y., and Aragaki, M. 1980. Chemical stimulation of oospore formation in *Phytophthora capsici*. Mycologia 72:1103–1108.

Udupa, S. M., and Baum, M. 2001. High mutation rate and mutational bias at (TAA)n microsatellite loci in chickpea (*Cicer arietinum* L.). Mol. Genet. Genomics 265:1097–1103.

Vercauteren, A., Boutet, X., D'hondt, L., Van Bockstaele, E., Maes, M., Leus, L., Chandelier, A., and Heungens, K. 2011. Aberrant genome size and instability of *Phytophthora ramorum* oospore progenies. Fungal Genet. Biol. 48:537–543.

Wang, Z. Y., Langston, D. B., Csinos, A. S., Gitaitis, R. D., Walcott, R. R., and Ji, P. S. 2009. Development of an improved isolation approach and simple sequence repeat markers to characterize *Phytophthora capsici* populations in irrigation ponds in southern Georgia. Appl. Environ. Microb. 75:5467–5473.

Weber, J. L., and Wong, C. 1993. Mutation of human short tandem repeats. Hum. Mol. Genet. 2:1123–1128.

CHAPTER 3

# Evaluation of commercial bell pepper cultivars for resistance to Phytophthora blight (*Phytophthora capsici*)[2]


## *Abstract*

Phytophthora blight caused by *Phytophthora capsici* is an important disease of bell peppers, causing crown, root, and fruit rot, as well as foliar lesions. Substantial yield losses can result from either plant death or fruit rot. Host resistance is an effective management strategy for the root and crown rot phase of the disease, and a number of commercially-available pepper cultivars are described by the supplier as intermediately resistant. In field trials conducted over 5 years, the bell pepper cultivars Archimedes, Aristotle, Intruder, and Paladin were found to be the most resistant to a single isolate of *P. capsici* from New York State (NY 0664-1). Cultivars ACR285, Declaration, PS 09941819, Revolution, and Vanguard showed intermediate levels of resistance. Escalade, Karisma, Keystone Giant, King of the North, and Red Knight were highly susceptible to NY 0664-1. This information will be useful to growers selecting cultivars to plant in fields with a history of Phytophthora blight.


## *Introduction*

In 2012, bell peppers for the fresh and processing markets were grown on 55,500 acres, and valued at more than $625 million in the United States (USDA 2013). An important pathogen that can limit pepper production is the oomycete *Phytophthora capsici*, which causes root and crown rot, stem and leaf lesions, and fruit rot (Tompkins and Tucker 1941; Weber 1932).

[2] Reprinted from Dunn, A. R., Lange, H. W., and Smart, C. D. 2014. Evaluation of commercial bell pepper cultivars for resistance to Phytophthora blight (*Phytophthora capsici*). Plant Health Progress. 15:19-24. doi:10.1094/PHP-RS-13-0114.

Collectively, these symptoms are called Phytophthora blight and can result in considerable yield losses. Most other solanaceous vegetables, all cucurbits, and some legumes are also susceptible to Phytophthora blight (Granke et al. 2012). The sexual spores of the pathogen (oospores) can survive in soil for years, and serve as the primary inoculum in temperate growing regions (e.g. the northeastern United States) where peppers and other vegetable or weed hosts do not grow year-round (Bowers et al. 1990). Previous studies have shown that germination of oospores in the soil (and subsequent infection of plants) requires fluctuations in soil moisture (Bowers and Mitchell 1990; Hord and Ristaino 1992). The asexual sporangia are produced rapidly and release motile zoospores when sufficient water is present (Bernhardt and Grogan 1982). They can also spread quickly through a field via surface water (Café-Filho and Duniway 1995; Ristaino et al. 1993; Ristaino et al. 1997). Increased disease incidence has been associated with warm temperatures and excess soil moisture (Tompkins and Tucker 1941).

Effective management of Phytophthora blight requires an integrated approach, including cultural practices, crop rotation, fungicide application, and host resistance (Granke et al. 2012). Several wild peppers with high levels of resistance to Phytophthora blight have been identified, including a land race from Mexico (CM-334), which is a common source of resistance for both public and private breeding programs. While resistant bell pepper cultivars have been available for some time, the genetics controlling resistance to Phytophthora blight in pepper are not clearly understood. Evidence to date supports the hypothesis that multiple genes are involved, and that different genes are responsible for resistance to root rot, fruit rot, and leaf lesions (Monroy-Barbosa and Bosland 2008; Sy et al. 2005).

Previous studies have shown that isolates of *P. capsici* vary greatly in their virulence on various hosts, including different pepper genotypes (Foster and Hausbeck 2010b; Polach and

Webster 1972). While some studies have classified *P. capsici* isolates into races based on virulence to differential pepper genotypes (Glosier et al. 2008), there is currently no consensus on race classifications of this pathogen. Although no commercially-available pepper cultivars are immune to Phytophthora blight, previous studies conducted either in the greenhouse or in a single growing season have reported high levels of resistance in Paladin, and intermediate levels of resistance in Aristotle, Revolution, and Declaration (Babadoost 2006; Foster and Hausbeck 2010b; McGrath and Fox 2009). Levels of resistance varied among studies, and among isolates of *P. capsici*, but Red Knight was consistently highly susceptible. Intruder and PS 09941819 are newer cultivars that are also marketed as resistant to *P. capsici.*

The objective of this study was to identify commercially-available bell pepper cultivars that show high levels of resistance to an isolate of *P. capsici* in upstate New York. This information can be used by growers when selecting the best cultivar for their specific fields and markets. It will also be useful to researchers screening additional pepper cultivars for resistance to Phytophthora blight in the field.

### *Methods and Results*[3]

*Field site and plant material.* All trials were conducted at the Phytophthora Blight Farm, a nine-acre research field at Cornell University's New York State Agricultural Experiment Station in Geneva, NY. This facility was constructed in 2007 for the purpose of studying the biology and management of *P. capsici* in upstate New York, without impacting nearby research and commercial vegetable fields. The soil is Odessa silt loam. In each year, peppers were grown on raised beds (4 inches high, 3 ft wide, and 7 ft apart on center) covered with black plastic. At

[3] Papers submitted to the journal *Plant Health Progress* are required to combine the method, results, and discussion sections, with a brief conclusion at the end of the paper.

the time beds were built, 300 lb/A of 10-10-10 fertilizer was applied in the center of each bed and drip tape was laid beneath the plastic (slightly off-center). Commercial bell pepper cultivars were selected based on what is commonly grown on upstate New York farms, including some cultivars that are advertised as resistant to Phytophthora blight (Table 3.1).

**Table 3.1** Pepper cultivars screened for resistance to Phytophthora blight (*Phytophthora capsici*) in field trials conducted from 2008 through 2012.

| Cultivar[a] | Phytophthora resistance[b] | Source | |
|---|---|---|---|
| ACR285 | Intermediate | Abbott & Cobb | Feasterville, PA |
| Archimedes | Intermediate | Seminis | St. Louis, MO |
| Aristotle | NA | Seminis | St. Louis, MO |
| CM-334 | High | Land race | |
| Declaration | Intermediate | Harris Moran | Modesto, CA |
| Escalade | Intermediate | Abbott & Cobb | Feasterville, PA |
| Intruder | Intermediate | Syngenta | Greensboro, NC |
| Karisma | NA | Harris Moran | Modesto, CA |
| Keystone Giant | NA | Johnny's Selected Seeds | Waterville, ME |
| King of the North | NA | Johnny's Selected Seeds | Waterville, ME |
| Paladin | Intermediate | Syngenta | Greensboro, NC |
| PS 09941819 | Intermediate | Seminis | St. Louis, MO |
| Red Knight | NA | Seminis | St. Louis, MO |
| Revolution | Intermediate | Harris Moran | Modesto, CA |
| Vanguard | Intermediate | Harris Moran | Modesto, CA |

[a] All listed cultivars are commercially-available bell peppers, with the exception of CM-334 which is a land race used as a source for Phytophthora resistance in many breeding programs.
[b] Resistance to Phytophthora blight, as advertised by the supplier, only (not as determined by previous studies). NA = not advertised as resistant to Phytophthora blight

The following cultivars were tested from 2008 through 2012: ACR285 (2009), Archimedes (2012), Aristotle (2008, 2009, 2011), Declaration (2008, 2009, 2010, 2011), Escalade (2009), Intruder (2011, 2012), Karisma (2009, 2010, 2011), Keystone Giant (2012), King of the North (2012), Paladin (2008, 2009, 2011, 2012), PS 09941819 (2012), Red Knight (2008, 2009, 2010, 2011, 2012), Revolution (2008, 2009, 2010, 2011), and Vanguard (2010,

2011). The land race CM-334 was included in 2008 because it is a common source of resistance to Phytophthora blight in breeding programs. Peppers were seeded in Cornell potting mix (peat, perlite, and vermiculite in a 4:1:1 ratio) and germinated in a greenhouse. When plants were 5 to 8 weeks old, they were transplanted into the field in a randomized complete block design with each cultivar replicated three or four times, depending on the year. Transplanting dates ranged from 10 Jun to 22 Jun, depending on weather conditions and when the field could be prepared. Each plot contained 5 (2008 and 2009) or 10 (2010, 2011, and 2012) plants, spaced 12 inches apart, except in 2011, when plants were spaced 18 inches apart. Throughout the season, natural rainfall was supplemented with trickle irrigation, as needed. Weeds were controlled by a combination of hand-weeding, and mowing between rows.

*Inoculation with* Phytophthora capsici. The *P. capsici* isolate used in inoculation (NY 0664-1) is a single-spore isolate obtained from a bell pepper plant grown on a commercial farm in central New York in 2006. Based on preliminary greenhouse assays, it is representative of other *P. capsici* isolates found in New York State in terms of virulence on pepper (Table 3.2).

**Table 3.2** Comparing virulence of select *Phytophthora capsici* isolates on pepper. All isolates were collected on New York Farms in 2006 from pepper (NY 06120-1 and NY 0664-1) or butternut squash (all others).

| | Incidence (%)[a] | |
|---|---|---|
| Isolate | 4 days pi | 7 days pi |
| Water | 0 | 0 |
| NY 06120-1 | 100 | 100 |
| NY 0664-1 | 100 | 100 |
| NY 06180-5 | 0 | 37.5 |
| NY 06180-8 | 0 | 12.5 |
| NY 06181-1 | 12.5 | 100 |
| NY 06181-2 | 0 | 87.5 |
| NY 06181-3 | 25 | 100 |
| NY 06181-4 | 25 | 100 |
| NY 06181-5 | 25 | 100 |
| NY 06181-6 | 37.5 | 87.5 |

[a] Eight plants were inoculated with 5 ml ($1x10^5$ - $3x10^5$ zoospores/ml) of a zoospore suspension of each isolate. Percent of plants with symptoms (wilted or dead) 4 and 7 days post inoculation.

The same isolate was used in each season (as has been done previously; Foster and Hausbeck 2010a), so that results could be compared between years. The isolate was periodically inoculated onto a host in the greenhouse and re-isolated in order to maintain virulence. Each year, peppers were inoculated multiple times (Table 3.3), and inoculum was prepared as previously described (Dunn et al. 2013). In the first years of the trial, plants were inoculated later in the season and with lower concentrations of zoospores at early inoculation dates (Table 3.3). This was done in order to avoid killing all plants immediately, since the soil at the trial site is heavy and poorly-drained. In subsequent years, plants were inoculated earlier in the season and with higher concentrations of zoospores, in order to achieve a stringent test of host resistance. Weather conditions in each year also impacted the inoculation dates.

**Table 3.3** Details of pepper plant inoculation with the Phytophthora blight pathogen (*Phytophthora capsici*). Inoculation timing and number of zoospores used is listed for field trials conducted from 2008 to 2012.

| | First inoculation | | | | |
|---|---|---|---|---|---|
| Year | Plant age (weeks) | Date | Number of inoculations | Zoospores applied per plant[a] | Inocuation dates |
| 2008 | 16 | 6 Aug | 3 | $5x10^3$ - $1x10^6$ | 6 Aug, 8 and 26 Sep |
| 2009 | 11 | 14 Jul | 5 | $5x10^2$ - $5x10^5$ | 14 and 27 July, 3, 18 and 31 Aug |
| 2010 | 7 | 7 Jul | 6 | $5x10^3$ - $5x10^5$ | 7, 16, 23 and 30 July, 5 and 13 Aug |
| 2011 | 8.5 | 26 Jun | 3 | $2x10^5$ - $5x10^5$ | 24 Jun, 6 and 19 July |
| 2012 | 8 | 19 Jun | 2 | $5x10^5$ | 19 Jun, 10 July |

[a] Zoospores were applied using a hand-pump sprayer and the quantity of zoospores was increased in subsequent inoculations throughout the season in all years except 2012.

At each inoculation, 5 ml of a suspension of zoospores of *P. capsici* (see Table 3.3 for concentrations) was applied with a 1.5-gallon hand-pump sprayer at the crown of each plant. As the season progressed, higher concentrations of zoospores were applied per plant (except in 2012), and inoculum was directed higher up the stem (where stem tissue was still green) as plants aged and stems became lignified.

*Evaluation of resistance to Phytophthora blight in bell peppers.* Beginning approximately 1 week after inoculation, the number of plants showing symptoms of Phytophthora blight (wilting or death) was counted two to three times per week and used to calculate incidence in each plot on each rating date. Plants were rated through the remainder of the growing season (2 to 3 months, depending on the season and when plants were inoculated). Disease incidence throughout the season was used to calculate area under the disease progress curve (AUDPC) for each cultivar in each year, which was then divided by the total number of days over which ratings were taken to calculate the relative AUDPC (RAUDPC; Madden et al. 2007). This was done so that comparisons could be made between years, since the magnitude of AUDPC is directly related to the number of rating dates. A higher RAUDPC value corresponds to both greater disease incidence and more rapid plant death. Significant differences in RAUDPC among cultivars in a single year were tested using an analysis of variance (ANOVA) in the R statistical software, the most recent version of which is available online from the R Foundation for Statistical Computing. Means were then separated with a Tukey's Honestly significant differences test (alpha = 0.05) using the 'agricolae' package, which is published by the International Potato Center and available online through the Comprehensive R Archive Network.

Results comparing final disease incidence (not RAUDPC) among cultivars from 2011 have been previously published (Dunn et al. 2013), and RAUDPC results from that year are included here in order to make comparisons among years. Of the cultivars planted in these trials over five years, Aristotle, Intruder, and Paladin consistently had the lowest RAUDPC values and therefore the most resistance to the isolate of *P. capsici* used in this study (Fig. 3.1). Archimedes also showed high levels of resistance (mean RAUDPC not significantly different from Intruder and Paladin), but was only included in 2012. The land race CM-334 remained symptomless

throughout the season in 2008, while low levels of disease were observed on the other highly resistant cultivars in most years. Cultivars Karisma and Red Knight had the highest RAUDPC values over multiple years. They were therefore considered highly susceptible and are not recommended for fields with a history of Phytophthora blight. Although they were only tested in one year, the cultivars Escalade, Keystone Giant, and King of the North had RAUDPC values not significantly different from Karisma and Red Knight in those years, and therefore are not likely to perform well in fields with a history of Phytophthora blight.

Cultivars with mean RAUDPC values significantly larger than those observed for resistant cultivars, but significantly smaller than those observed for susceptible cultivars in most years were considered to have 'intermediate resistance' to this isolate of *P. capsici*. Based on these criteria, the cultivars ACR285, Declaration, Revolution, PS 09941819, and Vanguard were considered intermediately resistant, although ACR285 and PS 09941819 were only planted in one year, each. In the case of Declaration and Revolution, resistance compared to Red Knight varied between years. Because the *P. capsici* isolate used in these trials is representative of isolates found on New York farms, these results are a good indication of how these cultivars will likely perform in New York fields infested with *P. capsici*. However, since resistance to Phytophthora blight in pepper is known to vary depending on the isolate (Foster and Hausbeck 2010b; Polach and Webster 1972), results may differ in other states. The inclusion of Paladin and Red Knight (widely reported to be highly resistant and highly susceptible, respectively; Babadoost 2006, Foster and Hausbeck 2010b, McGrath and Fox 2009) in most years of this trial should facilitate comparison with results from trials conducted in other states with local isolates of *P. capsici*.

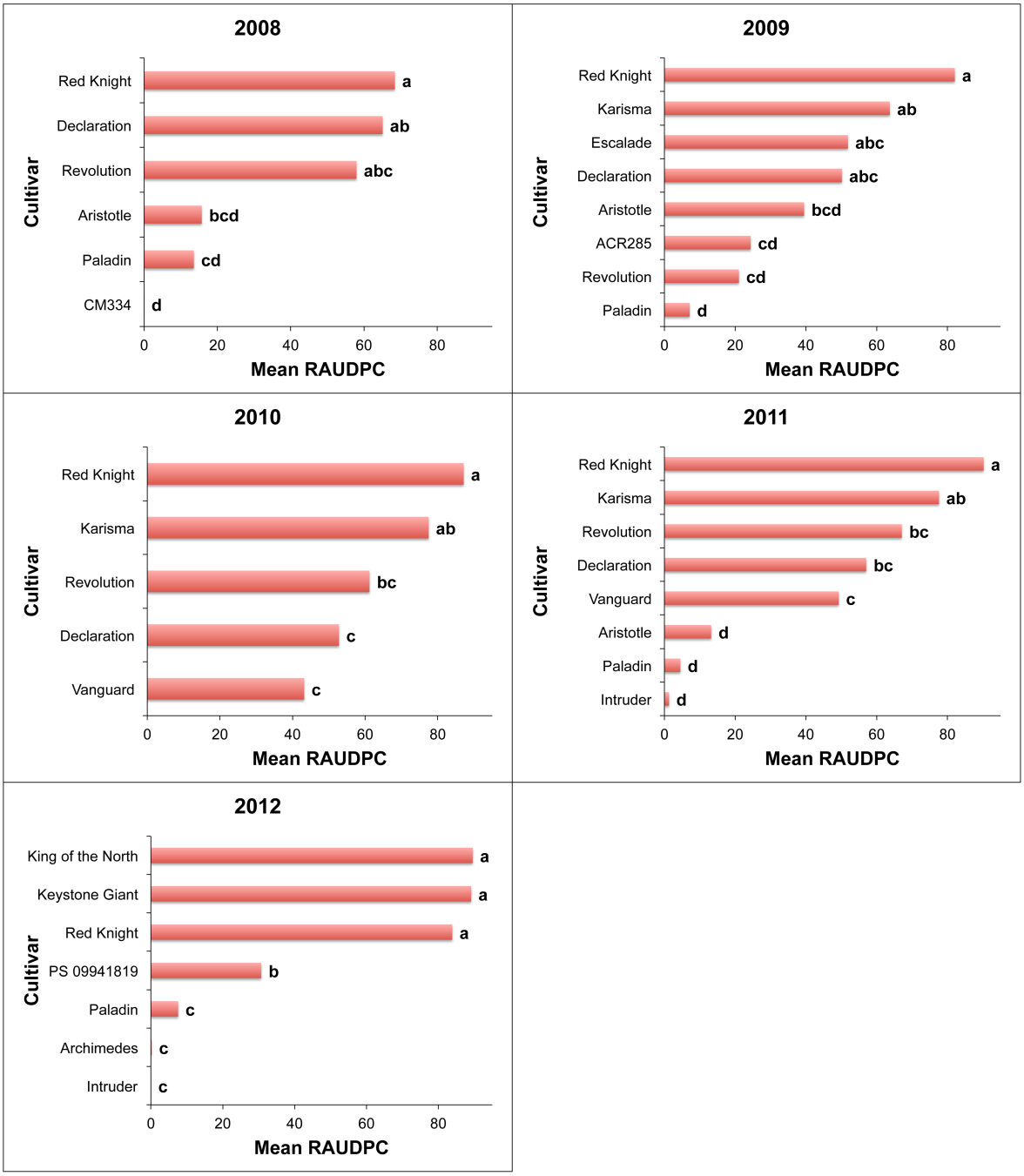



**Figure 3.1** Testing bell pepper cultivars for resistance to Phytophthora blight (*Phytophthora capsici*) in inoculated field trials conducted from 2008 through 2012. Disease is reported as mean relative area under the disease progress curve (RAUDPC), which was calculated from disease incidence ratings. Larger RAUDPC values correspond to higher incidence and more rapid plant death. Within each year, means followed by the same letter are not significantly different based on a Tukey's Honestly significant differences test, $P = 0.05$.

*Variation in temperature and rainfall among years.* Each season, weather data was collected at a weather station located less than two miles from the trial location. Data collected from this station were either averaged for the months of June, July and August, or summed over these three months in each year. These data included: average, maximum, and minimum daily air temperatures (°F), average daily pan evaporation per month (inches), total monthly rainfall (inches), total number of days per month without precipitation, the maximum consecutive days without precipitation during all three months, and the cumulative growing degree days (GDD; base 50°F) during these three months each year (Table 3.4).

Based on growing degree days and total monthly rainfall for June, July, and August in each year, the 2008 growing season had moderate temperatures and was relatively wet. The 2009 season was cool and wet, and 2010 was warm and very wet. Both the 2011 and 2012 growing seasons were warm and dry (especially July 2011). The mean RAUDPC for the most susceptible pepper cultivar varied greatly among years, with lower values in 2008, and higher values in 2009 through 2012 (Fig. 2.1). This was probably due largely to timing of inoculation (both plant age and weeks post transplant), the number of times plants were inoculated, and inoculum concentration (Table 2.3). These and other factors are known to impact incidence of Phytophthora blight (Hord and Ristaino 1992; Kim et al. 1989; Reifschneider et al. 1986; Tompkins and Tucker 1941).

**Table 3.4** Temperature and rainfall for the months of June, July and August 2008 through 2012. Measurements were made at a weather station located within two miles of the field site where peppers were screened for resistance to Phytophthora blight (*Phytophthora capsici*).

| | 2008 | | | 2009 | | |
|---|---|---|---|---|---|---|
| Temperature and rainfall | June | July | Aug | June | July | Aug |
| Max. air temp (°F)[a] | 78.6 | 80.3 | 75.9 | 73.3 | 75.9 | 79.0 |
| Min. air temp (°F)[b] | 59.5 | 61.1 | 57.4 | 53.7 | 57.8 | 59.7 |
| Avg. air temp (°F)[c] | 69.0 | 70.7 | 66.6 | 63.5 | 66.8 | 69.4 |
| Pan evaporation[d] | 0.22 | 0.22 | 0.20 | 0.21 | 0.22 | 0.19 |
| Monthly rainfall[e] | 3.45 | 4.99 | 4.16 | 4.80 | 3.61 | 3.28 |
| Days without rain[f] | 11 | 13 | 18 | 17 | 13 | 15 |
| Max. days no rain[g] | | 5 | | | 8 | |
| Cumulative GDD[h] | | 1763.8 | | | 1572.8 | |

| | 2010 | | | 2011 | | |
|---|---|---|---|---|---|---|
| Temperature and rainfall | June | July | Aug | June | July | Aug |
| Max. air temp (°F)[a] | 76.7 | 82.6 | 78.7 | 77.0 | 84.7 | 78.9 |
| Min. air temp (°F)[b] | 58.7 | 63.4 | 61.1 | 58.1 | 63.8 | 59.0 |
| Avg. air temp (°F)[c] | 67.7 | 73.0 | 69.9 | 67.6 | 74.3 | 69.0 |
| Pan evaporation[d] | 0.21 | 0.24 | 0.20 | 0.24 | 0.29 | 0.23 |
| Monthly rainfall[e] | 6.62 | 5.16 | 4.47 | 2.34 | 0.72 | 2.62 |
| Days without rain[f] | 11 | 19 | 17 | 14 | 26 | 14 |
| Max. days no rain[g] | | 10 | | | 19 | |
| Cumulative GDD[h] | | 1863.8 | | | 1903.1 | |

| | 2012 | | |
|---|---|---|---|
| Temperature and rainfall | June | July | Aug |
| Max. air temp (°F)[a] | 76.7 | 84.3 | 81.3 |
| Min. air temp (°F)[b] | 57.2 | 63.5 | 59.6 |
| Avg. air temp (°F)[c] | 66.9 | 73.9 | 70.5 |
| Pan evaporation[d] | 0.26 | 0.27 | 0.21 |
| Monthly rainfall[e] | 2.59 | 2.80 | 2.26 |
| Days without rain[f] | 17 | 21 | 22 |
| Max. days no rain[g] | | 11 | |
| Cumulative GDD[h] | | 1909.2 | |

[a] Daily maximum air temperatures averaged over each month
[b] Daily minimum air temperatures averaged over each month
[c] Daily average air temperatures averaged over each month
[d] Daily pan evaporation (inches) averaged over each month
[e] Total rainfall (inches) for each month
[f] Total number of days without rainfall for each month
[g] Maximum number of consecutive days without rainfall over the months of June, July, and Aug
[h] Cumulative growing degree days (base 50) 1 Jun through 31 Aug

High RAUDPC values observed in 2010 are not surprising. Warm temperatures are known to favor Phytophthora blight, and high rainfall and humidity (as measured by pan evaporation) are expected to promote sporulation and secondary spread. In addition, plants were inoculated six times in 2010, beginning at a young age (7 weeks). Since inoculations in 2011 and 2012 were timed to coincide with sufficient moisture (and natural rainfall was augmented with irrigation prior to inoculation), the high RAUDPC values for susceptible cultivars in these years were also expected. The warm temperatures promote pathogen growth, and drier weather (as evidenced by higher pan evaporation) places additional stress on plants, resulting in earlier wilting and death following infection by *P. capsici*.

In 2008 and 2009, initial inoculations were made with similarly low concentrations of zoospores, followed by higher concentrations at later inoculation dates. Although 2008 was a much warmer growing season than 2009 (based on accumulation of GDD between 1 Jun and 31 Aug), and rainfall was similar between the two years, there was substantially less-severe disease on Red Knight in 2008 compared to 2009. A variety of factors likely contributed to this difference, including age at inoculation (11 weeks in 2009, compared to 16 weeks in 2008), timing of the first inoculation (14 Jul in 2009, compared to 6 Aug in 2008), and number of times the plants were inoculated (five times in 2009, compared to three times in 2008). Plants were even younger, and the first inoculation occurred earlier in the season in 2010 through 2012 (7 to 8 weeks old). During these last three years, RAUDPC values for the most susceptible cultivars were also high.

Decreased susceptibility to Phytophthora blight with age of pepper plants has been previously reported in greenhouse trials (Hwang et al. 1996; Kim et al. 1989; Reifschneider et al. 1986), and we have seen similar trends when inoculating pepper seedlings in the greenhouse.

Further work would be needed to elucidate the relative effects of weather, plant genotype, and plant age on incidence of Phytophthora blight on pepper in the field. However, even if pepper plant resistance increases with plant age, pepper fruit remain susceptible throughout the season. Currently-available commercial bell pepper cultivars only have resistance to Phytophthora root rot, not fruit rot, and high incidence of fruit rot can still occur in resistant cultivars like Paladin (McGrath and Fox 2009). Thus, the results of this trial should not be interpreted to mean that Phytophthora blight is a disease that only requires control early in the season.

***Conclusion***

Although plants were inoculated at very different ages and times during the growing season, similar trends were still observed between years. Of the cultivars included in this trial, Paladin, Aristotle, and Intruder consistently showed the highest levels of resistance to this isolate of *P. capsici* over multiple years, while Red Knight and Karisma consistently showed the highest susceptibility. In 2008, RAUDPC values for Paladin and Aristotle were not significantly different from the standard resistant land race CM-334, however CM-334 was not included in subsequent years of the trial (2009-2012), and therefore Paladin, Aristotle, and Intruder could not be compared to CM-334 in these years. The variable performance of Revolution and Declaration from year to year (compared to Red Knight) is likely due to variation in environmental conditions among years. The fact that these two cultivars did not perform better in a year with lower disease pressure supports this hypothesis.

***Acknowledgements***

The authors thank S. Reiners for horticultural advice. Support for A. R. Dunn was provided by a fellowship from Cornell University College of Agriculture and Life Sciences. The project was funded through a grant from the NY State Department of Agriculture and Markets Specialty Crop Block Grant.

***References***

Babadoost, M. 2006. Performance of bell pepper cultivars in a commercial field infested with *Phytophthora capsici*. HortScience. 41:981.

Bernhardt, E. A., and Grogan, R. G. 1982. Effect of soil matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica*, *Phytophthora capsici*, and *Phytophthora cryptogea*. Phytopathology. 72:507–511.

Bowers, J. H., and Mitchell, D. J. 1990. Effect of soil-water matric potential and periodic flooding on mortality of pepper caused by *Phytophthora capsici*. Phytopathology. 80:1447–1450.

Bowers, J. H., Papavizas, G. C., and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Dis. 74:771–777.

Café-Filho, A. C., and Duniway, J. M. 1995. Dispersal of *Phytophthora capsici* and *P. parasitica* in furrow-irrigated rows of bell pepper, tomato and squash. Plant Pathol. 44:1025–1032.

Dunn, A. R., Wyatt, L. E., Mazourek, M., Reiners, S., and Smart, C. D. 2013. Performance and tolerance to Phytophthora blight of bell pepper varieties. HortTechnology. 23:382–390.

Foster, J. M., and Hausbeck, M. K. 2010a. Managing Phytophthora crown and root rot in bell pepper using fungicides and host resistance. Plant Dis. 94:697–702.

Foster, J. M., and Hausbeck, M. K. 2010b. Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24–30.

Glosier, B. R., Ogundiwin, E. A., Sidhu, G. S., Sischo, D. R., and Prince, J. P. 2008. A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. Euphytica. 162:23–30.

Granke, L. L., Quesada-Ocampo, L. M., Lamour, K., and Hausbeck, M. 2012. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. Plant Dis. 95:1588–1600.

Hord, M. J., and Ristaino, J. B. 1992. Effect of the matric component of soil water potential on infection of pepper seedlings in soil infested with oospores of *Phytophthora capsici*. Phytopathology. 82:792–798.

Hwang, B. K., Kim, Y. J., and Kim, C. H. 1996. Differential interactions of *Phytophthora capsici* isolates with pepper genotypes at various plant growth stages. Eur. J. Plant Pathol. 102:311–316.

Kim, Y. J., Hwang, B. K., and Park, K. W. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. Plant Dis. 73:745–747.
Madden, L. V., Hughes, G., and van den Bosch, F. 2007. The study of plant disease epidemics. The American Phytopathological Society, St. Paul, MN.
McGrath, M. T., and Fox, G. M. 2009. Evaluation of Phytophthora-resistant bell pepper cultivars with and without a fungicide program, 2008. Plant Dis. Manag. Rep. 3:V132.
Monroy-Barbosa, A., and Bosland, P. W. 2008. Genetic analysis of Phytophthora root rot race-specific resistance in Chile pepper. J. Amer. Soc. Hort. Sci. 133:825–829.
Polach, F. J., and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. Phytopathology. 62:20–26.
Reifschneider, F. J. B., Café-Filho, A. C., and Rego, A. M. 1986. Factors affecting expression of resistance in pepper (*Capsicum annuum*) to blight caused by *Phytophthora capsici* in screening trials. Plant Pathol. 35:451–456.
Ristaino, J. B., Larkin, R. P., and Campbell, C. L. 1993. Spatial and temporal dynamics of *Phytophthora* epidemics in commercial bell pepper fields. Phytopathology. 83:1312–1320.
Ristaino, J. B., Parra, G., and Campbell, C. L. 1997. Suppression of Phytophthora blight in bell pepper by a no-till wheat cover crop. Phytopathology. 87:242–249.
Sy, O., Bosland, P. W., and Steiner, R. 2005. Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. J. Amer. Soc. Hort. Sci. 130:75–78.
Tompkins, C. M., and Tucker, C. M. 1941. Root rot of pepper and pumpkin caused by *Phytophthora capsici*. J. Agric. Res. 63:417–426.
USDA-NASS. 2013. Vegetables summary 2012. Online. United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS), Washington, DC. <http://usda01.library.cornell.edu/usda/current/VegeSumm/VegeSumm-01-29-2013.pdf>
Weber, G. F. 1932. Blight of peppers in Florida caused by *Phytophthora capsici*. Phytopathology. 22:775–780.

CHAPTER 4

## Performance and tolerance to Phytophthora blight of bell pepper varieties[4]


### *Abstract*

In 2011, total marketable yield, fruit size and number of lobes, fruit discoloration due to silvering, and plant structure were compared among eight commercial green bell pepper (*Capsicum annuum*) varieties and four breeding lines at three field sites in central New York. Tolerance to Phytophthora blight (*Phytophthora capsici*) was also assessed at one of these sites. No wilting or plant death due to Phytophthora blight was observed on the four breeding lines. Paladin, Intruder, and Aristotle had the highest levels of tolerance to Phytophthora blight, among the commercial varieties and maintained their yields in the presence of disease. In the absence of Phytophthora blight, yields from these three varieties were comparable to susceptible varieties, but fruit tended to be smaller, and incidence of silvering was high in Paladin and Intruder. Less silvering was observed on 'Aristotle' fruit. Total marketable yields from the breeding lines and percent of fruit with four lobes was comparable to the commercial varieties, and some breeding lines also had a low incidence of silvering, but fruit were smaller and set later in the season. Overall, this study suggests that Paladin, Intruder, and Aristotle will yield well in fields with a history of severe Phytophthora blight, but new large-fruited varieties with low incidence of silvering and good tolerance to Phytophthora blight are needed.


[4] Dunn, A. R., Wyatt, L. E., Mazourek, M., Reiners, S., and Smart, C. D. (2013) Performance and tolerance to Phytophthora blight of bell pepper varieties. HortTechnology. 23(3):382-390.

***Introduction***

In the United States, 56,200 acres of bell pepper were planted in 2011, with a total production value of just under $685 million (USDA, 2012b). However, desirable traits vary from region to region, based on a variety of factors, including the growing conditions in the region (e.g., climate and predominant soil type), major diseases affecting the region, the market for which peppers are grown (e.g., fresh versus processing), and whether fruit will be harvested green, or when reaching their mature color (e.g., red or yellow; Crosby, 2008). This results in different varieties being preferred by growers in different parts of the country. For example, 'Revolution' yielded well in a Pennsylvania trial (Sánchez et al., 2011), but performed poorly in Maine where the growing season is shorter and generally cooler (Hutton and Handley, 2007).

In New York State, the majority of fruit are harvested green. Production was valued at $9.9 million in 2010 (the last year for which data are available) on 1200 acres (including upstate and Long Island), and all are grown for the fresh market (USDA, 2012a). For fresh market, not only total yield, but also fruit size, shape, and appearance affect the value of the crop, and are important characteristics when New York growers select a bell pepper variety. For the highest grades, USDA standards require that fruit be at least 2.5 (for "U.S. No. 1" grade) or 3 (for "U.S. Fancy" grade) inches in diameter, free from damage or injury, and not misshapen (USDA, 2005). In addition, early-yielding plants with fruit that are blocky, larger (4 inches in diameter), thick-walled, and have four lobes are increasingly desirable for fresh market production in the United States (Crosby, 2008).

One abiotic disorder that can reduce the value of bell pepper fruit or make it completely unmarketable is skin separation, often called "silvering". These mottled patches of white or silver on the fruit surface are superficial, but can reduce marketability, especially if they cover

large areas of the fruit (Kline et al., 2011). One study reported up to 66% of fruit affected by silvering on a single harvest date (Wyenandt and Kline, 2006). Previous work has shown that incidence of silvering can vary by harvest date, field site, and the rate and type of fertilizer applied, but pepper variety appears to be the most important and consistent factor (Kline et al., 2011). Interestingly, fruit silvering tends to be more common on bell pepper varieties with tolerance to the disease Phytophthora blight, while it is less common on more susceptible varieties (Kline et al., 2011).

Phytophthora blight is a soilborne disease caused by the oomycete *Phytophthora capsici* (Leonian, 1922). Once Phytophthora blight is on a farm, it is essentially impossible to eradicate from the soil, and can result in severe yield losses in pepper due to fruit rot and plant death (Granke et al., 2012). This disease may have been introduced to New York State as early as the mid 1930s, when infected watermelon (*Citrullus lanatus* var. *lanatus*) fruit shipped from Colorado were observed in New York City markets (Wiant and Tucker, 1940). It is now present on many New York vegetable farms (Dunn et al., 2010), in vegetable production regions across the United States, and around the world (Granke et al., 2012). Because of the large host range [including eggplant (*Solanum melongena*), tomato (*Lycopersicon esculentum*), all cucurbits (family *Cucurbitaceae*), and snap beans (*Phaseolus vulgaris*)] and the increasing prevalence of insensitivity to fungicides (Dunn et al., 2010; Jackson et al., 2012; Keinath, 2007), host resistance is a key component to successful management of Phytophthora blight. A number of commercial bell pepper varieties with varying levels of tolerance to Phytophthora blight are available, including Paladin (highly tolerant, but not completely immune), Revolution, and Aristotle (both showing intermediate levels of tolerance) (Foster and Hausbeck, 2010; McGrath and Fox, 2009). The genetic basis of this tolerance is not clearly understood, but involves

multiple genes (Ortega et al., 1992), with tolerance to different disease symptoms (i.e., stem and root rot, fruit rot, or leaf lesions) being conferred by different genes (Sy et al., 2005).

Like all plant diseases, the severity of Phytophthora blight in any year depends on inoculum abundance, the presence of a susceptible crop, and the occurrence of favorable environmental conditions. These conditions include warm air temperatures (Mchau and Coffey, 1995) and periodic flooding or soil saturation events (due to heavy rainfall, over-irrigation, or poor soil drainage; Bowers and Mitchell, 1990). Therefore, predicting disease pressure before the season begins is difficult. If bell pepper varieties with tolerance to Phytophthora blight have less desirable traits (e.g., fruit silvering or smaller fruit size), then growers will need to weigh this information against the history of disease severity (and probable inoculum level) in their fields. The objectives of this study are to (i) determine which commercial bell pepper varieties have the best combination of yield, fruit quality traits, and tolerance to Phytophthora blight when grown under conditions similar to those on vegetable farms in upstate New York (i.e., relatively heavy soil and moderate temperatures), in the presence and absence of disease pressure, and (ii) compare Phytophthora-tolerant pepper breeding lines to commercially available varieties.

### *Materials and Methods*

*Field sites*. These experiments were conducted at three field sites: two located at the New York State Agricultural Experiment Station in Geneva, NY, and one at the East Ithaca research farm of Cornell University in Ithaca, NY. The Ithaca field site (hereafter referred to as "Ithaca") and one field site in Geneva ("Geneva") are located on vegetable research farms with no history of Phytophthora blight and the soil types are Arkport sandy loam and Lima loam, respectively. At the second Geneva site (hereafter referred to as "Geneva-P"), vegetables have been inoculated

with *P. capsici* annually since 2007, but no overwintering population of the pathogen is present. The soil is Odessa silt loam. The Geneva site was treated with Treflan at the labeled rate to control weeds 3.5 weeks prior to transplanting. At all sites, raised beds were 4 inches high and 3 ft wide, with 7 ft between centers, and covered with 1.25-mil black embossed plastic mulch (Belle Terre Irrigation, Sodus, NY). At the time beds were built, 300 lb/A 10N-4.4P-8.3K fertilizer (Phelps Supply, Phelps, NY) was applied under the mulch and drip tape (12 inches between holes and flow rate of 0.45 gal/min per 100 ft; Toro, Bloomington, MN) was laid under the mulch, slightly off center.

*Pepper varieties and culture.* Five commercially-available bell pepper varieties described by the supplier as being "intermediate" in their tolerance to Phytophthora blight and three varieties not described as tolerant (although one of these - Aristotle - is known to have tolerance to Phytophthora blight, as discussed above) were selected for this experiment. All eight varieties are grown commercially in New York State for the fresh market and usually harvested green. In addition, four breeding lines were also included (Table 4.1). Pepper seedlings were grown in a greenhouse under supplemental and natural light, using heated mats to improve germination. On 8 and 10 June 2011, (Geneva site, and Geneva-P and Ithaca sites, respectively) 6.5-week-old seedlings were transplanted in single rows with 18-inch spacing between plants. At each site, plants were arranged in a randomized complete block design, with each block containing 10-plant plots of each variety (with the exception of Pcap-NY8007-1, for which only five or six plants were available per plot at the Geneva and Geneva-P sites, due to limiting seed and germination). At all sites, blocks were replicated four times across adjacent rows in the field. At transplant, a total of 1.6 lb soluble fertilizer (Peters Excel 21N-2.2P-16.6K; JR Peters, Inc., Allentown, PA) was applied across the four blocks via a water wheel transplanter at an

approximate rate of 0.07 gal/row ft (2 lb fertilizer per 50 gal water). Total monthly rainfall at Geneva and Geneva-P sites was 2.34, 0.72, and 2.62 inches for June, July, and August, respectively. At the Ithaca site, total monthly rainfall was 2.59, 1.99, and 4.63 inches for June, July, and August, respectively. Plants were irrigated using drip tape to supplement rainfall as needed.

**Table 4.1** Bell pepper varieties and breeding lines used and tolerance to Phytophthora blight. Tolerance information is provided only for commercially available varieties, and is reported as advertised by the supplier. NA = not advertised by supplier as tolerant to Phytophthora blight (regardless of whether tolerance has been demonstrated in research trials).

| Variety or breeding line | Phytophthora tolerance | Source | |
|---|---|---|---|
| 'Declaration' | Intermediate | Harris Moran Seed Co. | Modesto, CA |
| 'Intruder' | Intermediate | Syngenta | Greensboro, NC |
| 'Paladin' | Intermediate | Syngenta | Greensboro, NC |
| 'Revolution' | Intermediate | Harris Moran Seed Co. | Modesto, CA |
| 'Vanguard' | Intermediate | Harris Moran Seed Co. | Modesto, CA |
| 'Aristotle' | NA | Seminis | St. Louis, MO |
| 'Karisma' | NA | Harris Moran Seed Co. | Modesto, CA |
| 'Red Knight' | NA | Seminis | St. Louis, MO |
| Pcap-NY8001-1 | | Cornell breeding line | |
| Pcap-NY 8002-3 | | Cornell breeding line | |
| Pcap-NY 8006-1 | | Cornell breeding line | |
| Pcap-NY 8007-1 | | Cornell breeding line | |

*Inoculation with Phytophthora blight*. A New York isolate of *P. capsici* (NY 0664-1; obtained from pepper in 2006) was used to inoculate the Geneva-P site. To induce sporulation, the isolate was cultured on plates of V8-juice agar at room temperature under fluorescent lights 15 h per day for 7 to 10 d. Plates were then flooded with sterile distilled water and sporangia (asexual spores) were dislodged using an L-shaped spreading rod. On 24 June (plants were 8.5 weeks old), sporangia from 17 plates were collected (total volume of approximately 300 mL) and this suspension was incubated at room temperature for approximately 30 min to allow zoospore

release. The concentration of zoospores was quantified using a hemocytometer, and approximately 5 mL of a $4x10^4$ zoospores per milliliter suspension were applied at the crown of each plant using a 1.5-gal hand-pump sprayer. On 6 and 19 July, the inoculation was repeated with 5 mL of a $1x10^5$ zoospores per milliliter suspension following similar procedures, but inoculum was applied higher up the stem of the plants (where stem tissue was still green) and allowed to run down the stem to the soil surrounding the plant. Beginning 29 June, the proportion of plants in each plot showing symptoms of Phytophthora blight (either wilting or death) was recorded approximately twice weekly, with the final disease rating on 21 Sept.

*Harvest*. Beginning 28 July and 2 and 11 Aug. at the Ithaca, Geneva, and Geneva-P sites, respectively, mature green fruit were harvested approximately every 2 weeks for a total of four harvests at each site. Harvests at the Geneva-P site included mature fruit from symptomatic plants, if fruit were present. At all sites, height, width, and height to first fruit for each plant were measured just prior to the first harvest. These data were not collected for dead plants at the Geneva-P site. In addition, uprightness of plants was rated on a per plot basis using a scale from 1 (completely prostrate) to 3 (upright) at the first harvest date (all sites), and midway through harvest (Ithaca site) or at the last harvest date (Geneva and Geneva-P sites). At the first harvest date, only, the ease with which fruit could be picked was also rated at all sites on a scale from 1 (many branches break off during harvest) to 3 (fruit can be harvested easily without breaking branches).

After harvest, unmarketable fruit were sorted into the following categories: affected by silvering (covering at least one square inch of the surface of the pepper), having symptoms of Phytophthora blight (at Geneva-P site, only), or otherwise unmarketable (fruit misshapen or damaged by blossom end rot or insects). Marketable fruit were sorted by maximum diameter

(less than 2 inches, 2-2.5 inches, 2.5-3 inches, 3-3.5 inches, 3.5-4 inches, and greater than 4 inches), and fruit with diameters less than 2 (Geneva and Geneva-P sites) or 2.5 (Ithaca site) inches, were designated unmarketable. At all harvest dates, the total weight of fruit in each of the above categories was recorded for each plot, and the total weight of marketable fruit (adjusted to weight per 10 plants) was calculated for each plot at each harvest date. Yields at the Geneva-P site were adjusted per 10 plants that were healthy at the first inoculation date (24 June), in order to account for yield loss due to plant death. At the first harvest, the total number of marketable fruit per plot was counted and used to obtain an average weight per marketable fruit and average number of fruit per plant. At all harvests, 10 marketable fruit per plot (if available) were arbitrarily selected from all marketable size categories and the following measurements were made on each fruit: number of lobes, length, width at stem end, and width at blossom end. The number of lobes per fruit was then used to calculate the percent of marketable fruit with four lobes (most desirable for fresh bell peppers for local markets).

*Statistical analysis*. Because all plants in the fourth block at the Geneva site were markedly stunted compared to plants in the other three blocks (probably due to a problem with irrigation), data from this block were excluded, and all analyses for this site were performed with data from three blocks, rather than four. The R statistical software (R Development Core Team, 2012) was used to conduct analysis of variance (ANOVA) on all data. In addition, the "car" package (Fox and Weisberg, 2011) was used to do a repeated measures ANOVA on total weight of marketable fruit (with harvest date and variety as independent variables) to determine if harvest date was a significant factor. If it was, then total marketable fruit weight was also analyzed on each harvest date, separately. Following all significant ANOVAs ($P < 0.05$), means were separated with a Tukey's Honestly Significant Difference (HSD) test (at alpha = 0.05)

using the "agricolae" package (de Mendiburu, 2012). For each site, the following data were summed across all four harvest dates prior to analysis: weight of total marketable fruit, weight of large fruit (at least 3 inches in diameter, which is the minimum diameter for fruit to be graded as "U.S. Fancy"; USDA, 2005), and percent (by weight) of fruit affected by silvering. Average fruit weight and average number of fruit per plant were analyzed on only the first harvest date (Geneva and Geneva-P sites) or the second harvest date (Ithaca site), and data from the first and second harvest dates (Geneva and Geneva-P sites), and second and third harvest dates (Ithaca site) were combined for analysis of fruit dimensions and lobe number. At the Geneva-P site, ANOVA followed by a Tukey's HSD test was conducted on disease incidence from the final rating date.

***Results***

Differences in weight of total marketable fruit (summed over all marketable size categories and harvest dates) among varieties and breeding lines were only significant at the Geneva-P site (Fig. 4.1), where yield closely paralleled disease tolerance (Fig. 4.2). Yields from 'Vanguard', 'Declaration', 'Karisma', and 'Revolution' at the Geneva-P site did not differ significantly from 'Red Knight' (which had no marketable yield). Therefore, these five varieties were considered highly susceptible to Phytophthora blight and excluded from analyses of mean percent large fruit, percent of marketable yield affected by silvering, fruit size and lobe number, at the Geneva-P site, only. These varieties will hereafter be referred to as 'susceptible,' in spite of advertised tolerance in Vanguard, Declaration, and Revolution (Table 4.1).

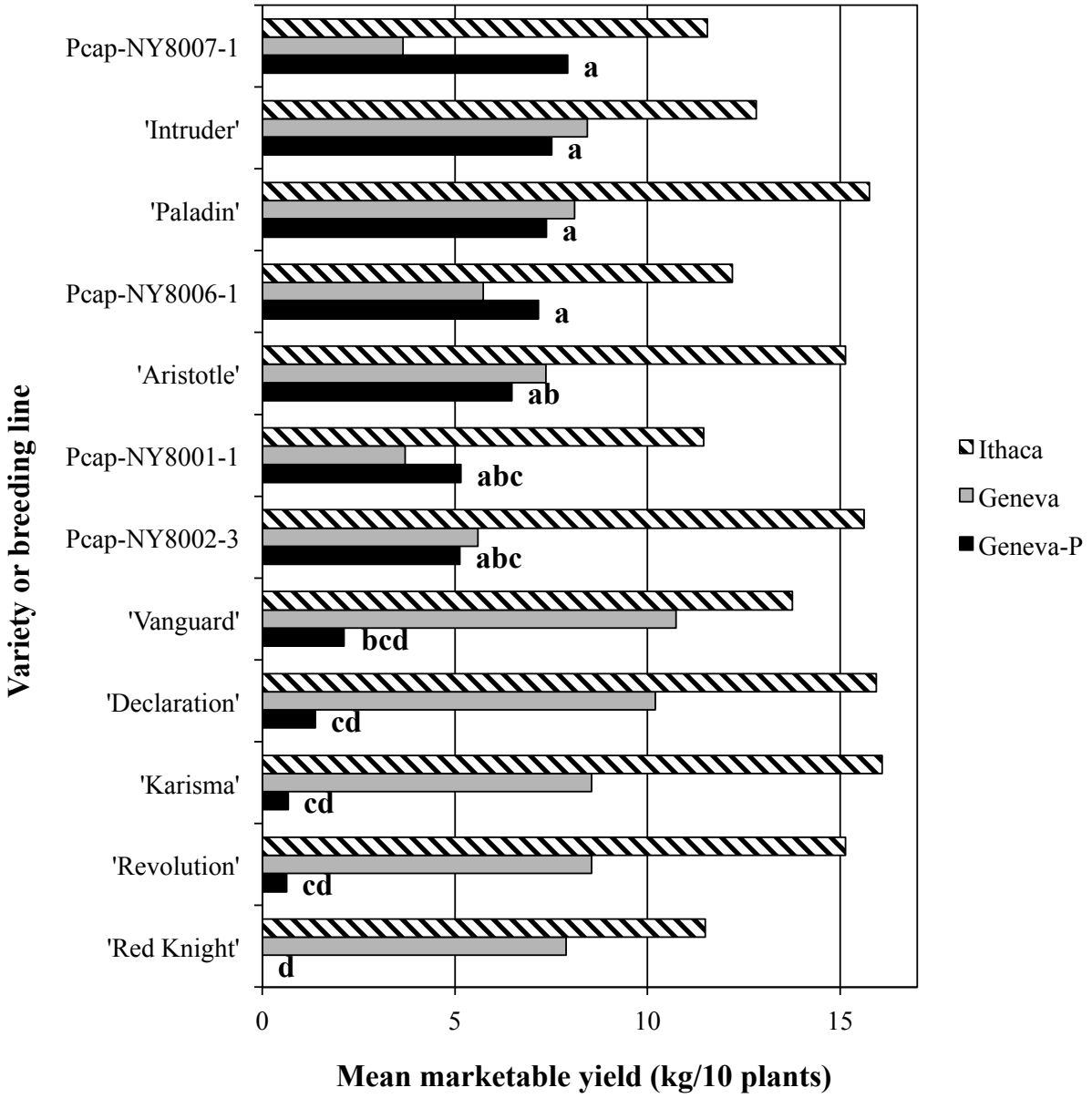



**Figure 4.1** Total marketable yield of green bell pepper varieties and breeding lines at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen *Phytophthora capsici* on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated. Yields are total weight (1 kg = 2.2046 lb) of fruit from all marketable size categories summed across four harvest dates at each site, and are adjusted per 10 plants (or per 10 plants healthy prior to inoculation with Phytophthora blight at the Geneva-P site) to account for plots which had fewer than 10 plants. Marketable fruit were defined as being greater than 2 or 2.5 inches (5.1 or 6.35 cm) in diameter at Geneva and Geneva-P, or Ithaca sites, respectively, and without blemishes from diseases, insects, or abiotic disorders. At the Geneva-P site, bars followed by the same letter are not significantly different at $P < 0.05$ (ANOVA $P = 6.66E\text{-}8$). Differences between varieties at the Geneva and Ithaca sites were not significantly different at $P < 0.05$.

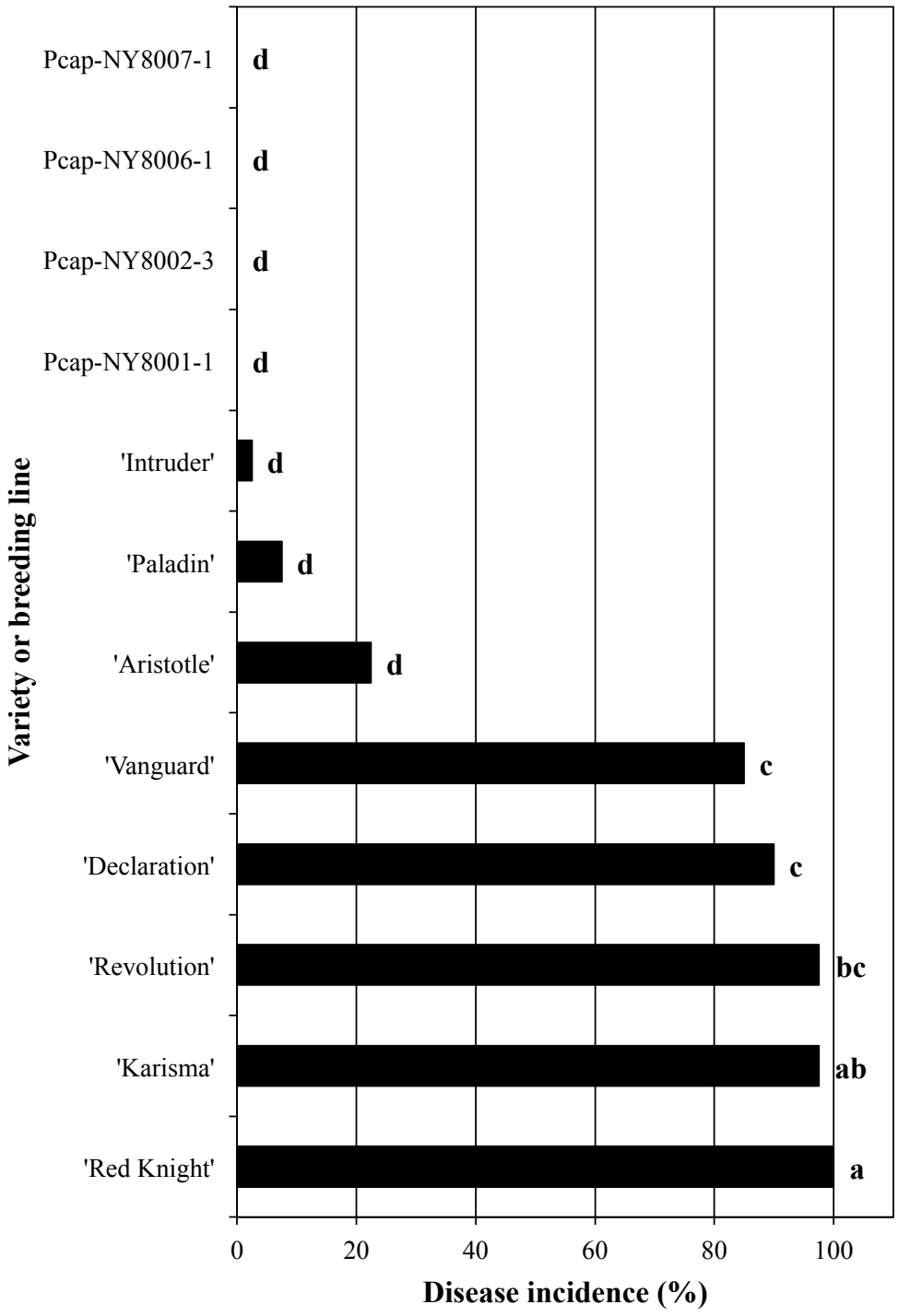



**Figure 4.2** Incidence of Phytophthora blight on green bell pepper varieties and breeding lines grown at the Geneva-P site (inoculated with the plant pathogen *Phytophthora capsici* on 24 June). Disease incidence is the proportion of plants per plot showing symptoms of Phytophthora blight (wilting or plant death) on 21 Sept. Means followed by the same letter are not significantly different at $P < 0.05$ (ANOVA $P < 2.2E\text{-}16$).

Under heavy disease pressure, yields from Pcap-NY8007-1, Intruder, Paladin, and Pcap-NY8006-1 were significantly larger than from all five susceptible varieties. In addition, 'Aristotle', Pcap-NY8001-1, and Pcap-NY8002-3 produced significantly larger yields than 'Red Knight'. 'Intruder', 'Paladin', and 'Aristotle' will subsequently be referred to as 'tolerant' to Phytophthora blight, in spite of the fact that 'Aristotle' is not marketed as such (Table 4.1). Possibly due to limited rain events during peak fruit production, we observed very few fruit with symptoms of Phytophthora blight, so no comparisons of fruit rot incidence were made among varieties and breeding lines. There were no significant differences among varieties in mean number of marketable fruit per plant at any of the three sites (Table 4.2).

**Table 4.2** Mean number of marketable fruit per plant at three field sites. At one site (Geneva-P), plants were inoculated with *Phytophthora capsici* on 24 June, but not at the other two sites (Geneva and Ithaca). Data for only the seven most tolerant varieties and breeding lines are shown for the Geneva-P site since susceptible varieties produced little or no yield at this site due to disease pressure. Data shown are from the first (Geneva-P and Geneva sites) or second (Ithaca site) harvest, only. ANOVAs testing for significant differences among varieties and breeding lines were not significant (at $P = 0.05$) at any site.

| | Mean fruit/plant | | |
|---|---|---|---|
| Vareity | Geneva-P[z] | Geneva[z] | Ithaca[z] |
| 'Aristotle' | 2.7 | 3.5 | 5.2 |
| 'Declaration' | | 3.1 | 4.8 |
| Pcap-NY8002-3 | 2.2 | 2.1 | 3.9 |
| 'Paladin' | 3.2 | 3.2 | 3.7 |
| 'Intruder' | 2.9 | 2.5 | 3.7 |
| 'Karisma' | | 2.3 | 3.5 |
| 'Revolution' | | 3.3 | 3.4 |
| Pcap-NY8006-1 | 2.8 | 2.4 | 3.3 |
| 'Vanguard' | | 1.9 | 3.0 |
| Pcap-NY8007-1 | 2.7 | 1.9 | 2.9 |
| Pcap-NY8001-1 | 1.7 | 2.1 | 2.8 |
| 'Red Knight' | | 2.3 | 2.2 |

[z] Plots from which fewer than 7 marketable fruit were harvested were excluded from the analysis.

While differences in total weight of marketable fruit were not significant in the absence of pressure from Phytophthora blight, there were significant differences in the proportion of fruit classified as "large" (at least 3 inches in diameter). At all sites, the four breeding lines tended to produce fewer large marketable fruit than did the commercial varieties, although these differences were not always significant (Table 4.3). Under pressure from Phytophthora blight, 'Paladin' produced the highest proportion of large fruit; significantly more than two of the breeding lines, but not significantly more than 'Aristotle' or 'Intruder'. In the absence of disease pressure, there were no significant differences in proportion of large fruit among commercial varieties at either the Geneva or Ithaca sites. However, at both sites, the commercial varieties with the highest proportion of large fruit had significantly more large fruit than at least two of the breeding lines. In general, more large fruit were produced at the Ithaca site than at either the Geneva or Geneva-P sites.

Similarly, marketable fruit from the breeding lines numerically tended to have smaller mean weights, and to be shorter than marketable fruit from commercial varieties (Table 4.4). Differences in mean weight were not significant at either the Geneva or Geneva-P sites, but at the Ithaca site, Pcap-NY8007-1 and Pcap-NY8001-1 produced marketable fruit that weighed significantly less than fruit from 'Revolution', 'Paladin', 'Declaration', 'Intruder', and 'Karisma'. All breeding lines had average fruit weights significantly less than that of 'Revolution' at this site. Under heavy disease pressure at the Geneva-P site, all breeding lines produced significantly shorter fruit than the three tolerant varieties (Paladin, Intruder, and Aristotle). At the Geneva and Ithaca sites, these differences were not always significant, but trends were similar.

**Table 4.3** Percent of marketable fruit classified as large [at least 3 inches (7.6 cm) in diameter] harvested over four dates at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen *Phytophthora capsici* on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated.

| Variety or breeding line | Large fruit (%)[z] | | |
|---|---|---|---|
| | Geneva-P[y] | Geneva | Ithaca |
| 'Declaration' | | 66.7 a | 98.0 a |
| 'Vanguard' | | 64.8 a | 97.8 a |
| 'Revolution' | | 58.1 ab | 99.0 a |
| 'Red Knight'[y] | | 52.6 ab | 95.5 a |
| 'Intruder' | 41.8 ab[x] | 52.0 ab | 96.2 a |
| 'Aristotle' | 36.8 abc | 40.4 ab | 95.0 ab |
| 'Paladin' | 49.7 a | 37.0 ab | 96.0 a |
| 'Karisma' | | 35.4 ab | 96.8 a |
| Pcap-NY8001-1 | 23.4 abc | 31.5 ab | 85.3 bc |
| Pcap-NY8007-1 | 22.5 abc | 21.4 b | 94.1 ab |
| Pcap-NY8002-3 | 21.4 bc | 21.2 b | 84.0 c |
| Pcap-NY8006-1 | 13.4 c | 20.5 b | 91.5 abc |
| *P* value[w] | 0.00327 | 7.47E-4 | 2.00E-5 |

[z] Percent by weight of total marketable fruit [greater than 2 or 2.5 inches (5.1 or 6.35 cm) in diameter at Geneva and Geneva-P, or Ithaca sites, respectively, and without blemishes from diseases, insects, or abiotic disorders] classified as large.
[y] At the Geneva-P site, only data from the seven most tolerant varieties and breeding lines were included in the analysis, since very few fruit were harvested from more susceptible varieties.
[x] Within a column, means followed by the same letter are not significantly different at $P < 0.05$.
[w] *P* value for ANOVAs testing for significant differences among varieties and breeding lines.

Percent of fruit with four lobes differed significantly among varieties only at the Geneva-P site, with Pcap-NY8002-3 having a significantly higher percent of marketable fruit with four lobes compared to the three tolerant commercial varieties (Paladin, Intruder, and Aristotle). There were no significant differences among commercial varieties in fruit weight, percent of fruit with four lobes, or fruit length. Differences in mean fruit width at the stem end were only significant at the Geneva-P and Ithaca sites, but differences were small in magnitude (Table 4.5). There were no significant differences in mean fruit width at the blossom end among varieties or breeding lines at any of the three sites.

**Table 4.4** Fruit characteristics of bell pepper varieties and breeding lines at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen *Phytophthora capsici* on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated. Data for only the seven most tolerant varieties and breeding lines are shown for the Geneva-P site since susceptible varieties produced little or no yield at this site due to disease pressure. All measurements are from marketable fruit, defined as greater than 2 or 2.5 inches (5.1 or 6.35 cm) in diameter at Geneva and Geneva-P, or Ithaca sites, respectively, and without blemishes from diseases, insects, or abiotic disorders.

| Variety or | Mean fruit wt (g)[z,y] | | | Mean fruit length (cm)[z,x] | | | Mean fruit with four lobes (%)[w] | | |
|---|---|---|---|---|---|---|---|---|---|
| breeding line | Geneva-P | Geneva | Ithaca | Geneva-P | Geneva | Ithaca | Geneva-P | Geneva | Ithaca |
| 'Revolution' | | 73 | 184 a[v] | | 6.4 abc | 8.1 abc | | 50.0 | 63.8 |
| 'Paladin' | 114 | 85 | 170 ab | 8.2 a | 7.6 a | 8.6 a | 27.5 b | 38.0 | 56.3 |
| 'Declaration' | | 75 | 169 ab | | 6.6 abc | 8.0 abc | | 55.0 | 61.3 |
| 'Intruder' | 128 | 75 | 169 ab | 8.1 a | 6.8 abc | 8.6 a | 32.5 b | 56.7 | 57.5 |
| 'Karisma' | | 88 | 166 abc | | 7.5 ab | 8.4 ab | | 55.0 | 61.3 |
| 'Red Knight' | | 70 | 164 abcd | | 6.6 abc | 8.4 ab | | 65.0 | 67.5 |
| 'Aristotle' | 112 | 72 | 157 abcde | 8.1 a | 7.4 ab | 8.6 a | 26.4 b | 35.0 | 43.8 |
| 'Vanguard' | | 66 | 155 abcde | | 6.6 abc | 8.2 abc | | 56.7 | 57.5 |
| Pcap-NY8002-3 | 109 | 79 | 137 bcde | 6.9 b | 6.3 abc | 7.7 abc | 60.0 a | 45.0 | 61.3 |
| Pcap-NY8006-1 | 81 | 60 | 129 cde | 6.4 b | 5.8 c | 7.2 c | 34.3 ab | 41.7 | 46.3 |
| Pcap-NY8007-1 | 89 | 66 | 127 de | 6.3 b | 6.1 bc | 7.5 bc | 51.3 ab | 51.6 | 56.3 |
| Pcap-NY8001-1 | 107 | 75 | 124 e | 6.7 b | 6.2 abc | 7.2 c | 40.0 ab | 58.0 | 64.7 |
| *P* value[u] | NS | NS | 4.10E-4 | 2.50E-10 | 7.31E-4 | 5.85E-07 | 0.0033 | NS | NS |

[z] 1 g = 0.0353 oz, 1 cm = 0.3937 inch.

[y] Average weight per marketable fruit from the first harvest date (Geneva-P and Geneva sites) or the second harvest date (Ithaca site).

[x] At the Geneva-P and Geneva sites, mean fruit length is based on 7-10 fruit per plot selected arbitrarily from all marketable fruit, and data from the first and second harvests were combined for analysis. Plots from which fewer than 7 marketable fruit were harvested were excluded from the analysis. At the Ithaca site, mean fruit length is based on 10 fruit per plot selected arbitrarily from all marketable fruit, and data from the second and third harvests were combined for analysis.

[w] Percent of marketable fruit which had four lobes.

[v] Within a column, means followed by the same letter are not significantly different at $P < 0.05$. The absence of letters indicates that there were no significant differences between varieties at this P value.

[u] *P* value for ANOVAs testing for significant differences among varieties and breeding lines. NS indicates $P \geq 0.05$.

**Table 4.5** Widths of bell pepper fruit harvested from varieties and breeding lines grown at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen Phytophthora capsici on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated. Data for only the seven most tolerant varieties and breeding lines are shown for the Geneva-P site since susceptible varieties produced little or no yield at this site due to disease pressure. All measurements are from marketable fruit, defined as greater than 2 or 2.5 inches (5.1 or 6.35 cm) in diameter at Geneva and Geneva-P, or Ithaca sites, respectively, and without blemishes from diseases, insects, or abiotic disorders.

| | Fruit width at stem end (cm)[z,y] | | | Fruit width at blossom end (cm)[z,y] | | |
|---|---|---|---|---|---|---|
| Vareity | Geneva-P | Geneva | Ithaca | Geneva-P | Geneva | Ithaca |
| Intruder | 7.3 a[x] | 6.9 | 7.3 abcd | 5.3 | 5.5 | 5.8 |
| Paladin | 7.2 ab | 7.0 | 7.3 abcd | 5.3 | 5.5 | 5.9 |
| Aristotle | 7.1 ab | 6.8 | 7.2 abcd | 5.3 | 5.2 | 5.5 |
| Pcap-NY8002-3 | 7.1 ab | 6.7 | 6.6 d | 5.8 | 5.8 | 5.8 |
| Pcap-NY8001-1 | 7.0 ab | 7.0 | 6.7 cd | 5.7 | 5.9 | 5.8 |
| Pcap-NY8007-1 | 6.5 b | 6.2 | 6.8 bcd | 5.3 | 5.4 | 5.9 |
| Pcap-NY8006-1 | 6.4 b | 6.4 | 6.6 d | 5.5 | 5.6 | 5.8 |
| Declaration | | 7.0 | 7.6 ab | | 6.0 | 6.4 |
| Karisma | | 6.8 | 7.5 abc | | 5.3 | 6.0 |
| Red Knight | | 6.9 | 7.4 abcd | | 5.6 | 6.2 |
| Revolution | | 7.1 | 7.9 a | | 5.9 | 6.6 |
| Vanguard | | 6.8 | 7.9 a | | 5.3 | 6.2 |
| *P* value[w] | 0.00279 | NS | 2.50E-8 | NS | NS | NS |

[z] 1 cm = 0.3937 inch.

[y] At the Geneva-P and Geneva sites, mean fruit length is based on 7-10 fruit per plot selected arbitrarily from all marketable fruit, and data from the first and second harvests were combined for analysis. Plots from which fewer than 7 marketable fruit were harvested were excluded from the analysis. At the Ithaca site, mean fruit length is based on 10 fruit per plot selected arbitrarily from all marketable fruit, and data from the second and third harvests were combined for analysis.

[x] Within a column, means followed by the same letter are not significantly different at $P < 0.05$. The absence of letters indicates that there were no significant differences between varieties at this P value.

[w] P value for ANOVAs testing for significant differences among varieties and breeding lines. NS indicates $P > 0.05$.

Percent of otherwise marketable fruit affected by silvering varied significantly among varieties and breeding lines at all three sites, but patterns were not always consistent across sites (Table 4.6). While a comparatively large percentage of fruit from Pcap-NY8001-1 were affected by silvering at the Geneva site, comparatively small percentages of Pcap-NY8001-1 fruit were

affected by silvering at the other two sites. Similarly, comparatively little silvering was observed on fruit from Pcap-NY8007-1 at the Geneva and Geneva-P sites, but the percent of Pcap-NY8007-1 fruit with silvering at the Ithaca site was not significantly different from 'Paladin', which had the highest percent of fruit affected by silvering at this site. At all three sites, Paladin and Intruder tended to have more fruit affected by silvering, and only at the Ithaca site did Aristotle have significantly less fruit affected by silvering compared to these two varieties. In general, silvering was more common at the Ithaca site, with high levels observed in the Phytophthora-susceptible varieties Red Knight and Vanguard, in addition to Paladin and Intruder (Table 4.6).

**Table 4.6** Silvering in bell pepper varieties and breeding lines at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen *Phytophthora capsici* on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated.

| Variety or breeding line | Mean silvering (%)[z] | | |
|---|---|---|---|
| | Geneva-P[y] | Geneva | Ithaca |
| 'Paladin' | 12.9 a[x] | 2.6 ab | 25.4 a |
| 'Red Knight' | | 3.4 ab | 24.6 a |
| 'Intruder' | 14.4 a | 4.1 ab | 21.8 ab |
| Pcap-NY8007-1 | 3.2 b | 0 b | 20.2 abc |
| 'Vanguard' | | 0 b | 10.4 bcd |
| 'Karisma' | | 0 b | 8.4 bcd |
| Pcap-NY8001-1 | 1.6 b | 4.5 a | 7.7 cd |
| 'Aristotle' | 6.1 ab | 0.8 ab | 3.6 d |
| Pcap-NY8006-1 | 1.1 b | 0 b | 3.0 d |
| 'Declaration' | | 0.3 ab | 1.9 d |
| 'Revolution' | | 0.7 ab | 1.7 d |
| Pcap-NY8002-3 | 2.2 b | 0.8 ab | 1.3 d |
| *P* value[w] | 8.13E-5 | 0.0048 | 2.53E-4 |

[z] Percent (by weight) of otherwise marketable fruit with at least one square inch of the fruit surface affected by silvering. Marketable fruit were defined as being greater than 2 or 2.5 inches (5.1 or 6.35 cm) in diameter at Geneva and Geneva-P, or Ithaca sites, respectively, and without blemishes from diseases, insects, or abiotic disorders.
[y] Data for only the seven most tolerant varieties and breeding lines are shown for the Geneva-P site since susceptible varieties produced little or no yield at this site due to disease pressure.
[x] Within a column, means followed by the same letter are not significantly different at $P < 0.05$.
[w] *P* value for ANOVAs testing for significant differences among varieties and breeding lines.

Although harvest date was a significant factor in predicting marketable yield at all field sites based on the repeated measures analysis, differences in marketable yield among varieties and breeding lines within each harvest date were not always significant. At the Geneva site, there were no significant differences between varieties at any of the harvest dates (Table 4.7). At the Ithaca site, yield per 10 Paladin plants was significantly larger than for any other variety or breeding line at the first harvest date, but at all other harvest dates differences among varieties were not significant (Table 4.8). At the Geneva-P site, yields were largest among tolerant commercial varieties (Paladin, Intruder, and Aristotle) at the first two harvest dates, but yields from the breeding lines were either relatively consistent over the four harvest dates, or in some cases increased at later dates (Table 4.9). Yields from the five susceptible varieties (Vanguard, Declaration, Revolution, Karisma, and Red Knight) tended to be smaller across all harvest dates at the Geneva-P site compared to Paladin, Intruder, and Aristotle (the most tolerant varieties). However, these differences were not significant at the third and fourth harvest dates, or for 'Vanguard' at any of the harvest dates.

**Table 4.7** Marketable yields from bell pepper varieties and breeding lines at four harvest dates at the Geneva field site. This site had not been inoculated with the plant pathogen *Phytophthora capsici*, and there were no significant differences among varieties at any of the harvests.

| Variety or breeding line | Mean marketable yield (kg)/10 plants[z] | | | |
|---|---|---|---|---|
| | Havest 1 | Harvest 2 | Harvest 3 | Havest 4 |
| 'Paladin' | 2.64 | 2.63 | 2.51 | 0.32 |
| 'Aristotle' | 2.59 | 2.83 | 1.68 | 0.27 |
| 'Revolution' | 2.44 | 2.61 | 2.49 | 0.99 |
| 'Declaration' | 2.31 | 3.14 | 3.65 | 1.11 |
| 'Intruder' | 1.97 | 2.90 | 3.00 | 0.57 |
| 'Karisma' | 1.90 | 3.19 | 2.89 | 0.57 |
| Pcap-NY8002-3 | 1.68 | 1.78 | 1.44 | 0.70 |
| Pcap-NY8001-1 | 1.56 | 1.31 | 0.50 | 0.33 |
| 'Red Knight' | 1.55 | 2.29 | 3.30 | 0.75 |
| Pcap-NY8006-1 | 1.46 | 2.81 | 1.25 | 0.21 |
| 'Vanguard' | 1.27 | 3.33 | 5.22 | 0.91 |
| Pcap-NY8007-1 | 1.22 | 0.99 | 0.94 | 0.49 |

[z] 1 kg = 2.2046 lb. Marketable fruit were defined as being greater than 2 inches (5.1 cm) in diameter, and without blemishes from diseases, insects, or abiotic disorders.

**Table 4.8** Marketable yields from bell pepper varieties and breeding lines at four harvest dates at the Ithaca field site. This site had been inoculated with the plant pathogen *Phytophthora capsici*.

| Variety or breeding line | Mean marketable yield (kg/10 plants) on four harvest dates[z] | | | |
|---|---|---|---|---|
| | Harvest 1 | Harvest 2 | Harvest 3 | Harvest 4 |
| Paladin | 2.46 a[y] | 6.04 | 5.01 | 2.26 |
| Pcap-NY8002-3 | 0.53 b | 4.95 | 6.38 | 3.76 |
| Declaration | 0.50 b | 7.56 | 5.03 | 2.85 |
| Pcap-NY8001-1 | 0.49 b | 3.52 | 4.67 | 2.78 |
| Revolution | 0.45 b | 5.84 | 5.97 | 2.89 |
| Red Knight | 0.40 b | 3.69 | 4.84 | 2.57 |
| Pcap-NY8007-1 | 0.32 b | 3.63 | 4.29 | 3.32 |
| Aristotle | 0.29 b | 7.91 | 4.75 | 2.20 |
| Intruder | 0.25 b | 5.90 | 4.72 | 1.95 |
| Karisma | 0.18 b | 5.59 | 6.63 | 3.69 |
| Vanguard | 0.00 b | 4.58 | 5.75 | 3.43 |
| Pcap-NY8006-1 | 0.00 b | 3.94 | 4.86 | 3.40 |
| *P*-values[x] | 8.20E-11 | 0.013 | NS | NS |

[z] 1 kg = 2.2046 lb. Marketable fruit were defined as being greater than 2.5 inches (6.35 cm) in diameter, and without blemishes from diseases, insects, or abiotic disorders.

[y] Within a column, means followed by the same letter are not significantly different based on a Tukey's Honestly Significant Differences test at alpha = 0.05. If no letters are present in the column, then there were no significant differences among varieties.

[x] *P* value for ANOVAs testing for significant differences among varieties and breeding lines.

**Table 4.9** Marketable yields from bell pepper varieties and breeding lines at four harvest dates at the Geneva-P field site. Peppers at this site were inoculated with the plant pathogen *Phytophthora capsici* on 24 June.

| Variety or breeding line | Mean marketable yield (kg/10 plants)[z] | | | |
|---|---|---|---|---|
| | Harvest 1 | Harvest 2 | Harvest 3 | Harvest 4 |
| 'Paladin' | 3.65 a[y] | 2.24 a | 0.87 ab | 0.62 bc |
| 'Intruder' | 3.58 a | 1.92 ab | 1.08 ab | 0.93 abc |
| 'Aristotle' | 2.42 ab | 1.73 abc | 1.44 ab | 0.89 abc |
| Pcap-NY8006-1 | 1.85 ab | 1.67 abc | 1.70 ab | 1.95 ab |
| Pcap-NY8002-3 | 1.85 ab | 1.09 abcd | 0.90 ab | 1.29 abc |
| Pcap-NY8001-1 | 1.67 ab | 1.15 abcd | 0.93 ab | 1.41 abc |
| Pcap-NY8007-1 | 1.31 ab | 1.43 abcd | 2.90 a | 2.30 a |
| 'Vanguard' | 1.28 ab | 0.46 bcd | 0.32 b | 0.07 c |
| 'Declaration' | 1.06 b | 0.18 cd | 0.12 b | 0.02 c |
| 'Revolution' | 0.41 b | 0.11 cd | 0 b | 0.11 c |
| 'Karisma' | 0.37 b | 0.24 cd | 0.04 b | 0.03 c |
| 'Red Knight' | 0 b | 0 d | 0 b | 0 c |
| P value[x] | 5.88E-5 | 2.56E-5 | 4.5E-4 | 6.89E-6 |

[z] Yields per 10 plants were based on the number of plants which were healthy when they were first inoculated with *Phytophthora capsici* on 24 June. 1 kg = 2.2046 lb. Marketable fruit were defined as being greater than 2 inches (5.1 cm) in diameter, and without blemishes from diseases, insects, or abiotic disorders.

[y] Within a column, means followed by the same letter are not significantly different at $P < 0.05$.

[x] P value for ANOVAs testing for significant differences among varieties and breeding lines.

There were few significant differences among varieties and breeding lines in plant height or height to first fruit at either the Geneva or Ithaca sites, although the breeding lines tended to be shorter (numerically) than the commercial varieties (Table 4.10). However, this was not true at the Geneva-P site. Among Declaration plants which survived to the first harvest at this site, plant height and height to first fruit were significantly reduced compared to one of the commercial varieties and three of the breeding lines, whereas Declaration plants were not significantly shorter than other varieties or breeding lines at either the Geneva or the Ithaca site. Also, at the Geneva site, 'Red Knight' produced its first fruit significantly closer to the ground than did 'Intruder'. While this difference was not significant at the Ithaca site, the same trend

was present. With the exception of two of the breeding lines (Pcap-NY8006-1 and Pcap-NY8007-1), plants inoculated with Phytophthora blight tended to be shorter than uninoculated plants at the two healthy sites.

Plants of all varieties and breeding lines were generally upright, and little lodging was observed during the 8-week harvest period at either the Geneva or Geneva-P site. At the Ithaca site, plots of Declaration plants tended to receive lower scores (i.e., more lodging) compared to other varieties or breeding lines at the mid-harvest rating, but differences were not statistically significant. At both the Geneva and Geneva-P sites, fruit were consistently very easy to harvest from breeding line plants, while ease of harvest from commercial varieties was variable between plots of the same variety. At the Ithaca site, ease of harvest was similar among varieties, although some plots (primarily breeding lines) had no mature fruit ready for harvest at the first harvest date, when data were collected.

**Table 4.10** Plant height and height to first fruit of bell pepper varieties and breeding lines grown at three field sites. At one site (Geneva-P), plants were inoculated with the plant pathogen *Phytophthora capsici* on 24 June, and at the other two sites (Geneva and Ithaca), plants were not inoculated. No data are available for 'Karisma' and 'Red Knight' from the Geneva-P site because all plants had already been killed by Phytophthora blight in two and four of the blocks, respectively, by the time data were collected.

| | Mean plant ht (cm)[z] | | | Mean ht to first fruit (cm)[z] | | |
|---|---|---|---|---|---|---|
| Variety or breeding line | Geneva-P | Geneva | Ithaca | Geneva-P | Geneva | Ithaca |
| 'Vanguard' | 39.7 abc[y] | 50.9 a | 52.6 a | 15.8 ab | 16.5 ab | 11.1 |
| 'Declaration' | 32.5 c | 51.4 a | 51.5 ab | 6.6 b | 17.2 ab | 9.5 |
| 'Aristotle' | 37.6 abc | 46.2 ab | 49.4 abc | 13.4 ab | 16.2 ab | 8.3 |
| 'Revolution' | 35.8 bc | 49.5 a | 49.2 abcd | 12.5 ab | 15.5 ab | 7.5 |
| 'Karisma' | | 49.1 ab | 48.5 abcd | | 17.0 ab | 8.8 |
| 'Paladin' | 38.9 abc | 45.4 ab | 48.5 abcd | 15.2 ab | 16.1 ab | 7.3 |
| 'Intruder' | 42.2 ab | 53.7 a | 47.5 bcde | 16.6 a | 23.9 a | 12.5 |
| 'Red Knight' | | 50.3 a | 47.1 bcde | | 13.8 b | 7.5 |
| Pcap-NY8006-1 | 43.8 a | 44.4 ab | 46.2 cde | 20.7 a | 16.3 ab | 8.6 |
| Pcap-NY8002-3 | 40.4 ab | 49.0 ab | 46.0 cde | 18.1 a | 21.4 ab | 10.6 |
| Pcap-NY8007-1 | 40.9 ab | 38.6 b | 44.9 de | 16.1 ab | 18.8 ab | 7.4 |
| Pcap-NY8001-1 | 38.9 abc | 44.5 ab | 43.6 e | 20.2 a | 18.1 ab | 9.7 |
| P value[x] | 0.00272 | 0.00291 | 3.53E-05 | 0.00295 | 0.0452 | NS |

[z] 1 cm = 0.3937 inch.

[y] Within a column, means followed by the same letter are not significantly different at $P < 0.05$. The absence of letters indicates that there were no significant differences between varieties at this P value.

[x] P value for ANOVAs testing for significant differences among varieties or breeding lines. NS indicates $P > 0.05$.

***Discussion***

With an increase in the prevalence of Phytophthora blight in the northeastern United States, choosing a bell pepper variety with both disease tolerance and excellent horticultural characteristics is critical. Results from this study suggest that commercial varieties and breeding lines included in this trial were equally high-yielding in weight but not fruit size, in the absence of Phytophthora blight. The most tolerant commercial varieties maintained their yields under heavy disease pressure from a single New York isolate of the pathogen. Interestingly, although Revolution has been described as intermediately-tolerant (Foster and Hausbeck, 2010; McGrath and Fox, 2009), and is frequently grown in commercial New York fields with a history of Phytophthora blight, in this trial it did not yield any better than the susceptible variety Red Knight in the presence of Phytophthora blight, and had only slightly lower disease incidence. This could be related to the high level of disease pressure in the trial, or to the specific isolate used in inoculation. Isolates of Phytophthora blight can vary widely in their virulence on different pepper varieties, and some have been shown to be virulent on Revolution, as well as Paladin and Aristotle (Foster and Hausbeck, 2010). It should also be noted that, while 'Paladin' showed high levels of tolerance in the present study, Ristaino and Johnston (1999) reported that it is susceptible to foliar infection by Phytophthora blight. In on-farm and research trials, we have seen fruit rot and foliar symptoms of Phytophthora blight on all the varieties and breeding lines included in this trial, in some cases including latent infections that may result in post-harvest symptom development (A.R. Dunn and C.D. Smart, unpublished). Using fungicide applications in combination with pepper varieties tolerant to root and stem rot can improve both plant survival and yield (McGrath and Fox, 2009).

As discussed previously, total weight of marketable fruit is not the sole criterion for selecting a bell pepper variety for fresh market production. While differences among commercial varieties were not significant in this trial, some of the most tolerant commercial varieties (especially Paladin, and Aristotle) did tend to produce smaller proportions of large fruit, compared to the more susceptible varieties. In a two-year study conducted in three fields across Pennsylvania, Sánchez, et al. (2011) reported that 'Revolution,' 'Intruder,' 'Aristotle,' 'Karisma,' and 'Paladin' were all comparable in terms of total marketable yield. However, Revolution surpassed these varieties (and all others in the trial) in terms of quantity of large fruit (defined as greater than 4 inches in diameter) produced. While differences were not significant in our trial, we saw similar trends in terms of percent of fruit with diameters greater than 3 inches, and average fruit weight of Revolution compared to the other four varieties this trial had in common with the Pennsylvania study.

Similar to previous reports of increased silvering in Phytophthora-tolerant bell pepper varieties (Kline et al., 2011), Paladin and Intruder did have comparably high levels of silvering at all three sites in this trial, while incidence of silvering in Aristotle fruit was high at only one of the three sites. In spite of this, total marketable yields from Paladin and Intruder were not significantly lower than yields from other commercial varieties with less silvering. Thus, in this study, marketable yields from Paladin and Intruder were still competitive with other commercial varieties, in spite of losses due to silvering. Incidence of silvering was low in 'Revolution' at both the Geneva and Ithaca sites. It is not clear why differences in silvering among varieties were not consistent across the three sites in the present study, or why much higher incidence of silvering was observed at the Ithaca site than at the other two sites. However, Wyenandt and Kline (2006) previously reported differences in percent of fruit affected by silvering between two

locations (although cultural practices differed between the locations). Kline et al. (2011) also speculated that environmental conditions (including soil moisture, soil and air temperature, or their fluctuation over time) may contribute to levels of silvering. While variety is likely not the only factor that influences fruit silvering in pepper, it is an important factor, and bell pepper varieties that are less-prone to silvering are needed.

The fact that some stunting was observed in all varieties and two of the four breeding lines in the presence of Phytophthora blight suggests that the disease is affecting even plants that do not wilt or die following inoculation. It seems likely that this stunting would affect marketable yield, although this trial provides no evidence of that. However, since uninoculated plants were grown in different fields than inoculated plants (with different soil types), it is difficult to make yield comparisons between inoculated and uninoculated plants. While disease incidence was high in 'Declaration', plants that did survive were also shorter and bore fruit closer to the ground than 'Intruder' and three of the breeding lines. This could result in increased vulnerability of fruit on surviving 'Declaration' plants to spores of Phytophthora blight splashed from the soil during heavy rain or overhead irrigation.

The four breeding lines included in this trial have excellent tolerance to Phytophthora blight, with total marketable fruit weights comparable to commercial varieties (with and without disease pressure). In addition, some had comparable proportions of fruit with four lobes (relative to commercial varieties), and Pcap-NY8006-1 and Pcap-NY8002-3 showed consistently low incidence of silvering across all three field sites. Fruit from the breeding lines tended to be smaller (in terms of diameter, weight, and length). Although they are not suited to production of early, extra-large fruit, their high degree of Phytophthora tolerance results in extended harvests when grown in infested soil.

*Conclusion.* The results of this trial suggest that 'Intruder', 'Paladin', and 'Aristotle' are good choices for fresh market green bell pepper production on farms in the northeastern United States with a history of heavy disease pressure from Phytophthora blight, although silvering is a concern, especially for 'Intruder' and 'Paladin'. All three varieties yielded well in the presence and absence of Phytophthora blight, suggesting that they will perform well even in fields where Phytophthora blight severity varies from year to year. In addition, it is possible that a field population of Phytophthora blight could overcome the host tolerance in a pepper variety if that variety is extensively and continuously planted in a field. Therefore, fields with a history of Phytophthora blight should be carefully monitored for increased disease incidence on previously-tolerant varieties. Especially in fields with high inoculum levels, an integrated approach that utilizes host tolerance, cultural practices (including crop rotation), and fungicides is recommended for successful management of this disease. This study also highlights the need for additional Phytophthora-tolerant bell pepper varieties that produce large fruit and are less prone to silvering.

***Acknowledgements:***

We thank H. W. Lange and M. Fink-Brodnicki for technical assistance. Support for A. R. Dunn was provided by a fellowship from Cornell University College of Agriculture and Life Sciences. The project was funded through a grant from the NY State Department of Agriculture and Markets Specialty Crop Block Grant. Support for L. E. Wyatt was provided by a Cornell University Presidential Life Sciences Fellowship and USDA National Needs Graduate Fellowship Competitive Grant No. 2008-38420-04755 from the National Institute of Food and Agriculture.

***References***


Bowers, J. H. and Mitchell, D. J. 1990. Effect of soil-water matric potential and periodic flooding on mortality of pepper caused by *Phytophthora capsici*. Phytopathology 80:1447–1450.

Crosby, K. M. 2008. Pepper, p. 221-248. In: Prohens, J. and Nunez, F. (eds.). Vegetables II. Springer, New York, NY.

de Mendiburu, F. 2012. Statistical procedures for agricultural research. Version 1.1-3. International Potato Center, Lima, Peru. <http://tarwi.lamolina.edu.pe/~fmendiburu>.

Dunn, A. R., Milgroom, M. G., Meitz, J. C., McLeod, A., Fry, W. E., McGrath, M. T., Dillard, H. R., and Smart, C. D. 2010. Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York State. Plant Dis. 94:1461–1468.

Foster, J. M. and Hausbeck, M. K. 2010. Resistance of pepper to *Phytophthora* crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24–30.

Fox, J. and Weisberg, S. 2011. An R companion to applied regression, 2nd ed. Sage Publications, Thousand Oaks, CA.

Granke, L. L., Quesada-Ocampo, L., Lamour, K., and Hausbeck, M. K. 2012. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. Plant Dis. 95:1588-1600.

Hutton, M. G. and Handley, D. T. 2007. Bell pepper cultivar performance under short, variable growing seasons. HortTechnology 17:136–141.

Jackson, K. L., Yin, J., and Ji, P. 2012. Sensitivity of *Phytophthora capsici* on vegetable crops in Georgia to mandipropamid, dimethomorph and cyazofamid. Plant Dis. 96:1337–1342.

Keinath, A. P., 2007. Sensitivity of populations of *Phytophthora capsici* from South Carolina to mefenoxam, dimethomorph, zoxamide, and cymoxanil. Plant Dis. 91:743–748.

Kline, W. L., Wyenandt, C. A., Ward, D. L., Sudal, J. F., and Maxwell, N. L. 2011. Evaluation of six nitrogen fertility programs on marketable yield and development of skin separation in bell pepper fruit. HortTechnology 21:323–328.

Leonian, L. H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopathology 12:401–408.

McGrath, M. T. and Fox, G. M. 2009. Evaluation of Phytophthora-resistant bell pepper cultivars with and without a fungicide program, 2008. Plant Dis. Manag. Rep. 3:V132.

Mchau, G. R. A. and Coffey, M. D. 1995. Evidence for the existence of two subpopulations in *Phytophthora capsici* and a redescription of the species. Mycol. Res. 99:89–102

Ortega, R. G., Español, C. P., and Zueco, J. C. 1992. Genetic relationships among four pepper genotypes resistant to *Phytophthora capsici*. Plant Breeding 108:118–125

R Development Core Team. 2012. R: A language and environment for statistical computing. <http://www.R-project.org/>.

Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of *Phytophthora* blight on bell pepper. Plant Dis. 83:1080–1089.

Sánchez, E. S., Butzler, T. M., Bogash, S. M., Elkner, T. E., Oesterling, R. E., Orzolek, M. D. and Stivers, L. J. 2011. Pennsylvania statewide bell pepper cultivar evaluation. HortTechnology. 21:384–390.

Sy, O., Bosland, P. W., and Steiner, R. 2005. Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. J. Amer. Soc. Hort. Sci. 130:75–78.

U.S. Department of Agriculture. 2005. United States standards for grades of sweet peppers. <http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5050318>.
U.S. Department of Agriculture. 2012a. New York annual statistics bulletin 2012. <http://www.nass.usda.gov/Statistics_by_State/New_York/Publications/Annual_Statistical_Bulletin/2012/2012-bulletin.htm>.
U.S. Department of Agriculture. 2012b. Vegetables summary 2011. <http://usda01.library.cornell.edu/usda/current/VegeSumm/VegeSumm-01-26-2012.pdf>.
Wiant, J. S. and Tucker, C. M. 1940. A rot of Winter Queen water-melons caused by *Phytophthora capsici*. J. Agr. Res. 9:73–88.
Wyenandt, C. A. and Kline, W. L. 2006. Evaluation of skin separation (silvering) in fruit of bell pepper cultivars. HortScience. 41(3):494.

CHAPTER 5

# Transformation of *Phytophthora capsici* with genes for green and red fluorescent protein for use in visualizing plant-pathogen interactions[5,6]


## *Abstract*

One of many uses of fluorescent proteins in plant pathology is their constitutive expression in a pathogen in order to facilitate microscopic visualization of host-pathogen interactions. However, if such transformants are to be useful, it is important that they be similar to wild type isolates in their ability to cause disease. The vegetable pathogen *Phytophthora capsici* was transformed to stably and constitutively express genes for either a green (*pgfp*) or red (*tdTomato*) fluorescent protein. All transformants fluoresced in all life stages, but varied in their intensity and contained one, two, or five copies of *pgfp* or *tdTomato*, as determined by Southern analysis. One transformant labeled with green fluorescent protein had reduced growth on artificial medium, produced smaller lesions on detached pepper fruit, and was reduced in virulence on pepper seedlings, compared to the wild type isolate. For these reasons, it is unsuitable for use in studies of host-pathogen interactions. Based on their intense fluorescence and similarity to the wild type isolate in growth and virulence, the other four transformants will be useful in future microscopy studies of interactions between *P. capsici* and its various hosts.


## *Introduction*

The oomycete *Phytophthora capsici* causes Phytophthora blight on many high-value vegetable crops, including sweet and hot pepper, tomato, eggplant and all cucurbits (Hausbeck

[5] Reprinted with kind permission from Springer Science and Business Media; Dunn, A. R., Fry, B. A., Lee, T. Y., Conley, K. D., Balaji, V. Fry, W. E., McLeod, A., and Smart, C. D. (2013) Transformation of *Phytophthora capsici* with genes for green and red fluorescent protein for use in visualizing plant-pathogen interactions. Australasian Plant Pathology 42:583-593.

[6] Transformation of *P. capsici* performed by B. A. Fry, T. Y. Lee, W. E. Fry, and A. McLeod.

and Lamour 2004), as well as snap bean (Gevens et al. 2008), lima bean (Davidson et al. 2002), and Fraser fir (Quesada-Ocampo et al. 2009). In addition, several common agricultural weed species have also been identified as hosts (French-Monar et al. 2006). Symptoms vary by host, and may include plant wilting and eventual death (as a result of root or crown rot), stem or leaf lesions, and fruit rot (Café-Filho and Duniway 1995; Erwin and Ribeiro 1996; Hausbeck and Lamour 2004). *Phytophthora capsici* reproduces rapidly under warm, wet conditions via asexual sporangia which release motile zoospores when exposed to sufficient water (Bernhardt and Grogan 1982; Crossan et al. 1954; Hausbeck and Lamour 2004). If both mating types (A1 and A2) of this heterothallic oomycete are present in a field, long-lived oospores are produced that can survive for years in the absence of a host crop (Lamour and Hausbeck 2003; Ristaino and Johnston 1999). Although none of these spores is wind-dispersed, they can be moved between fields in soil, infected plant materials, or water, and can be spread through a field by cultivation, heavy rains, and excess water in the field (Granke et al. 2009; Hausbeck and Lamour 2004).

Since the cloning of the gene for the wild type green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (Prasher et al. 1992), new and modified fluorescent proteins of various colors have been employed to study the interactions of microorganisms with their environments, including many plant pathogens (Czymmek et al. 2004; Larrainzar et al. 2005). This work has been facilitated by the modification of the wild type proteins in order to optimize them for use in various studies (Larrainzar et al. 2005). For example, changes at amino acid position 65 in wild type GFP (referred to as the S65T mutation) increased brightness, and altered the wavelength required for peak excitation, thereby substantially reducing photobleaching (Cubitt et al. 1995; Heim et al. 1995; Lorang et al. 2001). Several studies have described the utility of this modified GFP in plants (Chiu et al. 1996; Pang et al. 1996). Similarly, tdTomato is

a red fluorescent protein (RFP) derived from the wild type protein DsRed, but is a dimer, rather than a tetramer, and has increased brightness and a reduced maturation time compared to DsRed (Shaner et al. 2004).

Fluorescent proteins have been utilized to answer a variety of basic and applied questions about fungal and oomycete plant pathogens and how they interact with their hosts. Plant pathogens labeled with a fluorescent protein have been used to quantify pathogen growth in host plants (e.g., Chen et al. 2003; Si-Ammour et al. 2003), to facilitate observation of the infection and colonization of susceptible and non-susceptible plants (e.g., Li et al. 2011; Maor et al. 1998; Njoroge et al. 2011; Vallad and Subbarao 2008), and to visualize the timing or location of pathogen gene expression (e.g., Dumas et al. 1999; Sexton and Howlett 2001). Ah-Fong and Judelson (2011) also labeled various organelles in *Phytophthora infestans* with green, red, cyan, and yellow fluorescent proteins, and others have labeled plant cell components with fluorescent proteins in order to visualize the host response to pathogens (Takemoto et al. 2003). Relatively recently, Schornack et al. (2010) transformed *P. capsici* with a plasmid containing *tdTomato* or with the *P. infestans Avr3a* avirulence gene fused to *gfp*, in order to study localization of this protein during infection. The primary goal of this study was to use *P. capsici* as an efficient delivery system for oomycete effectors in order to study the role of these effectors in the infection process, rather than to investigate the physiological interactions between *P. capsici* and *N. benthamiana*. The authors did not report comparisons of transformants to the wild type isolate in terms of growth or virulence on *N. benthamiana* before selecting single transformants for use in these studies.

Transformants tagged with either GFP or RFP would be very useful for microscopically visualizing the interactions of *P. capsici* with its various hosts, but in such a study it is

imperative that transformants be similar to wild type isolates in both growth and virulence. Several studies have reported negative impacts on some proportion of stable transformants. For example, while growth of *Leptosphaeria maculans* GFP transformants on artificial medium was similar to that of the parent isolate, some transformants produced smaller lesions on *Brassica* spp. (Sexton and Howlett 2001). When Riedel et al. (2009) transformed *P. ramorum* with GFP, only one of the five transformants that fluoresced brightly was as virulent as the wild type isolate, and it also grew more slowly *in vitro*. Si-Ammour et al. (2003) reported that many of their brightly-flourescing *P. infestans* and *P. brassicae* transformants labeled with GFP did not grow as well *in vitro* as did the corresponding wild type isolates. However, they also reported that transformants selected for comparable *in vitro* growth caused similar symptoms on their respective hosts compared to the wild type isolates. Bailey et al. (1991) reported that *P. capsici* transformants resistant to hygromycin B caused similar symptoms on Serrano pepper seedlings compared to the wild type isolate. Others have also reported no loss of virulence or pathogenicity following transformation of various fungi and oomycetes (Judelson et al. 1991; Li et al. 2011; Nahalkova 2003; Vallad and Subbarao 2008; Visser et al. 2004). However, in some studies only one transformant was tested for virulence or pathogenicity ( Judelson et al. 1991; Vallad and Subbarao 2008), and in another case, transformants were pre-selected for normal growth and colony morphology prior to virulence or pathogenicity tests (Nahalkova 2003).

Thus, previous studies suggest that transformation can reduce virulence or growth of plant pathogens, and prior transformation of *P. capsici* with genes for fluorescent proteins has focused on using this pathogen to deliver effectors of other oomycetes to a plant of interest, rather than microscopic investigations of the physical interactions between pathogen and plant. This work was initiated with the goal of producing transformants suitable for microscopic

visualization of the infection and colonization processes of *P. capsici* on its numerous vegetable hosts. The objectives of this study were (i) to produce isolates of *P. capsici* that stably express genes for either green or red fluorescent protein in all life stages of the pathogen, and which fluorescence brightly, and (ii) to determine if any of these transformants have reduced growth or virulence compared to the wild type isolate, rendering them unsuitable for further studies of the colonization and infection processes.

***Materials and Methods***

Phytophthora capsici *isolate and vectors used in transformation*. *Phytophthora capsici* isolate NY 0664-1, a single-zoospore isolate collected from a pepper growing in Monroe county (central New York), was used in all transformation experiments. The isolate is A1 mating type and is sensitive to the fungicide mefenoxam. It was transformed with one of three vectors, pTORtdTomato, pSAMHAMGFP, or pSAMDBGFP. Vector pTORtdTomato (Steven Whisson and Severine Grouffaud, The James Hutton Institute, Dundee, Scotland, UK) contains the selectable marker gene *neomycin phosphotransferase* (*nptII*) fused to the promoter and terminator sequences of the *hsp70* gene (Judelson et al. 1991), and the improved red fluorescent protein gene *tdTomato* (Shaner et al. 2004) that is under control of the promoter and terminator sequences of the *ham34* gene ( Judelson et al. 1991). The gene *tdTomato* is used with the permission of Dr. Roger Tsien, HHMI Investigator at the University of California, San Diego (UCSD Docket No. 2001-A63-3). Hereafter, the protein is referred to as RFP. Vector pSAMHAMGFP was constructed from vector pSAM (Mauch et al. 2009); GenBank accession GI161347204). It contains the *nptII* gene fused to the promoter and terminator sequences of the *hsp70* gene, and the green fluorescent protein gene *pgfp*, with the S65T mutation (Pang et al.

1996), followed by the *ham34* terminator. Hereafter, the protein is referred to as GFP. Vector pSAMHAMGFP was constructed by cloning the *ham34* promoter into the *SacII* site of pSAM. Vector pSAMDBGFP differs from pSAMHAMGFP in that the *Piexo1/ham34* promoter, instead of the *ham34* promoter, drives expression of *pgfp*. The vector was constructed by PCR amplification of the *Piexo1/ham34* promoter fragment from plasmid pDBHAMT35G (McLeod et al. 2008), and blunt-end cloning it into the *Sac*II restriction site of pSAM.

*Protoplast production*. Protocols for producing *P. capsici z*oospores were similar to those that have been described previously (Foster and Hausbeck 2010). Zoospore suspensions obtained from 5-7 day-old cultures of *P. capsici* NY 0664-1 growing on five 90-mm Petri plates (yields approximately 60 ml zoospore suspension) was poured through Miracloth (Calbiochem Corp., La Jolla, CA) into a 1 L Erlenmeyer flask containing 100 ml of 2x pea broth (220 g frozen peas autoclaved in 1L distilled water and strained through two layers of cheese cloth, followed by the addition of 2 g $CaCO_3$ and autoclaving). The flask was incubated overnight at 25°C. The germinated zoospores (mycelia) were harvested onto Miracloth in a funnel, and washed with 0.8 M mannitol. Protoplasts were released from the harvested mycelia using a protocol modified slightly from McLeod et al. (2008). The mycelia from one Erlenmeyer flask were added to 40 ml of enzyme digestion solution containing 2.25 μg/ml cellulase (Sigma-Aldrich, St. Louis, Missouri) and either10 μg/ml lysing enzyme (Sigma-Aldrich), or 10 μg/ml glucanase (InterSpex Products, San Mateo, CA; no longer commercially available). Mycelia were digested for 45 – 60 min. at 28°C at 50 rpm until abundant protoplast release occurred. After washing protoplasts in W5 buffer, the re-suspended protoplasts were placed on ice for 30 min., prior to re-suspension in MMg solution (0.4 M mannitol, 15 mM $MgCl_2$, 4 mM MES pH 5.7) to a concentration of 2 x $10^7$ protoplasts/ml and incubated at room temperature for 10 min.

*Protoplast transformation and regeneration*. Protoplasts were transformed with vector DNA using a slightly modified method of McLeod et al. (2008). Protoplasts (500 µl) were transformed with 20 µg vector DNA, by first incubating the protoplast/DNA solution with gentle mixing on ice for 15 min. Subsequently, a 40% PEG solution (16 g PEG 4000 (Fluka, Sigma-Aldrich), 12 ml sterile distilled water, 10 ml 0.8 M mannitol, 4 ml 1M $CaCl_2$) was added in three aliquots (0.5 ml, 0.5 ml, and 1 ml) with gentle mixing following each addition. The tubes were kept on ice for 20 min., followed by the addition of 2 ml of 0.5 M mannitol/half-strength potato dextrose broth (PDB; Merck, Whitehouse Station, NJ) and a 2 min incubation. The suspension was brought to a total volume of 12 ml with 0.5 M mannitol/half-strength PDB broth. The content of each tube was added to a 90-mm Petri plate containing 10 ml of 0.5 M mannitol/half-strength PDB broth, and the protoplasts were regenerated for 5-6 h at 25°C.

*Selection of transformants*. The regenerated protoplasts from each plate were pelleted by centrifugation at 750 x *g* for 10 min. in a swinging bucket centrifuge. The pellet was washed with sterile distilled water and re-suspended in 1 ml sterile distilled water before plating 200 µl aliquots onto PDA plates containing 10 µg/ml geneticin (G418; Invitrogen Inc., Carlsbad, CA). Plates were incubated for 5-7 days, until colonies were visible. Hyphal tips from young colonies were transferred to new PDA+geneticin plates, followed by another hyphal tip sub-culturing onto the same medium. Transformants that grew after these two sub-culturings were screened visually for fluorescence using an epifluorescent Zeiss Axioscope microscope (West Germany), and the brightest transformants were retained. A single zoospore isolate was obtained for each transformant. Isolates were stored by transferring 5-10 small plugs (2-5 mm) from the growing edge of a young colony on V8 agar (with or without antibiotics) to 1 ml of sterile distilled water

in a 1.5 ml microcentrifuge tube (Schmitthenner and Bhat 1994). Stored isolates were kept in the dark at room temperature and re-stored approximately annually.

*Copy number of pSAMDBGFP, pSAMHAMGFP, and pTORtdTomato*. To confirm integration of pSAMDBGFP, pSAMHAMGFP, or pTORtdTomato into the *P. capsici* genome and to determine the copy number in each transformant, Southern blot analysis was performed following protocols from Sambrook and Russell (2001). Genomic DNA was isolated from the wild type isolate and transformants using the DNeasy kit from Qiagen (Valencia, CA), and 15 µg of genomic DNA were digested with *HindIII* or *BamHI* (for RFP transformants), or *EcoRI* or *XbaI*, (for GFP transformants). Digested DNA was separated on a 1% agarose gel, transferred to Hybond+ nylon membrane (GE Healthcare, Piscataway NJ), and fixed to the membrane by UV cross linking. The membrane was stored at room temperature prior to hybridization with probes labeled with DIG-dUTP using the Gene Images AlkPhos Direct Labeling kit and detected with CDP-*Star*, following the manufacturer's protocol (GE Healthcare). The probes were produced by PCR amplification using primers designed to amplify a 742 bp region of *tdTomato* (forward primer 5'-GCGAGGAGGTCATCAAAGAG and reverse primer 5'-TGATGACGGCCATGTTGTTG-3') or a 562 bp fragment of *pgfp* (forward primer 5'-GGAACTGGATGGTGATGTGAAC-3' and reverse primer 5'-CGTGGACAGGTAATGGTTGTC-3'). Results were visualized using Kodak Biomax Light film (Sigma-Aldrich).

*Production of* P. capsici *mycelia, sporangia, zoospores, and oospores for microscopic observation and virulence tests*. Zoospore suspensions were produced in order to determine the relative emitted fluorescence of each transformant and to inoculate pepper plants in the virulence assay described below. Mycelia, sporangia, zoospores, and oospores of transformants were

produced in order to document fluorescence in all life stages of the pathogen. All life stages were produced by culturing isolates on 15% V8 agar. Aerial mycelia were harvested by scraping them from the surface of cultures. For maximum sporangia production, plugs were cut from cultures that were less than two months old and transferred to V8 agar. These fresh cultures were incubated (unwrapped) under fluorescent light for approximately 15 hours per day in order to induce formation of sporangia. Zoospores were produced by scraping sporangia from 7-14 day old sporulating cultures, suspending them in 1 ml sterile distilled water in a microcentrifuge tube, and incubating them for approximately 30 minutes at room temperature. Oospores were produced following protocols used to determine mating type of *P. capsici* isolates (Dunn et al. 2010). Since all transformants were produced from an A1 mating type isolate, they were paired with isolate NY 06180-4, which is an A2 mating type single zoospore isolate of *P. capsici*. These plates were sealed with parafilm and incubated in the dark (for up to three months). Oospores were released from the agar by blending a 1 $cm^2$ piece of agar containing oospores with sterile distilled water in a microcentrifuge tube.

Fluorescence in all life stages was visualized as previously described (Hoch et al. 2005) using an Olympus BX61 microscope connected to a Confocal Laser Scanning Microscope (CLSM) system (Olympus Fluoview FV-300, Melville, NY). An Argon laser (488 nm excitation) or a Green Helium Neon laser (543 nm excitation) was used to excite the green or red fluorescent protein, respectively. Images were captured using the Fluoview system software.

*Measurement of emitted fluorescence from transformants.* In order to quantify fluorescence emitted from each transformant, sporangia were scraped from 7-14 day old cultures on V8 agar and zoospores were produced as described above. Zoospore suspensions were quantified using a hemacytometer (Fisher Scientific Inc., Pittsburgh, PA) and adjusted to a

concentration of $1x10^4$ or $1x10^6$ zoospores per ml for RFP or GFP transformants, respectively. Emitted fluorescence from 100 µl of each zoospore suspension was measured in a 96-well black microtiter plate (Fisher Scientific Inc.) with a fluorescence microplate reader (Synergy 2 from BioTek Instruments, Inc., Winooski, VT). A 485 nm (20 nm bandwidth) excitation filter and an 530 nm (25 nm bandwidth) emission filter were used for GFP transformants, and a 530 nm (25 nm bandwidth) excitation filter and a 590 nm (35 nm bandwidth) emission filter were used for RFP transformants. A suspension of zoospores from the wild type isolate NY 0664-1 was included as a control with both GFP and RFP transformants, and sterile distilled water was used to determine background fluorescence. The mean fluorescence emitted from wells containing sterile distilled water was subtracted from fluorescence measurements of all isolates prior to analysis. Four technical replicates were measured per isolate, and the entire experiment was repeated. In the second biological replicate of GFP transformants, a concentration of $1x10^5$ zoospores per ml was obtained for each isolate.

*Colony growth assay*. In order to determine whether growth rate was affected by transformation, a 7 mm agar plug of each transformant or the untransformed isolate NY 0664-1 was placed in the center of a 90-mm Petri plate containing V8 agar, with three replicate plates per isolate. After five days of growth at room temperature under lab lighting, two perpendicular measurements of colony diameter were made and averaged together. The entire experiment was repeated.

*Plant virulence assay*. Virulence of transformants was tested on bell pepper seedlings of the susceptible variety 'Red Knight' (Seminis Inc., St. Louis, MO). Plants were seeded into Cornell potting mix (composed of peat, perlite, and vermiculite in a 4:1:1 ratio) and germinated and grown in the greenhouse in 200 cell flats for 14-21 days before being transplanted into 4

inch pots prior to inoculation with transformants, the wild type isolate NY 0664-1, or sterile water as a control. Five 3-week old plants per isolate were inoculated with 2 ml of a zoospore suspension (1 x $10^4$ zoospores/ml) applied to the soil around the crown. As has been done previously with *P. capsici* (Ristaino 1991), disease severity was quantified by recording the proportion of symptomatic plants daily for 9 to 14 days following inoculation, rather than by rating individual plants. The experiment was repeated three times, and data were pooled for statistical analysis so that there were three replicates per treatment.

*Fruit virulence assay*. To test the virulence of transformants on detached pepper fruit (variety 'Declaration' Harris Moran, Modesto, CA), an assay was conducted using a protocol adapted from Foster and Hausbeck (2010) and Gevens et al. (2006). Prior to inoculation, pepper fruit were washed by soaking in a 10% bleach solution for 10 minutes, then double rinsed in distilled water and allowed to air dry. Room-temperature fruit were arranged in solid-bottom greenhouse trays and each fruit was wounded to a depth of approximately 0.5 cm with a sterile 1 mm dissecting needle. Three fruit were inoculated per transformant using 7 mm agar plugs cut with a sterile cork borer from the edge of two-day old cultures grown on V8 media under lab lighting (approximately 10 hours of fluorescent light per day). The untransformed isolate NY 0664-1 and sterile V8 agar plugs were used as positive and negative controls, respectively. A small amount of Vaseline was used to seal the cap of a 1.5 ml microcentrifuge tube over each plug, and two paper towels moistened with sterile distilled water were placed in each tray to maintain humidity. Trays were covered with clear plastic lids and incubated at room temperature under fluorescent lights (15 hours per day). After four days, two perpendicular diameter measurements were made of the resulting water-soaked lesion and these measurements were multiplied to estimate lesion area. The entire experiment was conducted twice. Sporangia and

hyphae were scraped from the surface of at least one lesion per transformant and checked with the CLSM to confirm that they were still fluorescent.

*Statistical analysis*. All data were analyzed using the R statistical software, the most recent version of which is available online from the R Foundation for Statistical Computing. For the plant virulence assay, daily incidence (percent of plants with symptoms) was used to calculate the area under the disease progress curve (AUDPC; (Madden et al. 2007), and because plants were not rated for the same number of days in all three replicates, these values were standardized by dividing by the total number of days over which incidence ratings were taken (Ristaino 1991). The resulting relative area under the disease progress curve (RAUDPC) values were used in further analyses. Since all data were continuous, significant differences among isolates in emitted fluorescence, lesion area, RAUDPC, and colony diameter were tested using an Analysis of Variance (ANOVA). In most cases data were normal, but if they were not, an equivalent non-parametric test (Kruskal-Wallis) was conducted to confirm the results of the ANOVA. When differences were significant ($P < 0.05$), means were then separated by a Tukey's Honestly Significant Differences test ($P = 0.05$) and standard errors were calculated using the R package 'agricolae' (published by the International Potato Center and available online through the Comprehensive R Archive Network).

***Results***

*Recovery of stable fluorescent transformants*. Subculturing of colonies from the primary transformation plate did not always yield stable transformants, which could have been due to a mixture of transformant and wild-type nuclei being present in these colonies. Therefore, it was important to make hyphal tip cultures followed by single zoospore cultures of putative transformant colonies onto new geneticin-amended medium, to ensure that stable transformants

were obtained. Using these procedures, seven brightly-fluorescing transformants (two GFP and five RFP) were obtained. However, after approximately one year of subculturing, two of these isolates (both containing three copies of *tdTomato*, based on Southern analysis, data not shown) had stopped fluorescing, even though they still grew on geneticin medium. The remaining five fluorescent transformants were used in all subsequent experiments. Transformants fluoresced either red (those transformed with pTORtdTomato) or green (those transformed with either pSAMHAMGFP or pSAMDBGFP) in all life stages (Fig. 5.1), although not all oospores from crosses between a fluorescent transformant and the non-fluorescent A2 isolate NY 06180-4 were fluorescent. In general, GFP transformants did not appear as bright as RFP transformants when viewed with the CLSM, and the wild type isolate did not fluoresce at all when viewed with the CLSM (data not shown).

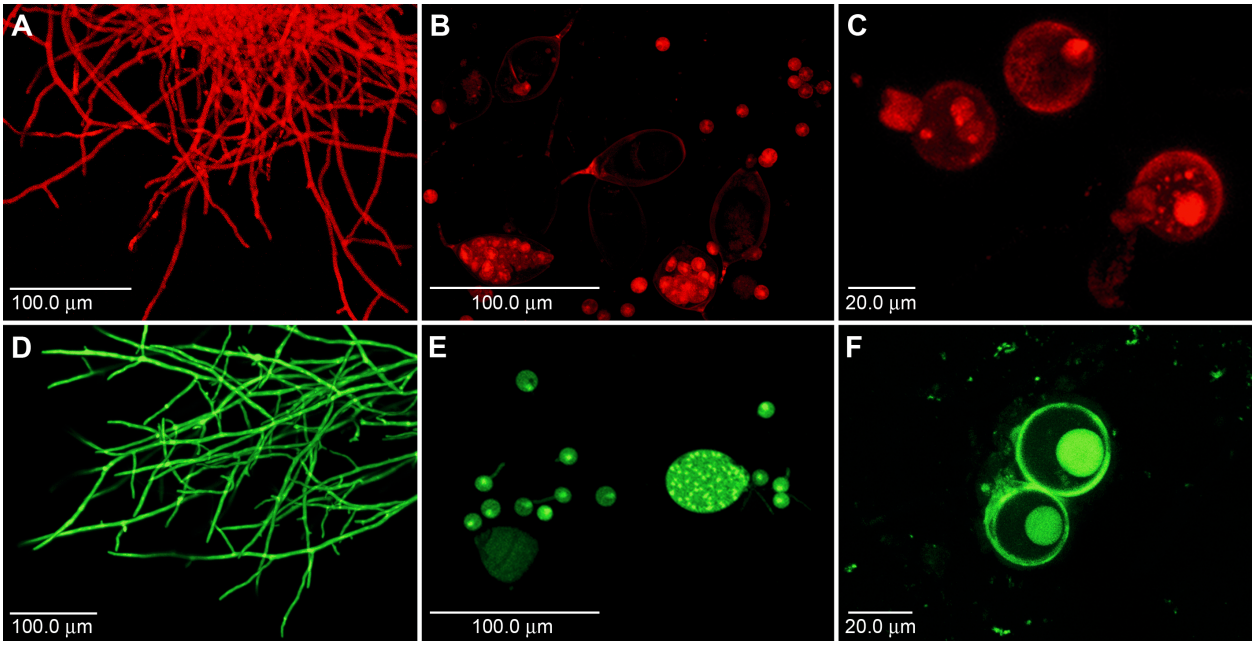



**Fig. 5.1** Mycelia (A and D), sporangia and zoospores (B and E), and oospores (C and F) of *Phytophthora capsici* transformants expressing a gene for red or green fluorescent protein

*Copy number and quantification of emitted fluorescence*. Results from Southern analyses indicated that transformants PcapR-c, PcapR-a, and PcapR-b have one, two, and five copies of *tdTomato*, respectively, while transformants PcapG-a and PcapG-b each contain two copies of *pgfp* (Table 5.1). The level of emitted fluorescence varied significantly among RFP transformants, with transformants PcapR-a and PcapR-b emitting significantly more fluorescence than either PcapR-c or the wild type isolate NY 0664-1 using the same excitation and detection filters (Table 5.1). Results were similar in both biological replicates. The two GFP transformants differed from each other and the wild type isolate (Table 5.1). The fluorescence emitted by each transformant was somewhat related to the number of copies of either *pgfp* or *tdTomato*. Transformants PcapR-a and PcapR-b (containing two and five copies of *tdTomato*, respectively) emitted significantly more fluorescence than did transformant PcapR-c, which only contained a single copy. However, PcapR-b did not emit significantly more fluorescence than did PcapR-a, even though it had more than twice as many copies of the gene. The two GFP transformants contained the same number of copies of *pgfp* (two), but transformant PcapG-b was significantly brighter, based on measurement of emitted fluorescence (Table 5.1).

**Table 5.1.** Fluorescence and copy number of *Phytophthora capsici* transformants. Plasmid used in transformation, copy number of gene for fluorescent protein, and mean emitted fluorescence of each tranformant compared to the untransformed wild type isolate (NY 0664-1).

| Isolate | Plasmid[b] | Fluorescent protein | Copy number[c] | Emitted fluorescence[a] | | |
|---|---|---|---|---|---|---|
| | | | | Mean | | SE[d] |
| PcapR-a | pTORtdTomato | RFP | 2 | 407 | a | 48.0 |
| PcapR-b | pTORtdTomato | RFP | 5 | 486 | a | 44.6 |
| PcapR-c | pTORtdTomato | RFP | 1 | 220 | b | 19.2 |
| NY 0664-1 (wt) | | na | 0 | 151 | b | 12.6 |
| | | | | | | |
| PcapG-a | pSAMHAMGFP | GFP | 2 | 1233 | b | 29 |
| PcapG-b | pSAMDBGFP | GFP | 2 | 1964 | a | 40 |
| NY 0664-1 (wt) | | na | 0 | 78 | c | 56 |

[a] Mean emitted fluorescence from four replicates detected after excitation at 485 nm or 530 nm, for isolates labeled with GFP or RFP, respectively. Measurements were made on suspensions of $1x10^4$ or $1x10^6$ zoospores per ml for RFP and GFP transformants, respectively, and wild type isolate zoospore suspensions at the same concentrations were included as controls. The mean fluorescence of wells containing only sterile distilled water was subtracted from each measurement before analysis was done. ANOVAs to test for significant differences among transformants and the wild type isolate were significant ($P < 0.001$) and means followed by the same letter are not significantly different at $P = 0.05$ using a Tukey's Honestly Significant Differences test. Results shown are from one biological replicate; results from a second replicate were similar.

[b] Plasmid vector used in transformation. The pTORtdTomato plasmid contains a gene (*tdTomato*) for a red fluorescent protein (RFP), and the pSAMHAMGFP and pSAMDBGFP plasmids contain a gene (*pgfp*) for a green fluorescent protein (GFP).

[c] Copy number as determined by Southern blot

[d] SE = standard error for each mean value. Relevant degrees of freedom for emitted fluorescence measured at 530 nm (RFP transformants) and 485 nm (GFP transformants) were 12 and 9, respectively.

*Colony growth and virulence on pepper plants and fruit*. In the *in vitro* colony growth assay, transformant PcapG-b was significantly reduced in colony growth compared to wild type isolate NY 0664-1 (Table 5.2). Transformant PcapR-c produced significantly larger colonies than the other RFP transformants (PcapR-a and PcapR-b), but did not differ significantly from the wild type control (Table 5.2).

**Table 5.2.** Growth and virulence of *Phytophthora capsici* transformants. Copy number of gene for fluorescent protein, *in vitro* growth, and virulence of transformants tagged with a red (RFP) or green (GFP) fluorescent protein. Isolate NY 0664-1 is the wild type isolate from which the transformed isolates PcapR-a, PcapR-b, PcapR-c, PcapG-a, and PcapG-b were generated.

| | | | Culture diameter (mm)[a] | | | RAUDPC on pepper plants[b] | | | Fruit lesion area ($mm^2$)[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Fluorescent protein | Copy number[d] | Mean | | SE[e] | Mean | | SE[e] | Mean | | SE[e] |
| NY 0664-1 (wt) | na | 0 | 82.5 | ab | 1.26 | 82.5 | a | 7.43 | 3589 | a | 255 |
| PcapR-a | RFP | 2 | 77.3 | b | 1.59 | 86.7 | a | 4.71 | 3232 | a | 277 |
| PcapR-b | RFP | 5 | 77.0 | b | 1.32 | 61.4 | a | 8.91 | 3062 | a | 303 |
| PcapR-c | RFP | 1 | 83.7 | a | 1.09 | 79.5 | a | 4.68 | 3475 | a | 188 |
| PcapG-a | GFP | 2 | 81.3 | ab | 0.73 | 75.6 | a | 4.09 | 3069 | a | 181 |
| PcapG-b | GFP | 2 | 51.2 | c | 1.64 | 29.4 | b | 2.27 | 1353 | b | 201 |
| water | na | | | | | 0 | c | 0 | 0 | c | |

[a] Mean colony diameter (mm) of three replicate plates per isolate (calculated from two perpendicular measurements per plate) on V8 agar after 5 days of growth; results of the second biological replicate were similar. ANOVA was significant at $P < 0.001$, and means followed by the same letter are not significantly different at $P = 0.05$ using a Tukey's Honestly Significant Differences test.

[b] Mean relative area under the disease progress curve (RAUDPC) calculated from incidence data. Incidence values were expressed as a percent of five pepper plants, and each treatment was replicated three times. ANOVA was significant at $P < 0.001$, and means followed by the same letter are not significantly different at $P = 0.05$ using a Tukey's Honestly Significant Differences test.

[c] Mean lesion area of three replicate pepper fruit per isolate (estimated by multiplying two perpendicular measurements of lesion diameter on each fruit). ANOVA was significant at $P < 0.001$, and means followed by the same letter are not significantly different at $P = 0.05$ using a Tukey's Honestly Significant Differences test. Results are shown from one biological replicate; results were similar in a second biological replicate.

[d] Copy number as determined by Southern blot

[e] SE = standard errors for each mean value. Relevant degrees of freedom for culture diameter, RAUDPC, and fruit lesion area were 12, 14, and 14, respectively.

In the pepper plant virulence assay, symptoms appeared in as few as three days after plants were inoculated, and included stem necrosis, girdling, wilting, and defoliation, followed by eventual plant death. Transformant PcapG-b was significantly less virulent than the other transformants or the wild type isolate NY 0664-1, based on RAUDPC. All other transformants did not differ significantly from the wild type isolate in their ability to cause disease on pepper

seedlings. No symptoms were observed on control seedlings treated with sterile water (Table 5.2).

Transformant PcapG-b was also significantly less virulent on detached pepper fruit, based on lesion area. All isolates produced water-soaked lesions and at least some sporulation within four days of inoculation. However, the mean area of the lesion produced by transformant PcapG-b was significantly smaller than the lesion areas produced by the other transformants or the wild type isolate (Table 5.2). Mean lesion areas produced by the other four transformants were not significantly different from the lesion area produced by the wild type isolate, and no lesion was produced on control fruit inoculated with sterile V8 agar. Observations with the CLSM confirmed that mycelia and sporangia collected from the inoculated pepper fruit were still fluorescent.

Preliminary observations of infected pepper stem and root tissue with the CLSM showed that pepper tissue autofluoresces under the same conditions used to excite RFP transformants (using a 543 nm HeNe laser), but autofluorescence of pepper tissue was not as bright as RFP *P. capsici* transformants. With the exception of xylem and stomates, pepper stem and root tissue did not autofluoresce when excited with the 488 nm Argon laser (used to excite GFP transformants). Therefore, using the CLSM, dual-channel images could be obtained showing pepper tissue colonized by GFP or RFP-tagged *P. capsici* transformants (Fig. 5.2). When plants were inoculated with a GFP transformant, channels were set to collect emitted light excited at 488 nm (pathogen) and 543 nm (host). When plants were inoculated with RFP isolates, channels were set to collect transmitted light (host) and emitted light excited at 543 nm (pathogen). Similar autofluorescence of host tissue has previously been used to facilitate observation of host-pathogen interactions in other crops (Lagopodi et al. 2002; Li et al. 2011).

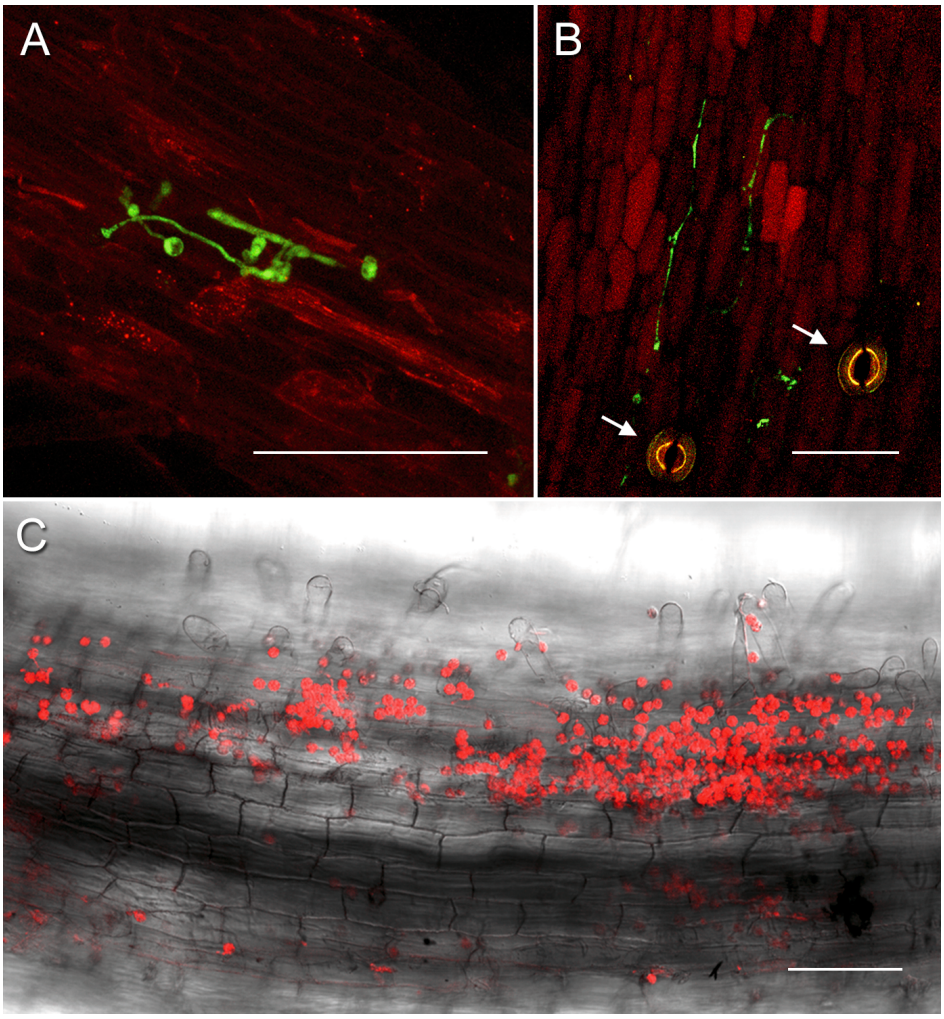



**Fig. 5.2** Fluorescent *Phytophthora capsici* transformants colonizing pepper plants. GFP transformant PcapG-a colonizing roots of susceptible pepper variety 'Red Knight' with plant cell walls autofluorescing red when excited at 543 nm (A). Pepper guard cells (indicated by arrows) appear yellow because they autofluoresce both red and green when excited at 543 nm and 488 nm, respectively (B). Zoospores of PcapR-a ensysted on root of 'Red Knight' pepper (C). Scale bars = 100 μm.

***Discussion***

Here we report the stable transformation of *P. capsici* using plasmid vectors containing *pgfp* or *tdTomato,* resulting in bright GFP or RFP fluorescence in all life stages of the pathogen. While transformants with more copies of the gene for the respective fluorescent protein tended to have higher emitted fluorescence, these differences were not always significant, and in the case

of the two GFP transformants, emitted fluorescence differed significantly between the two transformants, even though both had two copies of *pgfp*. Without additional transformants differing in copy number of *pgfp* or *tdTomato*, it is difficult to draw conclusions about the role of gene copy number in variation of fluorescent intensity among transformants. The location of the reporter gene within the genome of *P. capsici* could have an impact on gene expression and fluorescence intensity, as has been suggested previously (Judelson et al. 1993). These authors reported that GUS levels produced by transformed *P. infestans* varied both among promoters used to drive *GUS* expression, and also among transformants containing the same promoter. In addition, they did not find that copy number of *GUS* and GUS activity were directly related, but did find that the levels of GUS activity increased as quantity of *GUS* mRNA increased, as measured by Northern analysis. Based on these results, they hypothesized that the location of the reporter gene within the *P. infestans* genome could be impacting gene expression and GUS activity (Judelson et al. 1993). van West et al. (1999) also reported higher levels of *gfp* expression (as quantified by Northern analysis) in *P. palmivora* transformants corresponding to brighter fluorescence, but did not report how either the quantity of *gfp* mRNA or fluorescence intensity correlated with copy number of *gfp*.

Similar to what has been reported in other transformed oomycetes (Riedel et al. 2009; Sexton and Howlett 2001; Si-Ammour et al. 2003), one of the five stable *P. capsici* transformants described here was significantly reduced in virulence compared to the wild type isolate, as quantified by *in vitro* colony growth, lesion diameter on pepper fruit, and disease severity on pepper seedlings. Si-Ammour et al. (2003) noted that reduced *in vitro* growth of transformants was not correlated to the intensity of GFP fluorescence, and therefore hypothesized that the *gfp* gene itself was not responsible for the reduced virulence. They did not

report how either reduced virulence or intensity of GFP fluorescence was related to copy number of *gfp*. Keller et al. (1990) investigated whether transformation of *C. heterostrophus* reduced fitness (not virulence) and found that, while transformants were significantly less fit than the wild type isolate, neither the size of the plasmid used as a vector in transformation, nor the number of times the plasmid was inserted into the genome, nor the location of the insertion in the genome affected the fitness of transformants. While these factors did not affect fitness in *C. heterostrophus*, we cannot rule out the possibility that one or more of them could be affecting virulence in transformed *P. capsici*. Keller et al. also reported that when the transformation protocol was used on the wild type isolate, but without the addition of plasmid DNA, these "mock transformed" isolates were also less fit than the wild type isolate, suggesting that the transformation protocol alone has an impact on fitness. Similarly, variation in both virulence and morphology among *Rhizoctonia solani* isolates regenerated from protoplasts has also been reported, suggesting that the formation of, and regeneration from protoplasts can impact virulence (Yang et al., 1994). In *P. infestans*, both genetic variation (Abu-El Samen et al. 2003a) and variation in virulence (Abu-El Samen et al., 2003b) were observed among single zoospore isolates obtained from the same hyphal-tipped field isolate. The authors hypothesized that some combination of spontaneous mutation, gene conversion, mitotic crossing over, or extra-chromosomal elements could be responsible for this variation among asexual progeny (Abu-El Samen et al. 2003a). Thus, the reduction in virulence and growth of transformant PcapG-b could be explained by the fact that it was regenerated from a protoplast, or it could be that a less virulent, slow-growing variant happened to be selected when a single zoospore isolate was obtained following transformation. It should also be noted that in this study, transformant PcapG-b is the only one containing the pSAMDBGFP plasmid, so we cannot rule out the

possibility that this plasmid is affecting the virulence of this transformant. Additional studies would be needed to elucidate the cause of the observed reduction in colony growth and virulence of this transformant.

Although transformant PcapG-b had reduced virulence compared to the wild type, it was still able to cause disease on host plants and fruit; *in vitro* growth and symptom development were merely slower. Thus, this transformant, while still pathogenic, would be less-desirable for studies of *P. capsici* infection and colonization of plants. Interestingly, while transformant PcapG-b was reduced in both colony growth and virulence, transformant PcapR-c produced significantly larger colonies than the other RFP transformants, but differences in virulence on pepper plants or fruit were not significant. It is possible that more subtle differences exist among these transformants in virulence or growth, but have not been detected in these assays. It is also possible that *in vitro* growth and virulence on hosts are not necessarily related in *P. capsici*. These observations emphasize the fact that, while *in vitro* growth assays are useful in identifying transformants with similar characteristics to the wild type isolate, additional *in planta* assays should also be conducted. Multiple assays of virulence are likely necessary in order to identify transformants that most closely resemble the wild type isolate in their interactions with hosts.

In other pathosystems, the availability of isolates expressing fluorescent proteins has greatly facilitated microscopic investigation of host-pathogen interactions. For example, Vallad and Subbarao (2008) used a GFP transformed isolate of *Verticillium dahliae* to compare colonization of susceptible and resistant lettuce cultivars. Also, Njoroge et al. (2011) employed a long-spored isolate of *V. dahliae* tagged with GFP to investigate why broccoli (*Brassica oleracea* var. *italic* subvar. *cyamosa*) is not susceptible to this pathogen, while cauliflower (*B. oleracea* var. *botrytis* subvar. *cauliflora*) is. The stable *P. capsici* transformants described here -

tagged with GFP or RFP and not distinguishable from the wild type isolate in growth or their ability to cause disease - will facilitate similar studies in this pathogen. Ultimately, this information will contribute not only to an improved understanding of interactions between *P. capsici* and its hosts, but also to improved disease management.

***Acknowledgements:***

This project was supported by the Agriculture and Food Research Initiative Competitive Grant No. 2012-67011-19690 from the USDA National Institute of Food and Agriculture, and by the New York State Department of Agriculture and Markets through a NY Specialty Crops Block Grant. Support for A. R. Dunn was also provided by a fellowship from Cornell University College of Agriculture and Life Sciences. The authors thank H. C. Hoch for assistance with the confocal laser scanning microscope; H. Nihart for technical assistance; and the Provost of Hobart and William Smith Colleges for support of H. Nihart.

***References***

Abu-El Samen F. M., Secor G. A., Gudmestad N. C. 2003a. Genetic variation among asexual progeny of *Phytophthora infestans* detected with RAPD and AFLP markers. Plant Pathol. 52:314-325.

Abu-El Samen F. M., Secor G. A., Gudmestad N. C. 2003b. Variability in virulence among asexual progenies of *Phytophthora infestans*. Phytopathology 93:293-304.

Ah-Fong, A. M. V., and Judelson, H. S. 2011. Vectors for fluorescent protein tagging in *Phytophthora*: tools for functional genomics and cell biology. Fungal Biol-Uk. 115:882–890.

Bailey, A. M., Mena, G. L., and Herrera-Estrella, L. 1991. Genetic transformation of the plant pathogens *Phytophthora capsici* and *Phytophthora parasitica*. Nucleic Acids Res. 19:4273–4278.

Bernhardt, E. A., and Grogan, R. G. 1982. Effect of soil matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica*, *Phytophthora capsici*, and *Phytophthora cryptogea*. Phytopathology. 72:507–511.

Café-Filho, A. C., and Duniway, J. M. 1995. Dispersal of *Phytophthora capsici* and *P. parasitica* in furrow-irrigated rows of bell pepper, tomato and squash. Plant Pathol. 44:1025–1032.

Chen, N., Hsiang, T., and Goodwin, P. H. 2003. Use of green fluorescent protein to quantify the growth of *Colletotrichum* during infection of tobacco. J. Microbiol. Methods. 53:113–122.
Chiu, W., Niwa, Y., Zeng, W., Hirano, T., Kobayashi, H., and Sheen, J. 1996. Engineered GFP as a vital reporter in plants. Curr. Biol. 6:325–330.
Crossan, D. F., Haasis, F. A., and Ellis, D. E. 1954. Phytophthora blight of summer squash. Plant Dis. Rep. 38:557–559.
Cubitt, A. B., Heim, R., Adams, S. R., Boyd, A. E., Gross, L. A., and Tsien, R. Y. 1995. Understanding, improving and using green fluorescent proteins. Trends Biochem. Sci. 20:448–455.
Czymmek, K. J., Bourett, T. M., and Howard, R. J. 2004. Fluorescent protein probes in fungi. Method. Microbiol. 34:27–62.
Davidson, C. R., Carroll, R. B., Evan, T. A., and Mulrooney, R. P. 2002. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatis*) in the mid-Atlantic region. Plant Dis. 86:1049.
Dumas, B., Centis, S., Sarrazin, N., and Esquerre-Tugaye, M. T. 1999. Use of green fluorescent protein to detect expression of an endopolygalacturonase gene of *Colletotrichum lindemuthianum* during bean infection. Appl. Environ. Microbiol. 65:1769–1771.
Dunn, A. R., Milgroom, M. G., Meitz, J. C., McLeod, A., Fry, W. E., McGrath, M. T., Dillard, H. R., and Smart, C. D. 2010. Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York State. Plant Dis. 94:1461–1468.
Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora diseases worldwide*. St. Paul, Minnesota: The American Phytopathological Society.
Foster, J. M., and Hausbeck, M. K. 2010. Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24–30.
French-Monar, R. D., Jones, J. B., and Roberts, P. D. 2006. Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. Plant Dis. 90:345–350.
Gevens, A. J., Ando, K., Lamour, K. H., Grumet, R., and Hausbeck, M. K. 2006. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. Plant Dis. 90:1276–1282.
Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. Plant Dis. 92:201–209.
Granke, L. L., Windstam, S. T., Hoch, H. C., Smart, C. D., and Hausbeck, M. K. 2009. Dispersal and movement mechanisms of *Phytophthora capsici* sporangia. Phytopathology. 99:1258–1264.
Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Dis. 88:1292–1303.
Heim, R., Cubitt, A. B., and Tsien, R. Y. 1995. Improved green fluorescence. Nature. 373:663–664.
Hoch, H., Galvani, C., Szarowski, D., and Turner, J. 2005. Two new fluorescent dyes applicable for visualization of fungal cell walls. Mycologia. 97:580–588.
Judelson, H. S., Dudler, R., Pieterse, C. M. J., Unkles, S. E., and Michelmore, R. W. 1993. Expression and antisense inhibition of transgenes in *Phytophthora infestans* is modulated by choice of promoter and position effects. Gene. 133:63–69.
Judelson, H., Tyler, B., and Michelmore, R. 1991. Transformation of the oomycete pathogen, *Phytophthora infestans*. Mol. Plant-Microbe Interact. 4:602–607.

Keller, N. P., Bergstrom, G. C., and Yoder, O. C. 1990. Effects of genetic transformation on fitness of *Cochliobolus heterostrophus*. Phytopathology. 80:1166–1173.

Lagopodi, A., Ram, A., Lamers, G., Punt, P., Van den Hondel, C., Lugtenberg, B., and Bloemberg, G. V. 2002. Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Mol. Plant-Microbe Interact. 15:172–179.

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

Larrainzar, E., O'Gara, F., and Morrissey, J. P. 2005. Applications of autofluorecent proteins for in situ studies in microbial ecology. Annu. Rev. Microbiol. 59:257–277.

Li, C., Chen, S., Zuo, C., Sun, Q., Ye, Q., Yi, G., and Huang, B. 2011. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp *cubense* race 4. Eur. J. Plant Pathol. 131:327–340.

Lorang, J. M., Tuori, R. P., Martinez, J. P., Sawyer, T. L., Redman, R. S., Rollans, J. A., Wolpert, T. J., Johnson, K. B., Rodriguez, R. J., Dickman, M. B., and Ciufetti, L. M. 2001. Green fluorescent protein is lighting up fungal biology. Appl. Environ. Microbiol. 67:1987–1994.

Madden, L. V., Hughes, G., and van den Bosch, F. 2007. The study of plant disease epidemics. The American Phytopathological Society, St. Paul, MN.

Maor, R., Puyesky, M., Horwitz, B., and Sharon, A. 1998. Use of green fluorescent protein (GFP) for studying development and fungal-plant interaction in *Cochliobolus heterostrophus*. Mycol. Res. 102:491–496.

Mauch, F., Torche, S., Schläppi, K., Branciard, L., Belhaj, K., Parisy, V., and Si-Ammour, A. 2009. *Phytophthora brassicae* as a pathogen of arabidopsis. In Oomycete genetics and genomics: diversity, interactions, and research tools, eds. Lamour, K. H. and Kamoun, S. Wiley-Blackwell, Hoboken, NJ. p. 574.

McLeod, A., Fry, B. A., Zuluaga, A. P., Myers, K. L., and Fry, W. E. 2008. Toward improvements of oomycete transformation protocols. J. Eukaryot Microbiol. 55:103–109.

Nahalkova, J. 2003. Red fluorescent protein (DsRed2) as a novel reporter in *Fusarium oxysporum* f. sp. *lycopersici*. FEMS Microbiol. Lett. 225:305–309.

Njoroge, S. M. C., Vallad, G. E., Park, S.Y., Kang, S., Koike, S. T., Bolda, M., Burman, P., Polonik, W., and Subbarao, K. V. 2011. Phenological and phytochemical changes correlate with differential interactions of *Verticillium dahliae* with broccoli and cauliflower. Phytopathology. 101:523–534.

Pang, S. Z., DeBoer, D. L., Wan, Y., Ye, G., Layton, J. G., Neher, M. K., Armstrong, C. L., Fry, J. E., Hinchee, M. A., and Fromm, M. E. 1996. An improved green fluorescent protein gene as a vital marker in plants. Plant Physiol. 112:893–900.

Prasher, D. C., Eckenrode, V. K., Ward, W. W., Prendergast, F. G., and Cormier, M. J. 1992. Primary structure of the *Aequorea victoria* green-fluorescent protein. Gene. 111:229–233.

Quesada-Ocampo, L. M., Fulbright, D. W., and Hausbeck, M. K. 2009. Susceptibility of Fraser Fir to *Phytophthora capsici*. Plant Dis. 93:135–141.

Riedel, M., Calmin, G., Belbahri, L., Lefort, F., Götz, M., Wagner, S., and Werres, S. 2009. Green fluorescent protein (GFP) as a reporter gene for the plant pathogenic oomycete *Phytophthora ramorum*. Journal. Eukaryot. Microbiol. 56:130–135.

Ristaino, J. B. 1991. Influence of rainfall, drip irrigation, and inoculum density on the development of Phytophthora root and crown rot epidemics and yield in bell pepper. Phytopathology. 81:922–929.
Ristaino, J. B., and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. Plant Dis. 83:1080–1089.
Sambrook, J., and Russell, D. 2001. Molecular cloning: A laboratory manual. Third Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schmitthenner, A. F., and Bhat, R. G. 1994. Useful methods for studying *Phytophthora* in the laboratory. Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.
Schornack, S., van Damme, M., Bozkurt, T. O., Cano, L., Smoker, M., Thines, M., Gaulin, E., Kamoun, S., and Huitema, E. 2010. Ancient class of translocated oomycete effectors targets the host nucleus. P. Natl. Acad. Sci. 107:17421–17426.
Sexton, A., and Howlett, J. 2001. Green fluorescent protein as a reporter in the *Brassica-Leptosphaeria maculans* interaction. Physiol. Mol. Plant Pathol. 58:13–21.
Shaner, N. C., Campbell, R. E., Steinbach, P. A., Ben N G Giepmans, Palmer, A. E., and Tsien, R. Y. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. Nat. Biotechnol. 22:1567–1572.
Si-Ammour, A., Mauch-Mani, B., and Mauch, F. 2003. Quantification of induced resistance against *Phytophthora* species expressing GFP as a vital marker: beta-aminobutyric acid but not BTH protects potato and *Arabidopsis* from infection. Mol. Plant Pathol. 4:237–248.
Takemoto, D., Jones, D. A., and Hardham, A. R. 2003. GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of *Arabidopsis* by oomycete pathogens. Plant J. 33:775–792.
Vallad, G. E., and Subbarao, K. V. 2008. Colonization of resistant and susceptible lettuce cultivars by a green fluorescent protein-tagged isolate of *Verticillium dahliae*. Phytopathology. 98:871–885.
van West, P., Reid, B., Campbell, T. A., Sandrock, R. W., Fry, W. E., Kamoun, S., and Gow, N. A. R. 1999. Green fluorescent protein (GFP) as a reporter gene for the plant pathogenic oomycete *Phytophthora palmivora*. FEMS Microbiol. Lett. 178:71–80.
Visser, M., Gordon, T., Wingfield, B., Wingfield, M., and Viljoen, A. 2004. Transformation of *Fusarium oxysporum* f. sp *cubense*, causal agent of Fusarium wilt of banana, with the green fluorescent protein (GFP) gene. Australas. Plant Pathol. 33:69–75.

CHAPTER 6

# Interactions of *Phytophthora capsici* with resistant and susceptible pepper cultivars[1]


## *Abstract*

Host resistance is a key management strategy for root and crown rot caused by *Phytophthora capsici* in pepper. However, the cellular mechanisms underlying this resistance are not clearly understood. An isolate of *P. capsici* constitutively expressing a gene for green fluorescent protein was used to investigate pathogen interactions with roots and crowns of a susceptible (Red Knight) and two resistant (Paladin and CM-334) pepper cultivars. Zoospores attached to and germinated on roots of all three cultivars equally well at 30 and 120 minutes post inoculation (pi), respectively. At 3 days pi, significantly more secondary roots had lesions on Red Knight plants compared to Paladin and CM-334 plants, although hyphae had colonized tips of at least some secondary roots of all cultivars. By 4 days pi, necrotic lesions had formed on the primary root of Red Knight, but not Paladin and CM-334 plants. Although hyphae were visible in the crown tissue of Red Knight plants by 3 days pi, no hyphae were observed in crowns of Paladin or CM-334 plants, even after 10 days pi. Expression of four defense genes in stems and leaves of inoculated Red Knight, Paladin, and CM-334 plants at 8 and 10 hours pi was quantified, but no differences were observed among cultivars at either time point.


## *Introduction*

Various sources of resistance to the oomycete pathogen *Phytophthora capsici* have been identified in pepper (*Capsicum annuum*; e.g. (Guerrero-Moreno and Laborde 1980; Kimble and

[1] Dunn, A. R. and Smart, C. D. Interactions of *Phytophthora capsici* with resistant and susceptible pepper cultivars. Phytopathology. *Submitted*.

Grogan 1960; Palloix et al. 1990; Saini and Sharma 1978). The genetic basis of this resistance appears to be complex, involving multiple genes (Monroy-Barbosa and Bosland 2008; Ortega, et al. 1991; Reifschneider et al. 1992), with different genes controlling the various symptoms caused by the pathogen (Sy et al. 2005; Walker and Bosland 1999). Furthermore, virulence on individual pepper genotypes can vary greatly among different isolates of *P. capsici* (Foster and Hausbeck 2010; Polach and Webster 1972). Criollos de Morelos line 334 (CM-334; also sometimes referred to as Serrano Criollos de Morelos) is an inbred pepper line derived from a wild pepper collected in Mexico (Guerrero-Moreno and Laborde 1980). It is highly resistant to root and crown rot caused by diverse isolates of *P. capsici* and is frequently used in pepper breeding programs, or as a resistant control (Foster and Hausbeck 2010; Glosier et al. 2008; Guerrero-Moreno and Laborde 1980; Palloix et al. 1990). In addition, CM-334 is resistant to some potyviruses (Dogimont et al. 1996) and some nematode species (Djian-Caporalino et al. 1999). The commercially-available bell pepper cultivar Paladin also has high levels of resistance to many isolates of *P. capsici*, and CM-334 is likely part of its genetic background (Babadoost 2006; Dunn et al. 2014; Foster and Hausbeck 2010).

In other oomycetes, the relationship between early host-pathogen interactions and host resistance varies. Mitchell and Deacon (1986) showed that zoospores of two *Pythium* sp. pathogenic on grasses were less likely to encyst on wild non-grass species than on wild grass species, while zoospores of two generalist *Pythium* sp. encysted at similar frequencies on wild grass or non-grass species. However, this reduced encystment rate of grass-specific *Pythium* sp. was not observed on cultivated dicotyledonous plants (including tomato). Enkerli et al. (1997) investigated interactions of two *Phytophthora sojae* isolates with a resistant and a susceptible soybean cultivar, reporting no difference in zoospore encystment or germination on soybean

roots between compatible and incompatible interactions. They did, however, observe differences at later stages of the infection.

Kim and Kim (2009) observed roots of susceptible (cultivar Bugang) and resistant (CM-334) pepper plants 1 and 3 days post inoculation with *P. capsici*. They found that pathogen hyphae were still limited to the epidermal or cortical root tissue of CM-334 plants after 3 days, while hyphae had reached the vascular tissue of Bugang by this time. Furthermore, thickening of the middle lamellae between cells, and cell wall appositions where hyphae contacted cells appeared to block further hyphal growth in CM-334 plants. Lee et al. (2000) observed similar defense mechanisms in stem tissue of the susceptible pepper cultivar Hanbyul when resistance to *P. capsici* was chemically induced, or when plants were inoculated with a non-virulent isolate of *P. capsici*. Hwang et al. (1989) also reported the formation of cell wall appositions where hyphae contacted root cells (susceptible cultivar Hanbyul), but this did not halt infection, and hyphae were observed growing in xylem vessels 2 days post inoculation.

Not surprisingly, many studies have demonstrated the upregulation of pepper defense genes during incompatible pepper-*P. capsici* interactions (i.e. resistant pepper cultivars, or avirulent *P. capsici* isolates), including peroxidase, β-1,3-glucanase, sesquiterpene cyclase, PR-1, and 5-*epi*-aristolochene synthase (Fernández-Herrera et al. 2012; Silvar, Merino, and Diaz 2008; Wang et al. 2013). Understanding both the physiological interactions between *P. capsici* and pepper and the genetic responses of the plant will yield a more complete understanding of both compatible and incompatible interactions in this pathosystem. Ultimately, this could contribute to the development of new tolerant pepper cultivars, and possibly suggest improvement to disease management practices, such as critical plant growth stages for applying protectant fungicides, or cultural practices to limit contact between the pathogen and particularly

susceptible host tissues. The availability of a *P. capsici* isolate constitutively expressing a gene for green fluorescent protein facilitates investigation of pathogen attachment, germination, and colonization of susceptible and resistant peppers, without the need for staining or lengthy preparation of tissue sections (Dunn et al. 2013). The specific objectives of this study were to determine whether (i) *P. capsici* zoospores attach and germinate at different rates on the roots of the susceptible pepper cultivar Red Knight compared to the resistant cultivars Paladin or CM-334, (ii) *P. capsici* colonization of roots and crowns differs among Red Knight, Paladin and CM-334 plants three and four days after inoculation, and (iii) pepper defense genes are induced earlier or to a greater extent in Paladin and CM-334 plants compared to Red Knight plants, following inoculation.

***Materials and Methods***

*Plant material and culture*. The inbred pepper line CM-334, and the commercially-available bell pepper cultivars Paladin (Syngenta, Greensboro, NC) and Red Knight (Seminis, St. Louis, MO) were used in all experiments. All plants were grown from untreated seed in Cornell potting mix (peat, perlite, and vermiculite in a 4:1:1 ratio) in 50-cell flats. Seeds were germinated in the greenhouse under natural and artificial light, and heat mats were used to improve germination. Five-week-old plants were used in all experiments.

*Attachment and germination of* P. capsici *zoospores on pepper roots*. Zoospore inoculum was prepared as previously described from 7-day-old cultures of a *P. capsici* isolate (PcapG-a) that stably expresses a gene for green fluorescent protein in all life stages (Dunn et al. 2013). Potting mix was gently removed from the roots of a pepper plant, and the roots were incubated at room temperature in 40 ml of a $1x10^4$ zoospore per ml solution in a 50-ml Falcon tube (Corning,

Corning, NY) for 30 min. At the end of this time, roots were removed and dipped three times in sterile distilled water to wash off unattached zoospores. Three 1-cm pieces were cut from the tips of secondary or tertiary roots of the plant, and images were collected using a confocal laser scanning microscope (CLSM) system, as previously described (Hoch et al. 2005). Four z-series images (each of approximately 800 μm-long sections of root) were collected along each 1-cm root piece, using the 20x objective of the microscope and a green helium neon laser (543 nm) to excite autofluorescence of the pepper root, and an argon laser (488 nm) to excite the enhanced green fluorescent protein in the *P. capsici* isolate. Images were collected and assembled using the Fluoview software distributed with the CLSM system by compiling images taken at 2 μm increments over a depth of 14 μm, which included the root surface and the area just above the surface. All root pieces were incubated at 4°C until images could be collected (within 1 hour after they were cut from the plant) in order to slow zoospore germination and hyphal growth. The number of zoospores attached to the pepper root was summed among all four images collected from each 1-cm root piece. Three 1-cm root pieces were examined from each plant, and three plants were examined per cultivar, for a total of nine 1-cm root pieces per cultivar. The entire experiment was repeated.

In order to quantify zoospore germination 120 min post inoculation (pi), a separate set of plants was incubated for 30 min in 40 ml of a $1x10^4$ zoospore per ml solution, as described above, but after removal of unattached zoospores, the plants were incubated for an additional 90 min at room temperature in a sterile petri dish containing filter paper moistened with sterile distilled water. Three 1-cm root tip pieces were cut from each plant and four images per root piece were collected, as described above, except that a thicker z-series (26 μm deep, with images taken at 2 μm increments) was collected in order to accommodate the hyphal growth that had

occurred. Again, root pieces were incubated at 4°C until images could be collected (within 1 hour after they were cut from the plant) in order to prevent further germination of zoospores. Three 1-cm root pieces were examined from each plant, and three plants were examined per cultivar, for a total of nine root pieces per cultivar. The entire experiment was repeated.

*Colonization of pepper roots and crown by* P. capsici *3 days post inoculation*. Paladin, Red Knight, and CM-334 seedlings were grown in 50-cell flats, as described above. When seedlings were 5 weeks old, 10 plants per cultivar (only eight plants of CM-334 in the first biological rep, due to limited seed availability and germination) were inoculated with *P. capsici* isolate PcapG-a. Prior to inoculation, seedlings were watered so that the potting mix was near saturation, and the potting mix around the stem of each plant was then drenched with 3 ml of a suspension of $1x10^5$ zoospores per milliliter (Dunn et al. 2013). Plants were incubated in a greenhouse with natural and artificial light (16 hours of light/8 hours of dark) following inoculation. Nighttime air temperatures in the greenhouse were relatively constant across both biological replicates (lows between 14.4 and 15.6 °C), but daytime air temperatures were more variable (highs between 22.2 and 27.8 °C and between 26.7 and 42.2 °C in the first and second biological replicates, respectively).

After 3 days, the potting mix was gently washed from the roots of half of the plants (four or five plants per cultivar), and secondary roots attached to the primary root within 5 mm of the soil line were examined on each plant. Preliminary studies indicated that these roots were most likely to be colonized by *P. capsici*, using this inoculation protocol. The total number of secondary roots attached to the primary root in this region, and the number of these roots with brown lesions were counted. In addition, roots with lesions were examined for pathogen hyphae, using a mercury arc lamp and an FITC filter (on the same microscope used with the CLSM

system) to visualize the GFP-tagged pathogen. The CLSM was then used to collect representative images of colonized roots (over a 39 μm z-series, with images collected at 3 μm increments). Thin longitudinal sections of the crown tissue of each plant were made by hand (2-5 mm in length) and the mercury arc lamp and FITC filter, as well as the CLSM were used to visualize hyphae of *P. capsici* in the crown tissue. Images were collected as for the colonized root tips. The remaining seedlings (four or five plants per cultivar) were kept in the greenhouse and observed for symptoms of *P. capsici* infection (wilting or death). Ten days post inoculation, potting mix was gently washed from the roots of these seedlings, root systems were observed, and crown tissue was checked for hyphal growth. Seedlings inoculated with sterile distilled water were examined as a control, and the entire experiment was repeated (this time, with 10 plants per cultivar).

*Colonization of pepper roots and crown by* P. capsici *4 days post inoculation*. A new set of 8 (CM-334) or 10 (Paladin and Red Knight) seedlings per cultivar were grown and inoculated as described for the 3 day pi time point, and examined 4 days pi. Again, nighttime air temperatures in the greenhouse were relatively constant across both biological replicates (lows between 14.4 and 16.1 °C), but daytime air temperatures were more variable (highs between 23.9 and 36.7 °C and between 26.7 and 42.2 °C in the first and second biological replicates, respectively). After washing potting mix from roots, the length of the brown lesion on the primary root of four (CM-334) or five (Paladin and Red Knight) plants was measured. In addition, cross-sections of crown tissue were collected and examined using a dissecting microscope (to visualize discoloration of tissue) and the CLSM (to visualize hyphae). The remaining four or five seedlings per cultivar were observed in the greenhouse for 10 days pi, at which point roots and crown tissue were examined, as described for the 3 day pi time point.

Seedlings inoculated with sterile distilled water were examined as a control, and the entire experiment was repeated (this time, with 10 plants per cultivar).

*Quantification of pepper defense gene expression*. Quantification of pepper defense genes *β*-1,3-glucanase (*CABGLU*), peroxidase (*CAPO*1), PR-1 (*CABPR*1), and sesquiterpene cyclase (*CASC*1) was conducted as described by Silvar, et al. (2008) with minor modifications. Expression of defense genes was normalized to expression of genes for ubiquitin-conjugating protein (*CaUbi*3) and eukaryotic initiation factor 5A2 (*CaEif*5*a*2), using primers described by Wan et al. (2011), with amplification protocols from Silvar et al. These genes were selected as reference genes because preliminary studies indicated that both their primer efficiencies and expression levels were similar across uninoculated and inoculated Paladin, Red Knight, and CM-334 plants, under the same amplification conditions used for the defense genes (data not shown).

Plants were grown and inoculated as described for the root colonization assay above (with air temperatures ranging from approximately 15 °C at night to 32 °C during the day), but each plant was inoculated with 1 ml of a $1x10^5$ zoospores per ml suspension of isolate PcapG-a or 1 ml sterile distilled water, as a control. Stem and leaf tissue collected at 8 and 24 h pi was placed in microcentrifuge tubes, flash frozen in liquid nitrogen, and stored at -80° C for approximately nine or 12 months until RNA was extracted from stem and leaf tissue, respectively. RNA was extracted from 25-68 or 15-67 mg of stem or leaf tissue, respectively using a Quick-RNA MiniPrep kit with on-column DNase treatment (Zymo Research Corporation, Irvine, CA). In the first step, tissue in lysis buffer was disrupted with 2 sterile 5 mm stainless steel grinding beads (Qiagen, Valencia, CA) in a TissueLyser (Retsch, Newtown, PA) at 30 Hz for 4-6 min. Extraction was followed by an additional DNase treatment using the DNA-free RNA Kit (Zymo Research). Quantity and quality (260/280 ratio) of DNase-treated RNA was

determined with a NanoDrop ND 1000 (NanoDrop Technologies, Wilmington, DE), prior to reverse-transcription of 1000 ng RNA per sample as described by Silvar et al. (2008). To confirm that no genomic DNA was present in RNA samples prior to reverse transcription, all products of reverse transcription reactions (with and without reverse transcriptase) were used as template in a traditional PCR using the actin primers (Silvar et al. 2008) and the following reagents: 1X buffer (New England BioLabs, Ipswich, MA; 1.5 mM $MgCl_2$), 0.2 mM dNTPs (Invitrogen, Carlsbad, CA), 0.2 µM each primer (Integrated DNA Technologies, Coralville, IA), 0.5 units *Taq* polymerase (New England BioLabs), and approximately 800 ng template cDNA in a total reaction volume of 25 µl.

Quantitative PCR (qPCR) was performed on a C1000 Touch Thermal Cycler connected to a CFX96 Real-Time System (both from BioRad, Hercules, CA), in a 20 µl reaction volume, as recommended by the manufacturer, and with 60 ng cDNA as template. Each reaction was run in duplicate. Amplification efficiency ($E = 10^{-1/slope}$) was calculated for each primer pair from standard curves using the software supplied with the qPCR machine, and were averaged over two replicate plates for each primer pair. The template for standard curve reactions was five, 10-fold serial dilutions (starting at 80 ng) of DNA extracted from each of the three pepper cultivars with a Qiagen DNeasy Plant Mini kit. Each standard curve reaction was run in triplicate. Because not all samples could be run with all primers on a single plate, 60 ng of cDNA each from uninoculated Paladin, Red Knight, and CM-334 plants were run in duplicate on each plate with the *CaUbi*3 primers as inter-run calibrators (IRCs; Hellemans et al. 2007). Using amplification efficiency values calculated from the standard curves, the IRCs, and the Gene Study functionality of the CFX Manager software (BioRad), expression levels of each target gene were quantified using the ΔΔ Cq method.

*Statistical analysis*. Data on zoospore attachment and germination, as well as root colonization were analyzed using the R statistical software (R Development Core Team 2013). Because results were similar between the two repetitions of the experiment, data from the two replicates were analyzed together, and means reported are across both experiments. A Shapiro-Wilk test and a Bartlett test were used to test assumptions of normality and homogeneity of variance, respectively. An analysis of variance (ANOVA) was conducted to test for significant differences among cultivars in number of zoospores attached per 1 cm section of root at 30 min pi, percent of attached zoospores that had germinated 120 min pi, percent of secondary roots attached within 0.5 cm of the soil line with lesions 3 days pi, and length of lesion on primary root 4 days pi. If data were not normal, then a nonparametric Kruskal-Wallis test was also conducted to confirm the results of the ANOVA. If the ANOVA was significant at $P < 0.05$, then means were separated by a Tukey's Honestly Significant Differences test using the 'agricolae' package (de Mendiburu 2012).

Gene expression data from stem and leaf tissue were analyzed separately. Normalized expression levels obtained from the CFX Manager software were used to calculate fold increase in expression in each inoculated plant relative to the mean expression in uninoculated plants of the same cultivar, at each time point. Differences in fold increase of each defense gene were then compared among cultivars at each time point using an ANOVA followed by a Tukey's Honestly Significant Differences test, as described above.

## *Results*

*Attachment and germination of* P. capsici *zoospores on pepper roots*. Thirty minutes pi, the mean number of *P. capsici* zoospores attached per root section was 60, 59, and 51 for Red

Knight, Paladin, and CM-334 plants, respectively, with no significant differences among cultivars at $P = 0.05$ (Fig. 6.1 A-C). Interestingly, at this time point, some of the zoospores had already germinated. By 120 min pi, nearly all of the attached zoospores had germinated on all pepper roots examined (98.6%, 99.1%, and 98.6% on Red Knight, Paladin, and CM-334 roots, respectively), and there were no significant differences among cultivars at $P = 0.05$.

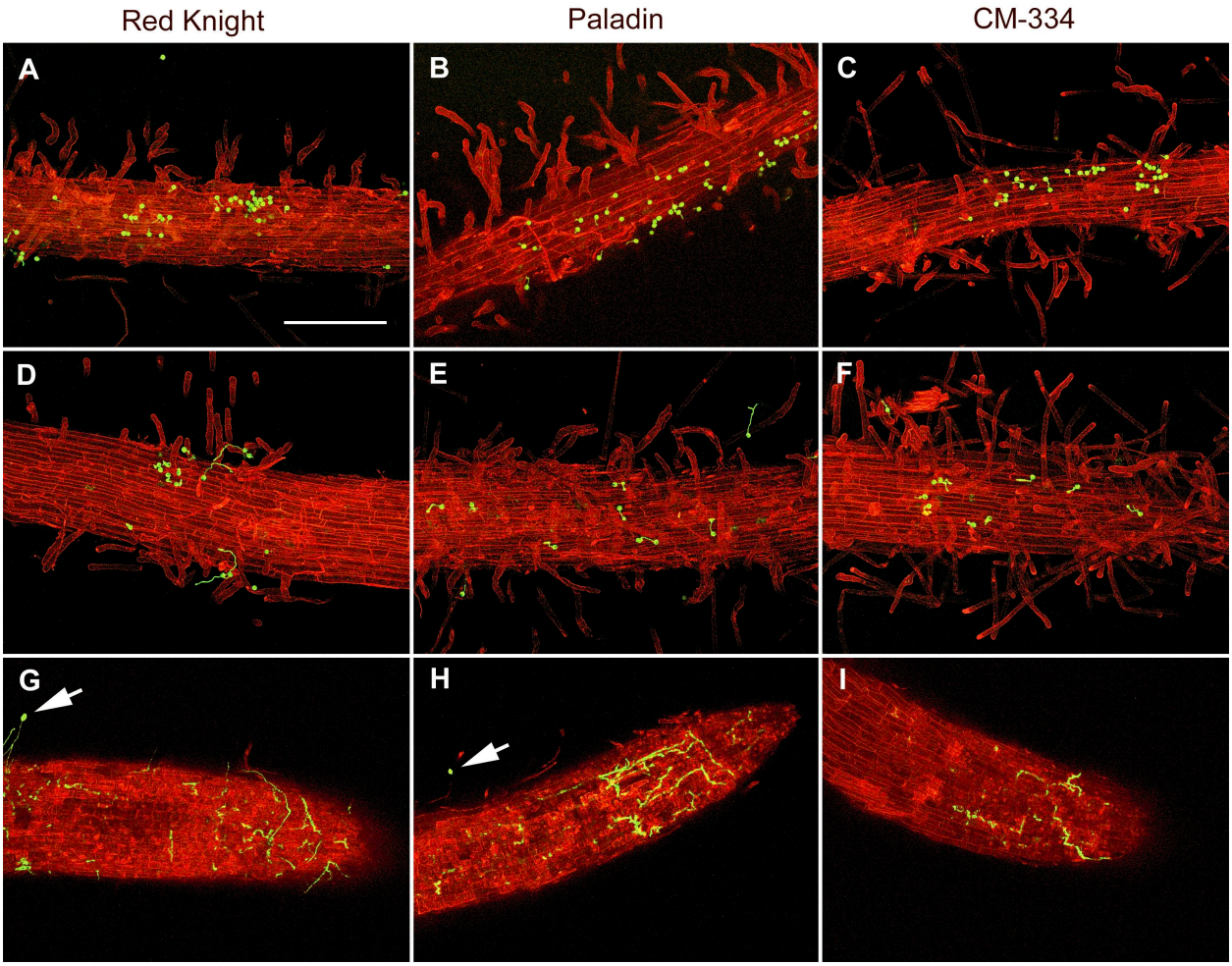



**Figure 6.1** Zoospore attachment (A-C) and germination (D-F) and early hyphal colonization (G-I) by a GFP-tagged isolate of *Phytophthora capsici* on pepper roots of susceptible Red Knight (A, D, and G), resistant Paladin (B, E, and H), and resistant land race CM-334 (C, F, and I) plants. Images were collected with a confocal laser scanning microscope 30 min (zoospore attachment), 120 min (zoospore germination), or 3 days (hyphal growth on secondary root tips with brown lesions) post inoculation. Sporangia are visible on Red Knight and Paladin roots (G and H; white arrows). Scale bar represents 200 µm, and is the same for all images.

At both 30 min and 120 min pi, the proportion of germinated zoospores was similar between the first root section imaged and the third root section imaged (after up to 1 h incubation at 4°C), therefore storing inoculated roots for short periods of time at 4°C was an effective way to pause pathogen growth immediately following inoculation. Because wounds on the roots tended to have large numbers of attached zoospores, unwounded root sections were selected for the purpose of comparing zoospore attachment among pepper cultivars.

*Root and crown colonization by* P. capsici. Three days pi, all plants looked healthy, but significantly more of the secondary roots attached within 0.5 cm of the soil line had brown lesions on Red Knight plants, compared to either Paladin or CM-334 plants (Table 6.1, Fig. 6.2 A). However, the presence of macroscopic lesions on these roots was not necessarily associated with visible (via confocal microscope) growth of *P. capsici* hyphae on the roots. While hyphae were visible on nearly all secondary roots with lesions from Red Knight plants, hyphae were visible on only about half of the secondary roots with lesions from Paladin plants, and less than half of the secondary roots with lesions from CM-334 plants (Table 6.1). Sporangia had frequently formed on roots of Red Knight plants by 3 days pi, but were only rarely observed on Paladin or CM-334 plants (Fig. 6.1 G and H).

GFP-tagged hyphae were visible in longitudinal sections of the crowns of all Red Knight plants, but none of the Paladin or CM-334 plants (Fig. 6.3 A-C). Some hyphae appeared to be growing through vascular tissue in the crown (Fig. 6.3 A) and roots (Fig. 6.3 G) of Red Knight plants. Lesions similar to those observed on the secondary roots of inoculated plants were not observed on uninoculated controls, and no hyphae were observed in longitudinal sections of crowns from uninoculated plants.

**Table 6.1.** Colonization of roots of three pepper cultivars by *Phytophthora capsici* 3 and 4 days post inoculation (pi).

| | | 3 days pi | | | | | | 4 days pi | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of roots[a] | | | Percent roots with lesions[b] | | | Primary root lesion length (mm)[c] | | |
| Cultivar | N[d] | Total | With lesions (%) | Hyphae[e] | Mean | | SE | Mean | | SE |
| Red Knight | 10 | 236 | 118 (50%) | 107 (91%) | 63.2 | a | 5.66 | 10.7 | a | 1.13 |
| Paladin | 10 | 192 | 57 (30%) | 30 (53%) | 34.5 | b | 5.51 | 0 | b | 0 |
| CM-334 | 9 | 245 | 59 (24%) | 20 (34%) | 24.2 | b | 4.12 | 0 | b | 0 |

[a] Secondary roots attached to the primary root within 0.5 cm of the soil line

[b] Percent (per plant) of secondary roots attached to primary root within 0.5 cm of the soil line that had brown lesions 3 days pi; means (of 9 or 10 plants) followed by the same letter are not significantly different at $P = 0.05$ based on a Tukey's Honestly Significant Differences test

[c] Length of lesion observed on primary roots 4 days pi; means (of 9 or 10 plants) followed by the same letter are not significantly different at $P = 0.05$ based on a Tukey's HSD test

[d] Number of plants observed per cultivar from two biological replicates

[e] Roots with lesions and on which growth of GFP-tagged hyphae was visible using the confocal laser scanning microscope (percent of roots with hyphae out of all roots with lesions in parentheses)

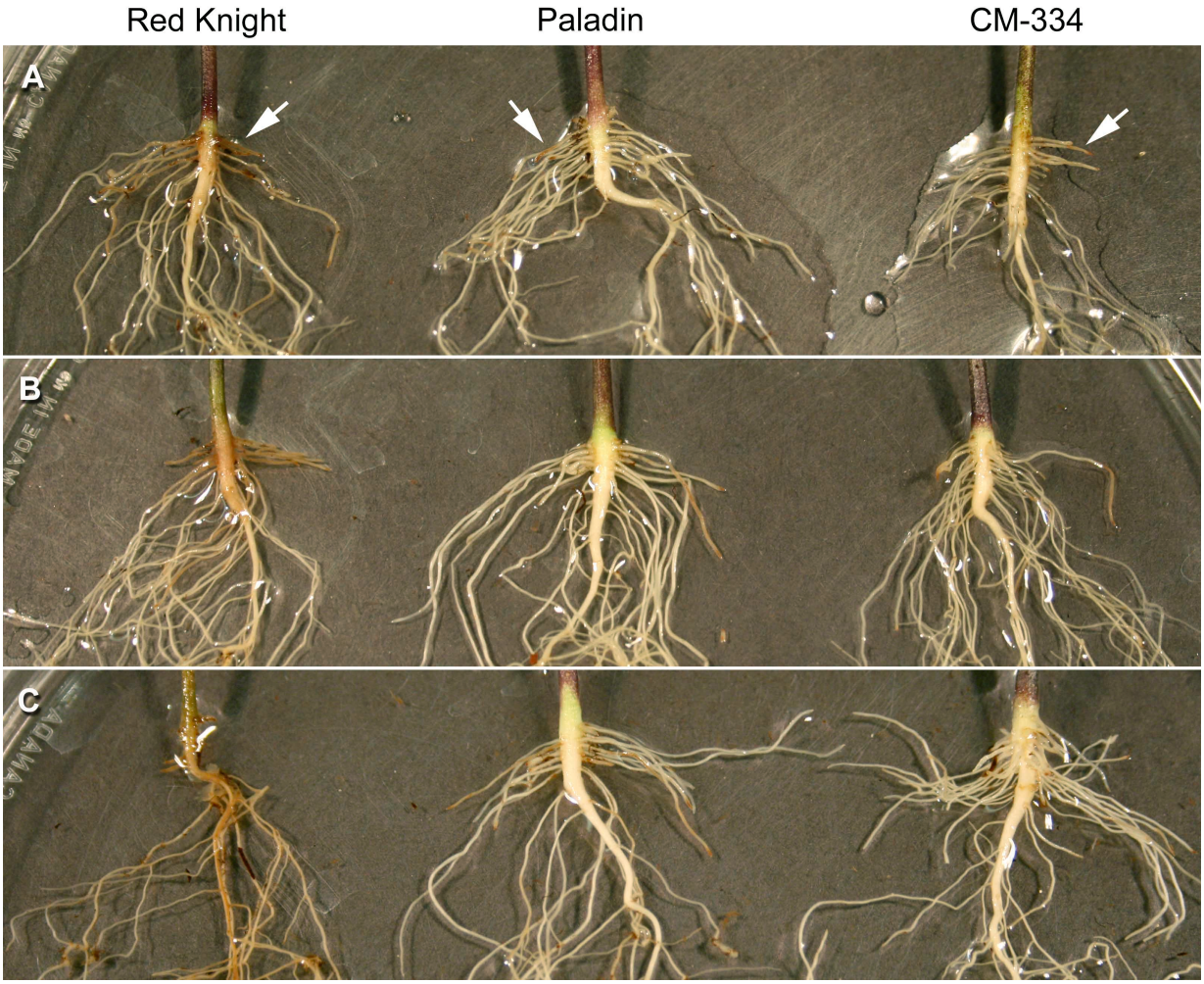



**Figure 6.2** Roots of susceptible Red Knight, resistant Paladin, and resistant land race CM-334 pepper plants 3 days (A), 4 days (B), or 10 days (C) post inoculation with a GFP-tagged isolate of *Phytophthora capsici*. The number of secondary roots attached within 0.5 cm of the soil line and having brown lesions (white arrows) were counted at 3 days post inoculation (A), and the length of the lesion on the primary root of each plant was measured 4 days post inoculation (B).

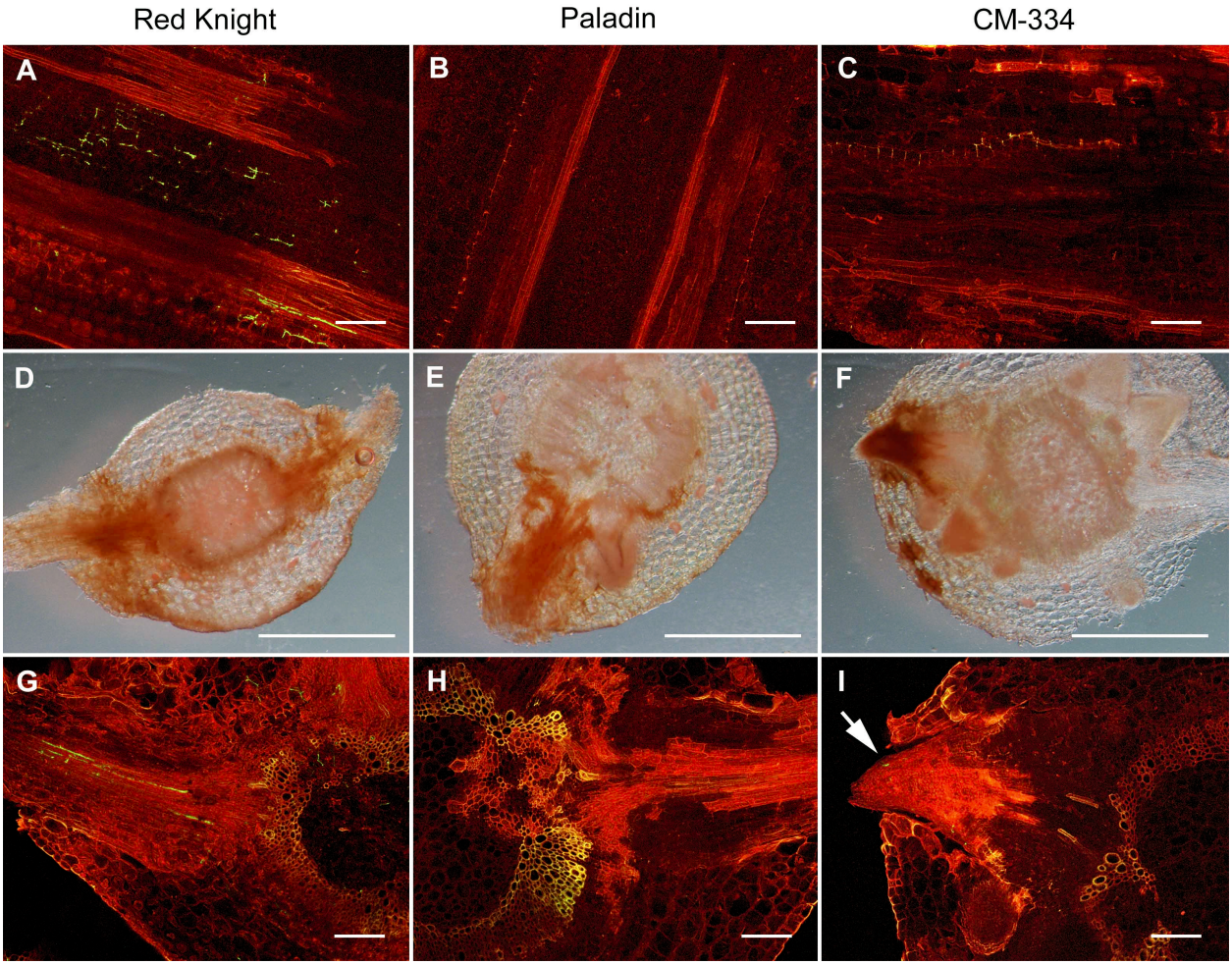



**Figure 6.3** Colonization by a GFP-tagged isolate of *Phytophthora capsici* of pepper crown tissue from susceptible Red Knight (A, D, and G), resistant Paladin (B, E, and H), and resistant land race CM-334 (C, F, and I) plants. Images collected with a confocal laser scanning microscope of either longitudinal (A-C 3 days pi) or cross (G-I; 4 days pi) sections show hyphae growing in the crown tissue of Red Knight but not Paladin and CM-334 plants. Images collected with a dissecting microscope of cross sections (D-F; 4 days pi) show extensive discoloration in crown tissue of Red Knight, but less discoloration in Paladin and CM-334. White arrow marks limited hyphal growth on a young secondary root of CM-334 (I). Scale bars in A-C and G-I represent 200 µm, and scale bars in D-F represent 1 mm.

By 4 days pi, Red Knight plants had begun to lose cotyledons. Lesions on the primary root of Red Knight plants were significantly longer than on Paladin or CM-334 (on which no lesions were observed on the primary root; Table 6.1, Fig. 6.2 B). Extensive brown discoloration was observed (especially on young secondary roots) in cross-sections of crown tissue taken at

this time point on Red Knight (Fig. 6.3 D). Discoloration was less extensive on Paladin and CM-334 (Fig. 6.3 E and F). While extensive hyphal growth was observed throughout cross sections of Red Knight crowns with the CLSM, hyphae were not observed in the crown tissue of Paladin and CM-334 plants (Fig. 6.3 G-I). In one instance, hyphae were observed on epidermal tissue of a young secondary root of CM-334 (Fig. 6.3 I). Lesions similar to those observed on the primary roots of inoculated plants were not observed on uninoculated controls, and no hyphae were observed in cross sections of uninoculated crowns.

By 10 days pi, Red Knight plants were completely dead, but Paladin and CM-334 plants still appeared healthy, and no hyphae were visible in the crowns of Paladin and CM-334 plants. While some secondary roots on Paladin and CM-334 plants had lesions, new roots had grown, and no lesions were visible on the primary roots of these plants (Fig. 6.2 C).

*Defense gene expression in peppers*. In both stem and leaf tissue collected from Red Knight, Paladin, and CM-334 seedlings, at either 8 or 24 hours post inoculation, there were no substantial fold increase in expression of the defense genes *CABGLU*, *CABPR*1, *CAPO*1, or *CASC*1 which were consistently significant across both biological replicates. In general, expression in inoculated plants relative to control plants was higher in stem tissue than in leaf tissue, but there was high variability among plants within a treatment.

## *Discussion*

These experiments demonstrate that the high levels of resistance observed in the bell pepper cultivar Paladin and the land race CM-334 are not a result of the inability of *P. capsici* zoospores to attach to or germinate on root tissue within 120 minutes pi. Furthermore, some hyphal growth, and even sporulation are possible on the roots of these highly resistant plants by

3 days pi. Differences were observed among susceptible and resistant cultivars in terms of lesion formation on secondary roots (3 days pi) and primary roots (4 days pi), as well as colonization of the crown tissue (3 and 4 days pi). These differences between cultivars were consistent across both replicates at both the 3 and 4 day pi time points, in spite of substantially warmer air temperatures in the second biological replicates. Longer primary root lesions and slightly more severe symptoms (macroscopic water-soaked lesions at the crown and some senescence of true leaves) were observed on Red Knight plants 4 days pi, in the second biological replicate. This is not surprising, since *P. capsici* is known to be favored by warm temperatures (Bowers et al. 2007; Cooke et al. 2000; Tompkins and Tucker 1941).

The higher proportion of secondary roots with lesions, the colonization of crown tissue by 3 days pi, and the formation of lesions on the primary root by 4 days pi in susceptible Red Knight plants compared to resistant Paladin and CM-334 plants are consistent with previous observations of *P. capsici* interactions with resistant and susceptible pepper cultivars. However, unlike previous studies, we quantified the attachment and germination of zoospores on roots of susceptible and resistant plants within the first two hours following inoculation. Kim and Kim (2009) and Lee et al. (2000) investigated interactions between pepper and *P. capsici* 1, 2 or 3 days pi. Kim and Kim (2009) reported differences in the root tissues colonized by *P. capsici*, with the pathogen eventually reaching the cortical and vascular tissue in the susceptible cultivar, but limited to epidermal and outer cortical tissue in CM-334. Lee et al. (2000) made similar observations in stem tissue 24 hours pi following inoculation of a susceptible cultivar (Hanbyul) with either a virulent (with and without chemical induction of host resistance) or avirulent *P. capsici* isolate. They also reported formation of hyphae and sporangia on the surface of pepper stems 48 hours pi in the compatible interaction, but only hyphae in the incompatible interactions.

This is similar to our observation of rare sporangia production on CM-334 and Paladin roots, compared to Red Knight. Similar to what we found, Café-Filho and Duniway (1996) reported lesion formation on the roots of both a susceptible (Yolo Wonder) and a resistant (Adra) cultivar following inoculation of root tips with *P. capsici* zoospores, but lesions grew more slowly on Adra roots compared to Yolo Wonder roots and never reached the crown of the plant. We made similar observations on Red Knight, Paladin, and CM-334 plants, but also related these differences to presence of hyphae on root tips and in crown tissue.

Interestingly, Pegard et al. (2005) observed similar host resistance responses when CM-334 plants were inoculated with root-knot nematodes (*Meloidogyne* spp.). Fewer J2 stage nematodes were able to penetrate the roots of CM-334 compared to a susceptible pepper cultivar, and nematodes never progressed to the root vascular tissue. A hypersensitive response appeared to have played an important role in halting the further movement of the nematodes in the roots. In addition, no egg masses were found on CM-334 plants, indicating that nematodes could not reproduce on these plants.

Based on the similarity in zoospore attachment and germination on all three pepper cultivars by 120 minutes pi, and the differences in lesion formation on secondary roots and hyphal colonization of crown tissue by 3 days pi, the critical events which determine susceptibility or resistance of the pepper plant likely occur within this window. Previous studies have indicated that differential expression of pepper genes associated with plant defense could be involved (Fernández-Herrera et al. 2012; Silvar et al. 2008; Wang et al. 2013). However, the fact that we did not detect substantial and consistent differences in expression of four defense genes in stem or leaf tissue among Red Knight, Paladin, and CM-334 plants at either 8 or 24 hours pi, does not provide evidence that these particular defense genes play a major role in preventing

infection of Paladin and CM-334 plants by *P. capsici*. This was surprising, since Silvar et al. (2008) reported substantial (up to 40-fold) up-regulation of the same genes in pepper stem tissue, at the same time points, in the resistant lines PI201234 and CM-331, compared to a susceptible cultivar (Yolo Wonder).

There are two possible explanations for the different results observed in our study. First, specific defense genes involved in host resistance to *P. capsici* and time of peak expression in stem tissue may vary between the two resistant cultivars used by Silvar et al. (PI201234 and CM-331) and the resistant cultivars used in our study (Paladin and CM-334). While the land races CM-331 and CM-334 originated from the same collection made in the Morelos state of Mexico (Guerrero-Moreno and Laborde 1980), and may therefore be related to each other, defense gene expression in these two land races may not be identical. However, previous studies have reported upregulation of defense genes in CM-334 following inoculation with *P. capsici* (Fernández-Herrera et al. 2012; Richins et al. 2010). Fernández-Herrera (2012) reported only slight (one- to three-fold ) upregulation of *CABPR*1, *CABGLU*, and *CAPO*1 in roots of inoculated CM-334 plants 6 hours pi, with expression at 24 hours pi between 5- and 11-fold greater than in uninoculated plants, and peak expression at 48 hours pi (up to a 13-fold increase relative to uninoculated plants). However, they did not compare these expression levels to those of a susceptible pepper cultivar. Since Wang et al. (2013) reported that expression of *CABPR*1, *CABGLU*, and *CAPO*1 was higher in leaves than in roots in an incompatible pepper-*P. capsici* interaction, we were surprised not to see higher levels of defense gene expression in leaf tissue (compared to stem tissue) of Paladin and CM-334 plants. Quantification of expression at additional time points post inoculation would be needed to conclusively demonstrate that these four genes are not upregulated in Paladin and CM-334 in response to *P. capsici*.

Another possible explanation for these different results is that our study was conducted in a greenhouse, where all plants were exposed to additional environmental stresses, including air movement caused by fans associated with the ventilation system, and greater fluctuations in air temperature. Conversely, previous studies maintained plants in growth chambers at 25-28 °C, with either 14 or 16 hours of light and 10 or 8 hours of dark each day. Although in our study environmental stresses were consistent among inoculated and uninoculated plants of all cultivars, the plants' responses to these stresses may have masked more subtle differences in defense gene expression due to inoculation with *P. capsici*. While these differences may be detectable under highly controlled experimental conditions, they may not be substantial enough to detect under greenhouse conditions.

The use of resistant cultivars is currently a key tool for successfully managing *P. capsici* in peppers. The knowledge that attachment and germination of *P. capsici* zoospores is not significantly different between the resistant and susceptible pepper cultivars tested, but rather differences are observed as lesion formation begins (by 3 days pi) advances our understanding of this pathosystem. However, even highly resistant cultivars are not completely immune to infection by *P. capsici*, especially under favorable environmental conditions (saturated soil, warm temperatures, and high levels of inoculum) (Dunn et al. 2014; Foster and Hausbeck 2010; McGrath and Fox 2009). This raises the question: what changes result in a small proportion of resistant plants eventually succumbing to the pathogen? Potential answers could include plant wounding or environmental stress, and the methods used here could help to answer this question in the future.

***Acknowledgements***

This project was supported by the Agriculture and Food Research Initiative Competitive Grant No. 2012-67011-19690 from the USDA National Institute of Food and Agriculture, and by the New York State Department of Agriculture and Markets through a NY Specialty Crops Block Grant. Support for A. R. Dunn was also provided by a fellowship from Cornell University College of Agriculture and Life Sciences. The authors thank H. C. Hoch for assistance with the confocal laser scanning microscope.

***References***

Babadoost, M. 2006. Performance of bell pepper cultivars in a commercial field infested with *Phytophthora capsici*. HortScience. 41:981.

Bowers, J. H., Martin, F. N., Tooley, P. W., and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology. 97:492–503.

Café-Filho, A. C., and Duniway, J. M. 1996. Effect of location of drip irrigation emitters and position of *Phytophthora capsici* infections in roots on Phytophthora root rot of pepper. Phytopathology. 86:1364–1369.

Cooke, D., Drenth, A., Duncan, J., Wagels, G., and Brasier, C. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet. Biol. 30:17–32.

de Mendiburu, F. 2012. Statistical procedures for agricultural research. Version 1.1-3. Lima, Peru: International Potato Center. <http://tarwi.lamolina.edu.pe/~fmendiburu>.

Djian-Caporalino, C., Pijarowski, L., Januel, A., Lefebvre, V., Daubéze, A., Palloix, A., Dalmasso, A., and Abad, P. 1999. Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). Theor. Appl. Genet. 99:496–502.

Dogimont, C., Palloix, A., Daubze, A.-M., Marchoux, G., Selassie, K. G., and Pochard, E. 1996. Genetic analysis of broad spectrum resistance to potyviruses using doubled haploid lines of pepper (*Capsicum annuum* L.). Euphytica. 88:231–239.

Dunn, A. R., Fry, B. A., Lee, T. Y., Conley, K. D., Balaji, V., Fry, W. E., McLeod, A., and Smart, C.D. 2013. Transformation of *Phytophthora capsici* with genes for green and red fluorescent protein for use in visualizing plant-pathogen interactions. Australas. Plant Path. 42:583–593.

Dunn, A. R., Lange, H. W., and Smart, C. D. 2014. Evaluation of commercial bell pepper cultivars for resistance to Phytophthora blight (*Phytophthora capsici*). Plant Health Prog. 15:19–24.

Enkerli, K., Hahn, M. G., and Mims, C. W. 1997. Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. Can. J. Botany. 75:1493–1508.

Fernández-Herrera, E., Rojas-Martínez, R. I., Guevara-Olvera, L., Rivas-Dávila, M. E., Valadez-Moctezuma, E., and Zavaleta-Mejía, E. 2012. Defense in chili CM-334 inoculated with *Phytophthora capsici* and infected by *Nacobbus aberrans*. Nematropica. 42:96–107.

Foster, J. M., and Hausbeck, M. K. 2010. Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. Plant Dis. 94:24–30.

Glosier, B. R., Ogundiwin, E. A., Sidhu, G. S., Sischo, D. R., and Prince, J. P. 2008. A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. Euphytica. 162:23–30.

Guerrero-Moreno, A., and Laborde, J. A. 1980. Current status of pepper breeding for resistance to *Phytophthora capsici* in Mexico. Proceedings of the IVth meeting of the Eucarpia Capsicum Working Group in Wageningen, Netherlands, pp. 52–56.

Hellemans, J. M., Mortier, G., De Paepe, A., Speleman, F., and Vandesompele, J. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8:R19.

Hoch, H., Galvani, C., Szarowski, D., and Turner, J. 2005. Two new fluorescent dyes applicable for visualization of fungal cell walls. Mycologia. 97:580–588.

Hwang, B., Kim, W. B., and Kim, W. K. 1989. Ultrastructure at the host-parasite interface of *Phytophthora capsici* in roots and stems of *Capsicum annuum*. J. Phytopathol. 127:305–315.

Kim, S. G., and Kim, Y. H. 2009. Histological and cytological changes associated with susceptible and resistant responses of chili pepper root and stem to *Phytophthora capsici* infection. Plant Pathol. J. 25:113–120.

Kimble, K. A., and Grogan, R. G. 1960. Resistance to Phytophthora root rot in pepper. Phytopathology. 50:642–642.

Lee, Y., Hong, J., Hippe-Sanwald, S., and Hwang, B. 2000. Histological and ultrastructural comparisons of compatible, incompatible and DL-β-amino-*n*-butyric acid-induced resistance responses of pepper stems to *Phytophthora capsici*. Physiol. Mol. Plant Pathol. 57:269–280.

McGrath, M. T., and Fox, G. M. 2009. Evaluation of Phytophthora-resistant bell pepper cultivars with and without a fungicide program, 2008. Plant Dis. Manag. Rep. 3:V132.

Mitchell, R. T., and Deacon, J. W. 1986. Differential (host-specific) accumulation of zoospores of *Pythium* on roots of graminaceous and non-graminaceous plants. New Phytol. 102:113–122.

Monroy-Barbosa, A., and Bosland, P. W. 2008. Genetic analysis of Phytophthora root rot race-specific resistance in Chile pepper. J. Amer. Soc. Hort. Sci. 133:825–829.

Ortega, R. G., Espanol, C. P., and Zueco, J. C. 1991. Genetics of resistance to *Phytophthora capsici* in the pepper line SCM-334. Plant Breed. 107:50–55.

Palloix, A., Daubeze, A. M., Phaly, T., and Pochard, E. 1990. Breeding transgressive lines of pepper for resistance to *Phytophthora capsici* in a recurrent selection system. Euphytica. 51:141–150.

Pegard, A., Brizzard, G., Fazari, A., Soucaze, O., Abad, P., and Djian-Caporalino, C. 2005. Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum*. Annu. Rev. Phytopathol. 95:158–165.

Polach, F. J., and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. Phytopathology. 62:20–26.

R Development Core Team. 2013. R: A language and environment for statistical computing. <http://www.R-project.org/>.
Reifschneider, F. J. B., Boiteux, L. S., Dellavecchia, P. T., Poulos, J. M., and Kuroda, N. 1992. Inheritance of adult-plant resistance to *Phytophthora capsici* in pepper. Euphytica. 62:45–49.
Richins, R. D., Micheletto, S., and O'Connell, M. A. 2010. Gene expression profiles unique to chile (*Capsicum annuum* L.) resistant to Phytophthora root rot. Plant Sci. 178:192–201.
Saini, S. S., and Sharma, P. P. 1978. Inheritance of resistance to fruit rot (*Phytophthora capsici* Leon.) and induction of resistance in bell pepper (*Capsicum annuum* L.). Euphytica. 27:721–723.
Silvar, C., Merino, F., and Diaz, J. 2008. Differential activation of defense-related genes in susceptible and resistant pepper cultivars infected with *Phytophthora capsici*. J. Plant Physiol. 165:1120–1124.
Sy, O., Bosland, P. W., and Steiner, R. 2005. Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* L. J. Amer. Soc. Hort. Sci. 130:75–78.
Tompkins, C. M., and Tucker, C. M. 1941. Root rot of pepper and pumpkin caused by *Phytophthora capsici*. J. Agric. Res. 63:417–426.
Walker, S. J., and Bosland, P. W. 1999. Inheritance of Phytophthora root rot and foliar blight resistance in pepper. J. Amer. Soc. Hort. Sci. 124:14–18.
Wan, H., Yuan, W., Ruan, M., Ye, Q., Wang, R., Li, Z., et al. 2011. Identification of reference genes for reverse transcription quantitative real-time PCR normalization in pepper (*Capsicum annuum* L.). Biochem. Biophys. Res. Commun. 416:24–30.
Wang, J.-E., Li, D.-W., Zhang, Y.-L., Zhao, Q., He, Y.-M., and Gong, Z.-H. 2013. Defence responses of pepper (*Capsicum annuum* L.) infected with incompatible and compatible strains of *Phytophthora capsici*. Eur. J. Plant Pathol. 136:625–638.

# CONCLUSION

## Evolution of an experimental population of *Phytophthora capsici* in the field

To our knowledge, the research population of *P. capsici* established at the Phytophthora Blight Farm in Fall 2008 is the only population like it in the world established from only two single-spore isolates, in a field with no prior history of *P. capsici*, and where subsequent introductions of *P. capsici* can be controlled. Thus, this population provided a unique opportunity to study changing population structure in the first four years following infestation. The population is sexual, although, like other populations of *P. capsici*, asexual reproduction also occurs. It is also characterized by high levels of diversity, which did not dissipate substantially over the first four years, and four new alleles were identified at two of the five microsatellite loci studied, suggesting that high rates of mutation may play a role in maintaining the genetic diversity observed in other field populations. Finally, in F1 progeny from an *in vitro* cross between the same two parental isolates, combinations of observed alleles were consistent with loss of heterozygosity or some other non-mendelian inheritance mechanism. Some combinations of alleles were consistent with possible selfing (which has not previously been reported in *P. capsici*), although this cannot be determined conclusively with these markers.

Continuing to sample and characterize isolates from this field in subsequent years will provide valuable information about evolution of an isolated population of *P. capsici* over a longer time period. While characterization of this population does not immediately inform the use of host resistance to manage *P. capsici*, it lays important groundwork for a better understanding of how populations of *P. capsici* in individual fields might be changing and

evolving over time. It also provides a reference point against which populations of *P. capsici* in commercial vegetable fields (especially fields inoculated by flood waters) can be compared. Newer genotyping techniques (e.g. genotyping-by-sequencing or RNA-Seq) could provide a more detailed picture of what is happening in this research population or in other field populations of *P. capsici*. Subsequent studies either in this research field, or using isolates collected from this field could also provide valuable information about the potential for important pathogen traits (e.g., virulence on different hosts or cultivars, fungicide resistance) to change over time. The ultimate goal would be to link these pathogen traits to genetic markers, in order to be able to recommend specific management practices to a grower, based on a genetic profile of *P. capsici* isolates present in the field. These recommendations could include the specific hosts or varieties most likely to yield well in the field, or a prediction of how long a particular resistant cultivar could be planted in the field before the local *P. capsici* population evolves to overcome that resistance.

**Evaluation of bell pepper cultivars for Phytophthora resistance and yield**

Based on trials conducted over five years in upstate New York with a *P. capsici* isolate representative of isolates found in New York State, we can make good recommendations to growers about the bell pepper cultivars likely to show the highest levels of resistance to *P. capsici* in New York, as well as those cultivars which should be avoided in a field with a history of *P. capsici*. Yield and fruit quality characteristics for many of the commonly-grown bell pepper cultivars were also compared at three separate field sites (one inoculated with *P. capsici*, and two uninoculated). While total yields were similar among commercial cultivars (either susceptible or resistant to *P. capsici*) in the absence of the pathogen, the most resistant cultivars (Paladin,

Intruder, and Aristotle) tended to have smaller fruit, and Paladin and Intruder were more prone to fruit silvering.

These experiments have provided immediately-applicable information, which is being used by vegetable growers across New York State to manage *P. capsici*. They also point out the need for improved fruit size and quality traits in new resistant bell pepper cultivars. As new pepper cultivars become available, and as suitable cucurbit, tomato, and eggplant cultivars with Phytophthora resistance (hopefully) become available in the future, similar methods could also be effective in identifying the best cultivars for New York growers. Periodic greenhouse screens of the isolate (NY 0664-1) used in these studies should be able to confirm that this isolate is still representative of *P. capsici* isolates affecting New York growers, or whether a different isolate (or a combination of isolates) should be used in subsequent screens.

**Interactions of *Phytophthora capsici* with resistant and susceptible peppers**

The GFP- and RFP- tagged isolates of *P. capsici* with growth and virulence characteristics similar to the wild type isolate (NY 0664-1) are a valuable tool for understanding how *P. capsici* colonizes its hosts. So far, the GFP-tagged isolate PcapG-a has been used to show that zoospore attachment to and germination on pepper roots does not differ between the resistant pepper cultivars Paladin and CM-334 and the susceptible pepper cultivar Red Knight within the first two hours after inoculation. The stark differences in susceptibility between Paladin and CM-334 compared to Red Knight appear to be due to the pathogen's ability to spread through host tissue following zoospore germination and initial growth of hyphae on pepper roots. The critical time point for halting pathogen spread appears to occur between 2 hours and 3 days post inoculation, but we found no evidence that differences in susceptibility were related to increased

expression of four pepper defense genes in leaves or stems of the resistant cultivars at 8 and 24 hours post inoculation. Previous studies conducted in growth chambers have reported increased expression of these genes in resistant pepper cultivars (including CM-334) inoculated with *P. capsici*, whereas our experiment was conducted in a greenhouse, with more variation in air temperature and other environmental stresses compared to a growth chamber. This may explain why our results differed from previous studies. The fact that differences in defense gene expression were not detected in a greenhouse study raises the question whether differences in expression of these four defense genes contribute substantially to the ability of Paladin, CM-334 and other cultivars to resist infection by *P. capsici* in the field (where plants experience even more environmental stresses than in a greenhouse). Additional experiments would be needed to test this hypothesis, and RNA-seq could be employed to identify other candidate pepper genes involved in defense against *P. capsici*.

The fluorescently-tagged isolates of *P. capsici* and the protocols described here could be used to answer a variety of other questions about interactions between *P. capsici* and its various hosts. Other solanaceous vegetables are either less susceptible to root and crown rot than pepper (tomato and eggplant), or are not reported hosts of *P. capsici* (potato). Thus, it would be interesting to compare zoospore attachment and germination, as well as hyphal colonization on tomato, eggplant, and potato roots to what has been observed on pepper roots in the present study. Similar studies could also be done using non-host vegetables currently recommended for rotation with Phytophthora-susceptible crops (e.g. corn or cabbage), or weeds commonly found in New York vegetable fields. The fact that some sporangia were produced on Paladin and CM-334 roots raises additional questions about the ability of *P. capsici* to reproduce on resistant cultivars, non-host vegetables and weeds. These fluorescently-tagged isolates could be used to quantify the

formation of sporangia and oospores on roots of various plants, and this could have important implications for growers seeking to reduce *P. capsici* through rotation to non-host crops.

**Final thoughts**

Although *P. capsici* currently presents many challenges to vegetable growers, and is likely to continue to do so, progress is being made. Many growers around New York State and elsewhere are able to profitably produce susceptible vegetables using integrated management strategies. Additional resistant pepper cultivars continue to be released, and work is ongoing to develop resistant cucurbit, tomato, and eggplant cultivars, as well. The research presented in this dissertation provides some information that is already helping New York growers manage *P. capsici* better (i.e. recommendations on high-yielding resistant cultivars), and it advances our understanding of the biology of this pathogen in other areas (i.e. changing population structure in a field over time, and interactions with roots of susceptible and resistant pepper plants). It is hoped that this new information about the biology of *P. capsici* will eventually help growers to manage this pathogen more successfully and continue to produce healthy vegetables, in spite of *P. capsici* infestations.

APPENDIX I

**Isolates of *Phytophthora capsici* associated with a research field population established in Fall 2008**

**Table AI.1.** *Phytophthora capsici* isolates used to study a research population over time. Two isolates (NY 0664-1 and NY 06180-4) were crossed in the lab (*in vitro*) and in a previously uninfested research field. The field was sampled from 2009 to 2012. Isolates collected from the field were compared to isolates collected from a naturally infested grower field (CD-4) in Schenectady County, New York. Date isolates were collected, host (and cultivar, if available; w. squash = winter squash; s. squash = summer squash) from which isolates were obtained, mating type (MT) and allele sizes (bp) at five microsatellite loci are listed.

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| NY 0664-1 | | A1 | 241 | 241 | 434 | 446 | 277 | 277 | 377 | 386 | 186 | 186 | 8/14/06 | pepper | Karma |
| NY 06180-4 | | A2 | 241 | 241 | 434 | 446 | 274 | 291 | 359 | 359 | 186 | 189 | 9/18/06 | w. squash | |
| 68-01 | *In vitro* | A2 | 241 | 241 | 434 | 434 | 277 | 277 | 386 | 386 | 186 | 186 | | | |
| 68-02 | *In vitro* | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | | | |
| 68-03 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-04 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | | | |
| 68-05 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | | | |
| 68-07 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 189 | | | |
| 68-08 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-09 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | | | |
| 68-10 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | | | |
| 68-11 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | | | |
| 68-12 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | | | |
| 68-13 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-14 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 277 | 377 | 377 | 186 | 186 | | | |
| 68-15 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 386 | 186 | 189 | | | |
| 68-16 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | | | |
| 68-17 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | | | |
| 68-18 | *In vitro* | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | | | |
| 68-19 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 277 | 386 | 386 | 186 | 186 | | | |
| 68-20 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-21 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | | | |
| 68-22 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | | | |
| 68-23 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-24 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | | | |
| 68-25 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | | | |
| 68-26 | *In vitro* | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | | | |
| 68-27 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 277 | 386 | 386 | 186 | 186 | | | |
| 68-28 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-29 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | | | |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 68-30 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | | | |
| 68-31 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | | | |
| 68-32 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | | | |
| 68-33 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 189 | | | |
| 68-34 | *In vitro* | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 377 | 186 | 186 | | | |
| 68-35 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | | | |
| 68-36 | *In vitro* | A2 | 241 | 241 | 434 | 434 | 277 | 277 | 386 | 386 | 186 | 189 | | | |
| 68-37 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | | | |
| 68-38 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | | | |
| 68-39 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | | | |
| 68-40 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | | | |
| 68-41 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | | | |
| 68-42 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | | | |
| 68-43 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 189 | | | |
| 68-44 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | | | |
| 68-45 | *In vitro* | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | | | |
| 68-46 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | | | |
| 68-47 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | | | |
| 68-48 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | | | |
| 68-49 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 359 | 386 | 186 | 189 | | | |
| 68-50 | *In vitro* | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | | | |
| 68-51 | *In vitro* | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 386 | 186 | 189 | | | |
| 68-52 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | | | |
| 68-53 | *In vitro* | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | | | |
| 68-54 | *In vitro* | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | | | |
| 09PF-01A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-02A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-03A | Field 2009 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-04A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-05A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 6/22/09 | s. squash | Zucchini Elite |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 09PF-06A | Field 2009 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 359 | 377 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-07A | Field 2009 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-08A | Field 2009 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 189 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-09A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 377 | 186 | 186 | 6/22/09 | s. squash | Zucchini Elite |
| 09PF-10A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-11A | Field 2009 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 189 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-12A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-13A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-14A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-15A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-16A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-17A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-18A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-19A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-20A | Field 2009 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 189 | 6/25/09 | s. squash | Zucchini Elite |
| 09PF-21A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 359 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-22A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-23A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-24A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-25A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-26A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 189 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-27A | Field 2009 | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-28A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-29A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-31A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-32A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-33A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-34A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/1/09 | s. squash | Zucchini Elite |
| 09PF-35A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 7/5/09 | s. squash | Zucchini Elite |
| 09PF-36A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 7/5/09 | s. squash | Zucchini Elite |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 09PF-37A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 7/8/09 | s. squash | Zucchini Elite |
| 09PF38A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 7/8/09 | s. squash | Zucchini Elite |
| 09PF-39A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 7/8/09 | s. squash | Zucchini Elite |
| 09PF-40A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 189 | 7/8/09 | s. squash | Zucchini Elite |
| 09PF-41A | Field 2009 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 7/8/09 | s. squash | Zucchini Elite |
| 09PF-42A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-43A | Field 2009 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-44A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 274 | 377 | 377 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-45A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 274 | 377 | 377 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-46A | Field 2009 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-47A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 09PF-48A | Field 2009 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | 7/12/09 | s. squash | Zucchini Elite |
| 10PF-01A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | 6/9/10 | tomato | Celebrity |
| 10PF-02A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 6/11/10 | tomato | Celebrity |
| 10PF-03A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 6/14/10 | tomato | Celebrity |
| 10PF-06A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | 7/13/10 | s. squash | |
| 10PF-07A | Field 2010 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 189 | 7/13/10 | pumpkin | Mystic Plus |
| 10PF-08A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 7/13/10 | pumpkin | Mystic Plus |
| 10PF-09A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | tomato | Celebrity |
| 10PF-10A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 377 | 186 | 186 | 7/14/10 | s. squash | |
| 10PF-11A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 7/15/10 | s. squash | |
| 10PF-12A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-13A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-14A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-15A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 377 | 186 | 189 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-16A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-17A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 189 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-18A | Field 2010 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-19A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-20A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 10PF-22A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-24A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-25A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-26A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-27A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-28A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-30A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-32A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 7/15/10 | pumpkin | Mystic Plus |
| 10PF-33A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-34A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-35A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-36A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 359 | 386 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-37A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 386 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-38A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-39A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-40A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 386 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-41A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 359 | 377 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-42A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-43A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-44A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-45A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 7/20/10 | pumpkin | Mystic Plus |
| 10PF-46A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 189 | 7/19/10 | s. squash | |
| 10PF-47A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 189 | 7/27/10 | s. squash | |
| 10PF-48A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 189 | 7/27/10 | tomato | Celebrity |
| 10PF-49A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 189 | 7/27/10 | tomato | Celebrity |
| 10PF-50A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/27/10 | tomato | Celebrity |
| 10PF-51A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 7/27/10 | tomato | Celebrity |
| 10PF-52A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | 7/27/10 | tomato | Celebrity |
| 10PF-53A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 7/27/10 | tomato | Celebrity |
| 10PF-54A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/12/10 | tomato | Celebrity |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 10PF-55A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 186 | 8/12/10 | tomato | Celebrity |
| 10PF-56A | Field 2010 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 386 | 186 | 186 | 8/12/10 | tomato | Celebrity |
| 10PF-57A | Field 2010 | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/12/10 | tomato | Celebrity |
| 10PF-58A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/12/10 | tomato | Celebrity |
| 10PF-59A | Field 2010 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/12/10 | tomato | Celebrity |
| 10PF-60A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 9/7/10 | tomato | Celebrity |
| 10PF-61A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 9/7/10 | tomato | Celebrity |
| 10PF-62A | Field 2010 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 9/7/10 | tomato | Celebrity |
| 10PF-63A | Field 2010 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 9/7/10 | tomato | Celebrity |
| 10PF-64A | Field 2010 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 186 | 9/7/10 | tomato | Celebrity |
| 10PF-65A | Field 2010 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 189 | 9/7/10 | tomato | Celebrity |
| 10PF-66A | Field 2010 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 9/7/10 | tomato | Celebrity |
| 11PF-01A | Field 2011 | A1 | 241 | 241 | 446 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 6/27/11 | pepper | Red Knight |
| 11PF-02A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 7/1/11 | pepper | Red Knight |
| 11PF-03A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/1/11 | tomato | Celebrity |
| 11PF-04A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 7/18/11 | pepper | Revolution |
| 11PF-05A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 7/18/11 | pepper | Red Knight |
| 11PF-06A | Field 2011 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 189 | 7/26/11 | pepper | Red Knight |
| 11PF-07A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 7/26/11 | pepper | Revolution |
| 11PF-08A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/1/11 | pepper | Revolution |
| 11PF-09A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 8/1/11 | cucumber | Diva |
| 11PF-10A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 186 | 8/1/11 | pepper | Red Knight |
| 11PF-11A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 389 | 186 | 186 | 8/1/11 | cucumber | Diva |
| 11PF-12A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 186 | 8/5/11 | pepper | Red Knight |
| 11PF-13A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 8/5/11 | pepper | Revolution |
| 11PF-14A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 8/5/11 | pepper | Revolution |
| 11PF-15A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 8/5/11 | pepper | Revolution |
| 11PF-16A | Field 2011 | A1 | 241 | 241 | 434 | 434 | 277 | 277 | 377 | 377 | 186 | 186 | 8/9/11 | pepper | Red Knight |
| 11PF-17A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 186 | 8/12/11 | pumpkin | Mystic Plus |
| 11PF-18A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 359 | 186 | 189 | 8/12/11 | pepper | Red Knight |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 11PF-19A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 8/12/11 | pepper | Red Knight |
| 11PF-20A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 8/15/11 | cucumber | Diva |
| 11PF-21A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 386 | 186 | 186 | 8/15/11 | cucumber | Diva |
| 11PF-22A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 8/18/11 | pumpkin | Mystic Plus |
| 11PF-23A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/18/11 | pumpkin | Mystic Plus |
| 11PF-24A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 186 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-25A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 291 | 359 | 359 | 186 | 189 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-26A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 291 | 359 | 359 | 186 | 189 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-27A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 277 | 377 | 386 | 186 | 186 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-28A | Field 2011 | A2 | 233 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-29A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-30A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 274 | 377 | 386 | 186 | 186 | 8/22/11 | pumpkin | Mystic Plus |
| 11PF-31A | Field 2011 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 377 | 186 | 189 | 8/22/11 | s. squash | Zucchini Elite |
| 11PF-32A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 8/22/11 | pepper | Revolution |
| 11PF-33A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 359 | 377 | 186 | 186 | 8/22/11 | pepper | Red Knight |
| 11PF-34A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/22/11 | pepper | Red Knight |
| 11PF-35A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/22/11 | cucumber | Diva |
| 11PF-36A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 8/22/11 | cucumber | Diva |
| 11PF-37A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 386 | 186 | 189 | 8/22/11 | cucumber | Diva |
| 11PF-38A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-39A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 359 | 359 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-40A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-41A | Field 2011 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-42A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-43A | Field 2011 | A2 | 241 | 260 | 434 | 446 | 277 | 277 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-44A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-45A | Field 2011 | A1 | 241 | 241 | 446 | 446 | 274 | 291 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-46A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 274 | 277 | 386 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-47A | Field 2011 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 386 | 392 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-48A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 11PF-49A | Field 2011 | A2 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-50A | Field 2011 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-51A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-52A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 274 | 277 | 386 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-53A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 277 | 386 | 386 | 186 | 189 | 8/24/11 | tomato | Celebrity |
| 11PF-54A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 377 | 186 | 186 | 8/24/11 | tomato | Celebrity |
| 11PF-55A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 8/25/11 | eggplant | Nadia |
| 11PF-56A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 8/25/11 | eggplant | Nadia |
| 11PF-57A | Field 2011 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 186 | 8/25/11 | eggplant | Nadia |
| 11PF-58A | Field 2011 | A1 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 189 | 8/25/11 | eggplant | Nadia |
| 12PF-01 A | Field 2012 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 6/27/12 | pepper | Red Knight |
| 12PF-02 A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 377 | 386 | 186 | 186 | 6/27/12 | pepper | Red Knight |
| 12PF-03 A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 277 | 277 | 377 | 386 | 186 | 186 | 6/27/12 | pepper | Red Knight |
| 12PF-04 A | Field 2012 | A1 | 241 | 241 | 434 | 446 | 277 | 277 | 359 | 377 | 186 | 189 | 6/27/12 | pepper | Red Knight |
| 12PF-05 A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 274 | 291 | 359 | 377 | 186 | 189 | 6/27/12 | tomato | Celebrity |
| 12PF-06 A | Field 2012 | A2 | 241 | 260 | 434 | 446 | 277 | 291 | 359 | 386 | 186 | 186 | 7/2/12 | pepper | Red Knight |
| 12PF-07 A | Field 2012 | A1 | 241 | 241 | 434 | 446 | 274 | 291 | 359 | 359 | 186 | 186 | 7/6/12 | s. squash | Zucchini Elite |
| 12PF-08 A | Field 2012 | A2 | 241 | 241 | 434 | 446 | 277 | 277 | 377 | 377 | 186 | 186 | 7/12/12 | s. squash | Zucchini Elite |
| 12PF-09 A | Field 2012 | A2 | 241 | 241 | 434 | 446 | 277 | 277 | 377 | 377 | 186 | 186 | 7/19/12 | s. squash | Zucchini Elite |
| 12PF-10 A | Field 2012 | A2 | 241 | 241 | 434 | 434 | 277 | 277 | 386 | 386 | 186 | 186 | 7/19/12 | s. squash | Zucchini Elite |
| 12PF-11 A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 291 | 291 | 359 | 359 | 186 | 189 | 7/19/12 | s. squash | Zucchini Elite |
| 12PF-12 A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 277 | 377 | 386 | 186 | 189 | 7/23/12 | s. squash | |
| 12PF-13 A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 386 | 186 | 189 | 7/23/12 | pepper | Red Knight |
| 12PF-14 A | Field 2012 | A2 | 241 | 241 | 434 | 446 | 277 | 291 | 359 | 377 | 186 | 189 | 7/23/12 | pepper | Red Knight |
| 12PF-15A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 274 | 277 | 377 | 386 | 186 | 186 | 7/25/12 | s. squash | Zucchini Elite |
| 12PF-16A | Field 2012 | A2 | 233 | 241 | 434 | 446 | 291 | 291 | 359 | 386 | 186 | 186 | 7/31/12 | pepper | Revolution |
| 12PF-17A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 189 | 8/6/12 | pepper | Red Knight |
| 12PF-18A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 377 | 377 | 186 | 186 | 8/7/12 | pepper | Red Knight |
| 12PF-22A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 291 | 291 | 359 | 359 | 186 | 189 | 8/27/12 | pepper | Red Knight |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| 12PF-23A | Field 2012 | A1 | 241 | 241 | 446 | 446 | 277 | 291 | 359 | 377 | 186 | 186 | 8/27/12 | pepper | Red Knight |
| 12PF-24A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 8/30/12 | s. squash | Zucchini Elite |
| 12PF-25A | Field 2012 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 189 | 9/5/12 | pepper | Red Knight |
| 12PF-26A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/5/12 | tomato | Celebrity |
| 12PF-27A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/5/12 | s. squash | Zucchini Elite |
| 12PF-28A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/12/12 | tomato | Celebrity |
| 12PF-29A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 291 | 291 | 359 | 386 | 186 | 186 | 9/12/12 | pepper | Red Knight |
| 12PF-30A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/14/12 | tomato | Celebrity |
| 12PF-31A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/14/12 | tomato | Celebrity |
| 12PF-32A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/14/12 | tomato | Celebrity |
| 12PF-33A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 277 | 277 | 377 | 377 | 186 | 186 | 9/14/12 | tomato | Celebrity |
| 12PF-37A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 386 | 186 | 186 | 9/24/12 | pepper | Red Knight |
| 12PF-38A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/27/12 | pepper | Red Knight |
| 12PF-39A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 277 | 377 | 377 | 186 | 186 | 9/27/12 | pepper | Red Knight |
| 12PF-40A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/27/12 | tomato | Celebrity |
| 12PF-41A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 291 | 359 | 359 | 186 | 189 | 9/24/12 | tomato | Celebrity |
| 12PF-42A | Field 2012 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 186 | 9/24/12 | tomato | Celebrity |
| 12PF-43A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 9/24/12 | tomato | Celebrity |
| 12PF-44A | Field 2012 | A2 | 241 | 241 | 434 | 434 | 277 | 291 | 386 | 386 | 186 | 186 | 9/17/12 | tomato | Celebrity |
| 12PF-45A | Field 2012 | A1 | 241 | 241 | 434 | 434 | 277 | 277 | 377 | 377 | 186 | 186 | 9/17/12 | tomato | Celebrity |
| 12PF-47A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/4/12 | tomato | Celebrity |
| 12PF-48A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/4/12 | tomato | Celebrity |
| 12PF-49A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/4/12 | pepper | Red Knight |
| 12PF-50A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/4/12 | s. squash | Zucchini Elite |
| 12PF-51A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/4/12 | tomato | Celebrity |
| 12PF-52A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/9/12 | tomato | Celebrity |
| 12PF-53A | Field 2012 | A2 | 241 | 241 | 446 | 446 | 274 | 277 | 377 | 377 | 186 | 186 | 10/9/12 | tomato | Celebrity |
| SC-02A | CD-4 | A2 | 239 | 241 | 434 | 446 | 274 | 274 | 383 | 383 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-03A | CD-4 | A2 | 241 | 241 | 434 | 446 | 274 | 274 | 386 | 386 | 186 | 189 | ~9/25/07 | pumpkin | |
| SC-04A | CD-4 | A2 | 241 | 241 | 434 | 434 | 291 | 291 | 377 | 377 | 186 | 189 | ~9/25/07 | pumpkin | |

**Figure AI.1 (continued)**

| Isolate | Sample population | MT | Alleles at microsatellite loci | | | | | | | | | | Date collected | Host | Cultivar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pcap1 | | Pcap3 | | Pcap5 | | Pcap7 | | SSRPC26 | | | | |
| SC-05A | CD-4 | A2 | 233 | 241 | 434 | 434 | 291 | 291 | 377 | 383 | 186 | 189 | ~9/25/07 | pumpkin | |
| SC-06A | CD-4 | A2 | 241 | 241 | 434 | 440 | 274 | 291 | 377 | 377 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-07A | CD-4 | A1 | 241 | 241 | 434 | 440 | 274 | 274 | 383 | 383 | 186 | 189 | ~9/25/07 | pumpkin | |
| SC-08A | CD-4 | A1 | 241 | 241 | 434 | 440 | 274 | 274 | 377 | 377 | 183 | 189 | ~9/25/07 | pumpkin | |
| SC-09A | CD-4 | A2 | 233 | 241 | 434 | 434 | 274 | 291 | 377 | 377 | 186 | 189 | ~9/25/07 | pumpkin | |
| SC-10A | CD-4 | A2 | 241 | 241 | 434 | 440 | 274 | 299 | 377 | 377 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-11B | CD-4 | A1 | 241 | 241 | 434 | 440 | 274 | 274 | 377 | 377 | 183 | 186 | ~9/25/07 | pumpkin | |
| SC-12A | CD-4 | A2 | 241 | 241 | 434 | 440 | 274 | 291 | 377 | 377 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-13.1A | CD-4 | A2 | 241 | 241 | 434 | 440 | 274 | 302 | 380 | 386 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-13.2B | CD-4 | A1 | 241 | 241 | 434 | 434 | 274 | 291 | 377 | 377 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-14.1A | CD-4 | A1 | 239 | 239 | 434 | 440 | 274 | 274 | 380 | 380 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-14.2A | CD-4 | A1 | 241 | 241 | 434 | 434 | 274 | 274 | 386 | 386 | 189 | 186 | ~9/25/07 | pumpkin | |
| SC-15A | CD-4 | A1 | 241 | 241 | 434 | 434 | 274 | 274 | 386 | 386 | 183 | 186 | ~9/25/07 | pumpkin | |
| SC-17A | CD-4 | A2 | 241 | 241 | 434 | 434 | 274 | 291 | 377 | 377 | 186 | 189 | ~9/25/07 | pumpkin | |
| SC-18A | CD-4 | A2 | 241 | 241 | 434 | 434 | 274 | 291 | 383 | 383 | 183 | 189 | ~9/25/07 | pumpkin | |
| SC-19A | CD-4 | A1 | 241 | 241 | 434 | 434 | 274 | 274 | 383 | 383 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-20A | CD-4 | A2 | 241 | 241 | 434 | 434 | 291 | 299 | 374 | 380 | 189 | 189 | ~9/25/07 | pumpkin | |
| SC-21A | CD-4 | A1 | 239 | 239 | 434 | 440 | 274 | 274 | 380 | 380 | 183 | 186 | ~9/25/07 | pumpkin | |
| SC-22A | CD-4 | A2 | 241 | 241 | 440 | 446 | 274 | 274 | 383 | 383 | 186 | 186 | ~9/25/07 | pumpkin | |
| SC-23A | CD-4 | A2 | 241 | 241 | 434 | 440 | 274 | 274 | 380 | 383 | 186 | 189 | ~9/25/07 | pumpkin | |

APPENDIX II

## Additional details on an *in vitro* cross between *Phytophthora capsici* isolates NY 0664-1 and NY 06180-4

### *Oospore germination protocol*

As described in Chapter 2, the research field population of *Phytophthora capsici* was compared to F1 progeny of an *in vitro* cross between the same parental isolates (NY 0664-1 and NY 06180-4), following protocols adapted from Donahoo and Lamour (2008) and Pavon et al. (2008). A more detailed description of the protocol for germinating and collecting single oospore isolates is given here.

The isolates were paired on 15% V8 agar (Lamour and Hausbeck 2001), and plates were sealed with parafilm and incubated in the dark at room temperature for two to three months to allow oospores to mature. Agar containing mature oospores was then cut from the center of each plate, placed in a round-bottom 2 ml microcentrifuge tube with a 4.5 mm sterile zinc-plated steel BB (Daisy Outdoor Products, Rogers, AR) and approximately 0.5 ml sterile distilled water, and disrupted by shaking in a TissueLyser (Qiagen, Valencia, CA) at 30 Hz for 30 s, twice. Ground agar was rinsed from the microcentrifuge tube with sterile distilled water and filtered through two layers, then four layers of cheesecloth to remove mycelial debris. After confirming the presence of oospores by examining a drop of the filtrate under the microscope, *Trichoderma harzianum* lysing enzyme (Sigma Aldrich, St. Louis, MO) was added to the filtrate at a rate of 1 mg/ml and shaken overnight (16-20 hrs) at 22-28°C and 100-165 rpm to digest any viable mycelia and sporangia. If hyphae and sporangia were not completely digested and hyphal growth had occurred overnight, the suspension was filtered through four layers of cheesecloth to remove

mycelia. Oospores were then concentrated by centrifuging at 3000 x *g* for 1 min and removing most of the supernatant, then treated with 1% $KMnO_4$ to achieve a final concentration of 0.1% $KMnO_4$, and vortexed for 10 min on the lowest speed. Oospores were then washed with sterile distilled water by centrifuging at 3000 x *g* for 1 min, removing the supernatant, adding 1 ml sterile distilled water, and repeating three times, or until the supernatant was clear. The pellet was resuspended and the presence of oospores was confirmed using a compound microscope before being incubated at 4°C for 3 days. After incubating at room temperature under lab lighting for an additional 3-4 days, the oospore suspension was diluted and spread on water agar plates. Single germinating oospores were picked within 24 hrs with the aid of a dissecting microscope and transferred to PARP (Schmitthenner and Bhat 1994; with 0.025 g rather than 0.1 g pentachloronitrobenzene).

### *References*


Donahoo, R. S., and Lamour, K. H. 2008. Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. Mycologia. 100:911–920.

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

Pavon, C. F., Babadoost, M., and Lambert, K. N. 2008. Quantification of *Phytophthora capsici* oospores in soil by sieving-centrifugation and real-time polymerase chain reaction. Plant Dis. 92:143–149.

Schmitthenner, A. F., and Bhat, R. G. 1994. Useful methods for studying *Phytophthora* in the laboratory. Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.

**Table AII.1** Growth of some single oospore progeny from an *in vitro* cross between *Phytophthora capsici* isolates NY 0664-1 and NY 06180-4. For each isolate, a 4 mm plug was transferred to each of three replicate 100 mm plates of 15% V8 media and plates were incubated in the dark at room temperature. After 3 days, two perpendicular measurements of colony diameter were made and averaged.

| Isolate | Type[a] | Mean diameter (mm) [b] |
|---|---|---|
| 68-32 | other | 75.5 a |
| 68-31 | other | 66.8 b |
| 68-09 | other | 62.5 c |
| 68-23 | other | 60.3 cd |
| 68-20 | other | 56.8 de |
| NY 0664-1 | parent | 56.0 e |
| 68-25[c] | other | 54.3 ef |
| 68-24 | other | 54.2 ef |
| 68-21[c] | other | 53.2 ef |
| 68-02 | other | 51.0 fg |
| 68-27 | possible self | 50.5 fgh |
| 68-10 | other | 50.3 fgh |
| 68-04[c] | other | 49.0 gh |
| 68-08[d] | other | 49.0 gh |
| 68-54[c] | other | 49.0 gh |
| NY 06180-4 | parent | 48.8 gh |
| 68-05 | cross | 48.7 ghi |
| 68-07 | other | 48.2 ghij |
| 68-11 | cross | 46.7 hijk |
| 68-47[c] | other | 44.7 ijkl |
| 68-01 | possible self | 44.2 jkl |
| 68-03[d] | other | 43.0 kl |
| 68-50 | other | 41.5 l |

[a] Isolates designated "possible self" contained only alleles from the A1 parent (NY 0664-1) at two microsatellite loci (Pcap5 and Pcap7), and therefore could be consistent with selfing. Isolates designated "cross" contained alleles from both parental isolates at these loci. Isolates designated "other" contained an allele from each parent at locus Pcap5, but were homozygous for an allele from the A1 parent (NY 0664-1) at locus Pcap7.

[b] Diameters are means of three replicate plates per isolate. Means followed by the same letter are not significantly different at $P = 0.05$ based on an ANOVA followed by a Tukey's Honestly Significant Differences test.

[c] Isolates 68-25, 68-21, 68-04, 68-54, and 68-47 shared the same genotype (mating type and alleles at five microsatellite loci).

[d] Isolates 68-08 and 68-03 shared the same genotype (mating type and alleles at five microsatellite loci).

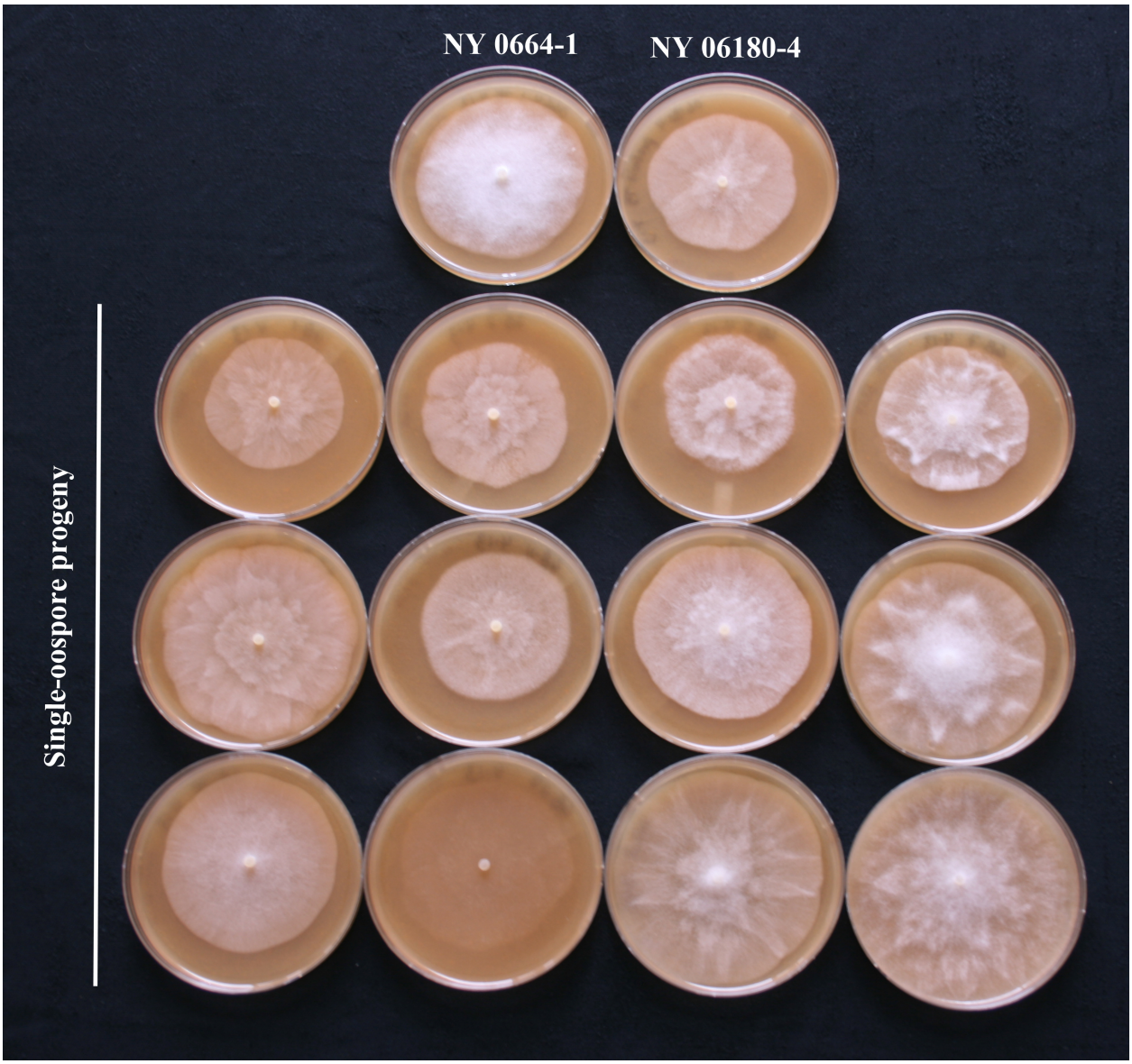



**Figure AII.1** Morphological variation among F1 progeny from the *in vitro* cross between *Phytophthora capsici* isolates NY 0664-1 and NY 06180-4. The parental isolates and 12 sample single-oospore progeny are shown after 4 days of growth at room temperature in the dark on V8 media. From left to right, single-oospore progeny are as follows: (top row) 68-1, 68-2, 68-5, 68-7; (middle row) 68-9, 68-11, 68-20, 68-23; (bottom row) 68-24, 68-27, 68-31, 68-32.